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The impact of sectors on agriculture based on artificial intelligence data: a case study on G7 countries and Turkiye

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DergiPark AKADEMİK

Abstract
The growing development of technology has had an impact on many sectors particularly business, communication, education and agriculture. In addition to its popularity, technology has brought many new concepts to the use of sectors, most of the important of which are cloud computing, artificial intelligence and cryptocurrencies. While the opportunities and concepts provided by technology have destroyed the existing job opportunities, they also introduced many positive opportunities like artificial intelligence, which can be considered as one of such positive innovations. The OECD artificial intelligence data of G7 countries and Turkey were used within the scope of this study. This study analyses the investment opportunities in agriculture and other sectors based on the artificial intelligence data. In addition to this study, both country-based and sectoral comparisons were made respectively. As a result, AI investments in the agricultural sector are generally at a lower level than other sectors. According to the analysis results, countries such as Türkiye and Canada are the countries that invest the most in the agricultural sector. This may reflect these countries' interest in agricultural potential and agricultural technology.

Keywords: Agriculture, G7 countries, artificial intelligence, venture capital investments, OECD.ai

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INTRODUCTION

The growing development and popularity of technology in time is a process that deeply affects human life (George and George, 2023). With the invention of the first computers, technology started to advance rapidly. Over time, computers have become smaller, more powerful and more accessible (Van Veldhoven and Vanthienen, 2022). With the widespread use of the Internet, access to information and communication has become easier accordingly (Khan et. al. 2020). The popularity of mobile technology has allowed people to stay connected anytime and anywhere. Smartphones, tablets and other portable devices have made people's daily lives more efficient and practical (Mayer, 2020). Moreover, the growth of social media platforms has changed the way people connect and share with each other (Alaimo et. al. 2020). On the other hand, the development of e-commerce and digital payment systems has radically changed shopping habits (Santos et. al. 2023). The spread of cloud computing technology has made data storage and processing processes more flexible and efficient (Atieh, 2021). New technologies such as artificial intelligence and machine learning have revolutionized business and industry (Bharadiya, 2023). Innovative products such as 3D printers, autonomous vehicles, smart home technologies are shaping the lifestyle of the future (Haktanır et. al., 2022). Overall, the development and popularity of technology has transformed the lives of humanity and drawn a promising roadmap for the future.

The impact of technology associated with its development has a vital importance in the modern world. Primarily, the advancement of technology in the healthcare sector has improved medical diagnosis and treatment methods, providing more effective care to patients (Paul et. al., 2023). With regard to the education sector, digital learning platforms and online resources have raised educational standards by providing students with access to a wider range of information (Akour and Alenezi, 2022). In the trade and retail sector, e-commerce and digital marketing techniques have transformed the shopping experience and increased customer satisfaction (Purnomo, 2023). The fintech innovations, payment systems and digital banking services emerged in the finance sector have

made financial transactions faster and more secure (Pazarbasioglu et. al., 2020). Automation and robotics technologies in the industry and manufacturing sector have turned production processes more efficient and cost-effective (Al Bashar et. al., 2024; Kahya and Özdüven, 2023). In the transport sector, autonomous vehicles and intelligent transport systems have provided safe and efficient transport while renewable energy technologies have increased energy efficiency by reducing environmental impacts (Iyer, 2021; Suman, 2021). Smart agricultural technologies emerged in the agriculture sector have supported the sustainability of agriculture by increasing productivity (Çağlar, 2024; Dhanya et. al., 2022). Furthermore, Figure 1 shows some of the benefits of artificial intelligence in the sectors.

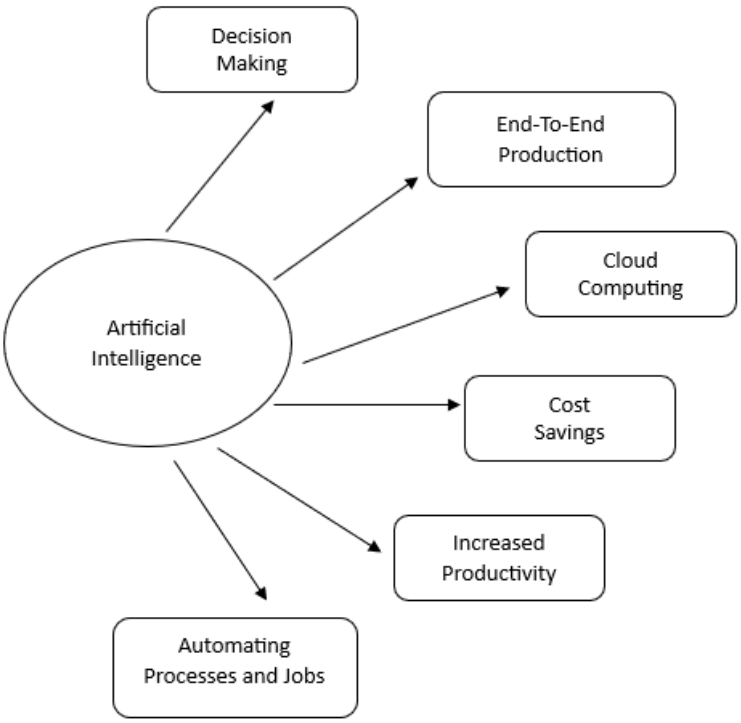

Figure 1. AI Benefits of Artificial İntelligence in Sectors

The agricultural sector experiences significant transformations with the impact of technological developments. Firstly, agricultural robots and automation systems have increased labor productivity by automating agricultural tasks (Mahmud et. al.,2020). Similarly, sensor technologies and smart farming applications monitor soil moisture, temperature and other important factors to improve the productivity of agricultural fields. In this way, water and fertilizer use is optimized and resources are used more efficiently (Ullo and Sinha, 2021). Climate forecasting and weather monitoring systems also provide farmers with important data to make the right decisions at the right time (Özbilge et. al.2020; Ceglar and Toreti, 2021). Advanced agricultural machinery and equipment also increase productivity, reduce workload and lower costs (Durai and Shamili, 2022; Atlı, 2023). Agricultural data analytics and artificial intelligence-based systems provide comprehensive data analysis for agri-businesses in making better decisions (Zhai et. al., 2020). Hence, it is possible to respond to market demands more quickly and flexibly. On the other hand, digital marketing and e-commerce platforms offer new opportunities in the marketing and sales of agricultural products (Ma and Zhang, 2022). Consequently, technological developments in the agricultural sector contribute to the development of more sustainable, efficient and competitive agricultural practices.

Among these innovative technologies, the opportunities provided by artificial intelligence are numerous. Artificial intelligence has offered so many possibilities that it has become revolutionary. Artificial intelligence has greatly facilitated the work of farmers by increasing efficiency and optimizing resource usage. In addition, it helps farmers in the early diagnosis of diseases and pests by monitoring plant health and growth processes thanks to precision agriculture practices. Data obtained using sensors or drones are analyzed with artificial intelligence algorithms and provide instant information about critical factors such as soil moisture, nutrient level and weather (Kamilaris & Prenafeta-Boldú, 2018). Prediction models are developed using artificial intelligence in data analysis. Analyzing many variables such as climate changes, market demands and crop cycles makes it easier for farmers to make strategic decisions (Wolfert et al., 2017). In addition, artificial intelligence-supported robots reduce labor costs by automating routine tasks such as weeding and harvesting in the fields (Shaikh et. al., 2022). Due to these unlimited advantages provided by artificial intelligence, it is thought that the agricultural sector's investment in artificial intelligence will yield positive results.

Literature Review

Upon the progressive development of technology and the increase in its usage areas, there have been developments in every sector in connection with such developments (Hervas-Oliver et. al., 2021). Therefore, new job opportunities have replaced the existing job opportunities. Yet this significant impact had a positive effect on all sectors in general. There have been very major positive effects especially in terms of time and money (Hu et. al., 2021).

In the literature, there are many studies regarding technology and sectors. This section reflects a number of studies on agriculture which is the research subject of the study, and artificial intelligence studies, which is one of the most popular technological developments.

The study titled "Agriculture and Artificial Intelligence" by Buğra GÜZEL and Ersan OKATAN (2022) from 2022, analyses with the effects and application areas of the use of artificial intelligence technologies in the agricultural sector. The study provides a comprehensive assessment of how artificial intelligence-based solutions can be used in different areas of agriculture where the authors discuss how artificial intelligence technologies in agriculture can contribute to factors such as productivity, sustainability and profitability. The study also highlights the current technological developments in the agricultural sector and the future potential of AI applications.

The article titled R&D and Innovation in the Agricultural Sector discusses the importance and impact of R&D (Research and Development) and innovation activities in the agricultural sector. The paper elaborates on the benefits of R&D and innovation in the agricultural sector in various aspects such as productivity growth, sustainability, competitiveness and market share gains. The research addresses the required policies and strategies for the promotion R&D and innovation in the agricultural sector and assesses the current situation respectively. Furthermore, the study also presents the effects of R&D and innovation activities in the agricultural sector with regard to producers, suppliers and consumers. It emphasizes the future potential and importance of R&D and innovation in the sector and argues that the relevant investments and support should be increased (Özaydin and Çelik, 2019).

This paper analyses the impact of artificial intelligence (AI) technology on company growth and product innovation. It uses various methods to analyze the impact of AI technology on companies' growth performance and product innovation. It concluded that the use of AI can increase the growth rate of companies and promote product innovation. The paper also explores the potential of AI technology to increase the competitive advantage of companies. It also addresses the effects of artificial intelligence technology on companies' marketing strategies and product development processes through underlining the importance for businesses to adopt and integrate AI technology (Babina et. al., 2024).

The article titled "Artificial Intelligence Investments in Turkey: An Evaluation in the Context of Strategic Management" analyses artificial intelligence investments in Turkey from a strategic management perspective. It discusses the Turkey's current situation and future potential in the field of artificial intelligence and the impact of artificial intelligence investments on the competitiveness of the country. The research analyses Turkey's strengths and weaknesses in the field of artificial intelligence and emphasizes the significance of artificial intelligence investments in terms of strategic management. Additionally, the study argues the Turkey's national policies and strategies in the related field and discusses the contribution of artificial intelligence investments to the economic and social development of the country. The article concludes the steps and strategic planning, which are required to enhance the Turkey's competitiveness regarding AI (Ercan, 2022).

METHODOLOGY

Within the framework of this study, OECD (Organization for Economic Co-operation and Development) data are used accordingly. The main objective of OECD is to coordinate economic and social policies among member countries, promote economic growth, increase welfare and contribute to the development of international trade (Canton, 2021).

OECD has developed a data platform called "OECD.ai", which provides comprehensive data and analyses on AI. This allows researchers, policy makers and other stakeholders to access information and make policy decisions on artificial intelligence (Anna et. al. 2022; Tricot, 2021). This platform includes various data sets covering the economic, social and ethical aspects of AI where the data set called "investments in AI and data" designed to help users better understand and make decisions about investments in AI and data (OECD.AI, 2024) was used for this study which analyses investments in AI and data technologies and visualizes their global trends and distribution. With this dataset, users can explore, compare and analyze AI and data investments from different countries, sectors or institutions. Hence, it is possible to learn about the size, distribution, sectoral focus and other important characteristics of investments in AI and data.

Upon using OECD.ai "Investments in AI and data" datasets, artificial intelligence investments in G7 countries were analyzed per specific sectors. From the perspective of this study, G7 countries and Turkey were compared on a sectoral basis. G7 countries were used in this study because they provide a strong basis for understanding global economic, political, technological and environmental dynamics. The policies and practices of these

countries provide valuable insights for predicting global trends and future developments (Koca, 2022; Yürükoğlu, 2021; Demir, 2021). In this context, Turkey's position among the G7 countries was evaluated and compared. While evaluating each G7 country according to its artificial intelligence investments, important sectors in the world were discussed. Firstly, artificial intelligence data in each G7 country and Turkey were analyzed from the general dimension under two factors "Sum of Investments (USD in millions)" and "Number of Investments" both of which were determined through the related dataset. On the other hand, artificial intelligence data in 5 different sectors from OECD.ai dataset were compared on the basis of each country as "1-Agriculture, 2-Energy, Raw Materials And Utility, 3-Environmental Services, 4-Logistic, Wholesale And Retail, 5-Media, Social Platforms, Marketing".

SPSS version 20 was used to analyse the artificial intelligence data set. Tests such as Anova and Tukey tests were performed to determine the differences or similarities between countries and sectors.

RESULTS AND FINDINGS

The main objective of this study is to compare G7 countries and Turkey with major sectors, particularly agriculture, through artificial intelligence data.

Table 1. Venture Capital Investment in AI

Venture Capital (VC) Investments in AI (Sum of Investments USD in millions)						
Items	N	Minimum	Maximum	Sum	Mean	Std. Deviation
SumVCinUSD*	96	0	114320	509795	5310,36	15663,47
AgriVCinUSD*	96	0	1441	7326	76,31	214,010
EnergyVCinUSD*	96	0	586	3898	40,60	86,427
EnvVCinUSD*	96	0	454	2462	25,65	65,364
LogVCinUSD*	96	0	2732	16893	175,97	480,227
SocVCinUSD*	96	0	15772	60706	632,35	2114,865
Venture Capital (VC) Investments in AI (Number of Investments)						
Items	N	Minimum	Maximum	Sum	Mean	Std. Deviation
SumVCNumber	96	0	2602	26185	272,76	520,431
AgriVCNumber	96	0	31	374	3,90	7,235
EnergyVCNumber	96	0	29	283	2,95	4,891
EnvVCNumber	96	0	22	181	1,89	3,769
LogVCNumber	96	0	91	704	7,33	15,347
SocVCNumber	96	0	326	3955	41,20	73,978

*SumVCinUSD= Sum of Venture Capital investment in USD, *AgriVCinUSD=Agriculture Venture Capital investment in USD, *EnergyVCinUSD= Energy, Raw Materials And Utility Venture Capital investment in USD, *EnvVCinUSD= Environmental Services Venture Capital investment in USD, *LogVCinUSD= Logistic, Wholesale And Retail Venture Capital investment in USD, *SocVCinUSD= MEDIA, SOCIAL PLATFORM, MARKETING Venture Capital investment in USD.

Table 1 provides the statistics of venture capital (VC) investments in artificial intelligence (AI) based on 96 different samples. In the first part, statistics on the total amount of VC investments (in millions in USD) are given where the minimum value of total VC investments is 0 and the maximum value is 114320 million USD. The average investment amount is 5310,36 million USD and the standard deviation is 15663,47 million USD. The second part presents statistics on the number of VC investments. Pursuant to these statistics, the total number of VC investments is 96 with a minimum of 0 and a maximum of 2602. The average number of investments is 272.76 and the standard deviation is 520.431. Additionally to table 1, the social sector consistently receives the highest investment amounts and number of investments, indicating a strong focus on AI applications in social contexts. Conversely, the environmental sector sees the lowest investment and fewer projects, suggesting a potential area for growth. The logistics sector, while not as prominent as the social sector, still shows substantial investment, reflecting the importance of AI in optimizing logistics operations.

Table 2. AI Investments Between Countries

Venture Capital (VC) Investments in AI (Sum of Investments USD in millions)								
Items	Germany	USA	UK	Italy	France	Japan	Canada	Türkiye
SumVCinUSD*	16351	427126	29873	1136	11033	8578	14730	968
AgriVCinUSD*	0	5539	644	7	216	367	539	14
EnergyVCinUSD*	388	2127	358	13	236	242	502	32
EnvVCinUSD*	8	1654	325	53	187	69	166	0
LogVCinUSD*	832	13579	472	16	960	350	682	2
SocVCinUSD*	1800	51048	2415	314	1540	1261	1677	651
Venture Capital (VC) Investments in AI (Number of Investments)								
Items	Germany	USA	UK	Italy	France	Japan	Canada	Türkiye
SumVCNumber	1130	17768	2651	187	1014	2020	1351	64
AgriVCNumber	0	237	30	1	17	34	49	6
EnergyVCNumber	25	153	25	2	20	20	31	7
EnvVCNumber	4	108	46	1	10	1	11	0
LogVCNumber	39	491	53	1	38	46	34	2
SocVCNumber	135	2667	411	46	194	280	213	9

*SumVCinUSD= Sum of Venture Capital investment in USD, *AgriVCinUSD=Agriculture Venture Capital investment in USD, *EnergyVCinUSD= Energy, Raw Materials And Utility Venture Capital investment in USD, *EnvVCinUSD= Environmental Services Venture Capital investment in USD, *LogVCinUSD= Logistic, Wholesale And Retail Venture Capital investment in USD, *SocVCinUSD= MEDIA, SOCIAL PLATFORM, MARKETING Venture Capital investment in USD.

Table 2 presents data on the total amount of venture capital (VC) investments in artificial intelligence (AI) (in millions of USD) and the number of investments. In the first part, the total amounts of VC investments in different countries are given. The second part of table shows the number of VC investments in different countries. Likewise, the distribution of VC investments by agriculture, energy, environment, logistics and social areas is presented separately. These data show the investment extent of different countries on artificial intelligence and their particular focus areas. Besides of these, the data clearly indicates that the USA is the leader in VC investments in AI across all sectors both in terms of the total investment amounts and the number of investments. Countries like Germany, the UK, and Japan also show significant activity, particularly in specific sectors like logistics and social applications. In contrast, countries like Italy and Turkey have much lower figures, highlighting the disparity in AI investment levels globally.

The table with data on Venture Capital (VC) investments includes the total amount and number of investments by year. The first section shows the total amount of VC investments (in millions of USD) by year. The investment amount, which was 2909 million USD in 2012, increased to 65507 million USD in 2023. Details of VC investments in agriculture, energy, environment, logistics and social areas are also provided. In the second section, the number of VC investments by year is given. The number of investments increased from 507 in 2012 to 2849 in 2023. Moreover, the number of VC investments in agriculture, energy, environment, logistics and social areas are also elaborated. Addition to these comments, the data underscores the increasing importance of AI across various sectors, driven by the potential for innovation and efficiency. The peak in 2021 likely reflects a culmination of technological advancements, investor confidence, and perhaps pandemic-driven digital transformation. Despite a slight decline post-2021, the investment levels remain high, indicating a strong future for AI technologies in driving global innovation and economic growth.

In table 4, values followed by the same letter or letters in same columns do not significantly differ from each other according to the Tukey's HSD (Honesty Significant Difference) test at $p<0,05$. The analysis reveals a clear global hierarchy in AI investments, with the USA leading both in the total sum and number of investments. Other countries like Turkey, Italy, Japan, France, Canada, Germany, and the UK are actively participating in the AI investment landscape but with lower volumes. This categorization underscores the USA's pivotal role in driving AI advancements and the collective efforts of other nations to foster AI development, albeit on a smaller scale. The varied investment levels across different sectors also highlight the strategic focus areas for each country, shaping their AI innovation trajectories.

Table 3. AI Investments Between Years

Venture Capital (VC) Investments in AI (Sum of Investments USD in millions)						
Year/Items	SumVCinUSD	AgriVCinUSD	EnergyVCinUSD	EnvVCinUSD	LogVCinUSD	SocVCinUSD
2012	2909	42	1	28	22	634
2013	4206	30	79	91	17	823
2014	13693	73	140	31	389	1632
2015	21549	108	175	32	847	2153
2016	18710	228	161	72	910	1889
2017	25755	524	230	142	873	2212
2018	38939	758	424	110	1392	3774
2019	50598	645	404	222	2500	5821
2020	56600	1215	256	186	1801	4343
2021	136832	1959	807	572	3481	13566
2022	74497	1025	595	632	3042	7342
2023	65507	719	626	344	1619	16517
Venture Capital (VC) Investments in AI (Number of Investments)						
Year/Items	SumVCNumber	AgriVCNumber	EnergyVCNumber	EnvVCNumber	LogVCNumber	SocVCNumber
2012	507	3	2	4	7	112
2013	751	10	6	4	12	173
2014	1054	12	14	8	26	217
2015	1385	15	12	6	37	269
2016	1696	23	10	10	45	309
2017	2073	31	19	9	55	361
2018	2571	34	30	7	63	401
2019	3026	47	32	15	80	452
2020	3136	51	27	23	81	448
2021	3827	59	50	27	129	481
2022	3310	45	45	39	94	365
2023	2849	44	36	29	75	367

Table 4. Tukey HSD Test

Venture Capital (VC) Investments in AI (Sum of Investments USD in millions)						
Countries/Items	SumVCinUSD	AgriVCinUSD	EnergyVCinUSD	EnvVCinUSD	LogVCinUSD	SocVCinUSD
Turkiye	b	b	b	b	b	b
Italy	b	b	b	b	b	b
Japan	b	b	b	b	b	b
France	b	b	b	b	b	b
Canada	b	b	b	b	b	b
Germany	b	b	b	b	b	b
UK	b	b	b	b	b	b
USA	a	a	a	a	a	a
Venture Capital (VC) Investments in AI (Number of Investments)						
Countries/Items	SumVCNumber	AgriVCNumber	EnergyVCNumber	EnvVCNumber	LogVCNumber	SocVCNumber
Turkiye	b	b	b	c	b	c
Italy	b	b	b	c	b	bc
Japan	b	b	b	c	b	bc
France	b	b	b	bc	b	bc
Canada	b	b	b	bc	b	bc
Germany	b	b	b	c	b	bc
UK	b	b	b	b	b	b
USA	a	a	a	a	a	a

Table 5. Percentage share

Items / Countries	Germany	USA	UK	Italy	France	Japan	Canada	Türkiye
PercentShareAgri	0.00% b	1.04% ab	1.02% ab	0.02% b	0.37% b	0.69% b	2.64% a	0.93% b
PercentShareEnergy	1.59% a	0.43% a	0.32% a	0.07% a	1.18% a	1.37% a	1.82% a	4.13% a
PercentShareEnv	0.02% a	0.56% a	0.58% a	0.10% a	0.48% a	0.04% a	0.47% a	0.00% a
PercentShareLog	3.82% a	2.94% a	0.97% a	0.03% a	3.35% a	1.73% a	3.92% a	5.03% a
PercentShareSoc	13.27% b	12.39% b	16.07% ab	38.79% a	17.66% ab	21.13% ab	16.66% ab	25.93% ab

Table 5 shows the percentage shares of VC investments in the agriculture, energy, environment, logistics and social sectors of different countries. In the field of agriculture, Canada's share is the highest with 2.64 percent, followed by the USA's 1.04 percent and the UK's 1.02 percent. This distribution highlights the strategic priorities of different countries in artificial intelligence investments; While Türkiye focuses strongly on Agriculture, Energy and Logistics, Italy focuses on Social Artificial Intelligence investments. USA, UK, France, Germany, Japan and Canada exhibit different investment models in these sectors.

Investments in the agricultural sector are generally at a lower level than other sectors. In particular, it is seen that investments in the agricultural sector are quite limited in European countries such as Germany, England and Italy. While it was expected that the agricultural sector in these countries would be subject to VC investments compared to other sectors, this did not happen. Countries such as Türkiye and Canada invest more in the agricultural sector. This may reflect these countries' interest in agricultural potential and agricultural technology. However, in general, investments in the agricultural sector appear to be less than in other sectors.

CONCLUSION

The effect of technology on sectors has significantly improved user experience and quality of life while increasing efficiency by improving business processes. However, the rapid advancement of technology has led to the change of traditional business models in some sectors and the emergence of new competitive environments. For instance, the abundance of data that comes with digital transformation has allowed businesses to make better decisions and gain competitive advantage. Thus, the speed and complexity of technological innovations have led to a lack of competence and infrastructure challenges in some sectors. Therefore, a continuous investment in training and infrastructure is required to reduce the digital divide between sectors. In conclusion, the impact of technology on sectors is a necessary factor to gain competitive advantage in the ever-changing business world and this process needs to be managed and adapted.

It is seen that investments made in the agricultural sector are generally lower than other sectors. It is noteworthy that investments in the agricultural sector are quite limited, especially in European countries such as Germany, England and Italy. However, it appears that Canada has a significant share in investments in the agricultural sector. It can be said that Canada's agriculture-based economy and its predisposition to agricultural technology affect this situation. On the other hand, a significant increase is observed in investments in Turkey's agricultural sector. Turkey's agricultural potential and developments in agricultural technology may be effective in increasing these investments.

Consequently, these findings provide many important benefits for developing and investing in new technologies. First, it guides strategic investment decisions and determines which sectors countries and companies should focus on. Optimizing resource allocation helps use resources in the most efficient way. Determining areas of technology development shows in which areas countries are leaders and in which areas they need development. It can accelerate global technological progress by unlocking opportunities for international cooperation and partnerships. Additionally, identifying market opportunities creates new business opportunities for entrepreneurs and investors. Governments can encourage AI investments by using this data in policy and regulatory developments. Companies and research organizations can optimize their strategies by conducting competitive analysis. In terms of training and talent development, educational institutions can determine in which areas they need to train more experts. Developing technological infrastructure is necessary to support AI investments. Finally, by planning long-term, countries and companies can more effectively plan their future technology and investment strategies. These findings are critical for developing and investing in new technologies and increasing competitive advantage.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author has no conflict of interest to declare.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Effect of entomopathogenic *Beauveria bassiana* (Balls.) Vuill. isolates on *Myzus persicae* (Sulzer) (Hemiptera: Aphididae)

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DergiPark AKADEMİK

Abstract

The aim of the study was to determine the lethal effect of 1 x 10^8 conidia mL^{-1} concentration of local *Beauveria bassiana* isolates (ET 10, BMAUM-M6-4, Bb 1) against *M. persicae* under laboratory conditions. For the spraying method, *B. bassiana* isolates was applied to *M. persicae* nymphs with a hand spray. In this context, the experiments were carried out in a randomized plots experimental design with ten replicates with ten nymphs in each Petri plate. After the applications, the number of live individuals was recorded by counting the 1st, 3rd, 5th and 7th days and the percentage mortality rate was calculated. On the third and fifth days of the experiment, the highest mortality rates of 64 and 95% were recorded for the Bb 1 isolate of *B. bassiana*, respectively. In the seventh day counts, 100% mortality rates were determined for Bb 1 and ET 10 isolates and 99% for the BMAUM-M6-4 isolate. In addition, the mortality date (LT_{50}) values were calculated as 3.62 days for ET 10 isolate, 3.60 days for BMAUM M6-4 isolate, and 2.93 days for Bb 1 isolates, respectively. As a result, it is thought that *B. bassiana* isolates can be used in biological control practices within the scope of integrated pest management program against *M. persicae*.

Keywords: Entomopathogenic fungi, *Beauveria bassiana*, *Myzus persicae*, Mortality, Biological control

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INTRODUCTION

Aphids (Hemiptera: Aphididae) are important pests of many vegetables and fruits (Tang et al., 2017). More than 5000 aphid species have been identified worldwide (Satrio Arinanto et al., 2024). The most damaging and widespread species of aphids include *Myzus persicae* (Sulzer, 1776); *Brevicoryne brassicae* L.; *Macrosiphum euphorbiae* Thomas; and *Aphis gossypii* Glover (Hemiptera: Aphididae) (Javed et al., 2019). Among these species, *M. persicae* is one of the most important aphid species that is harmful to agricultural areas and greenhouses. Green peach aphid *M. persicae* (Hemiptera: Aphididae) is a polyphagous insect pest, cause damage more than 400 plant species in more than 50 families and economic losses in many horticulture crops. Due to its rapid reproductive capacity, *M. persicae* prevents the growth rate and development of the plant from decreasing (Blackman and Eastop, 2000; Tang et al., 2017; Kumar and Paul, 2017). *M. persicae* individuals are harmful by sucking the sap of plants and release toxic substances into the plant during sucking. It also causes fumagine by releasing a honey-like substance. *M. persicae* is the vector of more than 100 plant viruses in approximately 30 different families (Diaz et al., 2009; Torres-Quintero et al., 2013). It is stated that the pest is resistant to many chemicals used in chemical control, including organophosphates, carbamates and pyrethroids (Devonshire et al., 1998). Chemical pesticides cause undesirable side effects on products, beneficial organisms, humans and the environment. Due to these negative effects, the need for sustainable alternative control methods is increasing (Kingsley Nwosu and John, 2022; Nicolopoulou et al., 2016). One of these changes is the use of entomopathogenic fungi (EPF). Entomopathogenic fungal pesticides are important in Integrated Pest Management Programs (IPM) and cause diseases in insects, suppressing their growth and reproduction rates (Thomas and Read, 2007). Considering the studies carried out; various EPFs such as *Lecanicillium* sp. (Jung et

al., 2006, Steenberg and Humber, 1999), *Beauveria bassiana* (Quesada-Moraga et al., 2006), *Metarhizium anisopliae* (Shia and Feng, 2004) and *Isaria farinosa* (Shia and Feng, 2004). They are reported to be effective biocontrol agents used in the control against aphids and many other pests. Although *B. bassiana* (Bals.) Vuill. one of these EPFs, is generally isolated from infected insects (Vega et al., 2008, Allegrucci et al., 2020), it is also obtained from various soil types such as peat bogs, mountain soil and desert soil (Zimmermann, 2007). It is reported that *B. bassiana* has 707 different hosts, including 521 genera, 149 families and 15 orders. *B. bassiana* causes disease in the orders Lepidoptera, Coleoptera, Hymenoptera, Diptera, Hemiptera, Orthoptera, Siphonaptera, Isoptera, Thysanoptera, Mantodea, Neuroptera, Dermaptera, Blattariae and Embioptera (Zimmermann, 2007). Entomopathogenic fungi have many benefits, such as being effective in all development stages of the insect, not creating resistance in pests, not being poisonous to mammals, controling in nature for a long time, being applied together with insecticides, and being cheap and easy to apply (Sevim et al., 2015). For these reasons, entomopathogenic fungi have a significant advantage in the control against harmful insects.

The objective of the present study was to evaluate the lethal effect of 1 x 10^8 conidia mL^{-1} concentration of ET 10, BMAUM-M6-4, Bb 1 isolates of the entomopathogenic fungus *B. bassiana* against *M. persicae* under laboratory conditions. Thus, it was discussed whether it is possible to use these EPF isolates within the scope of integrated pest management.

MATERIALS AND METHODS

Production of pepper plants and *Myzus persicae*

Mostar green pepper cv (*Capsicum annuum* L.) from the Solanaceae family was used for host plant production. Pepper seedlings obtained from a private company were transplanted into plastic pots (20 cm diameter) containing a mixture of soil, perlite and peat (1:1:1) and transplanted. Then, these pots which the pepper seedlings were planted in were kept in the growth chamber. No external fertilizer or pesticide application was made during the growing of the seedlings. However, diseased and harmful seedlings were removed from the plant growth chamber.

Aphids used in the experiment were grown on pepper plants in the plant growth chamber. Pepper leaves containing *M. persicae* were propagated by transferring them onto pepper seedlings that had reached a certain height and number of leaves. Fresh pepper seedlings were transferred periodically to ensure aphid stock culture. The production of *M. persicae* individuals and pepper plants was carried out in the plant growth chamber [25±1°C, 60±5% RH and photoperiod 16:8 h (L:D)] in the Entomology laboratory within the Department of Organic Farming Business Management, Faculty of Applied Sciences, Pamukkale University.

Preparation of EPF isolates and spore suspensions

The EPF isolates of *B. bassiana* used in this study are given in Table 1. Three local isolates of *B. bassiana* were obtained from different hosts and locations in Türkiye. All *B. bassiana* isolates were developed within the dark at 25±1°C for 7–15 days and after that subcultured on potato dextrose agar (PDA-Difco). Conidia were harvested from 7-15 days old entomopathogenic *B. bassiana* cultures by adding 10 mL of 0.01% Tween 20 to cultures on agar plates (100 mm) and gently scraping the surface of the cultures with a sterile inoculating loop to dislodge the conidia from the surface of the agar plates. Spor suspension was stirred by a magnetic shaker for 10 min. In order to calculate the spore density from the prepared suspension, a 10^{-2} dilution was made and counted with a Neubauer hemocytometer under light microscope, and spore suspensions with a density of 1 x 10^8 conidia mL^{-1} were prepared for each *B. bassiana* isolate (Fancelli et al., 2013).

Table 1. Fungal isolates, sources and locality of the EPF isolates used for bioassay study

Isolates	Source	Locality	References
Beauveria bassiana/ ET 10	*Sphenoptera antiqua*	Erzurum, Türkiye	Tozlu et al. (2017)
Beauveria bassiana/ BMAUM-M6-4	Field soil	Isparta, Türkiye	Baydar et al. (2016)
Beauveria bassiana/ Bb 1	Forest soil	Düzce, Türkiye	Erdoğan and Sağlan (2023)

Application of *B. bassiana* isolates to *M. persicae* nymph

In the experiments, thinly cut sponge and sterile blotting paper were placed on the bottom of plastic Petri plates (100 mm diameter). A pepper leaf was placed on the blotting paper and a ring (2 cm diameter) was placed in the middle of this leaf, to which the aphids could be transferred. There was a ring in each Petri plate, and the second stage nymphs of *M. persicae* were transferred into these rings under a binocular stereo microscope with the help of a fine-tipped brush. By spraying method, spore suspensions of *B. bassiana* isolates (ET 10, BMAUM-M6-4 and Bb 1) containing 1 x 10^8 conidia mL^{-1} were applied to the transferred aphid nymphs with a hand sprayer, 2 mL per petri plate. As a control, sterile distilled water containing 0.01% Tween 20 was sprayed onto the second stage nymphs. Experiments were carried out with 10 replicates and 10 nymphs in each

replication. Numbers of live nymph were recorded at 1st, 3rd, 5th, and 7th after spraying. The experiment was carried out in a plant growth chamber with 25±1°C temperature, 60±5% RH, photoperiod 16:8 (L: D) conditions.

Statistical analysis

One-way analysis of variance (One-Way ANOVA) was applied to the data obtained after angle transformation. The differences between the means were determined by the Tukey's multiple comparison test at the P≤0.05 significance level (Tukey, 1949). Statistical analyzes were carried out with the SPSS® 20.0 package program. ın addition, LT_{50} values were determined by the Probit analysis program (Throne et al., 1995).

RESULTS AND DISCUSSION

The mortality rates of ET 10, BMAUM-M6-4 and Bb 1 isolates of *B. bassiana* to second instar nymph of *M. persicae* by spraying method are given in Figure 1. After applying ET 10, BMAUM-M6-4 and Bb 1 isolates of *B. bassiana*, the differences between the mortality rates in *M. persicae* nymphs and the mortality rates in nymphs in the control group on the 1st, 3rd, 5th and 7th days were found to be statistically significant. On the first day after application, the highest mortality rate recorded in the BMAUM-M6-4 isolate with 16%, followed by ET 10 and Bb 1 isolates with a 12% mortality rate, and EPF isolates were statistically in the same group. The highest mortality rates were detected in the Bb1 isolate on the third and fifth days of application, with 64 and 95%, respectively. BMAUM-M6-4 (50-67%) and ET 10 (41-59%) isolates followed the Bb 1 isolate, respectively. EPF isolates were found to be statistically different in the 5th day counts. In the seventh day counts, a 100% mortality rate was recorded for Bb 1 and ET 10 isolates, and a 99% mortality rate for BMAUM-M6-4 isolate, and all EPF isolates were statistically in the same group (Figure 1). LT_{50} values of ET 10, BMAUM-M6-4 and Bb 1 isolates of *B. bassiana* applied to *M. persicae* nymphs exposed to 10^8 conidia mL^{-1} concentration are given in Figure 2. Percentage mortality rates increased with time after the application. LT_{50} values for ET 10, BMAUM-M6-4 and Bb 1 isolates were calculated as 3.62, 3.60 and 2.93 days, respectively.

The fungi causing pathogenecity in insects have been observed to cause mortality in insect pest populations and therefore studied for their use as biological control agents (Hesketh et al., 2008; Freed et al., 2012). Studies conducted on *Beauveria*, *Metarhizium* and *Isaria* spp. and some *Lecanicillium* spp. isolates were highly effective on aphids (Hayden et al., 1992; Vu et al., 2007; Kim and Kim, 2008). The entomopathogenic fungi potential against target aphid populations is different for different isolates and also varies from strain to strain. The study showed that *B. bassiana* is effective for the control of aphids on different crops at different concentrations. As a result of application of *B. bassiana* isolates 5493, JW-1 and GHA to the first instar nymphs of *M. persicae*, mortality rates of 91%, 100% and 56% were recorded (Jandricic et al., 2014). In another study, 100% mortality rate was recorded as a result of the application of *B. bassiana* isolate at a concentration of 1.0 x 10^8 conidia mL^{-1} to the third stage nymphs of *M. persicae* (de Loureiro and Moino, 2006). Berber and Birgücü (2020) recorded 98 and 92% mortality rates, respectively, nine days after treatment of second and third instar nymphs of *M. persicae* with LD.2016 and M6-4 isolates of *B. bassiana* at a concentration of 1.0 x 10^8 conidia mL^{-1}. It is reported that *B. bassiana* isolate applied at different concentrations throughout the study increased the percentage mortality value depending on the dose increase. Seven days after treatment of *M. persicae* individuals with *B. bassiana* ART41 and ART2580 isolates, mortality rates of 92 and 98% were recorded, respectively (Lefort et al., 2014).A mortality rate of over 80% occurred on the tenth day after the application of a dose of 10^7 conidia mL^{-1} of the *B. bassiana* isolate to *M. persicae*. In other studies, mortality rates of over 75% were recorded in adult *M. persicae* adults exposed to *Beauveria* isolates (Hesketh et al., 2008; Shan and Feng, 2010; Tesfaye and Seyoum, 2010). In another study, treatment of *M. persicae* individuals with Bb-72 and Bb-252 isolates of *B. bassiana* resulted in 91 and 95% mortality (Nazir et al., 2019). As a result of application of *B. bassiana* strains (Bb-202) (1.0×10^2 to 6.75×10^5 conidia mm^2) to *M. persicae* individuals, 100% mortality was detected (Bugti et al., 2018). As a result of another study conducted with the *B. bassiana* LPSC 1067 isolate, an increase in the mortality rate and a decrease in the formation of new nymphs were recorded in *M. persicae* adult individuals (Allegrucci, et al., 2020). LT_{50} values of LD.2016 and M6-4 isolates of *B. bassiana* were 6.19 and 5.5 days, respectively at 10^8 conidi mL^{-1} dose (Berber and Birgücü, 2020). Accordingly, the LT_{50} values of 1×10^5 , 1×10^6 , 1×10^7 , and 1×10^8 conidia/mL^{-1} concentrations of *Beauveria bassiana* strain 202 were calculated for *M. persicae* that were determined as 5.2~8.24, in days (Bugti et al., 2018). Our results are parallel to the results obtained in previous studies.

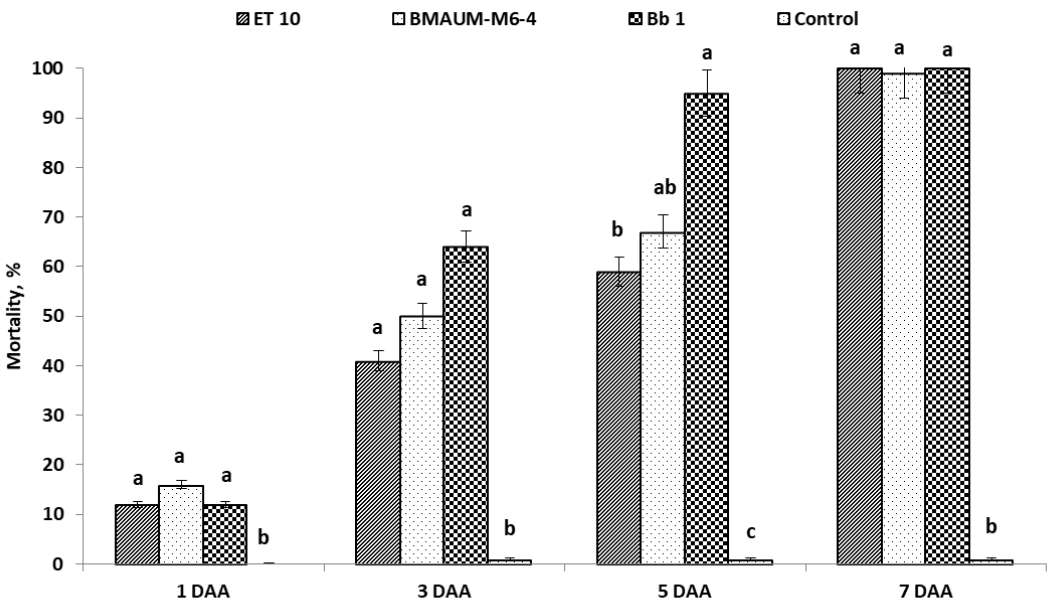

Figure 1. Mortality (%) of *Myzus persicae* nymphs inoculated with *Beauveria bassiana* isolates (1×10^8 conidia mL^{-1}. Different lowercase letters represent statistically significant differences among mortality, between treatments according to Tukey's HSD test ($P \leq 0.05$). DAA= days after application

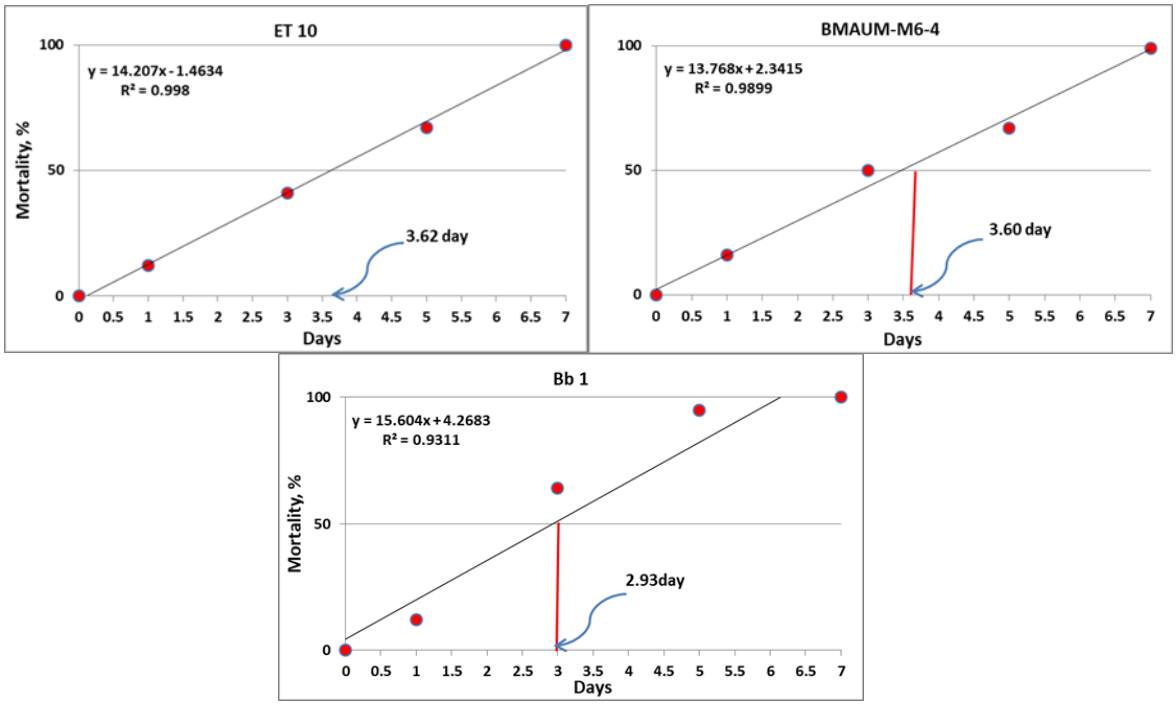

Figure 2. The mean LT_{50} values of ET 10, BMAUM-M6-4 and Bb 1 isolates of *Beauveria bassiana* applied to *Myzus persicae* nymphs

CONCLUSION

As a result of the study, ET 10, BMAUM-M6-4 and Bb 1 isolates of *B. bassiana* showed an effect against *M. persicae* from the 1st day of application, and this effect reached 99-100% on the 7th day. According to the results obtained from the study, the use of *B. bassiana* isolates in the control against *M. persicae* was found to be promising. Entomopathogenic fungi are a suitable alternative to chemicals since they do not have any toxic effects on mammals. However, efficacy trials need to be conducted with *B. bassiana* isolates under field and greenhouse conditions. Under field conditions, 60-80% reduction in *M. persicae* population was recorded after

the application of *B. bassiana* (CG-864 and PL-63) isolates (Filho et al., 2011). It is reported that relative humidity of 90% and above in greenhouse conditions increases the activity of *B. bassina* (Shipp et al., 2003). Additionally, temperature and other factors were found to be effective in the growth and speed of fungi (Orozco-Avitia et al., 2013). If these isolates are found successful in the studies, these isolates can be used in biological control applications within the scope of an integrated pest management program against *M. persicae*.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Assessing the influence of partial canopy cover and temperature variability on late-season dehydration in grape berries

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Abstract
Late-season dehydration (LSDN) is a physiological disorder affecting grape berry water content, resulting in dehydration. Vineyards in the Aegean Region of western Türkiye have experienced problems with LSDN, particularly during periods of high temperatures. This research examines how partial canopy covering materials affect temperature differentials inside and outside the canopy, including the determination of LSDN grape berries of Sultan 7 (*Vitis vinifera* L.). A partial shading net (PS) was employed to prevent LSDN in the grape berries, and shading net and polyethylene material (PSP) were deployed to assess the impact of increasing canopy temperatures on the occurrence of LSDN in grape berries. Although partial covering materials did not substantially affect grapevine yield, the control group produced the largest and the heaviest berries. In the second year, warmer conditions led to more clusters with LSDN-affected berries and increased sunburn damage on clusters. PS showed a high healthy cluster rate of 72.50%, while PSP and control showed lower rates of 63.60% and 58.10%, respectively. Throughout the study period, PS exhibited 9.02% LSDN berries, while the control and PSP showed 17.10% and 16.70% clusters with LSDN berries in the total harvested clusters, respectively. The study showed that PS treatment alleviated LSDN symptoms in clusters.

Keywords: Sultan 7, Raisin, Grape, Berry Shrivel, Quality

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INTRODUCTION

As a component of climate change, maximum temperatures are becoming a significant problem in the Mediterranean Region due to limited water availability and overheating in many areas (Cramer et al., 2018). Moreover, in recent years, the European viticulture industry has encountered significant challenges due to the adverse effects of climate change. Rising temperatures have adversely impacted grape cultivation and the sustainability of viticulture (Fraga et al., 2020; Santillán et al., 2020). New scenarios on climate change worldwide show that the rise in global greenhouse gas emissions is anticipated to result in a temperature increase beyond the 1.5°C threshold, thereby impeding efforts to restrict the temperature rise below 2°C beyond the year 2030 (IPCC, 2023). The elevated temperatures affect the growth cycle, including the flowering and ripening phase, which could cause a decline in the production of fresh grapes, raisins (Teker and Soltekin, 2023), and wine quality (van Leeuwen et al., 2019).

Previous studies in the Aegean Region, the western part of Türkiye, one of the warmest parts of the country where raisin production is prominent, have revealed that extreme temperatures during the flowering period and weather fluctuations in a month (both low and high temperatures) cause berry-shattering problems on clusters (Teker and Soltekin, 2023). Additionally, another study in the region has shown that sun exposure on berries can lead to sunburn issues, particularly on the west side of the vine canopy in north-south (NS) oriented vineyards during the hot summer afternoons (Teker, 2023). In light of these recent studies, it is evident that growers in this region may face many problems over many seasons due to adverse climate events, thus requiring them to take action.

Late-season dehydration (LSDN) is a type of berry shrivel observed in some grape cultivars in the Aegean Region (Teker and Altındişli, 2021) and is classified as a physiological disorder in grapevines (Krasnow et al., 2010). As for raisin production, many growers prefer to prune canes with many winter buds on grapevines for the following summer to obtain more clusters per vine, which leads to various problems. It sometimes triggers this physiological disorder depending on cultural practices and irrigation conditions, especially during the hot season for the Sultan 7 grape variety. Additionally, specific physiological responses of grapevines, such as stomatal conductance and midday leaf water potential, may indicate stress conditions, particularly in overloaded grapevines with excessive bud numbers. This problem could lead to dehydration of grape berries. The findings also suggest that environmental conditions may have accelerated this condition in grapes with LSDN (Teker and Altındişli, 2021).

The present study examined the effect of utilizing a shading net and polyethylene covering material on the temperature inside and outside the vine canopy. The study also investigated the effects on grape yield and quality and the effectiveness of this approach in mitigating the emergence of LSDN berries. Therefore, the study aims to investigate three primary questions: 1) How do partial shading and polyethylene covering materials influence the vine canopy and LSDN symptoms in grape berries? 2) How do variations in the inside and outside temperature values of vine canopies impact yield and quality? 3) Can partial covering of the vine canopy prevent sunburn damage to grape berries?

MATERIALS AND METHODS

Plant material and experimental site

The experiment was carried out in the 2019 and 2020 growing seasons in a trial vineyard with six-year-old vines (*Vitis vinifera* L. cv. Sultan 7) grafted onto 1103 Paulsen rootstock at the Viticulture Research Institute in Manisa, Türkiye (38° 37' North, 27° 24' East). The vines were planted with a spacing of 3.0 m X 2.0 m (row by vine), and the row orientation was north and south. Vines were trained using a goblet system on a V-shaped trellis with six wires and a cane pruning system positioned 100 cm above the soil surface, with approximately 18 buds per eight canes (corresponding to around 150 buds per vine). The vineyard soil was characterized as clay loam, moderately deep, and well-drained. A sub-surface drip irrigation system was installed at the experimental site. Emitters with a nominal flow rate of 4 L h^{-1} were spaced at 50 cm intervals on the lateral drip lines. Irrigation for all the vines in the study was started when water availability was reduced to 50% at an effective root depth of 0.90 meters. The irrigation period began with the berry set and continued until 15 days before harvest in 2019 and 2020. The total amount of water, excluding rainfall, was approximately 97 mm m^{-2} in 2019 and 118 mm m^{-2} in 2020.

Climatic conditions and phenological observations

The climate in the study area is typical of the Mediterranean. A warm-temperate climate prevails at the experimental site, with hot, dry summers and little rainfall (Teker and Altındisli 2021; Teker 2021). Mid-term data (1991–2020) shows that the average annual precipitation and temperature for Manisa province were 724.6 mm and 17.1°C, respectively (TMS, 2024). A weather station (iMETOS IMT280, Pessl Instruments, Weiz, Austria) was used to record temperature (T) and precipitation (P) data at the experimental site. To record the phenological development of grapevines, budburst, flowering, berry set, veraison, and harvest stages were determined using the modified Eichhorn & Lorenz (EL) system (Coombe, 1995).

Experimental setup

A completely randomized design with three treatments and six replications in three blocks was used; all treatments were assigned to each block. A guard row was left between treatments to eliminate shading effects on the cluster zone. Before the experimental setup, six soil samples were collected along the row to determine soil structure and composition differences. All soil samples showed similar results, and the slope of the field did not differ significantly. Therefore, the experimental conditions were the same for all vines and treatments.

In this study, three treatments were arranged in the vineyard: (A) control, (B) with a 35% partial shading net (PS), and (C) with a 35% partial shading net plus a plastic cover (PSP), positioned at 50 cm above the vine canopy (Figure 1). For all treatments, except the control (Figure 1A), green artificial shading nets (UV-resistant) were used at 35% for the shading cover, and transparent polyethylene (UV-resistant) was used for the plastic cover. Between berry set and harvest, a 35% shade net was applied for both PS and PSP treatments (Figure 1B and 1C), and for the PSP treatment, a plastic cover was also placed over the 35% shading net between veraison and harvest (Figure 1C).

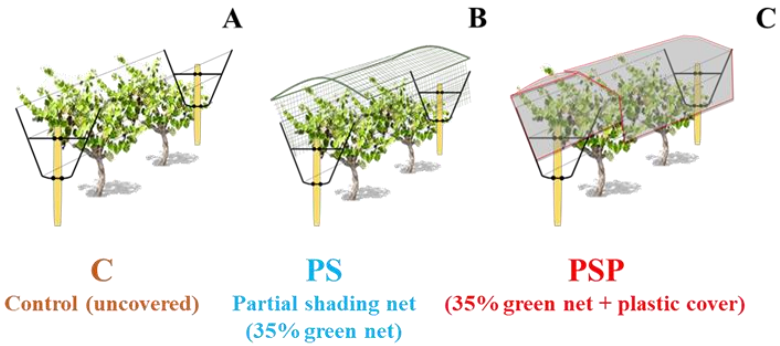

Figure 1. An illustration of the applications used in the study.

Canopy microclimate

Microclimate measurements of the vines were conducted between berry set and harvest during the 2019 and 2020 growing seasons. Temperature (°C) was monitored using portable data loggers (HOBOware, UX-100-003, Onset Computer Corp., MA, USA) at two sections of the canopies within each treatment: (1) above the canopy and (2) inside the canopy, specifically within the vine foliage in the cluster zone. Data loggers were positioned 35 cm above the canopy in the control (air temperature), PS (under shading net), and PSP treatments (under shading net and plastic cover film) to record temperatures (1). The others were installed 120 cm above the ground inside the canopy for all treatments (2). Mid-term temperatures were evaluated during three specific periods: from July 16th to July 31st, from August 1st to August 15th, and from August 16th until the harvest date (August 28th) for the 2019 season; and from July 23rd to July 31st, from August 1st to August 15th, and between August 16th and the harvest date (September 04th) for the 2020.

Yield parameters

The vine yield (kg vine^{-1}) and the number of clusters per vine were recorded at harvest. The cluster weight (g) was calculated by taking the per vine yield and dividing it by the number of clusters. To determine the berry weight (g), samples of 100 berries were used. Additionally, 20 samples in each replicate were measured for berry length (mm) and diameter (mm) using a digital vernier caliper (Mitutoyo Instruments. Illinois, USA).

Classification of harvested clusters

In this study, specific criteria were applied to classify grape clusters, considering their health and the presence of certain conditions. Clusters without any damage on berries were categorized as "healthy clusters." These clusters were deemed optimal, exhibiting no visible damage or abnormalities affecting the berries. Additionally, we identified clusters with berries that had suffered damage due to sunburn, which were distinctly labeled as "clusters with sunburned (SN) berries." Furthermore, we introduced a distinct category to address a different condition observed in some clusters. Berries in the final and third classifications experienced a loss of water content without being affected by sunburn. These clusters have been identified and classified as "late-season dehydration (LSDN) clusters" (Krasnow et al., 2010; Teker and Altındisli, 2021). The clusters above were counted, and the percentages of clusters in the total harvested clusters were calculated for each classification.

Statistical analyses

Statistical analyses were conducted using SPSS Statistics 21.0. software (IBM, Chicago, IL, USA). The normality of the data was assessed with the Shapiro-Wilk test, and homoscedasticity of variance was checked using Levene's test. The data was subjected to a two-way ANOVA to compare the means ($p \leq 0.05$). For canopy microclimate hourly temperature values, differences in mean values among the treatments were calculated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Weather conditions of the experimental site and phenological observations

The weather conditions at the experimental site during the growing seasons of 2019 and 2020 are depicted in Figure 2. The graph illustrates the monthly minimum, mean, and maximum temperatures and the recorded rainfall amount. During the 2020 growing season, temperatures were notably higher than in 2019. In the second year, there was an increase in heat conditions. Throughout the 2020 growing season from June to September, it was observed that temperatures surpassed critical thresholds of 40°C, 35°C, and 30°C on numerous occasions. More specifically, there were 22 days with temperatures exceeding 40°C, 44 days with temperatures surpassing 35°C, and 22 days with temperatures exceeding 30°C.

Based on the recorded rainfall data, a significant difference in the total precipitation between the two years of the study was observed. The total precipitation recorded during the first year amounted to 777.4 mm, while the figure for the second year was 533.2 mm. The experimental site recorded 196.4 mm of rainfall between March 1st and August 31st of 2020, significantly higher than the cumulative rainfall of 128.0 mm recorded over the same

period in the previous year. During the summer of 2020, there was a significant lack of rainfall. The month of June had 36.0 mm of precipitation, but the months of July and August did not experience any precipitation.

Regarding the phenological stages of grapevine, budburst (EL-4) was observed on March 26th and March 19th in 2019 and 2020, respectively. In 2019, flowering (EL-26) occurred on May 16th, veraison (EL-35) on July 16th, and the grape harvest based on grape maturity (EL-38) was achieved on August 28th. In the following year, 2020, flowering occurred on May 18th, followed by veraison on July 20th. The harvest was completed on September 4th.

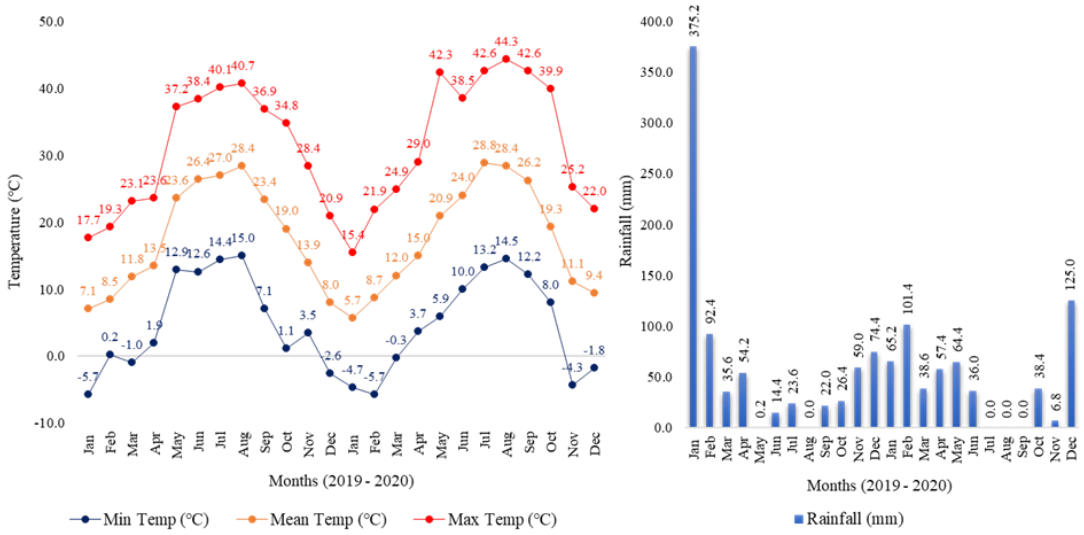

Figure 2. Minimum (Min Temp,°C), mean (Mean Temp, °C), maximum (Max Temp, °C), and rainfall (mm) values in the 2019 and 2020 growing seasons.

Canopy microclimate

Polyethylene covering material with shading nets on canopies in PSP treatment substantially increased the outside temperature of grapevine canopies. In both study years, the temperature outside the canopies significantly differed between the PSP and PS treatment and between the control (Figure 3A and 3B). Upon comparison with the control, it was observed that an average difference of approximately +2.30°C to +2.93°C occurred in 2019, and a difference of +3.18°C to +3.57°C was seen in 2020. This difference coincided with higher air temperature values, particularly in the second year of the study. On the other hand, shading nets (35%) used in the PS treatment also prevented an increase in outside canopy temperatures. It was found that there was no significant difference in the temperatures recorded outside the vine canopy between the PS treatment and the control. However, the temperature difference between the two experimental groups ranged from 0.5 to 1°C. In 2019, the mean temperatures for the control group showed variability within the range of 27.44°C to 28.45°C, while shading treatments displayed temperatures ranging from 26.36°C to 27.5°C. In the following year, temperature fluctuations were noted between 28.24°C and 29.79°C for the control group, while the PS treatment showed temperature variations between 27.81°C and 29.16°C (Figure 3A and 3B).

The findings indicate that the temperature inside the vine canopy significantly differs from outside in PSP treatment. The grapevine foliage in the canopy provided cooling shade and played a significant role in regulating the temperature. Applying partial shading net covers to vine canopies and leaving both sides of cluster zones open significantly reduced temperatures inside the canopy due to improved air circulation, compared to outside temperatures of the canopy, for PSP treatment. The temperature readings were lower in the control group and PSP treatments, particularly in 2019. Nevertheless, the foliage within the canopy offered some shading, resulting in temperature readings within the PSP treatment becoming more comparable to those in the control group.

Consequently, the temperature inside the PSP canopy was cooler than above the canopy when covered with the materials. Although no statistical significance was observed on many measurement days, the PS application caused a temperature difference ranging from 0.5°C to 1°C inside the canopy. Therefore, it was concluded that a 35% shading cover within the above vine canopy positively affected the microclimate temperature conditions (Figure 3C and 3D).

Temperature records were taken outside the vine canopy during the warmest parts of the day (from 12:00 h to 16:00h) to determine the thermal effect of the PSP treatment, which involved using polyethylene covering material. Our findings suggest that the PSP treatment had a significant thermal effect compared to the control group and PS treatment. The temperature readings obtained from the PSP treatment indicate that the highest temperatures were

recorded between 15:00h and 16:00h in the 2019 and 2020 growing seasons. Notably, in 2020, the peak temperature reached 46.34°C, surpassing the previous year's highest temperature record of 43.85°C outside the vine canopies (Figure 4A and 4B). In both years of our study, we observed a significant decrease in heat effect in PS treatment. Therefore, our findings indicate that using shading nets resulted in a more pronounced reduction in inside canopy temperatures compared to the control group (Figures 4C and 4D). It was determined that as the day reached its peak temperature, the temperature variance between the control group and PSP gradually diminished. This was considered due to the polyethylene covering utilized in the PSP treatment, which generates a temperature effect. After analyzing the inside canopy temperatures over two years, it was observed that the control group and PSP treatment had similar values.

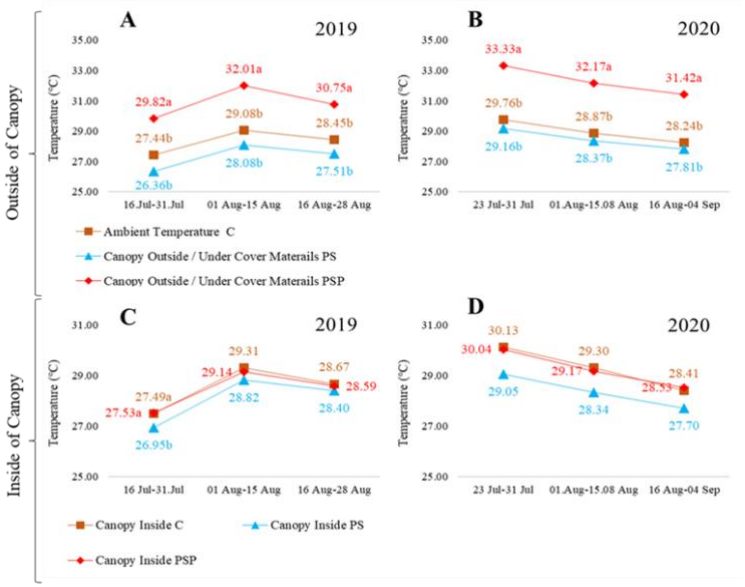

Figure 3. Temperature changes in the outside (A and B) and the inside (C and D) of vine canopy in the 2019 and 2020 growing seasons, respectively, for each treatment between veraison and harvest over a three-period. According to Duncan's test, lowercase letters indicate statistically significant differences ($p < 0.001$).

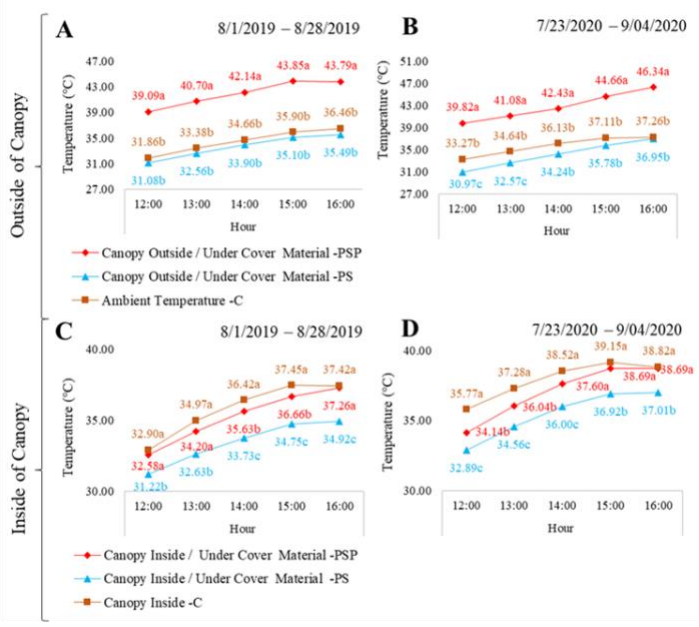

Figure 4. The graph displays the temperature fluctuations in time intervals (between 12:00h and 16:00h) both outside (A and B) and inside the vine canopy (C and D) from veraison to harvest over a three-period for each treatment in the 2019 and 2020 growing seasons. According to Duncan's test, lowercase letters indicate statistically significant differences ($p < 0.001$).

The present study has demonstrated that applying PS treatment has resulted in significant reductions in temperature levels at specific time intervals, thereby providing convincing evidence of its effectiveness in regulating the temperature inside the canopy. This study also found that the PS treatment could keep cooler canopy temperatures during specific periods when the heat was at its peak, even though there was a decrease in temperature difference between the PSP treatment and control group during the hottest hour of the day.

Yield parameters

The study results indicated that none of the treatments significantly impacted vine yield, number of clusters, and cluster weight values over the years. The average cluster weights for the control group, PS, and PSP treatments were 386.68 g, 416.43 g, and 398.72 g, respectively. However, berry weights were similar in the PS (1.74 g) and PSP (1.72 g) treatments, while the control group (1.85 g) had the highest values statistically. In both years, the control group consistently caused the highest berry diameter (12.37 mm) and length (15.45 mm) values. In the second year of the study, it was noted that the cluster number value was lower than expected despite a high cluster weight value. This difference was due to a high load per vine (150 buds per vine) during the first year, resulting in decreased bud fertility and lower yield in the following year. The findings of this study are supported by another study by Teker and Altındisli (2021). In addition, this study showed that shading covers, typically considered to have a negligible impact on yield, can result in variations in berry weight, like in the previous study (Miccichè et al., 2023).

Table 1. Effects of uncovered (C), partial shading net (PS), and partial shading net + polyethylene (PSP) on yield components of 'Sultan 7/ 1103P' grapevines[abc].

		Vine Yield (kg vine^{-1})	Cluster	Cluster Weight (g)	Berry Weight (g)	Berry Diameter (mm)	Berry Length (mm)
2019	C	17.83 ± 2.36	53.11 ± 5.91	337.19 ± 38.09	1.85 ± 0.13	12.03 ± 0.24	15.31 ± 0.31 ab
	PS	18.38 ± 2.21	50.65 ± 7.45	394.66 ± 32.74	1.77 ± 0.15	11.56 ± 0.54	14.72 ± 0.72 bc
	PSP	20.24 ± 2.63	57.26 ± 6.83	373.95 ± 36.30	1.74 ± 0.18	11.19 ± 0.37	14.19 ± 0.61 c
2020	C	18.24 ± 2.89	43.01 ± 6.38	436.18 ± 98.76	1.85 ± 0.12	12.72 ± 0.25	15.60 ± 0.28 a
	PS	19.54 ± 1.79	44.90 ± 4.01	438.21 ± 53.38	1.72 ± 0.06	12.26 ± 0.50	15.44 ± 0.59 a
	PSP	19.12 ± 2.98	45.63 ± 4.35	423.49 ± 83.22	1.70 ± 0.12	12.50 ± 0.21	15.69 ± 0.35 a
Mean (T)	C	18.04 ± 2.65	48.06 ± 7.93	386.68 ± 89.73	1.85 ± 0.12 A	12.37 ± 0.42 A	15.45 ± 0.33 A
	PS	18.96 ± 2.09	47.77 ± 6.26	416.43 ± 49.34	1.74 ± 0.12 B	11.91 ± 0.63 B	15.08 ± 0.75 A
	PSP	19.68 ± 2.90	51.45 ± 6.70	398.72 ± 72.34	1.72 ± 0.15 B	11.85 ± 0.72 B	14.94 ± 0.90 B
Years (Y)	2019	18.82 ± 6.97	53.67 ± 2.57^{A}	368.60 ± 43.14^{B}	1.78 ± 0.16	11.59 ± 0.53^{B}	14.74 ± 0.73^{B}
	2020	18.97 ± 5.14	44.51 ± 2.66^{B}	432.62 ± 80.94^{A}	1.75 ± 0.12	12.49 ± 0.39^{A}	15.57 ± 0.44^{A}
p-value	T	ns	ns	ns	*	*	*
	Y	ns	*	*	ns	*	*
	T*Y	ns	ns	ns	ns	ns	*

[a]Values in each column are indicated mean ± standard deviations of the mean.
[b]ns shows non-significant; * indicates p value ≤ 0.05
[c]T and Y indicate 'Treatments' and 'Years', respectively.

There is a divergence of opinions concerning the influence of partial cover or shading applications on vine yield. However, the reported studies do not present a universally applicable paradigm regarding the impact of shading nets on vine yields (Pallotti et al., 2023). Some findings of previous studies indicate that there was no statistically significant decrease in total yield in Cabernet Sauvignon grapevines when covered with a 60% shading net at full fruit set, despite only a 10% decrease in yield (Martínez-Lüscher et al., 2020). A study in Australia on Syrah grapevines indicated no difference in total yield with a 62% shading capacity treatment (white cloth above the canopy) compared to the control group (Caravia et al., 2016). Conversely, some studies claimed that covering material like green or white net decreases the vine yield (Miccichè et al., 2023).

On the other hand, some studies showed that shading covers exceeding 70% coverage on cluster zones are positively correlated with an increase in marketable yield (Cataldo et al., 2022). Scafidi et al. (2013) found that artificial shading increased berry weight in the Grillo cultivar. Findings in the present study indicate that the control group yielded the heaviest berries while covering treatments such as PS and PSP decreased berry weight.

Healthy and unhealthy clusters

As part of the research, three groups of grape clusters were analyzed, and one of them was kept as the control group while the other two were treated with PS and PSP. After the harvest, the results revealed that 58.1% of the grape clusters in the control group were healthy, whereas 26.40% showed sunburn (SN) symptoms and 17.1% displayed late-season dehydration (LSDN) symptoms (mean values of the years). According to the visual analysis, 72.5% of grape clusters in the PS group were found to be healthy, with 17.50% displaying SN symptoms and 9.02% showing LSDN symptoms. In contrast, the PSP group had 63.60% healthy grape clusters, while 19.6% exhibited SN damage and 16.7% displayed LSDN symptoms (Figure 5). Therefore, the main effects of the

treatments indicate that the PS treatment had the highest percentage of healthy clusters (72.50%), while the control group (58.10%) and PSP (63.60%) had the lowest percentage in the same category, statistically.

Additionally, the PS treatment showed the lowest incidence of grape berries affected by LSDN (9.0%) in both years. The study observed an increase in LSDN-affected berries in both the control group and PSP treatment. The control group showed the highest percentage of berries affected by SN damage, while both the PS and PSP treatments had similar SN damage levels, resulting in statistical grouping. In reviewing the data over the study years, it is evident that the number of healthy clusters in all treatments decreased from the first year to the second year. Similarly, there was an increase in clusters showing LSDN symptoms and SN damage. This variation can be attributed to the warmer and drier weather in 2020.

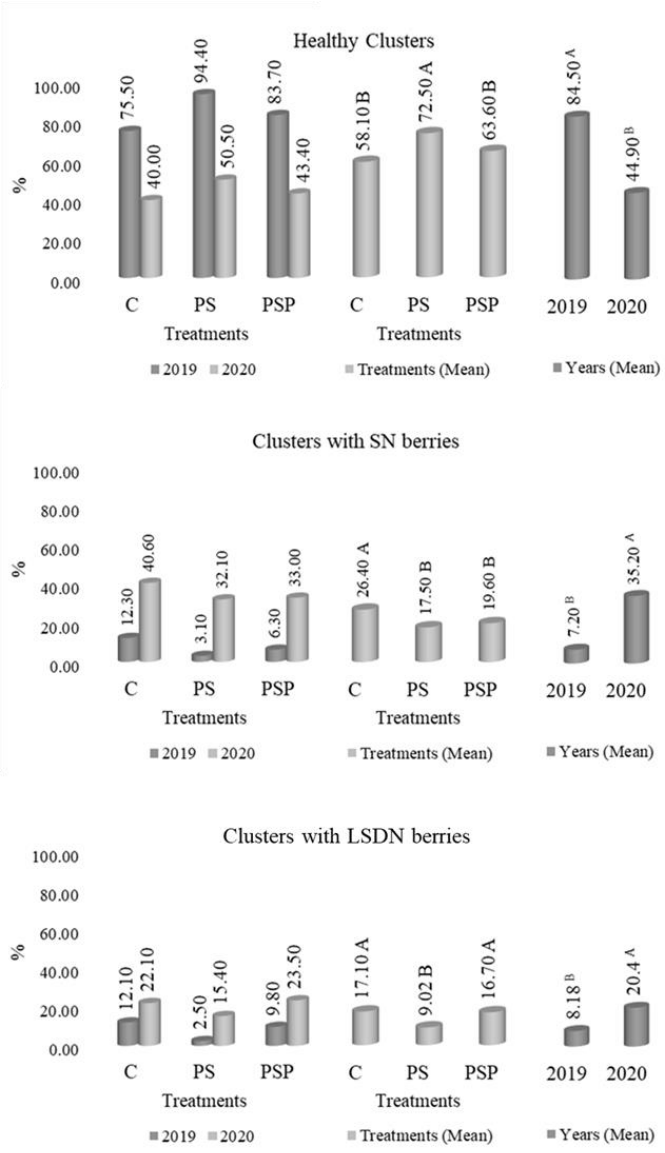

Figure 5. Comparison of uncovered, partially shading net (35%), and shading net (35%) + polyethylene (PSP) on healthy clusters, sunburned (SN) clusters, and LSDN clusters in the total harvested clusters, as a percentage (%). The uppercase values indicate statistically significant differences in treatments and study years ($p \leq 0.05$).

Previous studies showed that water loss in grape berries can be prevented by covering the cluster zone with shading material, effectively preventing the shriveling of grape berries (Lobos et al., 2015; Caravia et al., 2016). The quantity of berry dehydration was reduced by half when the Touriga Nacional vines cluster zone was shaded after fruit set and veraison (Oliveira et al., 2014). Likewise, it was noted that shaded Cabernet Sauvignon clusters exhibited significantly lower fruit damage and dehydration compared to exposed clusters (Martínez-Lüscher et al., 2020). As in these studies, the present study shows that PS treatment has a positive impact on reducing berry dehydration, particularly in comparison with the control group.

CONCLUSION

In recent years, Sultan 7 (*Vitis vinifera* L.) grapes have emerged as the preferred cultivar for seedless grape production. The grape variety is favored for its high yield in the Aegean Region of western Türkiye. However, the cultivar is prone to late-season dehydration during its final growth stages of grape berries. Previous studies indicated that this occurs because of an excessive cluster yield due to overloaded buds. Overloaded grapevines may be more susceptible to adverse weather conditions during the summer, particularly when temperatures reach 38°C or higher. As a result of this condition, grape berries may lose their water content by the end of the season, resulting in symptoms of late-season dehydration.

This research underscores the significance of preserving healthy grape clusters and shielding them from the adverse impacts of air and vine canopy temperatures. Compared to the first year of the study, there was a 39.6% decline in healthy clusters in 2020, the warmest year. Furthermore, there was a 12.2% rise in late-season dehydration issues and a 28.0% increase in clusters affected by sunburn across all applications. These shifts were linked to temperature factors, increasing late-season dehydration, and sunburn problems on grape berries. As such, viticulturists must adopt adaptive strategies to mitigate the adverse effects of high temperatures and other environmental stressors on vineyards.

Regulating optimal canopy temperatures, with particular attention to the vine canopy, is important to mitigate late-season dehydration in grape berries. The findings of the present study will be valuable for future research on exploring different ratios of shading materials exceeding 35%. Fully covered canopy systems may be more effective in preventing grapevine berries from late-season dehydration symptoms than partial coverings. It is also essential to assess the potential impact of covering the sides of the canopy (especially for cluster zones) to gain a more comprehensive understanding and mitigate late-season dehydration symptoms.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare no competing, actual, potential, or perceived conflict of interest.

Author contribution

TT conceived, designed, and performed the experiment; analyzed the data; wrote the paper. OS designed and performed the experiment analyzed the data, and reviewed the article. ETÖ assisted with the measurements in the experimental vineyard. All authors read and approved the manuscript.

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The effects of mixes of peat and olive pomace at various ratios on the vegetative growth of potted grapevine saplings

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Abstract

Numerous studies have been conducted in order to utilize the olive pomace, the solid waste left over from the oil processing of olives, which is widely cultivated, especially in Mediterranean countries, and to consider it a raw material that has added value instead of being considered waste. Some of these research are focused on establishing the re-utilization of olive pomace in agricultural production. Although some studies have reported that olive pomace can be utilized as fertilizer, soil improvement regulator, solid media culture, and even mulching material, this study was carried out due to the lack of sufficient scientific data on whether or not this material can be used as a growth medium in the cultivation of potted grapevine saplings. The study was carried out by growing ungrafted grapevine saplings of *Vitis vinifera* L. cv. Hatun Parmağı on media with peat and olive pomace at different ratios for six months, and then some vegetative growth parameters were examined. Due to the elevated olive pomace ratios in the growth medium, shoot and root growth of the grapevine saplings were restricted. The chlorophyll index and root fresh weight decreased dramatically when more than 25% (v/v) and 20% (v/v) crude pomace were available in the growth medium, respectively. Moreover, the availability of olive pomace in the medium significantly reduced shoot length, number of leaves, shoot weight, and leaf weight of grapevine saplings, regardless of the amount of olive pomace. However, it was found that 15% (v/v) or less of crude olive pomace could be utilized in growth medium mixtures when growing potted grapevine saplings; however, higher ratios would prevent the saplings from attaining marketable quality. While designing new studies, examining different grape varieties, fruit species, and lower olive pomace ratios would contribute to new and more comprehensive findings on the utilization of olive pomace in growing potted saplings.

Keywords: Olive Waste, Growing Media, *Vitis vinifera* L., Potted Sapling

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INTRODUCTION

Nowadays, research on the re-utilization of organic wastes left over from agricultural production and processing of agricultural products in agriculture and industry branches has been concentrated worldwide due to its economic contribution and the opportunity to save the cost of storage and the space allocated for storage (Taurisano et al., 2014; Obi et al., 2016; Harshwardhan and Upadhyay, 2017; Duque-Acevedo et al., 2020). Some of the studies on the re-utilization of agricultural wastes are carried out to establish whether they are eligible for re-utilization in agricultural production (Koul et al., 2022). The literature contains many studies reporting that

plant wastes can be re-utilized as feed, fertiliser, soil conditioner, seedling and sapling growth medium (El-Mashad et al., 2003; Gruda, 2019; Sharma et al., 2019; Adegbeye et al., 2020; Raza et al., 2022; Lasoń-Rydel et al., 2022). Some of these studies have shown that the waste material utilized can be re-utilized in crop production due to its organic structure, richness in minerals, and rapid dissolution qualities. Indeed, the prerequisite for successful plant cultivation is the provision of the environmental conditions required by plants. As well as climatic conditions, the presence of sufficient, balanced, and absorbable minerals in the plant root is highly important for growing healthy plants (Marschner, 1995; Ağaoğlu et al., 2010; Koç et al., 2021). In recent years, studies on the possibility of using low-cost organic materials free from diseases and pests in this production model have increased with the effect of the widespread use of soilless agriculture (Carlile et al., 2015; Chrysargyris et al., 2021; Gruda, 2022). Furthermore, the global depletion of peat and perlite reserves, extensively utilized in soilless cultivation, and the emergence of waste issues in materials such as rock wool and glass wool make it necessary to identify alternatives that can be utilized in solid media culture (Dönmez et al., 2016). Here, agricultural wastes come out as highly potential products. Indeed, many agricultural wastes (cocopeat, paddy husk, etc.) are currently utilized in soilless agriculture, and new materials alternative to them have been searched (Carlile et al., 2019; Altun, 2024).

Olive pomace—one of the organic wastes that can be reutilized in agricultural production— contains high amounts of phytochemical compounds and minerals that are released during the extraction of oil from olives (Buono et al., 2011; Chrysargyris et al., 2023). Despite the long dissolution process, the composition of the minerals it contains has led researchers who study in the world's leading olive oil producer countries to research whether olive pomace can be utilized in plant production or how it can be utilized (Ameziane et al., 2020; Boutasknit et al., 2020; Regni et al., 2020; Tüzel et al., 2020; Alma and Söylemez, 2022). Indeed, it has been estimated that in Türkiye alone, approximately 2 million tons of olives were processed into olive oil in 2022, and it has been suggested that 35–40% of the olives processed into oil, i.e., 700–800 thousand tons of crude olive pomace, was generated as waste (Albayrak, 2023; Kıcı and Saltan, 2020). When a similar calculation is made over the amount of oil olives produced worldwide (approximately 20 million tons per year), it appears that approximately 7-8 million tons of olive pomace are released as waste (Anonymous, 2022). Reintroducing large quantities of organic waste material into production is crucial. It has been considered that the use of olive pomace in plant production would allow access to a low-cost raw material, and the special fields allocated for the storage of this material could be utilized for different purposes (Alkhalidi et al., 2023; Muezzinoglu, 2023; Enaime et al., 2024). Some studies have found that the high polyphenol content in olive pomace causes phytotoxicity in plants and therefore inhibits plant growth (Omer and Mohamed, 2012; Pinho et al., 2017; Ladhari et al., 2021). On the other hand, it has been found that the application of olive pomace compost improves both the physical properties of the soil and increases the organic matter content and has positive effects on the development of plants due to the partial loss of polyphenols contained in the olive pomace during the composting process (Baddi et al., 2009; Ouzounidou et al., 2010). However, the finding that different plant wastes (hazelnut husk, tomato compost, tea compost, mushroom compost, tobacco dust compost, apple compost, grape compost, etc.) can be used as a growth medium in seedling-sapling production (Kütük et al., 1995; Durukan, 2004; Aydın and Demirsoy, 2020; Akay et al., 2021; Çiçek and Yücedağ, 2021; Kartal and Geboloğlu, 2023) has led to the suggestion that olive pomace should also be investigated for use in different plant species for such purposes. Moreover, the number of the studies in the literature on whether olive pomace can be used in the production of potted fruit saplings is quite limited. This study, which was carried out to contribute to the literature in this field and to determine the possibility of using olive pomace in the cultivation of potted grapevine saplings, examined the vegetative growth of saplings of the Hatun Parmağı grape variety in different peat-olive pomace mixtures.

MATERIALS AND METHODS

Material

This study was carried out in 2021 using the experimental fields of the Agricultural Practice and Land Management and Research Center (ADYUTAYAM) at Adıyaman University, the laboratories of the Science and Technology Practice and Research Center (HUBTAM) at Harran University, and the Faculty of Agriculture at Adıyaman University. The study utilized 1-year-old ungrafted (own-rooted) open-rooted saplings of the Hatun Parmağı grape variety as plant material. The climatic data of the experimental field where the grapevine saplings were grown during the study were acquired from the climate station (Metos, Pessl Instruments, Austria) and presented in Table 1.

To determine the usability of pomace, a solid organic waste left over from olive oil production, in the production of potted grapevine saplings, some physical and chemical properties and plant nutrient contents of pomace supplied from the Ebrulim olive oil factory at Harran University were identified through analyses done in the Science and Technology Practice and Research Center at Harran University (HUBTAM). The analysis results showed that the olive pomace used in the study contained dry matter of 31.07%, fixed fat of 5.96%, and nitrogen of 1.09% (Table 2).

In addition to this, the olive pomace used in the study contained 704.9 ppm potassium, 212.0 ppm calcium, 6.302 ppm iron, and 60.15 ppm magnesium (Table 3).

Table 1. Some climatic data from the experimental field where grapevine saplings were grown

Months	Ave. Temperature (°C)	Ave. Max. Temp. (°C)	Ave. Min. Temp. (°C)	Total Precipitation (mm/da)
April	17.0	31.7	3.6	1.8
May	24.6	37.6	12.1	14.4
June	27.2	39.4	15.9	0.2
July	32.5	41.7	21.3	-
August	32.0	41.7	22.0	4.0
September	26.0	37.0	13.8	-
October	20.2	32.4	11.6	20.6

Table 2. Some physical and chemical properties of the pomace used in the study

Ash (%)	Oil (%)	Dry Matter (%)	N (%)	C (%)	H (%)	S (%)
5.09	5.96	31.07	1.09	46.03	6.58	4.86

Table 3. Some nutrient content of pomace used in the study

P (ppm)	K (ppm)	Fe (ppm)	Ca (ppm)	Cu (ppm)	Mg (ppm)	Zn (ppm)	Mn (ppm)
0.239	704.9	6.302	212.0	0.731	60.15	0.692	0.554

Since the amount of plant nutrients contained in pomace is relatively low compared to other widely utilized growing materials and their dissolution rates and intake from the root medium by the active and passive transport of plants are predicted to be slow, this study did therefore not use pomace alone as a rooting medium but examined different ratios of peat-olive pomace mixtures. The peat used in this study was TS-1-type peat produced by Klasmann-Deilmann (Klasmann-Deilmann, Potground H, Germany). Table 4 shows the compositions of different mediums of peat-olive pomace mixture prepared for grapevine sapling cultivation in this study. A growth medium containing 100% peat was used as a control group, and the other growth media were prepared by gradually increasing the olive pomace content.

Table 4. Peat-olive pomace mediums, the effects of which were examined in the study

Growing Media	Mixing Ratio (v/v)
Control	100% Peat
6P-1OP	85% Peat + 15% Olive Pomace
4P-1OP	80% Peat + 20% Olive Pomace
3P-1OP	75% Peat + 25% Olive Pomace
2P-1OP	67% Peat + 33% Olive Pomace
1P-1OP	50% Peat + 50% Olive Pomace
1P-2OP	33% Peat + 67% Olive Pomace

Method

Before moving the saplings to the prepared rooting medium, the roots were pruned to cut off 70% of them. Also, the strongest shoot on the saplings was pruned over 2 buds, and the other shoots were cut off from the bottom and removed. Saplings were planted on 12/04/2021 in 5-litre black rubber tubes containing different growth medium mixtures (Figure *1a*). The tubes were punched with an equal number of holes at the same locations to provide ventilation and drainage of excess water. In order to examine the effects of growth medium, the study was carried out according to a randomized block design with 4 replicates. A total of 84 potted grapevine saplings were investigated in the study. After planting, saplings were irrigated until the tubes were saturated with water (Figure 1*b*).

Figure 1. Planting the saplings in tubes (*a*), irrigating the saplings (*b*), setting up the experimental plots (*c*), and a view of the saplings with single-shoot (*d*)

During the growth period, the saplings in all growth media were irrigated simultaneously, and periodically sprayed, weeded, and carried out other care practices. In May, saplings were forced to develop a single shoot by leaving the one with the best growth strength on the sapling and taking out the others from the bottom (Figure 1*d*). In order to prevent the formation of shoots other than the single (main) shoot left on the saplings during the experiment, the saplings were periodically checked, and fresh shoots were cut off when they were just beginning to shoot.

The chlorophyll index in the sapling leaves was determined by SPAD-502 (Konica Minolta Sensing, Inc., Japan) just before the saplings were uprooted in October (Erdogan et al., 2018). Chlorophyll measurements were made on three mature leaves of all saplings in each growing mixture. Subsequently, saplings were uprooted, and the number of leaves was identified (Kara and Fakhar, 2020). Then shoot lengths of the saplings were measured with a tape measure (Kamiloğlu and Güler, 2014). Total fresh weight of leaves , totalfresh weight of shoots, and total fresh weight of roots were measured with precision balance. Then, leaf, shoot and root samples were packaged separately and dried in a drying oven at +65 °C for 72 hours, and their dry weights were determined on a precision scale after drying (Müftüoğlu et al., 2006; Tunçel and Dardeniz, 2013; Güneş, 2015; Cangi and Etker, 2019). The analysis of variance was run to the results using the Minitab version 18.0 program and the differences between the means were determined through Tukey multiple comparison test.

RESULTS AND DISCUSSION

The physical structure, chemical content, and mineral content of olive pomace used in the preparation of growth medium for grapevine saplings in this study are similar to the findings of researchers investigating this material previously (Dermeche et al., 2013; Ilay et al., 2013; Kara et al., 2022).

Table 5 presents the findings on the effects of growth media of different peat-olive pomace mixtures on shoot length, leaf number, and chlorophyll index of Hatun Parmağı grape variety seedlings in this study, in which the effects of olive pomace on the development of potted grapevine saplings were examined. According to the findings, the longest shoots were formed in the control (100% peat) group. The shoot lengths of the saplings decreased ($p<0.01$) due to the increase in olive pomace rate in the growth medium mixtures examined. Similarly, the saplings in the control group, the only growth medium without olive pomace, yielded better results for the leaf number. However, regardless of the amount of olive pomace added to the growth medium, the presence of olive pomace in the medium had a statistically similar effect on the leaf number of grapevine saplings and there were dramatic decreases in the leaf number of saplings ($p<0.01$). The highest values in terms of chlorophyll index in the leaves of grapevine saplings were recorded from the control group, but the saplings in this group and the saplings grown in 6P-1OP, 4P-1OP, and 2P-1OP media were statistically ($p<0.01$) in the same group. The saplings grown in the 1P-2OP medium had the lowest chlorophyll index.

Table 6 shows the effects of the growth medium of different peat-olive pomace mixtures on the fresh weight of shoots, fresh weight of leaves, and fresh weight of roots of grapevine saplings. Accordingly, the highest values in terms of the fresh weight of shoots were recorded in the control group, while the lowest values were recorded in the 1P-2OP medium. Although the medium containing pomace was in the same statistical group in terms of the fresh weight of shoots, the shoot development of the saplings was significantly limited due to the increase in the amount of olive pomace. It was found that a mixture ratio of 85% peat and 15% olive pomace was the best for using olive pomace in the growth of potted grapevine saplings to promote shoot development. Hence, the analysis of the fresh weight of leaves revealed that the control group had the highest value, followed by the 6P-1OP group. It is quite remarkable that Control, 6P-1OP, and 4P-1OP were in the same statistical group for the fresh weight of the root. These growing mediums caused heavier roots to form in grapevine saplings compared to the other growth

media examined in the study *(p<0.01)*. 1P-1OP and 1P-2OP media resulted in saplings with lower values than the other mediums in terms of all three parameters.

Table 5. Effects of peat-olive pomace medium on shoot length, leaf number, and chlorophyll index of grapevine saplings

Growing Media	Shoot Length** (cm)	Leaf Number** (count/sapling)	Chlorophyll Index**
Control	73.50 ± 6.76^{a}	162.5 ± 56.1^{a}	41.29 ± 1.43^{a}
6P-1OP	47.00 ± 7.44^{b}	80.3 ± 28.1^{b}	37.61 ± 2.25^{a}
4P-1OP	41.75 ± 9.07^{bc}	49.0 ± 10.4^{b}	37.38 ± 2.90^{a}
3P-1OP	41.38 ± 11.18^{bc}	53.5 ± 31.0^{b}	36.53 ± 0.87^{ab}
2P-1OP	43.25 ± 4.50^{b}	49.0 ± 22.2^{b}	37.68 ± 1.83^{a}
1P-1OP	26.75 ± 5.01^{cd}	47.0 ± 15.7^{b}	31.38 ± 0.90^{bc}
1P-2OP	14.35 ± 2.47^{d}	53.7 ± 8.8^{b}	29.63 ± 3.92^{c}

**: There is a statistically significant (*p<0.01*) difference between the mean values in the same column.

Table 6. Effects of peat-olive pomace media on the fresh weight of shoots, leaves and roots of grapevine saplings

Growing Media	Shoot Fresh Weight** (g)	Leaf Fresh Weight** (g)	Root Fresh Weight** (g)
Control	29.12 ± 8.34^{a}	71.64 ± 11.28^{a}	71.84 ± 14.83^{a}
6P-1OP	11.31 ± 3.42^{b}	41.44 ± 8.38^{b}	72.30 ± 6.05^{a}
4P-1OP	6.85 ± 1.54^{b}	28.68 ± 3.71^{bc}	77.76 ± 8.20^{a}
3P-1OP	7.13 ± 3.90^{b}	28.30 ± 11.52^{bc}	53.35 ± 3.77^{ab}
2P-1OP	7.26 ± 1.25^{b}	25.95 ± 2.78^{bc}	53.05 ± 18.74^{ab}
1P-1OP	3.87 ± 0.64^{b}	18.80 ± 3.21^{c}	35.41 ± 9.75^{b}
1P-2OP	3.95 ± 0.37^{b}	15.40 ± 3.40^{c}	28.71 ± 6.20^{b}

**: There is a statistically significant (*p<0.01*) difference between the mean values in the same column.

Table 7 presents the results for the changes in the dry weights of shoots, leaves, and roots of ungrafted grapevine saplings according to the growth medium. The results showed that the saplings with the highest values for the dry weight of shoots were grown in the control medium, and the ones with the lowest values were grown in the 1P-1OP medium. However, the saplings grown in 1P-1OP and 1P-2OP media were in the same statistical group for the dry weight of shoots, and they differed from the saplings grown in the other studied medium with respect to this characteristic (*p<0.01*). Similar to the dry weight of shoots, the highest values for the dry weight of leaves were recorded in the saplings grown in the control medium, and the lowest values were recorded in the saplings grown in the 1P-2OP medium (*p<0.01*). Moreover, the saplings grown in 3P-1OP, 2P-1OP, 1P-1OP, and 1P-2OP media were statistically similar in terms of the dry weight of the leaves. Depending on the olive pomace ratio in the growth medium, the dry weight of the roots of grapevine saplings varied. Unlike the fresh weight of the root, the dry weight of the root values showed that the saplings grown in the 6P-1OP medium yielded higher values compared to those grown in the other growth media, and they differed according to this characteristic. Additionally, grapevine saplings grown in 1P-1OP and 1P-2OP media were statistically in the same group for the dry weight values of roots and had lower values than the mixture of other mediums analyzed.

Table 7. Effects of peat-olive pomace media on the dry weight of shoots, leaves, and roots of grapevine saplings

Growing Media	Shoot Dry Weight** (g)	Leaf Dry Weight** (g)	Root Dry Weight** (g)
Control	9.43 ± 2.32^{a}	22.11 ± 2.46^{a}	16.89 ± 3.44^{ab}
6P-1OP	4.49 ± 1.12^{b}	14.12 ± 2.13^{b}	19.44 ± 2.61^{a}
4P-1OP	2.65 ± 0.42^{bc}	9.74 ± 1.11^{bc}	17.06 ± 0.98^{ab}
3P-1OP	2.59 ± 1.29^{bc}	9.32 ± 3.61^{c}	11.31 ± 2.32^{bc}
2P-1OP	2.64 ± 0.63^{bc}	8.56 ± 1.52^{c}	11.51 ± 4.90^{bc}
1P-1OP	1.34 ± 0.25^{c}	6.30 ± 1.25^{c}	9.30 ± 2.80^{c}
1P-2OP	1.49 ± 0.14^{c}	5.21 ± 1.07^{c}	7.05 ± 1.62^{c}

**: There is a statistically significant (*p<0.01*) difference between the mean values in the same column.

Ilay et al., (2013) reported that the presence of olive pomace in sunflower and bean growth media, regardless of the amount, greatly inhibited plant growth (plant height, leaf number, fresh and dry weight). Rahil et al., (2021) determined that 100% olive pomace medium was not suitable for use in cucumber and eggplant cultivation due to its high salt concentration and low acidity, but if olive pomace was mixed with peat moss at a ratio of 1:1, it did not adversely affect plant growth and productivity. Varol et al., (2020) found that if olive pomace compost was used in olive sapling production, it would improve the macro- and micro-element content of the saplings. Kamel (2023) found that olive pomace treatment of lettuces grown in loamy soil conditions improved the fresh weight (yield), head width, and leaf number of the lettuces. In olive orchards, Camposeo and Vivaldi (2011) found that

the mulching of defatted olive pomace affected the canopy development of trees positively, while Nasini et al. (2013) found that the treatment of olive pomace as a soil conditioner positively affected the canopy development of trees. In contrast to short-term laboratory incubations, Innangi et al., (2017) found that soil organic matter coverage and biological activity improved when long-term studies were carried out on olive pomace-treated soils. On the other hand, Proietti et al., (2015) reported that olive pomace has a strong phytotoxic effect on plants, and this effect diminished after the composting process. Indeed, Alma and Söylemez (2022) reported that the presence of 5% (v/v) crude olive pomace in the growth medium restricted the growth of pepper seedlings (plant height, leaf, dry weight of leaves and roots, chlorophyll index) and decreased the vegetative growth parameters of plants with higher pomace content. Papafotiou et al., (2004) found that the height, dry weight of roots, number of bracts and the number of nodes where the first bract was formed reduced as the proportion of olive pomace compost gradually rose in peat-olive pomace compost medium in which rooted shoots of *Euphorbia pulcherrima* were grown. On the other hand, Ceglie et al., (2011) reported that composted olive pomace can be an alternative growth medium to peat for tomato seedlings. The findings of the present study are compatible with the findings of the researchers, who found that the vegetative growth of plants would be negatively affected if raw olive pomace was utilized in a seedling-sapling growth medium.

Table 8 presents the coefficients determined by running Pearson's correlation test on the results related to vegetative growth parameters of grapevine saplings grown for 5 months in growth media created by using peat and olive pomace at different ratios and the findings on their statistical significance under the study. The findings showed that all vegetative growth parameters were positively correlated with each other and statistically significant at the significance level of 1%. Additionally, the highest correlation coefficient ($r = 0.994$) was found between the leaf fresh weight (LFW) and the leaf dry weight (LDW). The lowest correlation coefficient ($r = 0.382$) was found between leaf number (LN) and the root fresh weight (RFW). In general, the detection of statistically significant and positive correlations between the vegetative growth parameters of grapevine saplings indicates that the reliability of the study findings from growth media of peat-olive pomace mixture at different ratios examined hereunder is also high. Indeed, different studies have found strong positive correlations between the leaf number of grapevine saplings and their root length, between leaf surface and leaf weight, and between the length of the mother shoot and the internode number (İşçi et al., 2019; Demirova, 2023; Atak and Çorak, 2024).

Table 8. Correlations between some growth parameters of grapevine saplings grown on medium with different ratios of peat-olive pomace mixtures

	LN	CI	SFW	LFW	RFW	SDW	LDW	RDW
SL	0.673**	0.786**	0.796**	0.832**	0.681**	0.833**	0.836**	0.589**
LN		0.513**	0.846**	0.846**	0.382**	0.860**	0.823**	0.387**
CI			0.582**	0.639**	0.712**	0.601**	0.655**	0.623**
SFW				0.967**	0.483**	0.987**	0.943**	0.463*
LFW					0.580*	0.983**	0.994**	0.576**
RFW						0.536**	0.610**	0.926**
SDW							0.968**	0.529**
LDW								0.618**

Abbreviations: SL: shoot length; LN: leaf number; CI: chlorophyll index; SFW: shoot fresh weight; LFW: leaf fresh weight; RFW: root fresh weight; SDW: shoot dry weight; LDW: leaf dry weight; RDW: root dry weight

**: Pearson's correlation coefficient was statistically significant ($p<0.01$).

Almost all of the studies in the literature found that the presence of crude pomace in the growth media negatively affected the vegetative growth of plants, although the examined plant species were different. However, the findings by Pinho et al., (2017), who reported that the physical and phytochemical content of olive pomace generated as waste from olive mills varied according to the extraction method of olive oil (2 phase-3 phase), suggest that it is necessary to take into consideration that the phytotoxic effects of olive pomace, which is planned to be used in the growth medium, may vary depending on the extraction method. Furthermore, the findings of the researchers who have reported that olive pomace composts contain relatively low amounts of polyphenols after the composting process, and therefore their phytotoxic properties are largely lost, indicate that olive pomace compost can be added to plant growth media (Altieri and Esposito, 2010; Karaca et al., 2015). Findings of the present study are compatible with the findings of other studies, which have reported dramatic reductions in the leaf number, shoot-stem length, biomass, root weight, chlorophyll content of leaves, and similar growth parameters of plants with a higher crude olive pomace content in growth media. However, since both crude olive pomace and pomace compost can have varying effects on plant growth, even at a limited level, it is necessary to conduct trials before production to determine the effects of these materials on the plant species and variety planned to be cultivated, as well as the most appropriate dose and rate of use.

CONCLUSION

This study, which was conducted to determine the potential for the reutilization of olive pomace, showed that the growth of grapevine saplings transferred to the growth medium to which olive pomace was added was limited, and their vegetative growth was significantly inhibited upon the increase in the amount of olive pomace added to the medium. Almost all the analyzed vegetative growth parameters showed that the control (100% peat) medium yielded better results than the other media. Additionally, it was determined that 6P-1OP (85% peat and 15% olive pomace) medium can be practically used for the marketability of potted grapevine saplings, a traded agricultural product. On the other hand, it was concluded that the addition of more than 15% olive pomace by volume to the growth medium of saplings was not eligible as it would cause negative effects on the vegetative growth of grapevine saplings and there would be problems in the marketing of those saplings. Furthermore, finding of the present study that the presence of proportionally low amounts of pomace in the growth medium of saplings may have a positive effect on the root dry weight may be due either to the storage of some of the phytochemicals contained in olive pomace by the grapevine saplings in their own roots to alleviate the stress factor in the root medium or to the transport to the roots of some compounds synthesized in order to regulate such a condition in the plant due to the inhibition of the uptake of one or more of the plant nutrients in the root medium by these compounds. However, it is necessary to establish similar experiments once again and to do more comprehensive chemical and biotechnological analyses in order to confirm the validity of these two hypotheses. Indeed, the findings that vegetative growth of grapevine saplings were limited or even diminished with the increase in the amount of olive pomace in the root medium may be due to the low tolerance of the grape variety (Hatun Parmağı) used as plant material in the study to some phytochemical compounds contained in pomace, or it may be due to the sensitivity of grape varieties belonging to the species *Vitis vinifera* L. to the presence of these phytochemical compounds in the root area. Further research on the use of other cultivated grapevine species and cultivars as plant material would contribute to a more comprehensive evaluation of the findings of this study on the use of olive pomace in the cultivation of potted grapevine saplings.

Consequently, the findings of the study indicate that olive pomace can be utilized in the preparation of root medium in the cultivation of grapevine saplings at volumetric ratios of 15% and below. Such use would not only allow the waste material generated in olive oil processing facilities to be reutilized but also reduce pollution occurring in nearby olive oil processing facilities.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Adsorption of crystal violet dye with selenium nanoparticles obtained by green synthesis from cherry (*Prunus avium* L.) fruit stalk

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DergiPark AKADEMİK

Abstract
The rapid development of the global production printing and dyeing industry has led to an increase in the demand for various dyes. Crystal violet (CV), a versatile dye, is widely used in the textile industry and other applications. The reason for its widespread use is its effectiveness and the vivid color it gives to fabrics.CV dye is a water-soluble, toxic, resistant organic dye that is quite dangerous for the ecosystem and causes environmental pollution. Therefore, it must be removed before being released into the recipient environment. This study synthesized selenium nanoparticles (Se NPs) from agricultural *Prunus avium* L. (*Pa*L.) wastes and removed CV dye. In batch adsorption tests, the effects of pH, amount of adsorbent, time, initial concentration, and temperature were investigated. In this study, where 3 different kinetic and isotherm models were tested, it was determined that the most suitable kinetic and isotherm models for the removal of CV dye with *Pa*L-Se NPs were Pseudo second order (R^2:0.999) and Langmuir (R^2:0.997), respectively. Additionally, the maximum adsorption capacity (qmax) was calculated as 142.61 mgCV/g *Pa*L-Se NP. Accordingly, it can be said that low-cost *Pa*L-Se NPs synthesized by environmentally friendly methods are a suitable alternative for the removal of CV dye.

Keywords: Agricultural waste, *Prunus avium* L., Green synthesis, Crystal violet, Nanoparticle, Adsorption

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INTRODUCTION
The acceleration of the production-based economy in the world in the last quarter, together with the progress in science and technology, also accelerated the growth of the textile industry. Due to this rapid growth, dye-based pollution has become a serious environmental problem faced by many developing countries (Ngoc Hoang et al., 2021; Zhao et al., 2019). Synthetic textile wastewater, which contains dyes and has a complex aromatic ring molecular structure, causes harmful effects on the environment and humans, such as allergies, cancer, irritation and even mutations (Du et al., 2011). CV dye, which is included in this toxicological dye group, is a cationic triphenylemethane dye that is widely used in many areas such as textile colorant, paper dye and biological dye (Kulkarni et al., 2017). The presence of the dye in the aquatic ecosystem disrupts the photosynthetic activity of water and affects the fauna and flora of water bodies causing ecological imbalance. For this reason, dye wastewater must be treated before being released into the aquatic ecosystem (Rehan et al., 2023).Therefore, in order to prevent further degradation of the natural ecosystem and protect public health, there is a great need to develop an effective approach to remove dye wastewater prior to discharge into the aquatic ecosystem (Kubra et al., 2021).Removal of azo dyes from wastewater is a major challenge because these dyes have a resistant and complex structure that makes them durable for long periods of time. Thanks to these structures, they are thought to be electron-deficient xenobiotic compounds that are resistant to degradation (Cheruiyot et al., 2019). Many methods such as flocculation, coagulation, membrane filtration, chemical oxidation, ion exchange, electrolysis and reverse osmosis are used for dye removal from aquatic environments (Yagub et al., 2014). In addition to these methods, the adsorption method is one of the most widely used methods in dye removal from aqueous solutions. (Moosavi et al., 2020).Recently, bio-supported production of nanostructured materials (plants, etc.) by researchers has stood

out as environmentally friendly methods that play an important role in minimizing pollution (Rahmat et al., 2023). Physical, chemical or green synthesis (biological methods) methods are used in the synthesis of nanomaterials. However, physical and chemical processes can produce both expensive and toxic products. For this reason, green nanobiotechnology method is frequently used in the production of nanomaterials (Nikam et al., 2022). Nanoparticle-based adsorbents have shown great success in removing dye wastes from aqueous solution due to high surface area, nanoscale size, and chemical composition, among other physical and chemical characteristics (Mashkoor et al., 2020). Therefore, in this study, selenium nanoparticles (*Pa*L-Se NP) were synthesized from agricultural *Prunus avium* L. (*Pa*L.) waste and crystal violet (CV) dye was removed. In batch adsorption tests, the effects of pH, adsorbent amount, time, initial concentration and temperature were investigated, and kinetic and isotherm studies were carried out. Promising results were obtained in the removal of CV dye in this sustainable study, which was carried out as an alternative to other treatment methods.

MATERIALS AND METHODS

Chemicals

All chemicals used in experimental studies are of analytical purity. Powder sodium selenite (Na_2SeO_3, 172.94 g/mol, Sigma-Aldrich), crystal violet dye in powder form ($C_{25}N_3H_{30}Cl$, 407.979 g/mol, Sigma-Aldrich), nitric acid in liquid form (HNO_3, 1.39 g/cm^3, 65%, Merck), sodium hydroxide in pellet form (NaOH, 40.00 g/mol, ≥99.0%, Sigma-Aldrich) and sulfuric acid in liquid form ($H_2(SO_4)_3$, 1.81 g/cm^3, ≥ 90-91 %, Merck) were used.

Collection of *Prunus avium* L. (*Pa*L) Plant

In this study, the stalks of cherries purchased commercially in June in Şeyhan neighborhood of Artuklu district of Mardin province were removed. Later, he was washed many times, first with tap water and then even with pure water. At the end of the process, the cherry stalks were dried in a room protected from sunlight and ground into a powder with a rotary mill (Device name: Ika Universal Muhle). The obtained stalks were stored at +4 ºC to be used in experimental procedures.

Extraction of *Pa*L wastes

In this study, 50 grams of previously dried cherry stalks were weighed. The weighed cherry stalks were placed in a 1000 mL beaker. 400 mL of analytical purity ethanol was added, and it was covered and kept for four days. Then, it was subjected to filtration and the filtrate was removed and the ethanol was removed in a Heidolp brand evaporator. Then, the pure extract remaining at the bottom of the flask was used in experimental procedures(Baran et al., 2023).

Biosynthesis of *Pa*L -Se NPs

500 mg of *Prunus avium* L. (*Pa*L) plant extract, previously pure cherry fruit stalks, were taken into erlenmeayer flask and dissolved in 200 mL of pure water. Then, 150 mL of 75 mM was added from the stock sodium selenite Na_2SeO_3 solution. The reaction process was allowed to stir at 75 ºC for 24 hours. Then, the dark-colored solution was taken and centrifuged (6000 rpm for 25 minutes). After centrifugation, the solid part was removed and placed on a glass pellet and dried at 80 °C for 72 hours. The obtained *Pa*L-SeNPs were preserved to be used in absorption processes. Production stages are presented in Figure 1.

Cherry *Pa*L stalk | Powdered form of *Pa*L stalk | Cherry *Pa*L extract | *Pa*L-SeNPs from *Pa*L | Powder form of *Pa*L-SeNPs stalk

Figure 1. Production scheme showing the synthesis of *Pa*L-SeNPs obtained from *Pa*L stalks

Adsorption studies

First of all, a stock solution of CV dye with a concentration of 1000 mg/L was prepared. Sequential dilute solutions were prepared from this stock solution and a calibration curve was created at a wavelength of 586 nm on a UV-Vis spectrophotometer (Hach DR6000, Germany). The equation $y = 0.1871x + 0.0054$ ($R^2 = 0.9993$) was used to measure dye concentrations in adsorption studies. Adsorption studies were carried out in an orbital shaker device (Heidolp, Unimax1010, Germany) at a stirring speed of 200 rpm. In addition, environmental parameters (effect of pH, adsorbent amount, time, and initial concentration) were also studied, and suitable conditions were determined for the adsorption of CV dye on *Pa*L-Se NPs.

Equations (1) and (2) were used to evaluate the numerical data obtained.

$$R(\%) = \frac{C_0 - C_e}{C_0} \times 100 \quad (1)$$

$$q_e = \frac{(C_0 - C_e) \times V}{m} \quad (2)$$

Here, the term *R* refers to the removal efficiency, while the terms C_0 and Ce refer to the dye concentrations (mg/L) at the beginning and end of the reaction, respectively. Additionally, the term *V* represents the solution volume (L) and the term *m* represents the amount of *Pa*L-Se NPs (g).

Adsorption kinetics

Kinetic experiments were conducted at room temperature (23±2 ^{0}C), 200 rpm and neutral pH, in a 50 mL volume beaker with an initial dye concentration of 10 mg/L. Dye concentrations were measured by taking samples at certain intervals and Pseudo first order (equation no. 3) (Lagergren, 1898), Pseudo second order (equation no. 4) (Ho et al., 1996) and Elovich (equation no. 5) (Kumar et al., 2011) models were tried to process the numerical values.

Pseudo first order: non-linear: $\frac{dq_t}{d_t} = K_1(q_e - q_t)$, linear: $Log(q_e - q_t) = Log\ q_e - \frac{K_1}{2.303} t$ (3)

Pseudo second order: non-linear: $\frac{dq_t}{d_t} = K_2(q_e - q_t)^2$, linear: $\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e} t$ (4)

Elovic: non-linear: $q_t = \beta \ln(\alpha\beta t)$, linear: $q_t = \frac{1}{\beta} \ln(\alpha\beta) + \frac{1}{\beta} \ln t$ (5)

Here, the terms q_e and q_t express the amount of pollutant (mg/g) removed per unit adsorbent at equilibrium and any time *t*, respectively, while K_1 and K_2 are the kinetic constants of the models. Additionally, the term α represents the initial adsorption rate (mg/g.min) and β represents the model constant (g/mg).

Adsorption isotherms

Isotherm experiments were carried out at room temperature (23±2 ^{0}C), 200 rpm and neutral pH 5 mg *Pa*L-Se NPs were added to 6 different tubes with a volume of 10 mL, with initial dye concentration ranging from 15.63 to 500 mg/L, and after centrifugation for 120 minutes, the upper phase water was measured in a UV-Vis spectrophotometer. It was read according to the prepared calibration curve. The obtained numerical values were processed in Freundlich (equation no. 5) (Wang & Guo, 2020), Langmuir (equation no.6) (Guo & Wang, 2019; Langmuir, 1916) and Temkin (equation no.7) (Temkin, 1940) isotherm models.

Freundlich: non-linear: $q_e = k_F C_e^{1/n}$ linear: $log(q_e) = log(k_F) + {}^1/_n log(C_e)$ (5)

Langmuir: non-linear: $q_e = \frac{q_m k_L C_e}{1 + k_L C_e}$ linear: $\frac{C_e}{q_e} = \frac{1}{q_{max} k_L} + \frac{C_e}{q_{max}}$ (6)

Temkin: non-linear: $q_e = \frac{RT}{b_T} ln(k_T C_e)$ linear: $q_e = \frac{RT}{b_T} ln(k_T) + \frac{RT}{b_T} ln(C_e)$ (7)

Here, the term q_e represents the amount of pollutant removed per unit adsorbent in equilibrium (mg/g), the term C_e represents the final pollutant concentration (mg/L), and K_f and n represent the Freundlich constants. In the Langmuir isotherm, the term q_m represents the maximum adsorption capacity (mg/g) and K_L represents the Langmuir adsorption constant (L/mg). The equation "$R_L = 1/(1 + a_L.C_0)$" is needed to find the dispersion constant (R_L) in the Langmuir isotherm. Here, the term a_L refers to the Langmuir constant and C_0 refers to the initial pollutant concentration of the reaction (mg/L). Additionally, in the Temkin equation, the term *B* refers to the model constant (J/mol). To calculate this, the equation "$B = R_T/b_T$" is used. Here, the term *R* refers to the universal gas constant (8.314 J/mol. K), the term *T* refers to the temperature (K), and b_T refers to the isotherm constant (kj/mol).

RESULTS AND DISCUSSION

Influence of environmental factors

In order to determine the effect of pH on removing the CV dye of *Pa*L-Se NPs, pH 3, 5, 7 and 9 were used in 4 different tubes with an initial CV concentration of 10 mg/L and an initial amount of *Pa*L-Se NPs of 10 mg, at a stirring speed of 200 rpm at room temperature. Experiments were carried out for 120 minutes. At the end of the period, 7.81, 6.98, 6.92 and 6.88 mg/L dye measurements were observed in the dye measurements made in the upper phase, respectively. In this case, it was determined that the dye removal efficiency reached from 21.9% to 31.2% with increasing pH. In this context, it can be stated that although the removal efficiency is low in strongly acidic conditions, there is an increase in efficiency in neutral pH and partially basic environments, but the

difference is not very high. When the literature was examined, it was reported that the removal efficiency of CV dye was carried out by coating Fe_3O_4 on biochar and that the removal efficiency was suitable at neutral and high pH (Sun et al., 2015). Similarly, in a different study in which CV dye was removed by synthesizing magnetic nanoparticles, it was reported that pH increases positively affected the yield (Samrot et al., 2021).

On the other hand, experiments were carried out under similar environmental conditions to determine in which direction the removal of CV dye was affected by varying amounts of *Pa*L-Se NPs. Again, in 5 different experiments with a volume of 10 mL, where the initial CV concentration was 10 mg/L, 5, 10, 20, 40 and 80 mg *Pa*L-Se NPs were added to each tube, and CV concentrations were measured at the end of the period. According to the data obtained, 84.7% efficiency was achieved at 5 mg dosage, while the efficiency reached 88.2% in the experiment where 10 mg *Pa*L-Se NP was added, and finally, the output dye concentration could not be observed in the experiment where 20 mg *Pa*L-Se NP was added. Therefore, it is clear that the removal efficiency increases as the amount of adsorbent increases. When the literature was examined, it was reported in a study in which CV dye was removed by loading silver nanoparticles on activated carbon that the increase in the amount of adsorbent contributed positively to the removal efficiency (AbdEl-Salam et al., 2017). Similarly, in a different study in which CV dye was removed with photogenic magnetic nanoparticles, it was reported that a decrease in the CV dye output concentration was observed because of the increase in the amount of adsorbent (Ali et al., 2018).

To determine the effect of the initial dye concentration on the removal efficiency in the adsorption process, experiments were carried out in 6 different test tubes with a volume of 10 mL, each with an adsorbent amount of 0.5 g/L, under the same environmental conditions, with the initial dye concentration ranging from 15.63 to 500 mg/L. The data obtained from the dye analyses performed at the end of the period are presented in Figure 2(a). Accordingly, at an initial concentration of 15.63 mg/L, the dye concentration remaining in the solution in the experimental unit was measured as 5.9 mg/L, the removal efficiency was calculated as 62.24% and qe was calculated as 19.45 mg/g. When the initial dye concentration was 125 mg/L, the exit concentration was measured as 68 mg/L, the removal efficiency was calculated as 45.6% and qe was calculated as 114 mg/g. Finally, when the initial dye concentration reached 500 mg/L, the output concentration, removal efficiency and qe values were calculated as 434 mg/L, 13.2% and 132 mg/g, respectively. Accordingly, it is said that the dye removal efficiency is inversely proportional to the increase in the initial dye concentration, while the amount of substance removed per unit adsorbent increased in direct proportion. When the literature was examined, it was reported that similar results were obtained in the removal of CV dye with magnetic nanoparticles (Amodu et al., 2015).

It is very important to determine the effect of contact time on removal efficiency in the adsorption process. To determine this, 25 mg *Pa*L-Se NPs were added to a 50 mL volume beaker with an initial CV concentration of 10 mg/L under the same environmental conditions, samples were taken at certain time intervals and CV dye analysis was performed in the upper phase liquid. The results obtained are presented in Figure 2(b). During the 300-minute experiment period, at the end of the 1st minute, the dye concentration decreased to 5.36 mg/L, in this case, the q*t* value was calculated as 9.28 mg/g and the removal efficiency was calculated as 46.4%. While no significant decrease was observed in the exit dye concentration as the reaction time was extended, the CV concentration was measured as 3.57 mg/L at the end of 300 minutes, the qt value was 12.86 mg/g and the removal efficiency was calculated as 64.3%. When the literature was examined, it was determined that CV and MB dyes were removed by synthesizing magnetic iron oxide nanoparticles from fig leaves, and a decrease in dye concentration was observed as the reaction time increased (Alizadeh et al., 2017).

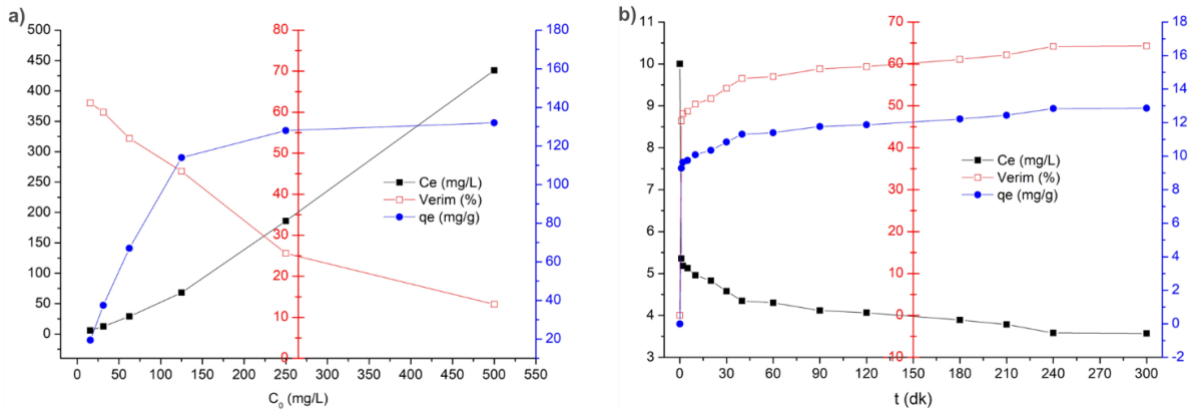

Figure 2. a) Effect of initial concentration; C_0:15.63-500 mg/L, V: 10 mL, m: 0.5 g/L, pH 7.0, T: 23^{0}C, mixing 200 rpm, Time: 120 min, b) Effect of contact time, V: 50 mL, m: 0.5 g/L, C_0:10 mg/L, pH 7.0, T: 23^{0}C, stirring 200 rpm.

Adsorption kinetics

To determine which of the Pseudo first order, Pseudo second order and Elovich kinetic models the numerical data obtained in the experiments conducted in the laboratory fit, the data obtained by removing the linear regression curves of the models are shown in Table 1 and Figure 3(a), (b) and (c). is also presented. In addition, the graph showing the change in time of the amounts of pollutants removed per unit adsorbent obtained from laboratory experiments and the values obtained from kinetic models is presented in Figure 3(d).

Thanks to kinetic models, information is obtained about how long it takes the adsorbent to remove the pollutant it is intended to remove. In addition, information is obtained about the step of the adsorbent in adsorbing the pollutant. The R^2 value is generally considered to determine which kinetic, and isotherm model the numerical data obtained from experimental studies are suitable for. When the table is examined, the R^2 values of the Pseudo first order and Elovich kinetic models were calculated as 0.962 and 0.947, respectively, while the R^2 value of the Pseudo second-order kinetic model was calculated as 0.999. Accordingly, the adsorption process of *Pa*L-Se NP particles to the CV dye occurs following the Pseudo second-order kinetic model. When the literature was examined, the removal of CV dye was achieved with nanoparticles obtained from corn starch and it was reported that it fit the Pseudo second order kinetic model with an R^2 value of 0.999 (Gad et al., 2019). On the other hand, in a study in which carbon nanotubes and nanoparticles were synthesized and the CV dye was removed, it was found that there was a Pseudo second order kinetic model with an R^2 value of 0.999 (Gabal et al., 2014). In another study, cv dye removal from aqueous solution was examined using a surfactant-modified magnetic nanoadsorbent and it was reported that it fit the Pseudo second-order kinetic model with an R^2 value of 0.993 (Muthukumaran et al., 2016).

In another reported study CV dye removal from aqueous solution was examined using *Spirulina*-based surfactant-modified iron oxide nanoparticles for the adsorptive removal of CV dye, and it was stated that it fit the Pseudo second-order kinetic model with an R^2 value of 0.964 (Bhukal et al., 2022).

Table 1. Kinetic and isotherm parameters were calculated for the adsorption of CV on *Pa*L-Se NPs.

			Parameter	R^2
Kinetic models	Pseudo first order	k_1 = 0.052		0 .962
	Pseudo second order	k_2 = 0.034	q_e = 12.407	0 .999
	Elovich	β = 1.544	α = 587384	0 .947
Isotherm models	Freundlich	k_F = 11,986	1/n = 0.447	0 .844
	Langmuir	k_L = 0.034	R_L = 0.172 q_{max} = 142.61	0 .997
	Temkin	B_T = 0.085	k_T = 0.374	0 .921

Adsorption isotherms

To determine the most appropriate isotherm model for the adsorption of CV dye on *Pa*L-Se NPs, calculations of Freundlich, Langmuir and Temkin models were made and presented in Table 1. In addition, linear regression curves were created and shown in Figure 4(a), (b) and (c), and the change of experimental and theoretical qt values against Ce values is shown in Figure 4(d).

In this context, the R^2 values of the Freundlich and Temkin isotherm models were calculated as 0.844 and 0.921, respectively, while the R^2 value of the Langmuir isotherm model was calculated as 0.997. Additionally, the maximum adsorption amount was calculated as 142.61 mg/g. Accordingly, it seems that the most suitable model for the adsorption of CV dye on *Pa*L-Se NPs is Langmuir. In the Langmuir isotherm model, it can be said that the adsorption process occurs in certain localized regions and there is single-layer adsorption (Vijayaraghavan et al., 2006). When the literature was examined, the removal of CV dye was achieved by obtaining nickel oxide nanocomposite with various nickel compounds, and it was reported that the most suitable model was Langmuir with an R^2 value of 0.999 and a q_{max} value of 53.16 mg/g (Bani-Fwaz et al., 2021). On the other hand, in a study in which F_3O_4 nanoparticle synthesis was carried out from weathered basalt stones, the CV dye was removed, and it was reported that the most suitable model was the Langmuir model with an R^2 value of 0.967 and a q_{max} value of 282.5 mg/g (Abu Sharib et al., 2021).

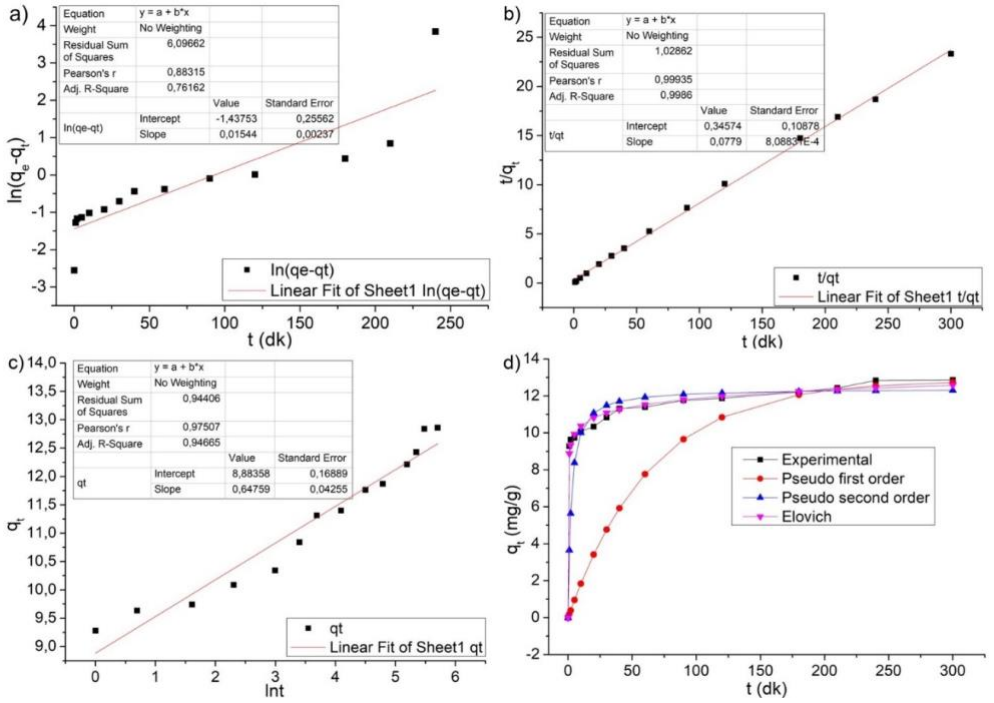

Figure 3. a) Pseudo first order model, b) Pseudo second order model, c) Elovic model, d) change graph of experimental and theoretical q_t values against t.

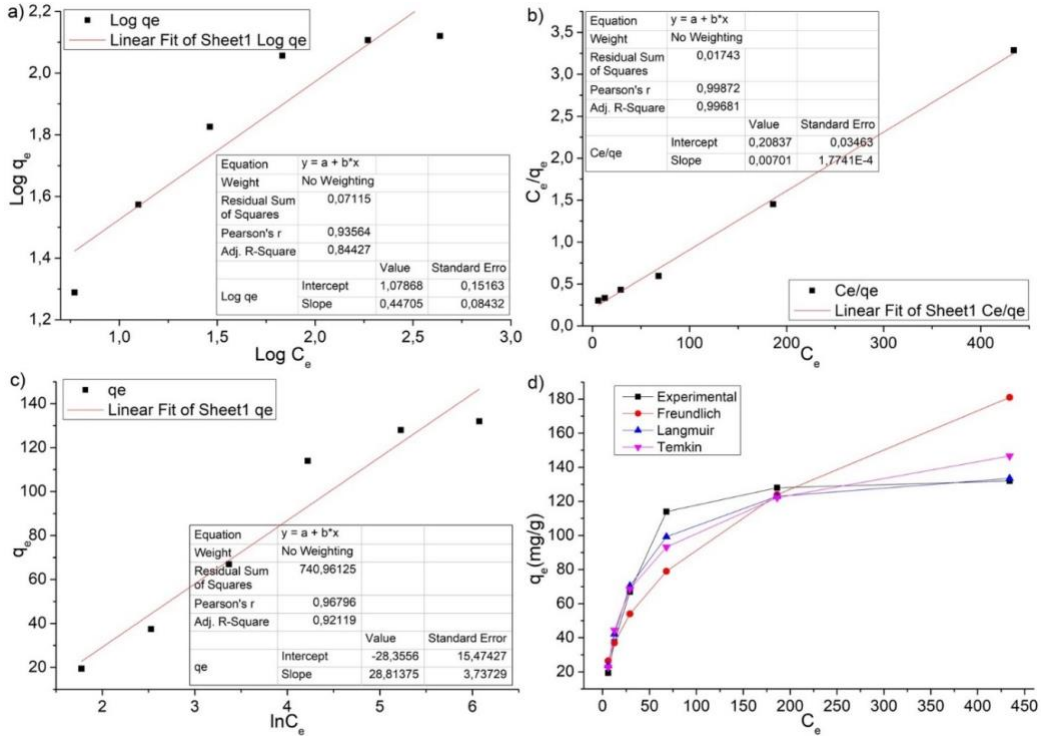

Figure 4. Regression curves of each isotherm model (a) Freundlich, b) Langmuir, c) Temkin and d) Variation of experimental and theoretical q_t values against C_e values.

Comparison of the study and the results obtained with the literature

A summary of the experimental conditions and results of this study is presented in Table 2, along with a comparison of similar studies in the literature. In the study carried out for the purpose of adsorption of CV onto functionalized multi-walled carbon nanotubes, it showed good agreement with langmuir and freundlich isotherm models and pseudo-second-order kinetic model; the maximum adsorption capacity was 90.52 mg/g (Sabna et al., 2016). In the study in which CV dye was removed with the nanomaterial obtained from chitin shrimp shell, the removal efficiency 79.13% and adsorption capacity were found to be 39.56 mg/g (Gopi et al., 2016). To remove the Congo red (CR) dye, zinc oxide (ZnO) in nanoflake form on the zeolite surface was used. In a different adsorption study, it was determined that the most suitable kinetic model was the pseudo-second order kinetic model, and the most suitable isotherm model was the langmuir isotherm model, and the maximum adsorption capacity was 161.3 mg/g (Madan et al., 2019). In a study in which CV dye was removed with *Citrus Fortinella*-Se NPs, it was determined that the most appropriate kinetic model was the pseudo-second order kinetic model with an R^2 value of 0.999, and the most appropriate isotherm model was freundlich with an R^2 value of 0.993. The maximum adsorption capacity was 23.55 mg/g (Solmaz et al., 2024). In this study, it was determined that the most suitable kinetic model was the pseudo-second order kinetic model with an R^2 value of 0.999, and the most suitable isotherm model was the langmuir isotherm model with an R^2 value of 0.997. The maximum adsorption capacity was 142.65 mg/g. As a result, the *Pa*L-Se NPs used in our study are seen as promising for environmental studies due to their adsorption capacity and environmental friendliness.

Table 2. Comparison of the results obtained with the literature.

	This study	(Sabna et al., 2016)	(Gopi et al., 2016)	(Madan et al., 2019)	(Solmaz et al., 2024)
NP Chemical	Se	Commercial product	Chitin-Fe_3O_4	ZnO	Se
NP Origin	*prunus avium*	multi-walled carbon nanotubes	shrimp shell	silica zeolitic	*citrus fortunella*
Experiment volume (mL)	50	20	40	50	50
Mixing speed (rpm)	200	-	600	-	200
Experiment time (min)	120	45	-	60	1500
Working pH's	3-9	3-8	-	3-12	7.0±0.5
pH adjusting agent	0.1 M H_2SO_4/NaOH	-	-	-	0.1 M H_2SO_4/NaOH
Working temperature (^{0}C)	23±2	-	Room	Room	23±2
Removed pollutant	CV	CV	CV	CR	CV
Working pollutants concentrations (mg/L)	15.63-500	25-150	-	25-500	6.25-200
Adsorbent amount (g/L)	0.5	0.25-1.25	-	0.025– 0.1 g	-
Best fitted kinetic model	pseudo-second-order	pseudo-second-order	-	pseudo-second-order	pseudo-second-order
Best fitted isotherm model	Langmuir	Langmuir-Freundlich	-	Langmuir	Freundlich
Max. adsorption capacity (mg/g)	142.61	90.52	39.56	161.3	23.55

CONCLUSION

In this study, the removal of CV dye was carried out with *Pa*L-Se synthesized from agricultural *Pa*L wastes. The effects of pH, adsorbent amount, initial dye concentration and contact time on CV dye removal were discussed. By comparing the values derived from the linear forms of 3 different isotherms and kinetic models, The most suited kinetic model was found to be Pseudo second order, and the most appropriate isotherm model was Langmuir. On the other hand, the maximum CV removal amount of *Pa*L-Se NPs was calculated as 142.61 mgCV/g *Pa*L-Se NPs. Studies were carried out between pH 3-9 to determine the effect of pH on the removal of CV dye from PaL-Se NPs. Additionally, studies were performed between pH 3-9 to determine the effect of pH on the removal of CV dye from PaL-Se NPs. In this case, it was determined that as the pH increased, the dye removal efficiency reached from 21.9% to 31.2%. Overall, these results indicate that green synthesis of selenium nanoparticles has the potential to be an effective, economical and environmentally friendly method for many scientific and technical applications in the future for the treatment of organic dye pollution in aquatic ecosystems.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that they have no competing, actual, potential or perceived conflict of interest

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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The use of biofertilizer contribution to plant development and yield in greenhouse broccoli cultivation

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Abstract

In this study, the effects of chemical fertilizers and fertilizers containing microorganisms on broccoli yield were examined. It is aimed to reduce the amount of chemical fertilizer by using microorganisms. Mundo F1 Broccoli variety was used as plant material. The research was established according to the randomized block trial design with 3 treatments and 3 replications, and 20 plants were used in each replication. Applications: 1. Control: 100% chemical fertilization (U1), 2. Treatment II: 70% chemical fertilization + Microorganisms (U2), 3. Treatment II: 100% chemical fertilization + Microorganisms (U3). The aim of the study is the effects of chemical fertilizers and microorganisms on plant growth and development; To examine the effect of plant height, stem diameter, number of leaves and yield. It was concluded that the number of leaves, plant height and stem diameter generally increased in the plots where microorganisms were applied. When the results were evaluated in terms of yield compared to the control treatment, U2 treatment increased yield by 20% and U1 treatment increased yield by 15%.

Keywords: *Brassica oleracea* var. *italica*, Chemical fertilizer, Fertilizer, Microorganism

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INTRODUCTION

As a result of the population increase in the world, it is clear that agricultural production must increase in order to meet the need for food, and fertilizer use, which is the most important input to increase the amount of production, will increase. As a result of the rapid increase in the world population, food and feed production will necessarily reach 3 billion tons in 2050. In this case, the most emphasized issue today is the decrease in natural resource reserves despite population growth and economic development (Karaçal & Tüfenkçi, 2019). In crop production, fertilizers are used intensively to achieve high yields and maximum growth. In vegetables, inorganic fertilizers are given more than necessary by producers in order to increase growth and development and consequently to obtain high yields (Badr & Fekry, 1998; Arisha & Bardisi, 1999; Dauda et al., 2008). However, although chemical fertilizers increase the amount of production, they pollute the soil and groundwater resources.

Living organisms have an important place in the natural structure of soil. These are divided into two as soil flora and soil fauna. Soil flora, i.e. plant organisms, ranks first in terms of activity. This group includes bacteria, fungi, actinomycetes and algae. Each of these has different benefits in terms of soil productivity. Dwindling fertilizer resources and expensive production costs in the world indicate that we will experience serious yield problems in crop production in the next 50 years (Ortas & Lal, 2011). For this reason, the use of these microorganisms, which reduce the demand for chemical fertilizers, are friendly to nature and the environment, and are from the ecology's own natural resources, in agricultural production is becoming increasingly important. In recent years, the interest of researchers has been directed towards studies to ensure agricultural sustainability by using beneficial microorganisms instead of chemical fertilizers and pesticides.

Preparations called "biofertilizers", which contain some bacteria, fungi and algae species, have started to be preferred by producers in recent years due to the benefits they provide. When used in cultivation, biofertilizers improve the soil by increasing biological activity and have a positive effect on the physical, chemical and biological structure of the soil. Apart from that, they help nutrient uptake and provide tolerance against

environmental stresses by increasing the availability and usefulness of plant nutrients through nitrogen fixation, phosphate and potassium solubilization or mineralization, release of plant growth regulators, antibiotic production and biodegradation of organic matter in the soil (Sinha et al., 2014; Sivakumar et al., 2013). As a result; it also increases yield and product quality by promoting plant growth and development. Biofertilizers differ from chemical and organic fertilizers in that they do not provide any direct nutrients to plants in agricultural production and are special cultures of bacteria and fungi, relatively simple and low installation costs.

Soil microorganisms are involved in many chemical changes in the soil and are important elements of soil fertility as they are involved in the cycling of nutrients necessary for plant growth, for example nitrogen and carbon. The beneficial microorganisms in bio-fertilizers promote plant growth directly and indirectly by establishing colonies in the rhizosphere and endorhizosphere of the plant (Saxena et al., 2005).

By asymbiotic nitrogen fixation, increasing the solubility of inorganic phosphorus and mineralization of organic phosphorus compounds, increasing the uptake of iron through siderophore production and some other trace elements through organic acid production, beneficial bacteria can improve the mineral nutrition of plants and promote growth. In addition, plant growth can be directly enhanced through the production of plant hormones such as auxins, gibberellins, cytokinins, inhibition of ethylene synthesis through 1-Aminocyclopropane-1-carboxylate (ACC) deaminase enzyme activity, reduction of environmental stress; harmony in the bacteria-plant relationship, vitamin synthesis, and increased root permeability (Esitken et al, 2005; Şahin et al., 2004; Zahir et al., 2004; Canbolat et al., 2006; Fuentes-Ramirez and Caballero Mellado, 2006; Aslantas et al., 2007; Çakmakçı et al., 2007 a; Cakmakci et al., 2007 b; Akgül and Mirik, 2008; Yildirim et al., 2011; Cakmakci et al., 2009). They stimulate the development of plant organs through cell division and expansion (Taiz and Zeiger, 2002) or by improving nutrient uptake (Chabot et al., 1996; Yanni et al., 1997). Through these mechanisms, plant growth-promoting bacteria benefit plant growth by increasing germination rate, root growth, yield, leaf area, chlorophyll content, Mg, N content, protein, hydraulic activity, drought resistance, shoot and root weights and delaying the formation of the abscission layer in leaves (Lucy et al., 2004).

Bacteria such as *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, *Azospirillum*, *Pseudomonas* and *Bacillus* have been reported to be present in India from desert ecosystems to acidic soils, saline soils to alkaline soils (Selvakumar et al., 2009, Upadhyay et al., 2009). The adaptation of microorganisms to different stress factors has occurred through many complex processes (Srivastava et al., 2008). While in some species that can survive under extreme environmental conditions (thermophiles and halophiles) the optimum conditions for metabolic activities such as enzymatic activities may be high temperature and salinity, other microorganisms have developed different adaptation mechanisms to cope with stress. Some bacteria, such as *Pseudomonas*, can survive under stress conditions because they produce exopolysaccharide (EPS). EPS protects microorganisms against water stress and flooding (Sandhya et al., 2009). Exopolysaccharide has a unique ability to retain and bind water and has important effects in regulating the structure and balance of soil aggregates and in the transport of nutrients dissolved in water to the plant root zone (Roberson and Firestone 1992; Tisdall and Oadea 1982).

Furthermore, microorganisms can play an important role in stress management due to their tolerance to unusual conditions and their ability to exist in many different climatic and soil conditions for the same reason. These organisms can also be used as an important model to unravel the mechanism of tolerance to different stressors. In this way, tolerance mechanisms against stressors such as cold damage, salinity, heavy metal damage and high temperature can also be developed. In the last century, it has been proven that bacteria belonging to different genera such as *Rhizobium, Bacillus, Pseudomonas, Pantoea, Paenibacillus, Burkholderia, Achromobacter, Azospirillum, Microbacterium, Methylobacterium, Variovorax, Enterobacter*, etc. increase the tolerance of host plants to different abiotic stress factors (Grover et al., 2011). These bacteria exert these effects through ACC deaminase enzyme activity. Polymeric ACC deaminase linked to pyridoxal 5-phosphate (PLP) was first studied with Pseudomonas by researchers, and especially the studies were directed towards the promotion of plant growth under stress conditions and the reduction of the negative effects of stress (Mayak et al., 2004; Madhaiyan et al., 2006).

Broccoli (*Brassica oleracea* var. *italica*), which is consumed raw or cooked as small green tubers, is among the vegetables of the *Brassicaceae* (*Cruciferae* family). In broccoli, it is important for market quality that the flower stalks do not open, the crown color maintains its green color when it reaches the harvest width and has a smooth shape. According to 2023 data, our broccoli production is 120 549 tons. Broccoli, which is grown in large areas in Europe and America and consumed fondly, has been rapidly increasing in production and consumption in our country in recent years. Especially its positive effects on health increase the demand for broccoli. Broccoli (*Brassica oleracea* var. *italica*) is widely consumed worldwide due to its high nutritional value (e.g., rich in vitamins A, B2, C, minerals (Anonymous, 2018; Glaser & Lehr, 2019), fiber and low calories (Anonymous, 2017). It is beneficial for health due to its high content in antioxidants and bioactive compounds such as atocopherol, β-carotene and isothiocyanates (Babalola, 2010). It has also been found to have anticancer properties in recent studies, especially in relation to specific phytochemicals such as organosulfuric compounds,

sulforaphane and glucosinolates (Cruz et al., 1993;Yadav & Sarkar, 2019; Zia et al., 2021). The increase in the living standards of people, the awareness of consumers, the importance of "healthy" and "high quality" food as well as being satisfying, and the emphasis on the effect of adequate and balanced nutrition on human health and development have caused producers to be more attentive during cultivation and to take many measures. With the development of technology, excessive use of fertilizers or pesticides has increased in intensive agricultural areas, which has negatively affected human health and environmental health. In vegetable cultivation, the use of fertilizers is more intensive due to the reasons such as more plants per unit area, especially the high yield of the varieties bred in recent years, thus removing more plant nutrients from the soil, and obtaining several times more yield from the unit area compared to open cultivation, especially in greenhouse cultivation. For this reason, research on practices that reduce the use of chemical fertilizers in vegetable production or products of organic origin that can be used in the nutrition of plants such as chemical fertilizers has been quite high in recent years. Especially due to the pandemic, healthy nutrition and products that strengthen the immune system have come to the forefront, and the importance of practices that will not adversely affect the environment and human health has increased even more.

In our research, a commercial biofertilizer containing many beneficial microorganisms was used in the cultivation of broccoli, a vegetable that stands out with the above-mentioned characteristics and is in constant demand in the market. No research has been conducted on broccoli under Malatya conditions before and the producers in the region are far from broccoli cultivation. Therefore, it was thought that the research would contribute to the producers of the region. The aim of the study was to determine whether the biofertilizer used in the experiment saves chemical fertilizer fertilizer in broccoli cultivation and its effect on plant growth and yield. In addition to being a fertilizer for plants exposed to different abiotic stresses in crop production due to climate change in recent years, the use of biofertilizers is also important to provide resistance against stress. Here, we used a biofertilizer that can be found commercially by the producers in the market, so that the results can be directly recommended to the producers.

MATERIALS AND METHODS

This study was conducted in a plastic greenhouse (270 m^2) belonging to the Faculty of Agriculture of Malatya Turgut Özal University between October/March 2020-2021 (Figure 1).

Figure 1. Appearance of broccoli plants planted in the experimental greenhouse.

Plant Material

Mundo F_1 (Asgen Tarım Tic. A.Ş.) Broccoli variety was used as plant material in the research. This variety is a medium late, 80-85 days old variety. It has a very tight head structure, dark green in color and round. The side heads after the top is cut are of the same quality. It is suitable for spring, fall and summer planting.

Fertilizers used in the study and their application

Sufficient studies have been carried out with single strains of bacteria isolated and propagated from the laboratory environment, and the benefits of the bacteria to the plant in single use have been reported by researchers. What needs to be done in the future is to bring together their combined use and the benefits they provide to the plant in different aspects. The biofertilizer we used in our study here is from Japanese oak and is a biofertilizer that can be easily supplied by commercial producers. It contains beneficial microorganism species for which research and development studies have been carried out and which will not be a problem in terms of production in the same solution.

Bio-fertilizer; Saion EM with its commercial name, contains beneficial microorganisms *Pseudomonas fluorescens, Rhizobium leguminosarum, Azotobacter chroococcum, Bacillus subtilis, Serratia aquatilis, Aspergillus oryzae, Penicillium chrysogenum* Biofertilizer application was done as recommended by the company. The number of live microorganisms guaranteed by the company: $1x10^7$ kob/ml. Liquid biofertilizer containing active microorganisms was started to be applied 13 days after planting. This process continued at 10-day intervals until the end of harvest. In this application, 3 liters/da was given to the plants.

Commercial fertilizers; commercial fertilizers used in conventional cultivation were given during the growing season at the doses recommended for broccoli cultivation according to the results of soil analysis. Commercial fertilizers used in the experiment were Triple Super Phosphate, Potassium Nitrate, Ammonium Sulphate. In addition, elemental sulfur was given to reduce soil pH and during seedling planting. Fertilization was calculated as 15 kg N, 20 kg P_2O_5 and 20 kg K_2O per decare and applied to the plots.

The research was established according to the randomized block design with 3 treatments and 3 replications, and 20 plants were used in each replicate. Broccoli seedlings were planted in the greenhouse on October 1 with 100 cm between rows and 30 cm above rows. Before planting, basic fertilization was given to all treatments during soil preparation. In order to determine the effect of the use of microorganisms on fertilizer saving, a treatment in which commercial fertilizers were reduced by 30% was also included in the experiment. All cultural operations were carried out regularly from planting to harvest.

Treatments;

1. Control: Plots where all recommended fertilizer amounts were applied in broccoli production (100% chemical fertilization). This is the type of fertilization used by producers in broccoli production in conventional cultivation.

2. Treatment I: Plots with 30% reduced rates of recommended fertilizer amounts and active microorganisms in broccoli production (70% chemical fertilization + Microorganisms). It is a treatment to reduce the use of chemical fertilizers in conventional farming and to improve the soil with the use of beneficial microorganisms.

3. Treatment II: Plots where all recommended fertilizer amounts and effective microorganisms were applied in broccoli production (100% chemical fertilization + Microorganisms). This application was made to see the contribution of using biofertilizers in addition to chemical fertilizers in conventional production without reducing chemical fertilizers to the soil and plants.

Measurements on plants

Plant growth and development measurements (plant height, stem diameter and number of leaves) started one month after planting the seedlings in the greenhouse and were carried out four times at four-week intervals. In addition, root length, root, stem, stem, leaf fresh and dry weights were taken on the plants that were uprooted twice during the young plant period (December 09) and at the end of the experiment (March 24). Broccoli heads of the expected size could not be obtained during the cold period in the unheated plastic greenhouse. Harvesting started on March 11 and ended on March 30, and a total of 4 harvests were made. The first harvest values of the treatments were evaluated as early yield and the effect of microorganism application on early yield was determined. Then, all harvest values were summed and total yield was obtained.

RESULTS AND DISCUSSION

Plant Growth Parameters: The results of plant growth parameters on broccoli plants on 4 different dates are presented in Table 1. When we look at the results of plant height; although there was no significant difference in the first measurements in the treatments using microorganisms, it was determined that microorganism treatments increased the plant height by 8-9% at the last measurement date. Plant height in plots with 30% fertilizer reduction (U1) was almost the same as in plots with 100% fertilizer + microorganisms (U2). The difference between the treatments was found to be statistically significant at the last measurement date. When we examined the stem diameter values, the stem diameter values of the plants in the plots where microorganisms were applied were higher on the first measurement date, and the stem diameters of the plants in the plots where 30% fertilizer was reduced (U1) were higher than the plots where 100% fertilizer + microorganisms were applied (U2) in the last measurements except the first two measurement dates. There was a statistically significant difference between the treatments only on the 3rd measurement date. At the last measurement date, microorganism application provided an increase of 7-11% in stem diameter. When we look at the number of leaves; it is seen that the plants formed more leaves in the plots where 20-30% microorganisms were applied in the first two measurement dates. Even the difference determined on the 2nd measurement date is at the level of statistical significance. In the following measurement dates, the lower old leaves were pruned as the plants grew and the number of leaves in all treatments were found to be close to each other.

Table 1. Effect of biofertilizer treatment on some growth parameters of broccoli measured at different dates

Date of Measurement	Treatments	Plant Height (cm)	Stem Diameter (mm)	Number of Leaves (number)
3 November (32 days after planting)	Control	37.36	10.66	10.39
	U-1	38.13	13.12	12.76
	U-2	38.29	11.52	13.73
	Std sp.	00.41	01.02	01.40
21 November (60 days after planting)	Control	51.59	23.23	14.15 b
	U-1	55.63	22.82	18.79 a
	U-2	52.93	22.16	18.66 a
	Std sp.	01.68	00.44	02.15
9 December (88 days after planting)	Control	57.53	23.76 b	21.49
	U-1	60.59	24.62 a	24.13
	U-2	58.46	25.66 a	21.43
	Std sp.	01.28	00.77	01.25
25 December (116 days after planting)	Control	61.63 b	23.92	24.76
	U-1	66.93 a	25.66	26.63
	U-2	66.46 a	26.65	24.56
	Std sp.	02.39	01.12	00.93

In addition to the measurements made on the plants in the greenhouse, the plants were uprooted on the measurement date on December 9, 88 days after planting, and some of the plant growth parameters were measured in the uprooted plants (Table 2). The effects of treatments on root length, root, stem and leaf fresh and dry weights were analyzed. The difference in leaf fresh weight was statistically significant except for the root and stem dry weight parameters. In the young period, microorganisms are more effective in plant growth. On December 9, when we examined the results of the plant uprooting, we found that the root length in the plots with 30% reduced fertilizer + microorganisms (U1) was about 20% longer than the control plant roots, and the plant roots in the plots with 100% fertilizer + microorganisms (U2) were about 47% longer. In parallel with these results, root dry weight values were 66% and 56% higher in the plots treated with microorganisms compared to the control plots. Root development was quite good in the plots treated with microorganisms and this was reflected positively on the above-ground parts. When we take stem+leaf dry weight values, 85% more dry matter accumulation was found in plots with 30% reduced fertilizer + microorganism (U1) and 50% more in plots with 100% fertilizer + microorganism (U2).

Table 2. Some measurement results of broccoli plants uprooted 88 days after planting (December 9)

Treatments	Root Length (cm)	Root Fresh Weight (g)	Leaves Fresh Weight (g)	Stem Fresh Weight (g)	Root Dry Weight (g)	Leaves Dry Weight (g)	Stem Dry Weight (g)
Control	23.66	42.16	346.94	89.11	12.54	37.55	24.14
U-1	25.83	44.99	324.52	70.96	11.49	22.57	21.80
U-2	34.66	48.02	315.47	107.59	10.17	32.75	22.78
Std sp.	04.76	02.39	13.23	14.95	00.97	6.25	00.96

The boosting effect of biofertilizers may be due to the fact that microorganisms increase root activity in the rhizosphere, initiate hormonal activity and thus increase the uptake of plant nutrients (Vessey, 2003; Itelima et al., 2018; Kamal et al., 2021). Many studies have reported the supportive effects of biofertilizers on plant growth. Rather et al. (2018) found that *Azotobacter* and *Azospirillum* bacteria increased the amount of IAA and root length, enhanced cytokinin formation and root branching, thus increasing nutrient uptake from the soil and accelerating plant growth.

The yield and plant growth improvement effects of the bacteria used in this study can be explained by their N_2-fixing and P-solubilization capacities. The positive effects of biofertilizers on yield and growth parameters (such as apricot, tomato, sugar beet and barley) are explained by their N_2-fixing ability, phosphate solubilizing capacity, indole acetic acid and antimicrobial production (Esitken et al., 2005; Rodríguez et al., 2006; Wilsion, 2006; Malik et al., 2001). In general, improvements in macro/micro nutrient contents were found to be higher in PGPR treatments. The increase in mineral uptake by plants is due to the contribution of biofertilizers to the plant. They reported that the use of N_2-fixing and P-solubilizing PGPR in barley (Rodríguez et al., 2006), tomato (Caballero-Mellado et al., 2007), and lettuce (Bar-Ness et al., 1992), increased macro-micro nutrient uptake in plants. According to the results of Valverde et al. (2015), the application of biofertilizer containing Azospirillum + Azotobacter (50% each) to broccoli during transplanting by root dipping method increased the yield as well as the functional biomolecules in the plant. Singh et al. (2014) and Choudhary & Paliwal (2017), showed that the integration of bio-organic and mineral fertilizers showed a significant effect in maximizing broccoli yield.

Total Yield: When the total yield values (Figure 2) were analyzed; similar results were obtained for plant growth parameters. Yield in plots treated with microorganisms was higher than the control. Yield in plots with 100% fertilization and microorganisms (U2) was 20% higher than the control and yield in plots with 30% reduced fertilization + microorganisms (U1) was 15% higher than the control.

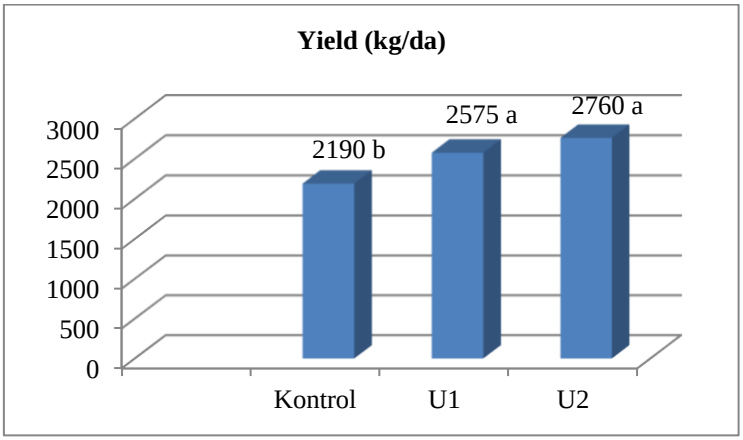

Figure 2. Effect of biofertilizer treatments on broccoli yield (std sp.: 237.42)

As in other cultivated plants, yield is the most important criterion in vegetable cultivation. Although it is a known practice to increase yield with chemical fertilization, the effective use of biofertilizers and the reduction of chemical fertilizer applications may necessitate the updating of fertilization practices. With this result in the yield parameter, environmental and economic gains can be achieved by reducing the use of chemical fertilizers. It can be said that this situation is due to the activity of microorganisms in the soil. Various studies have reported an increase in productivity and a decrease in the use of chemical fertilizers thanks to biofertilizers. Panda (2011) and Berg (2009) reported that biofertilizers have an effect on yield in the range of 35-65%. Some researchers have also determined that NPK use can be reduced with the use of microbial fertilizers (Chauhan and Bagyaraj, 2015; Yıldırım et al., 2011).

Some bacteria, such as *Bacillus* and *Azotobacter*, can synthesize organic acids and phosphates that convert the unavailable form of phosphorus into a usable form for plants (Tošić et al. 2016), while *Pseudomonas*, *Bacillus* and *Rhizobium* bacteria are known to be the most powerful phosphate solubilizing bacteria (Rodríguez and Fraga, 1999). Among the phosphorus-solubilizing microorganisms, phosphate-solubilizing bacteria have the potential to solubilize 1-50% of phosphorus (Chen et al., 2006) and phosphate-solubilizing bacteria secrete phosphate organic acid metabolites containing hydroxyl and carboxyl group chelates and convert them into a usable form by binding with cation bonds (Sagoe et al., 1998).

Microorganisms work more efficiently if there is a lack of nutrients in the environment. In the light of previous studies, we can say that microorganisms increase nutrient uptake in the soil and consequently increase yield. Recent research has shown that *Rhizobium leguminosarum*, *Rhizobium* spp. and *Bradyrhizobium* spp. increased plant biomass, yield and chlorophyll content in plants compared to non-inoculated plants. The highest increase was recorded in IRBG strains, which showed a 14% increase compared to uninoculated plants (Verma et al., 2019). Similarly, some Rhizobia strains increased the surface area, photosynthetic rate, water uptake capacity, yield and stomatal conductance of inoculated plants (Enebe & Babalola, 2018). In addition, a bacterial mixture of *Pseudomonas*, *Bacillus lentus* and *Azospirillum brasilense* was reported to increase chlorophyll content and antioxidant enzymes in plants under stress conditions (Brahmaprakash et al., 2017). Khalid et al. (2017) found that biofertilizer application increased growth, chlorophyll content, antioxidant activity, yield and phenolic compounds in spinach. Total phenolic compounds were reported to be 58% higher than uninoculated

spinach. Similarly, Arora et al. (2018) reported an increase in growth, yield, phenolic compounds, anthocyanins and carotenoid content of lettuce when inoculated with *Azotobacter chroococcum* and *Piriformospora indica*.

Hassen et al. (2016) reported an 80% increase in soybean yield when inoculated with nitrogen-fixing *Rhizobium* and *Bradyrhizobium.* Dicko et al. (2018) used plant growth-promoting bacteria in a study conducted by Dicko et al. (2018), which found that biofertilizer increased plant growth and yield, and increased corn yield. Recently, the effect of biofertilizer made from plant growth-promoting *Bacillus pumilus* strain TUAT-1 was evaluated on rice genotype, and it was revealed that biofertilizer made from Bacillus strain increased rice yield (Win et al., 2019). In the study conducted by Fathi (2017), in maize, biofertilizer containing phosphate solubilizing bacteria was used, and it was reported to increase maize growth and yield compared to the uninoculated control. Altuntaş (2018) reported that the highest total head yield was obtained by using Bacillus subtilis inoculations in broccoli. In addition to the increase in yield and crop quality in the use of biofertilizers, more importantly, soil nutrients are reduced as a result of different activities occurring in the soil, such as surface runoff, burning of crop residues and washing of agricultural soil. The nutrients in the soil travel through rain-induced runoff to the groundwater body, where they cause eutrophication and contamination of the groundwater body (Yu et al., 2019). This poses a major threat to the natural environment. Therefore, the application of nutrient-rich biofertilizers made from plant growth-promoting microorganisms with potentials such as nitrogen fixation, potassium solubilization and phosphate solubilization is essential in recovering soil nutrients to enhance plant growth and yield performance (Olanrewaju et al., 2019).

CONCLUSION

The use of microorganisms such as bacteria, fungi, actinomycetes and algae as biological fertilizers reduces the environmental risk in agricultural production. These preparations, which can be easily used in organic production, come to the forefront as an environmentally friendly technique with the fact that they do not cause environmental pollution, on the contrary, they improve the structure of the soil, in addition to the benefits they provide to plants in recent years when environmental awareness has developed. The results of our researches have revealed that the biofertilizer we use in broccoli cultivation positively affects plant growth and yield. Both the growth and development parameters of the plants in the greenhouse and the biomass measurements as a result of plant removal gave higher results in the applications using biofertilizer. In addition, the increase in yield (15% and 20%) shows that it is worth recommending the biofertilizer to the producer. Biofertilizer, which contains different microorganisms that enable plants to effectively utilize the given nutrients and contains different microorganisms in its content, is important both environmentally and economically with this increase in yield. Biofertilizer is used approximately 2.5 liters per decare during the production season. In this case, it is possible to improve the soil, especially biologically, and increase the yield with an environmentally friendly and cheap input. Recommending biofertilizers containing microoganisms in fertilizer recommendations in open and greenhouse vegetable cultivation will be beneficial in terms of plant development and soil improvement and increasing biological activity.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Impact of bio-pesticides and storage containers on lentil seed preservation and pre-sowing fungal treatment

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Abstract
We conducted a study to determine the most effective method of preserving lentil seeds for future sowing. The experiment involved six different types of storage containers: cotton cloth bags, tin containers, earthen pots, plastic containers, polythene bags, and gunny bags. We also used four plant extracts: Piper betel (Betel leaf), Azadirachta indica (Neem), Allium indica (Garlic), and Swietenia mahagoni (Mahagani). We measured the vigor index and germination percentage at 2, 4, and 6 months after storage, and then documented the fungal connection. In a separate experiment, we conducted a pre-sowing seed treatment using botanicals and biological agents such as garlic (5% w/v aqueous solution), datura (5% w/v aqueous solution), mehogoni leaf extract (5% w/v aqueous solution), mehogoni seed extract (5% w/v aqueous solution), and fern leaf extract (5% w/v aqueous solution). We treated the seeds with various substances to suppress seed-borne fungi, including ash coating (10 g kg-1 seed), fresh cow dung coating, a solution of cow urine (5% v/v water), Provax-200 (2 g kg-1 seed), and an untreated control group. In terms of germination, vigor index, and seed infection, the lentil seeds stored in a polythene bag with neem leaf extract significantly outperformed the other treatments. We found that the durability of lentil seeds significantly decreased as the storage time increased. The seed treatment fungicide Provax-200 had a significant impact on lowering the presence of fungus (by 87.41%) and boosting the germination percentage (by 39.49%) of lentil seeds.
Keywords: Lentil seed preservation, Storage containers, Plant extracts, Seed germination, Seed-borne fungi, Pre-sowing treatment

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INTRODUCTION
In Bangladesh, pulses are a crucial component of the daily diet, serving as a primary source of protein (Das et al., 2016). Among the several pulses available, lentils are the most widely consumed, surpassing mung beans, chickpeas, and black grams in popularity. In our country, the average daily consumption of lentils is only 4 grams per person. However, the World Health Organization (WHO) recommends a daily intake of 45 grams of pulses per person (Rahman et al., 2013). The principal area for lentil cultivation in the Mid-Western region of Bangladesh includes Jashore, Jhenidah, Magura, Faridpur, Rajbari, Kushtia, Chuadanga, Madaripur, Meherpur, and Pabna districts (Afzal et al., 1999).

Seeds generally degrade quickly when exposed to high temperatures and relative humidity during storage, which creates ideal conditions for the growth of pests and fungi that affect the seeds. This negatively affects the germination of the seeds. Farmers store the majority of seeds sown in the field (98.85%), while BADC supplies a small portion (1.15%). However, the storage conditions for the BADC-supplied seeds sometimes lack sufficient temperature and moisture control. Seeds are the primary and crucial component in crop production. The use of high-quality seeds significantly enhances agricultural productivity. The inadequate storage conditions led to a

decline in the quality of the seed, as reported by Fakir et al. (2007), which ultimately resulted in a low yield. In Bangladesh, the lentil yield is comparatively low (0.963 t ha-1) compared to other countries such as Australia (1.50 t ha-1), Canada (1.80 t ha-1), and Ethiopia (1.22 t ha-1) (BBS 2011, Matny 2015, Wang 2017) due to a scarcity of high-quality seeds (Kashem et al., 2005).

Proper storage conditions and seed maintenance are critical factors in obtaining high-quality seeds for sowing. The optimal storage conditions and proper care are crucial for preserving the high germination rate and seed vigor for the following year's planting. Currently, our country lacks sufficient supplies and facilities for seed storage at the farmer's level, despite its crucial role in crop production. Applying fungicides to seeds is an important method in crop management to prevent seed-borne diseases and ensure the production of healthy plants (Mortuza et al., 2002). The application of fungicidal treatment not only protects seedlings from soil-borne diseases but also improves plant development and vitality in lentils (Kovacicova, 1970). Pesticide application in the field is a highly successful and universally endorsed approach to disease control. Lentil farmers and researchers widely utilize chemical fungicides due to their convenience and efficacy in controlling field diseases. Several studies (Ahmed, 2011; Huq and Zaman, 2007; Gupta et al., 1996; Bakr and Ahmed, 1992; Iqbal et al., 1989) support this.

The use of plant extract for controlling seed-borne fungi is not as widely practiced as chemical control. However, several studies have shown that botanical extract can effectively control seed-borne fungi in other crop diseases (Bhatiya et al., 2007; Islam, 2005; Bowers and Locke, 2000; Sharma and Gupta, 1998). Plant extract is applied to treat seeds. Biological agents are more cost-effective, environmentally benign, and readily accessible compared to chemical agents (Karuna et al., 2012; Khan et al., 1999).

Recent studies highlight the potential of seed priming and biological treatments to improve seedling growth and resistance to pathogens. Anwar et al. (2020) demonstrated that seed priming enhances growth and nutrient content in cucumber seedlings. Damalas et al. (2019) showed hydropriming's positive effects on seed germination and field performance in faba beans. Devika et al. (2021) emphasized seed priming as a supplement in integrated resource management. Feng et al. (2016) explored the relationship between plant canopy characteristics and photosynthetic productivity in cotton, while Jain et al. (2012) highlighted microbial consortium-mediated defense in pea plants.

This research aims to determine the optimal technique for storing and treating seeds to preserve their long-term viability and health.

MATERIALS AND METHODS

Experimental Location

The study was conducted in the Plant Pathology Laboratory at the Regional Agricultural Research Station, Bangladesh Agricultural Research Institute (BARI), Jashore. The lentil variety used for the experiment was BARI Masur-6.

Preservation Containers

Six types of storage containers were utilized in the experiment:

1. Cotton cloth bag (C1)
2. Tin container (C2)
3. Earthen pot (C3)
4. Plastic container (C4)
5. Polythene bag (C5)
6. Gunny bag (C6)

Plant Extracts

Four plant extracts were tested for their efficacy:

1. Betel leaf (*Piper betel*) (T1)
2. Neem leaf (*Azadirachta indica*) (T2)
3. Garlic (*Allium sativum*) (T3)
4. Mahogany leaf (*Swietenia mahagoni*) (T4)

Preparation of Plant Extracts for Seed Storage

Leaves of *Piper betel*, *Azadirachta indica*, *Swietenia mahagoni*, and cloves of *Allium sativum* were cleaned, cut into small pieces, and blended in distilled water to prepare a 1% (w/v) solution. Each plant material (10 g) was blended with 1 liter of distilled water. These solutions were applied to the seeds using a hand sprayer. The seeds were then air-dried before storage, following the methodology of Tohmi et al. (2012).

Calculation of Vigor Index

Seedling vigor and germination percentages were recorded at 2, 4, and 6 months of storage. At the end of 6 months, a blotter test was conducted to assess germination and fungal infection rates. Seedling vigor was calculated using the formula:

VI = Dry weight of 10 seedlings X germination percentage (Kashem et al. 2005).

The experiment followed a Complete Randomized Design (CRD) with three replications. The treatment combinations were as follows: C1T1, C1T2, C1T3, C1T4, C2T1, C2T2, C2T3, C2T4, C3T1, C3T2, C3T3,

C3T4, C4T1, C4T2, C4T3, C4T4, C5T1, C5T2, C5T3, C5T4, C6T1, C6T2, C6T3, and C6T4. Germination percentage, fungal association, and vigor index were statistically analyzed using ANOVA in SPSS 15, with means compared using Duncan's New Multiple Range Test (DMRT).

Pre-Sowing Seed Treatments

The following treatments were applied to control seed-borne diseases in lentils:

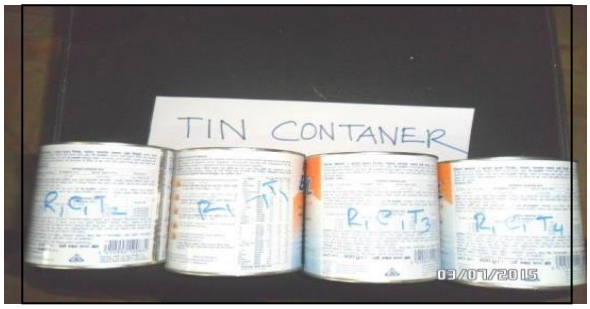
1. Garlic (5% w/v aqueous solution) (Tohami et al., 2002)
2. Datura (5% w/v aqueous solution) (Tohami et al., 2002)
3. Mahogany leaf extract (5% w/v aqueous solution) (Tohami et al., 2002)
4. Mahogany seed extract (5% w/v aqueous solution) (Tohami et al., 2002)
5. Fern leaf extract (5% w/v aqueous solution) (Tohami et al., 2002)
6. Ash coating (10 g/kg seed) (Meena, 2012)
7. Fresh cow dung coating (5 g/kg seed) (Meena, 2012)
8. Cow urine (5% v/v aqueous solution) (Meena, 2012)
9. Provax-200 (2 g/kg seed)
10. Control (untreated)

Preparation of Plant Extracts for Pre-Sowing Seed Treatment

Plant parts were cleaned, chopped, and blended with distilled water at a 1:1 ratio (100 g of plant material in 100 ml of water). The extracts were filtered through cheesecloth and stored at 4±1°C. Seeds were soaked in these extracts for 25 minutes, then air-dried. For ash and cow dung treatments, seeds were coated in a conical flask with the respective materials. Provax-200 was applied at 2 g/kg seed, and seeds were air-dried under cool conditions (Ahmed, 2011).

Blotter Test

Germination and fungal association were assessed using the blotter test method (ISTA, 2001). Four hundred seeds were randomly selected and placed on three-layered, sterilized, water-moistened blotter paper in sterilized glass petri dishes (9x12 cm), with 25 seeds per dish. Petri dishes were sterilized at 120°C for 12 hours before plating. The dishes were incubated at room temperature under a 12-hour light/dark cycle for seven days. The pre-sowing seed treatment experiment followed a CRD with four replications. Germination and seed infection percentages were recorded and analyzed using standard error and standard deviation formulas in MS Excel.



C_1

C_2

C_3

C_4

C_5 C_6

Figure 1. Different seed storing containers C1= Cloth bag, C2= Tin container, C3=Earthen pot, C4= Plastic container, C5= Polythene bag and C6= Gunny bag

T_1 T_2

T_3 T_4

Figure 2. Photograph of different botanicals T_1= Piper betel, T_2=Neem leaf T_3=Garlic and T_4= Mehogani leaf

Six containers and four plant extracts were used in this experiment. Germination percentage and vigor index after 2, 4 and 6 months showed significant difference amongthe treatments.

RESULTS

Germination percentage and Vigor Index (VI) at 2 months after storing

After 2 months of seed storage, a polythene bag containing neem leaf extract (C5T2) had the highest germination percentage (96.00%), followed by a polythene bag containing piper betel extract (C5T1) (92.00%). The results were statistically equivalent for both the polythene bag with mahogany extract (C5T4) (92.00%) and the polythene bag with garlic clove extract (C5T3) (91.00%). We reported the germination rate of seeds treated with garlic clove extract (C6T3) and stored in a gunny bag for 2 months as 80.67%, the lowest among all treatments. The polythene bag with neem leaf extract (C5T2) had the greatest vigor index (VI) at 105.60, followed by the polythene bag with garlic clove extract (C5T3) at 90.09. Table 1 shows that the application of mahogany leaf extract to gunny bags (C6T4) resulted in the lowest VI value of 59.28.

Germination percentage and Vigor Index (VI) at 4 months after storing

After 4 months, storing the seeds in a polythene bag with neem leaf extract (C5T2) resulted in the highest germination percentage (94.00%). A polythene bag containing mahogany leaf extract (C5T4) followed, exhibiting a germination percentage of 90.33%. The polythene bag with piper betel extract (C5T1) had a germination percentage of 90.00%, and the polythene bag with garlic clove extract (C5T3) had a germination percentage of 89.00%. Treating the seeds with garlic clove extract (C6T3) and storing them in gunny bags resulted in the lowest germination rate (78.67%). The polythene bag with neem leaf extract (C5T2) had the greatest vigor rating, measuring 102.44. The polythene bag with garlic clove extract (C5T3) followed with a vigor level of 88.11. The therapy that resulted in the lowest VI was the combination of gunny bag with neem leaf extract (C6T2), with a recorded value of 57.27 (Table 1).

Germination percentage, VI and seed infection at 6 months after storing

The germination percentage was highest (94.33%) after 6 months in the polythene bag containing neem leaf extract (C5T2), followed by the polythene bag containing garlic clove extract.We measured the efficiency of C5T3 at 90.33%, and the efficiency of the polythene bag containing mahogany leaf extract (C5T4) at 91.00%. Treating seeds with garlic clove extract (C6T4) and storing them in a gunny bag resulted in the lowest germination rate (64.00%). The polythene bag treated with neem leaf extract (C5T2) had the greatest vigor index, measuring 99.40. Garlic clove extract treated the gunny bag next. The gunny bag treated with mahogany leaf extract (C6T4) had the lowest VI value, 45.77. The highest rate of seed infection, at 39.17%, was observed in C1T2 (a cotton fabric bag treated with neem leaf extract), which was comparable to the infection rate of 38.23% in C1T4. C5T4, which used polythene bags containing mahogany leaf extract, had the lowest incidence of seed infection (5.87%). The seed infection rates in the C5T2 and C5T3 treatments were 7.37% and 7.44%, respectively, which were statistically indistinguishable from the infection rate in the C5T4 treatment. We also observed seed infection on the blotter after 6 months. C5T4 (5.87%) had the lowest seed infection rate, storing seeds in a polythene bag containing mahogany leaf extract. C5T2 (7.37%) and C5T3 (7.44%) followed, storing seeds in a polythene bag containing neem leaf extract and a polythene bag containing garlic clove extract, respectively. The seed infection rate was highest in C1T2, with a prevalence of 39.17%, followed by C1T4 with 38.23%, and C1T3 with 34.33%. The study found that the seed infection rate was the lowest when using polythene bags, as shown in Table 1.

Effect of pre-sowing seed treatment on fungal association

Various compounds employed in pre-sowing seed treatment exhibited varying responses against seed-borne fungi. The occurrence of Fusarium oxysporum varied from 0.50% to 6.67%. This fungus observed the highest incidence of seed infection in seeds treated with fresh cow dung and the control group (6.67%), followed by both mahogany leaf and seed extract (5.42%) and ash (5.41%). The lowest seed infection rate (0.50%) was found in seeds treated with Provax-200, followed by garlic clove extract (1.67%) in the case of F. oxysporum. The incidence of Alternaria tennuis infection was highest (5.75%) in the control group, followed by the seed coated with ash (4.25%) and fresh cow dung (3.67%). The seed treated with Provax-200 had the lowest infection rate (0.33%), followed by the garlic clove extract (1.08%). The prevalence of Stemphylium botryosum was highest in the control group (6.17%), followed by the groups treated with fern leaf extract (3.67%), mahogany leaf extract, and fresh cow dung (3.50%). The seed treated with Provax-200 had the lowest infection rate (0.17%), followed by the garlic clove extract (0.58%). Curvularia luanata caused the highest incidence of seed infection in the control group (5.25%), followed by the fern leaf extract group (2.33%) and the mahogany leaf extract group (2.25%). The seed treated with Provax-200 (0.50%) had the lowest incidence of fungal infection, followed by the garlic clove extract and the seed coated with ash (0.92%). The control group displayed the highest occurrence of Penicillium cladosporium association with seed (9.92%), followed by both mahogany seed extract and fern leaf extract (7.50%), and ash coating (6.42%). The fungus was least prevalent in the seed treated with Provax-200 (1.08%) and somewhat more prevalent in the seed treated with garlic clove extract (1.75%). Aspergillus niger caused the most significant seed infection in the seed treated with cow urine (9.33%), followed by fresh cow dung (9.00%) and mahogany seed extract (8.92%). The seed treated with Provax-200 had the lowest incidence of infection (2.83%), followed by the garlic clove extract treatment (3.65%). The control group had the highest prevalence of Aspergillus flavus (10.25%), followed by groups T7, T3, and T6. A. flavus caused the lowest seed infection in the Provax-200, with a rate of 2.92%, followed by garlic clove extract at 1.67%. The control group had the highest incidence of seed infection by Aspergillus ochraceous at 6.25%, followed by the mahogany leaf extract group at 4.50% and the mahogany seed extract group at 4.42%. The seed treated with Provax-200 had the lowest infection rate by this fungus, at 0.17%, followed by the garlic clove extract at 2.92%. The control group had the highest rate of Aspergillus parasiticus seed infection, with a prevalence of 3.50%, which was comparable to other groups. The sequence begins with the application of mahogany seed extract, followed by cow urine (3.41%). The seed treated with Provax-200 (0.08%) had the lowest incidence of infection by A. parasiticus, followed by the garlic clove extract treatment (1.50%). Both the control group and the fern leaf extract group showed the highest association of Aspergillus candidus (2.92%), followed by the datura leaf extract and ash group (2.42%). The seed treated with Provax-200 had the lowest association rate of 0.58%, whereas the garlic clove extract had a slightly higher association rate of 1.00%.. We computed the percentage increase in seed infection compared to the control. The

seed infection showed the greatest enhancement in Provax-200 (87.81%), followed by garlic clove extract (74.70%) and fresh cow dung-coated seed (60.64%). The seed infection showed the least improvement in mahogany seed extract (28.40%), followed by mahogany leaf extract (28.82%), as indicated in Table 2.

Table1. Effect of different container and plant extract on seed germination, seedling vigor and percent seed infection

Treatments	% Germination 2 months	% Germination 4 months	% Germination 6 months	VI at 2 months	VI at 4 months	VI at 6 months	% seed infectionat 6 months
C1T1	82.33ij	80.00jk	65.33h	65.87h	66.77hij	50.30hi	34.41b
C1T2	83.33hij	81.33jk	67.00gh	73.33e	71.57e-h	59.30g	39.17a
C1T3	84.00g-j	80.33jk	68.33gh	66.36gh	63.72ijk	54.05h	34.33b
C1T4	82.67ij	80.67jk	65.33h	64.77hi	62.92ijk	51.81h	38.23a
C2T1	87.00d-h	85.00e-i	71.33fg	78.30d	76.22c-f	63.90f	32.04bc
C2T2	88.00c-f	85.67d-h	74.67ef	78.32d	76.26c-f	65.90f	26.50efg
C2T3	87.33c-g	85.00e-i	73.67ef	70.74ef	68.85ghi	58.65g	26.05e-h
C2T4	87.00d-h	84.67f-i	77.33e	71.05ef	68.06ghi	63.99f	27.13def
C3T1	85.33e-i	83.33g-j	77.67e	68.28fgh	66.66hij	62.70fg	28.19de
C3T2	85.33e-i	83.33g-j	68.00gh	67.41fgh	63.18ijk	54.02h	24.18fgh
C3T3	84.33f-j	82.33h-k	66.33h	60.72j	59.72jk	50.28hi	23.60gh
C3T4	84.00g-j	82.33h-k	75.67ef	59.64j	64.04ijk	54.30h	22.83h
C4T1	90.67bcd	88.67b-e	86.67bcd	77.97d	78.03cde	72.35de	9.78jk
C4T2	90.33bcd	88.33b-f	85.33cd	78.90d	78.90cd	76.44cd	12.41j
C4T3	89.67bcd	88.00b-f	87.67bcd	80.10d	74.83c-g	77.52c	10.14jk
C4T4	88.33b-e	86.33c-g	84.00d	69.78efg	73.50d-h	71.54e	12.41j
C5T1	92.00b	90.00bc	89.00bc	83.72c	81.06c	87.28b	7.65kl
C5T2	96.00a	94.00a	94.33a	105.60a	102.44a	99.40a	7.37kl
C5T3	91.00bc	89.00bcd	91.00ab	90.09b	88.11b	83.71b	7.44kl
C5T4	92.00b	90.33b	91.00ab	70.84ef	69.56f-i	70.84e	5.87l
C6T1	81.67ij	79.67jk	66.00h	61.25ij	59.95jk	50.58h	30.42cd
C6T2	82.67ij	80.67jk	73.67ef	58.69j	57.27k	50.13hi	18.60i
C6T3	80.67j	78.67k	66.67h	62.13ij	60.57jk	51.23h	27.77de
C6T4	83.33ij	80.33jk	64.00h	59.28j	57.48k	45.77i	27.85de
CV (%)	2.32	2.34	2.26	2.86	5.44	4.10	8.37
F-test	**	*	**	*	*	*	**

N.B. VI= Vigor index, C1=Cloth bag, C2=Tin container, C3=Earthen pot, C4=Plastic container, C5=Polythene bag and C6=Gunny Bag; T1=Piper betel, T2=Neem leaf, T3= Garlic T4=Mahogany leaf. Mean(s) followed by common letter(s) do not differ significantly at 0.05 level. *=significant at 0.05 level, **=significant at 0.01 level.

Germination percentage influenced by pre-sowing seed treatment

The germination percentages were recorded and found to be highest in the seed treated with Provax-200 (93%), followed by garlic clove extract (81.42%) and datura leaf extract (79.17%). The seed treated with fresh cow dung exhibited the lowest germination percentage, which was 56.50% (Figure 3).

Table 2. Fungal infection in lentil seed treated with biological agents and fungicide

Treatmnts	% fungal infection											
	Fusarium oxysporum	*Alternaria tennuis*	*Stemphylium Botryosum*	*Curvularia Lunata*	*Penicillium cladosporium*	*Aspergillu sNiger*	*A. flavus*	*A. ochraceous*	*A. paraciticus*	*A. candidus*	Total	% Improve-ment
T1	1.67	1.08	0.58	0.92	1.75	3.65	2.92	0.83	1.50	1.00	15.90	74.70
T2	4.33	3.33	2.83	1.83	5.83	6.50	5.75	3.25	2.08	2.42	38.15	39.30
T3	5.42	3.42	3.50	2.25	6.33	8.75	6.42	4.50	2.48	1.67	44.74	28.82
T4	5.42	3.58	3.33	2.08	7.50	8.92	4.17	4.42	3.50	2.08	45.00	28.40
T5	3.83	2.58	3.67	2.33	7.50	8.08	5.42	2.67	3.08	2.92	42.08	33.04
T6	5.41	4.25	3.17	0.92	6.42	8.08	6.25	4.00	2.67	2.42	43.59	30.64
T7	6.67	3.67	3.50	1.50	5.50	9.00	6.58	2.83	2.50	1.92	43.67	60.64
T8	4.42	2.92	3.00	1.50	6.17	9.33	5.33	2.83	3.41	2.17	41.08	34.64
T9	0.50	0.33	0.17	0.50	1.08	2.83	1.67	0.17	0.08	0.58	7.91	87.41
T10	6.67	5.75	6.17	5.25	9.92	6.17	10.25	6.25	3.50	2.92	62.85	-
Mean±SE	4.43±0.78	3.09±0.64	2.99±0.67	1.91±0.25	5.80±0.84	7.13±0.77	5.48±0.72	3.18±0.71	2.48±0.45	2.01±0.45	-	-

N.B. T1=Garlic clove extract, T2=Datura leaf extract, T3= Mahogany leaf extract, T4= Mahogany seed extract, T5= Fern leaf extract, T6= Coated with ash,T7= Coated with fresh cow dung, T8= Cow urine, T9= Provax-200 and T10= Control (untreated)

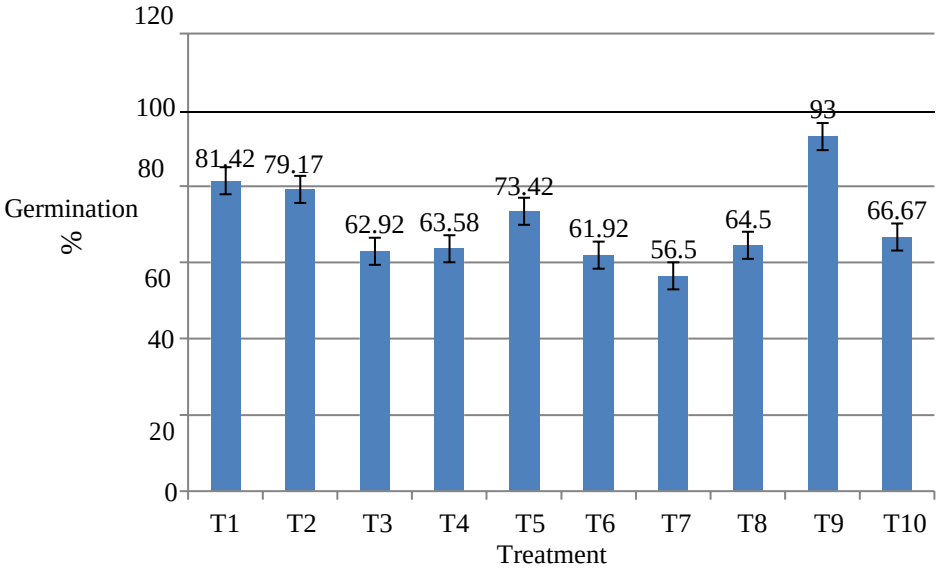


Figure 3. Effect of seed treatment by biological agents and fungicide on seed germination

DISCUSSION

It was clearly shown that lentil seeds stored in a polythene bag with neem leaf extract did better than other treatments when it came to germination, vigor index, and seed infection (Table 1). We found that the durability of lentil seeds significantly diminished as the storage time increased. Sadhu and Kar (2009) found comparable results when using neem extract to treat blackgram seeds. Additionally, researchers found that lentil seeds retain their vitality more effectively in a polythene bag than in other types of bags or containers. Kashem et al. (2005) observed in their study that storing lentil seeds in a polythene bag improved germination, plant growth, and seedling vigor. However, Prashant et al. (2007) discovered that piper betel is more efficient in preserving the germination and vigor of rice seeds. Both this study and earlier related studies demonstrate that storing seeds with the appropriate moisture content in an airtight container results in improved health. A container that lacks total air-tightness fails to prevent air from coming into contact with the seed. This results in the absorption of moisture from the air, which stimulates the seed's physiological processes and allows seed-borne fungus to cause the seed's health to decline over time.

Polythene bags prevent external air from entering the seed, whereas cloth and gunny bags do not. Presumably, external air manages to infiltrate the plastic and tin containers. The results clearly demonstrate that the seed-treating fungicide Provax-200 had a significant impact on lowering the presence of fungus by 87.41% and boosting the germination percentage of lentil seeds by 39.49% (Table 2 and Figure 3). Uddin's (2009) study found that Vitavax-200, formerly known as Provex-200, was the most effective in reducing seed-borne infections in lentils. Afzal et al. (1999) also found that applying Vitavax-200 to the seeds decreased the occurrence of foot and root rot in lentil plants. Mortuza et al. (2002) found that the use of Captan fungicide effectively suppressed Fusarium wilt in chickpeas, as well as the fungi Aspergillus niger and A. flavus. Garlic clove extract, datura leaf extract, and fern leaf extract decreased fungal association by 74.78%, 39.30%, and 33.04%, respectively, while increasing seed germination by 22.30%, 18.89%, and 10.15%, respectively. Hermansen et al. (1999) claimed that using biological agents to treat seeds had no impact on carrots. However, our study's findings contradict this claim and align with Alice and Rao's (1994) research. They found that using garlic clove extract effectively controlled seed-borne

diseases in rice caused by Drechslera oryzae. This investigation corroborates the findings of Khan and Kumar (1992) that garlic clove extract, ghagra, vatpata, and bishkatali leaf extract effectively decreased the occurrence of fungus in wheat seeds. In their study, Yasmeen and Saxena (1992) discovered that plant extracts can effectively inhibit seed-borne fungi. The majority of researchers have concluded that fungicides are the most efficacious means of controlling seed-borne fungus, surpassing other agents (Mortuza and Bhuiya 1988). Fungicides are potent chemical substances that possess the ability to effectively eliminate and inhibit fungus growth. Additionally, their residual properties provide protection to germinating seeds by preventing the transmission of pathogens present in the soil. Fungal infections were lowered by 28.82%, 28.40%, 30.64%, 60.64%, and 34.64% when mahogany leaf extract (T3), mahogany seed extract (T4), ash-coated seeds (T6), fresh cow dung-coated seeds (T7), and cow urine (T8) were used. However, the study also revealed a reduction in germination rates compared to the control group, with reductions of 5.62%, 4.63%, 7.12%, 15.25%, and 3.25%, respectively. The effects on germination exhibit inconsistency. These biological substances may have a detrimental effect on the lentil embryo, leading to a decrease in the percentage of germination. It was found that adding undecomposed cow dung to the soil cut down on fungal infections by 60.64% and germination by 15.25% compared to the control group, which had the biggest drop. According to Sherfudeen et al. (2015), undecomposed cow manure has the ability to kill bacteria and germs.

CONCLUSION

This study explored effective methods for preserving lentil seeds using various storage containers and botanical treatments to enhance germination, vigor, and reduce seed-borne fungal infections. Lentil seeds stored in polythene bags with neem leaf extract showed the highest germination rates, vigor indices, and lowest fungal infections over six months. Provax-200 fungicide reduced fungal infections by 87.41% and increased germination by 39.49%, while garlic clove extract also significantly improved seed health. These findings highlight the effectiveness of polythene bags with neem extract and fungicides like Provax-200 for seed preservation. Future research should examine the long-term impacts and sustainability of these treatments. Our study provides valuable guidelines for improving lentil seed preservation and agricultural productivity.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no conflict of interest to declare.

Author contribution

All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

Jahan Al Mahmud: Principal Investigator for the research, responsible for conceptualization, methodology, and overall project administration.

Mahtalat Ahmed: Co-supervisor, provided guidance on research design, data analysis, and interpretation.

Sanjoy Kumar Adhikary: Supervisor, provided oversight and critical revisions to ensure the research met academic and publication standards.

Md. Mamun Hossain: Contributed to drafting the manuscript, editing, and assisted in the final preparation for journal submission.

Md. Mahadi Morshed: Contributed to manuscript drafting, editing, and integrating revisions from reviewers, and incorporating reviewers feedback. Supported the publication process in the journal.

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Evaluation of the effects of storage time and temperature on the beverages

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Abstract
Beverages are an important part of the food sector their storage is also one of the most critical issues. This study investigated the effects of different storage temperatures and times on physicochemical properties, flavonoid and organic acid contents of soda, sherbet and ice tea produced by adding sour grape concentrate. Changes in the flavan-3-ol content of ice tea were also measured during the storage period. The beverages were stored in three different conditions, cold storage (~4°C), room temperature (~24°C) and controlled storage (20±1°C), for six months and analyzed every two months. Storage temperatures and time affected the total soluble solids and acidities of the beverages ($p \leq 0.05$). Tartaric acid decreased during storage, especially during the first two months in sherbets. Ice tea and soda drinks were found more stable than sherbets. The malic acid was found the major organic acid in beverages. Flavonoid content in ice tea was higher than others. The flavonoid concentrations of ice tea stored at 20 and 24°C and of sherbet at 4°C were statistically significant as a function of storage time while these values were not significant for ice tea stored at 4°C and of sherbet at 20 and 24°C ($p \leq 0.05$) . Concentrations of flavan-3-ols varied with storage conditions. The levels of epicatechin, epigallocatechin and epigallocatechin gallate in ice tea samples decreased between an average of 43.72 and 71.15% at the end of six months of storage. Principal component analysis separated two months storage from other storage periods and perfectly discriminated the studied flavan-3-ols except catechin. Soluble solid and brix-acid ratio also dissociated similarly to flavan-3-ols.

Keywords: Soft drinks, storage, Physico-chemical parameters, Flavonoids, Flavan-3-ols

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INTRODUCTION

Non-alcoholic drinks include bottled water, coffee and tea, soft drinks, juices and other similar products. The latest trends in this industry are consumer products that focus on health and wellness. Functional and fermented of non-alcoholic beverages goal to provide some benefit to the different consumer groups beyond thirst-quenching or flavour (Anonymous, 2024). In recent years, the increasing demand for functional beverages has accelerated product development efforts and the introduction of different products into the sector. In particular, several studies have been conducted on innovative functional drinks using different fruits and herbal extracts (Balaswamy et al., 2011; Jooyandeh, 2015; Nanasombat et al., 2019; Aguilar et al., 2018; Din et al., 2019; Idan et al., 2021; Bendaali et al., 2022; Paredes et al., 2022; Nikolaou et al., 2023).

Flavonoids having a protective role against human diseases such as inhibition of plasma platelet aggregation, radical scavenging activity and exhibiting antibacterial, antiviral and anti-allergenic effects are phenolic compounds and abundant in foods and beverages of herbal origin (Yang et al., 2009; de la Bastida et al., 2022;). The flavan-3-ols, flavonols and anthocyanins are in the flavonoid group. The sour grapes contain considerable amounts of polyphenols, as ripe grapes (Hayoğlu et al., 2009; Sabir et al., 2010). Polyphenol content varies

depending on maturity, culture, soil and environmental conditions (Sabir et al., 2010). Accordingly, phenolic compounds can also be found in considerable amounts in ripe grape and sour grape-based beverages. Sour grape juice or concentrate has a distinctive tartaric flavour with high acidity, polyphenol content. It can be used to acidify, source polyphenols and flavour drinks. On the other hand, tea drinks are also an important source of phenolic compounds (Wang et al., 2000; Henning et al., 2003; Serpen et al., 2012; Korkmaz et al., 2019; Thennakoon et al., 2022).

Ingredient stability is one of the most critical parameters of stored beverages. These ingredients are generally carbohydrates, proteins, acids, vitamins and phenolics. In particular, parameters such as pH, acidity and total soluble solid (TSS) are monitored to provide information on whether there is any microbial change in the beverages both during processing and storage. However, it is necessary to analyze the relevant components when monitoring of reduction of nutritional compounds. The effects of storage conditions on physical, chemical, microbial and sensory parameters of soft drinks being fruit-based soft drinks and tea drinks have been investigated by many researchers (Spanos and Wrolstad, 1990; Wang et al., 2000; Balaswamy et al.,2011; Genova et al., 2012; Yadav et al., 2013; Jooyandeh, 2015; Din et al., 2019; Omokpariola, 2022; Kumar et al., 2023). However, studies on the storage stability and changes in carbonated drinks, sorbets and ice tea produced with the addition of sour grape concentrate are limited. Furthermore, there are no studies on the changes of flavan-*3*-ols, which are found in significant amounts in tea-based beverages, under different storage conditions.

The aim of this study was to reveal the impacts of different storage temperatures and times on the physico-chemical properties, flavonoid and organic acid contents of sodas, sherbets and ice tea prepared by adding sour grape concentrate. In addition, the study aims to determine the changes in flavan-*3*-ols, one of the most important components of tea drinks, as a consequence of storage conditions.

MATERIALS AND METHODS

Preparation of the samples

The material of this study consists of drinks produced with the addition of sour grape concentrate. These drinks were soda (carbonated drinks or gaseous), sherbet and ice tea. The brix-acid ratios were used to design the drinks. These ratios were determined to commercial beverages and previous literature (Plestenjak et al., 2001; Balaswamy et al., 2011; Jooyandeh, H., 2015). The TSS of the sour grape juice concentrate used for beverage production was 45°Brix. The pH and acidity of this concentrate were 2.50 and 19.3 g/L, respectively. The soluble solids values were set at 10°Brix for the soda and sherbet samples and 8°Brix for the ice tea samples. For the production of sherbet samples, the brix-acid ratio was adjusted to 20 by adding sour grape concentrate and sugar syrup. The samples were then filled into 250cc bottles, closed with crown caps and pasteurized (85°C/15 min). To produce soda, sugar syrup and sour grape concentrate were mixed with carbonated water and the final brix-acid ratio was adjusted to 30. These mixtures were then bottled and sealed. In accordance with the Turkish Food Codex Communiqué on Non-Alcoholic Beverages (2007/26), sorbic acid (0.25 g/L) and benzoic acid (0.15 g/L) were used as preservatives. To prepare ice tea, black tea (0.75 kg Çaykur Kamelya, Türkiye) was infused (15 min) and diluted 2-fold with boiled aqua. Then sugar syrup, infused black tea and sour grape concentrate were mixed and the final brix-acid ratio was adjusted to 35. The iced tea produced was bottled and closed. The bottles were pasteurized at 85°C for 15 minutes.

Measurement of soluble solids, acidity and brix-acid

The soluble solids values of the samples were measured directly using a refractometer (Hanna HI96801, Romania). Total acidity was determined by the titration method. 10 mL sample was titrated by 0.1 N NaOH to pH 8.1 and the acidity value was expressed as tartaric acid equivalent (Ough and Amerine, 1988). The brix-acid ratio was calculated by proportioning the soluble solids value to the total acid.

Determination of flavonoids

The aluminium chloride colorimetric method was used to analyze the total flavonoids in the samples (Zhishen et al., 1999). The 1 mL of beverage was diluted with 4 mL of distilled water, and 300 µL of sodium nitrite (%5) was added to the diluted sample. After incubation for 5 minutes, 300 µL of %10 aluminium chloride was added to the mixture and incubated for 6 minutes. Then 2 mL sodium hydroxide (1 molar) was added and the final volume was adjusted to 10 mL with distilled water. A spectrophotometer (Thermo Sci., Multiskango, Finland) was used to measure the absorbance of the samples at 510 nm. Flavonoid results were expressed as catechin equivalents (mg/L).

Determination of organic acids

The analysis of tartaric and malic acids in the beverages was carried out with slight modifications of the method previously described by Castellari et al. (2000). An Agilent 1260 HPLC system (Quat pump, degas unit, autosampler, column oven, DAD detector and Lab advisor chemstation software) was used for the analysis. The 10 mL diluted sample was filtered through a 0.45 PTFE syringe filter and injected directly into the system. The column temperature was set at 30 °C and the elution time was 12 minutes. The measuring wavelength was 210 nm. The flow rate was set to 1 mL per minute for isocratic flow. Separation was performed on an Agilent ODS C18 column (250 x 4.6 mm, 5 µm). The mobile phase was 0.05 N H_2SO_4. The concentrations of tartaric and malic

acids in the samples were detected by comparing their retention times and spectra with those of analytical standard solutions. The analysis was conducted at a wavelength of 210 nm. Results were calculated using the calibration curves and presented as g/L.

Determination of flavan-*3*-ols

The HPLC analysis of flavan-3-ols was performed according to the method previously described by Porgalı and Büyüktuncel (2012) with slight modifications. The HPLC system (Agilent 1260, USA) consisted of a diode array detector (DAD), a quaternary pump, an autosampler, a degasser and a column oven. The software system was Agilent lab advisor chemstation. A C18 Inertsil ODS-3 reversed-phase column (250 x 4.6 mm, 5 µm) was used for the separation of flavan-3-ols. Beverage samples were adequately diluted with mobile phase and passed through a PTFE syringe filter (0.45 µm, Sartorius, Germany) before injection into the instrument. The mobile phase was methanol: HPLC water: formic acid (19.5:80.2:0.3) and the flow was isocratic. The column oven was set at 40 °C. The flow rate was 1 ml/min and the injection volume was 5 µL. Detection of flavan-3-ols was carried out at a wavelength of 280 nm. Chromatographic analysis was performed in triplicate and retention times and UV spectra of analytical phenolic standards were used for compound identification.

Storage conditions

The beverages were stored at different temperatures to determine the effect of storage conditions. Cold storage (~4°C), room conditions (~24°C) and temperature-controlled storage (20±1°C) were used for storage during six months. A refrigerator was used to create the cold storage conditions and the temperature was set at ~4°C. The beverages were stored a temperature-controlled storage and the temperature was set at 20±1°C. A normal temperature uncontrolled storage was used for storing beverages at room conditions (~24°C). Beverages were analyzed for physicochemical properties, organic acids, flavonoids and flavan-3-ols for every two months up to the end of storage.

Statistical analysis

In the current study, all analyses and treatments were carried out in triplicate. The mean values of the results were displayed with their standard deviations. The data were evaluated by one-way analysis of variance with Duncan's multiple comparison test using by SPSS IBM Statistics program ($p \leq 0.05$). Principal component analysis (PCA) was conducted to determine the relationships among the physicochemical parameters, organic acids and flavan-3-ols of the iced tea samples.

RESULTS AND DISCUSSION

In the current study, the effects of storage time and temperature on beverages were evaluated. The physico-chemical parameters of the soft drinks were presented in Table 1. Total acidity values ranged from 0.37 to 0.40% at 4°C, 0.38 to 0.51% at 20°C and 0.35 to 0.48% at 24°C for sodas depending on storage time. These values changed between 0.24 and 0.28% at 4°C, 0.24 to 0.26% at 20°C and 24°C for ice tea samples. Total acidity of sherbets was between 0.47 and 0.50%. The highest acidity variance with storage conditions was observed for sodas. The changes in acidity of the samples were also presented as a graphical on Figure 1.

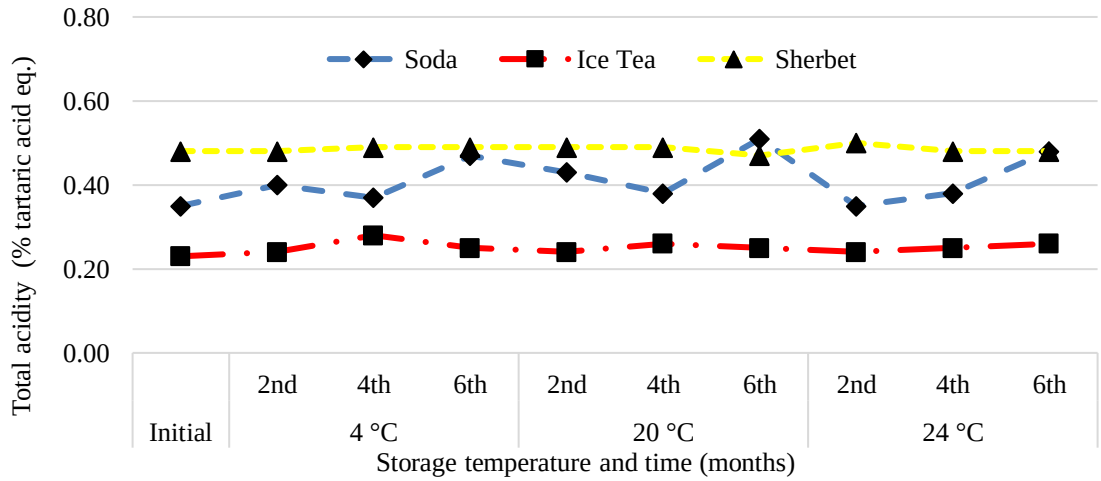

Figure 1. The changes in the total acidity of the soft drinks

*Cold storage: ~4 °C; temperature-controlled storage: 20°C; room conditions: ~24°C.

The TSS values varied between 10.67 and 11.30°Brix at 4°C, 10.77 and 11.47°Brix at 20°C and 10.80 and 11.80°Brix at 24°C for sodas during six months. These values ranged from 7.87 to 8.40°Brix for ice tea samples and 10.27 to 10.84°Brix for sherbets according to the storage temperature and time. There were no significant quantitative changes in TSS values among treatments for ice tea and sherbets, although the differences were

statistically significant at the $p \leq 0.05$ level. Soda TSS values showed a higher variation than other samples. The TSS changes of the soft drinks were graphically illustrated on Figure 2 also.

Table 1. The changes in physico-chemical parameters with storage conditions.

Soft Drinks	Storage temperature	Storage time, months	Total acidity, %	TSS, °Brix	°Brix/Acid
Soda		*Initial*	0.35±0.01	10.33±0.60	29.51±0.74
	Cold storage, 4 ºC	*2nd*	0.40±0.01^{b}	11.03±0.05^{b}	27.44±0.13^{b}
		4th	0.37±0.01^{c}	10.67±0.05^{c}	28.57±0.14^{a}
		6th	0.47±0.01^{a}	11.30±0.01^{a}	24.13±0.39^{c}
	Controlled storage, 20 ºC	*2nd*	0.43±0.01^{b}	11.47±0.10^{a}	26.95±0.01^{b}
		4th	0.38±0.01^{c}	11.10±0.17^{b}	29.29±0.01^{a}
		6th	0.51±0.01^{a}	10.77±0.19^{c}	21.30±0.01^{c}
	Room conditions, 24 ºC	*2nd*	0.35±0.01^{c}	11.80±0.08^{a}	34.15±0.24^{a}
		4th	0.38±0.01^{b}	10.80±0.01^{c}	28.47±0.31^{b}
		6th	0.48±0.01^{a}	11.67±0.05^{b}	23.44±0.30^{c}
Ice Tea		*Initial*	0.23±0.01	8.07±0.06	35.75±0.23
	Cold storage, 4 ºC	*2nd*	0.24±0.01^{c}	8.40±0.05^{a}	35.27±0.14^{a}
		4th	0.28±0.01^{a}	8.10±0.01^{c}	29.27±0.18^{c}
		6th	0.25±0.01^{b}	8.20±0.01^{b}	32.68±0.46^{b}
	Controlled storage, 20 ºC	*2nd*	0.24±0.01^{c}	8.30±0.01^{a}	34.32±0.30^{a}
		4th	0.26±0.01^{a}	8.17±0.05^{b}	31.53±0.10^{c}
		6th	0.25±0.01^{b}	8.20±0.01^{b}	32.39±0.23^{b}
	Room conditions, 24 ºC	*2nd*	0.24±0.01^{c}	8.23±0.05^{a}	34.68±0.25^{a}
		4th	0.26±0.01^{a}	7.87±0.05^{c}	29.92±0.34^{c}
		6th	0.25±0.01^{b}	8.07±0.05^{b}	31.95±0.28^{b}
Sherbet		*Initial*	0.48±0.01	10.40±0.0.01	21.76±0.18
	Cold storage, 4 ºC	*2nd*	0.48±0.01^{b}	10.87±0.05^{a}	22.65±0.23^{a}
		4th	0.49±0.01^{a}	10.33±0.05^{c}	21.12±0.11^{b}
		6th	0.49±0.01^{a}	10.57±0.05^{b}	21.49±0.23^{b}
	Controlled storage, 20 ºC	*2nd*	0.49±0.01^{a}	10.80±0.14^{a}	22.10±0.29
		4th	0.49±0.01^{a}	10.50±0.01^{b}	21.61±0.28
		6th	0.47±0.01^{b}	10.27±0.05^{c}	22.12±0.36
	Room conditions, 24 ºC	*2nd*	0.50±0.01^{a}	10.67±0.05	21.23±0.53
		4th	0.48±0.01^{b}	10.60±0.01	21.96±0.11
		6th	0.48±0.01^{b}	10.57±0.05	21.94±0.19

* Means followed by different letters within each column and beverage are significantly different at $p \leq 0.05$. TSS: Total soluble solids.

The differences in brix-acid ratios were statistically significant for all samples and storage conditions ($p \leq 0.05$) except for sherbets stored at 20 and 24°C temperatures ($p > 0.05$). These scores varied from 21.30 to 34.15 in sodas, 29.27 to 35.27 in ice tea samples and 21.12 to 22.65 in sherbets.

Preserving of the freshness and taste of the food is one of the purposes of storing products. The ratio of soluble solids to the total acidity is one of the most important parameters affecting the edibility and taste of foods, especially beverages. Changes in TSS and acidity provide information not only about the brix-acid, but also about the microbial activities in the soft drinks. The acidity and TSS values of ice tea and sherbet samples were statistically significant depending on the different storage conditions. However, the differences between these results were relatively minor in quantitative terms. This situation also affected the brix-acid ratios in the same direction. However, these values varied more in soda samples than in other samples. In particular, total acidity and TSS in soda samples increased depending on the storage time, and brix-acid ratios were affected accordingly. It is thought that this change may be due to a change in carbonation in soda samples. The increase in the acidity values of soda beverages with the storage period was compatible with the results of previous studies (Umeocho et al., 2021; Jooyandeh, 2015). Furthermore, Jooyandeh (2015) stated that the increase in acidity and decrease in pH were caused by the production of CO_2 forming weak acids upon dissolution in naturally carbonated fruit juices. Balaswamy et al. (2011) expressed that the changes in acidity and TSS were negligible after 6 months of storage period at room temperature in sour grape-based carbonated and non-carbonated beverages. The TSS findings of the current study are in agreement with the results of Balaswamy et al. (2011). It is thought that the acidity and TSS values of sherbet and ice tea as they were pasteurized changed less depending on different storage conditions compared to soda samples.

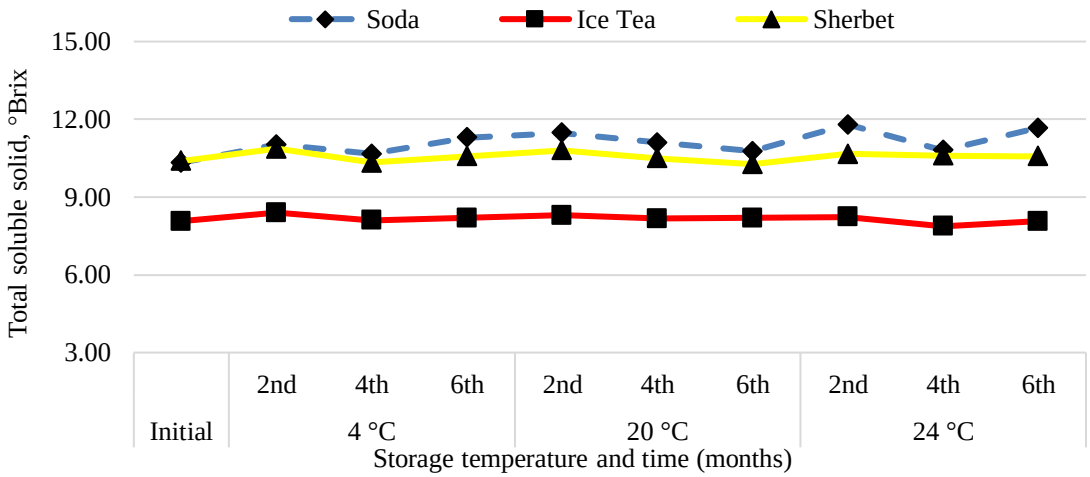

Figure 2. Total soluble solids change of the samples during storage
*Cold storage: ~4 °C; temperature-controlled storage: 20°C; room conditions: ~24°C.

The organic acids and total flavonoids of the samples were presented in Table 2. The Total flavonoid content in the sodas could not be detected because of the low concentration. Since the spectrophotometric method was used in the analysis of total flavonoids, low flavonoid values could no measured.

Table 2. Total flavonoids, malic and tartaric acids in soft drinks

Soft Drinks	Storage temperature	Storage time, months	Total flavonoid, mg/L	Tartaric acid, g/L	Malic acid, g/L
Soda		*Initial*	nd	0.12±0.05	3.00±0.34
	Cold storage, 4 ºC	*2nd*	nd	0.12±0.01^{b}	3.37±0.01^{a}
		4th	nd	0.14±0.01^{a}	2.89±0.01^{c}
		6th	nd	0.08±0.01^{c}	2.98±0.01^{b}
	Controlled storage, 20 ºC	*2nd*	nd	0.12±0.01^{b}	3.22±0.01^{a}
		4th	nd	0.11±0.01^{c}	3.16±0.01^{b}
		6th	nd	0.13±0.01^{a}	2.85±0.01^{c}
	Room conditions, 24 ºC	*2nd*	nd	0.11±0.01^{b}	2.94±0.01^{b}
		4th	nd	0.10±0.01^{b}	3.09±0.01^{a}
		6th	nd	0.14±0.01^{a}	2.93±0.01^{b}
Ice Tea		*Initial*	123.97±13.62	0.08±0.01	1.87±0.10
	Cold storage, 4 ºC	*2nd*	127.65±8.26	0.07±0.01^{a}	1.85±0.02
		4th	120.59±6.76	0.04±0.01^{b}	1.98±0.02
		6th	115.59±5.29	0.05±0.01^{b}	1.99±0.07
	Controlled storage, 20 ºC	*2nd*	102.45±4.60^{b}	0.07±0.01^{a}	1.83±0.01^{c}
		4th	117.35±10.81^{b}	0.04±0.01^{b}	1.97±0.01^{a}
		6th	144.70±8.80^{a}	0.05±0.01^{b}	1.89±0.01^{b}
	Room conditions, 24 ºC	*2nd*	120.10±2.37^{a}	0.10±0.04	1.79±0.04
		4th	128.82±8.83^{a}	0.05±0.01	1.95±0.01
		6th	98.38±9.26^{b}	0.04±0.01	1.79±0.15
Sherbet		*Initial*	4.34±0.25	0.42±0.01	4.13±0.03
	Cold storage, 4 ºC	*2nd*	5.14±0.17^{ab}	0.29±0.01^{a}	5.00±0.01^{a}
		4th	3.97±0.83^{b}	0.22±0.01^{b}	4.61±0.01^{b}
		6th	7.01±0.97^{a}	0.23±0.01^{b}	4.48±0.01^{c}
	Controlled storage, 20 ºC	*2nd*	5.76±0.66	0.30±0.01^{a}	4.77±0.01^{a}
		4th	4.73±0.36	0.23±0.01^{b}	4.54±0.01^{b}
		6th	6.54±0.71	0.23±0.01^{b}	4.37±0.01^{c}
	Room conditions, 24 ºC	*2nd*	4.93±0.11	0.30±0.01^{a}	4.69±0.02^{a}
		4th	6.01±1.01	0.22±0.01^{c}	4.59±0.01^{b}
		6th	7.51±0.72	0.23±0.01^{b}	4.44±0.01^{c}

* Means followed by different letters within each column and soft drink are significantly different at $p \leq 0.05$. nd: not detected.

The tartaric and malic acid concentrations of the soft drinks during the storage period were analyzed, and their changes were evaluated. The tartaric acid values were 0.08-0.14 g/L for sodas, 0.05-0.10 g/L for ice tea samples and 0.23-0.30 g/L for sherbets. While the tartaric acid content of the soda samples stored at 4°C decreased with increasing storage time, this decrease was not observed in the samples stored at 20 and 24°C. On the other hand, with increasing storage time, tartaric acid content decreased slightly in iced tea and sherbets. The changes in tartaric acid of the soft drinks were shown on Figure 3. The tartaric acid concentration of sherbet decreased dramatically during the storage period, in particular during the first two months. The ice tea and soda drink samples were more stable than sherbets in terms of tartaric acid contents. The changes in the tartaric acid composition of the beverages were no statistically significant as a function of storage temperature. However, the storage durations affected these values. In particular, the tartaric acid concentration of the sherbet samples decreased significantly with increasing storage time. This was due to the crystallization and sedimentation of tartaric acid as a consequence of storage duration.

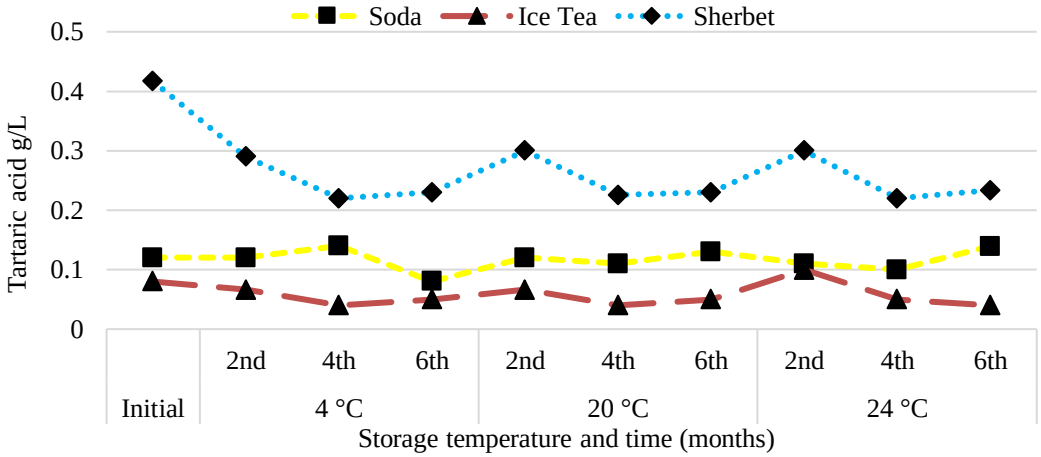

Figure 3. Tartaric acid changes in soft drinks during storage
*Cold storage: ~4 °C; temperature-controlled storage: 20°C; room conditions: ~24°C.

The main organic acid was malic acid in sodas, sherbets and ice tea samples produced by adding sour grape concentrate. The concentration of tartaric acid was lower than malic acid. Malic acid is a carboxylic acid and is found in many fruits and vegetables as the L-isomer form. It has a smooth taste and its DL-form is used as a flavouring and preservative in foods (Marques et al.,2020). The concentration of malic acid in each soft drink varied with storage temperature and time. The malic acid concentrations ranged from 2.98 to 3.37 g/L at 4ºC, 2.85 to 3.16 g/L at 20ºC and 2.93 to 3.04 g/L at 24ºC for sodas during storage period. These concentrations varied between 1.85 and 1.99 g/L at 4ºC, 1.83 and 1.97 g/L at 20ºC and 1.79 and 1.95 g/L at 24ºC for ice tea samples. While the change in the malic acid content of the soda samples was statistically significant for all three storage temperatures as a function of the storage time, it was only found to be statistically significant for the iced tea samples at the 20 ºC condition ($p \leq 0.05$). On the other hand, the malic acid content of the sherbets decreased depending on the storage time, especially after two-months storage. The malic acid concentation of ice tea was more stable than that of other soft drinks.

The flavonoid contents in ice tea samples were higher than in other soft drinks. In ice tea samples, the flavonoid contents ranged from 115.59 to 127.65 mg/L at 4 ºC, 117.35 to 144.70 mg/L at 20 ºC and 98.38 to 128.82 mg/L at 24 ºC. In sherbets, these values changed between 3.97 and 7.01 mg/L at 4 ºC, 4.73 and 6.54 mg/L at 20 ºC and 4.93 and 7.51 mg/L at 24 ºC. The changes in flavonoid content of the ice tea samples were showed on Figure 4. The differences in flavonoid content of ice tea stored at 20 and 24ºC were statistically significant according to storage temperatures ($p > 0.05$). In additon, the differences between sherbet flavonoid concentrations for 4ºC storage temperatures were statistically significant at the $p \leq 0.05$ level also. It is thought that the flavonoid content of ice tea samples decreased slightly with the precipitation occurring depending on the time stored at 4 ºC. This difference was no significant as statistical. However, storage in room conditions at 24 °C caused a time-dependent decrease in flavonoids, probably due to temperature changes under uncontrolled conditions. Flavonoids were better preserved in storage at 20ºC, which was determined as a controlled storage temperature. The increase of flavonoids depending on the duration may have been caused by the collapse of the turbidity factor components over time and accordingly, a percentage increase in the concentration of flavonoids in the unit dry matter.

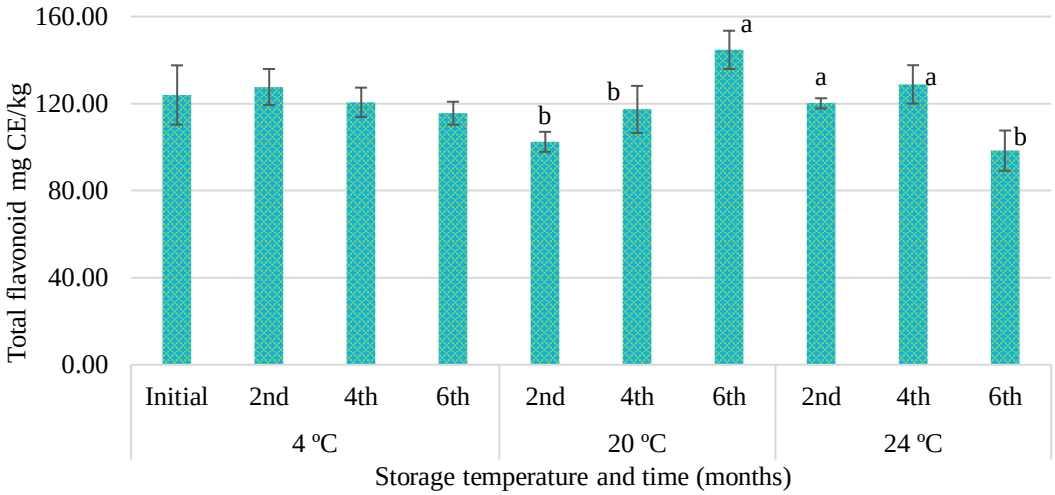

Figure 4. Total flavonoid changes in the iced tea samples during storage
*Cold storage: ~4 °C; temperature-controlled storage: 20°C; room conditions: ~24°C.

Flavonoids, consisting of flavan-*3*-ols, flavonols, and anthocyanins, are active polyphenols as biological and have high antioxidant power (Thennakoon et al., 2022). The flavonoid content of sherbet and soda were no remarkable compared to the ice tea when considering the soft drinks examined. According to these findings, almost all of the flavonoids in the produced beverages were originated by tea and its very low part by sour grape concentrate. In addition, the flavonoid content of iced tea was statistically more stable during storage than that of the sherbet. In a study comparing the properties of black and green tea, the flavonoid content varied between 0.47-5.15 mg QE/g in brewed black tea and 0.31-2.68 mg QE/g in green tea (Thennakoon ve ark., 2022). Aydemir et al (2023) found that the total flavonoid concentrations of 79 different black tea samples varied from 10.5 to 90.2 mg QE/g DW depending on the brewing time. In this study, the flavonoid findings obtained from ice tea samples were lower than in previous literature. It is thought that this situation may be caused by the tea variety and growing area, as well as the processing technique. In another study, Din et al. (2019) stated that the phenolic content of carbonated ice tea samples made from tea brewed in different proportions decreased significantly during the 90-day storage period. The results of the current study were no compatible with this literature. This difference may be caused by the process differences and the use of preservatives.

The variation of flavan-*3*-ols in iced tea samples as a consequence of storage condition was presented in Table 3. The flavan-3-ols in the ice tea samples were analyzed every two months during the storage period. The concentrations of (+)-catechin (CA), (-)-epicatechin (EC), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG) altered between 27.10 and 48.50 µg/mL, 36.81 and 60.15 µg/mL, 5.25 and 8.43 µg/mL and 6.87 and 19.11 µg/mL, respectively, depending on storage conditions.

Table 3. Alterations of flavan-*3*-ols in ice tea samples depending on storage conditions

Soft Drinks	Storage temperature	Storage time, months	CA, µg/mL	EC, µg/mL	ECG, µg/mL	EGCG, µg/mL
Ice Tea		*Initial*	33.78±2.21	78.81±4.98	14.67±2.90	30.54±0.76
	Cold storage, 4 ºC	*2nd*	28.65±2.25^{b}	60.15±6.59	6.85±1.65	15.90±2.15
		4th	48.50±1.70^{a}	36.83±4.71	6.64±1.23	7.29±1.70
		6th	46.36±1.74^{a}	45.17±6.63	6.39±0.84	7.26±1.74
	Controlled storage, 20 ºC	*2nd*	28.21±0.51^{b}	51.91±7.90	8.43±0.65^{a}	19.11±1.19^{a}
		4th	46.38±1.10^{a}	36.81±4.05	5.40±0.91^{b}	12.04±2.19^{b}
		6th	45.29±0.78^{a}	47.43±5.43	5.25±0.75^{b}	12.03±2.22^{b}
	Room conditions, 24 ºC	*2nd*	27.10±0.21^{b}	59.45±6.42	6.22±0.05	10.83±1.01^{a}
		4th	45.02±5.26^{a}	41.02±7.67	6.39±0.59	6.87±2.03^{b}
		6th	44.45±4.76^{a}	40.35±7.42	6.31±0.45	7.14±2.17^{b}

* Means followed by different letters within each column are significantly different at $p \leq 0.05$. nd: CA: (+)-Catechin, EC: (-)-Epicatechin, ECG: (-)-Epicatechin gallate, EGCG: (-)-Epigallocatechin gallate.

The alterations of flavan-3-ols in the ice tea samples were indicated graphically on Figure 5. EC, ECG and EGCG concentrations decreased with increased storage time, but CA slightly increased. These results revealed that the concentrations of flavan-3-ols having high antioxidant activity in ice tea could vary depending on the storage conditions. As particular, the concentration of epicatechin derivatives decreased dramatically with storage conditions in the present study. EC concentrations decreased by 42.68% for 4 ºC, 39.81% for 20 ºC and 48.80% for 24 ºC at the end of the storage. These declines were 56.44%, 64.21% and 56.99% for ECG cocentrations,

respectively. In addition, EGCG contents decreased between 60.61 and 76.62% during storage durations. The EGCG was the most sensitive component to storage conditions among the epicatechin derivatives investigated. The current study reveals that there may be a dramatic decrease in epicatechin derivates in the storage of ice tea beverages and this decrement rises from the second month of storage when all findings are taken into consideration. Furthermore, the increase in storage temperatures may influence the changes in their concentrations.

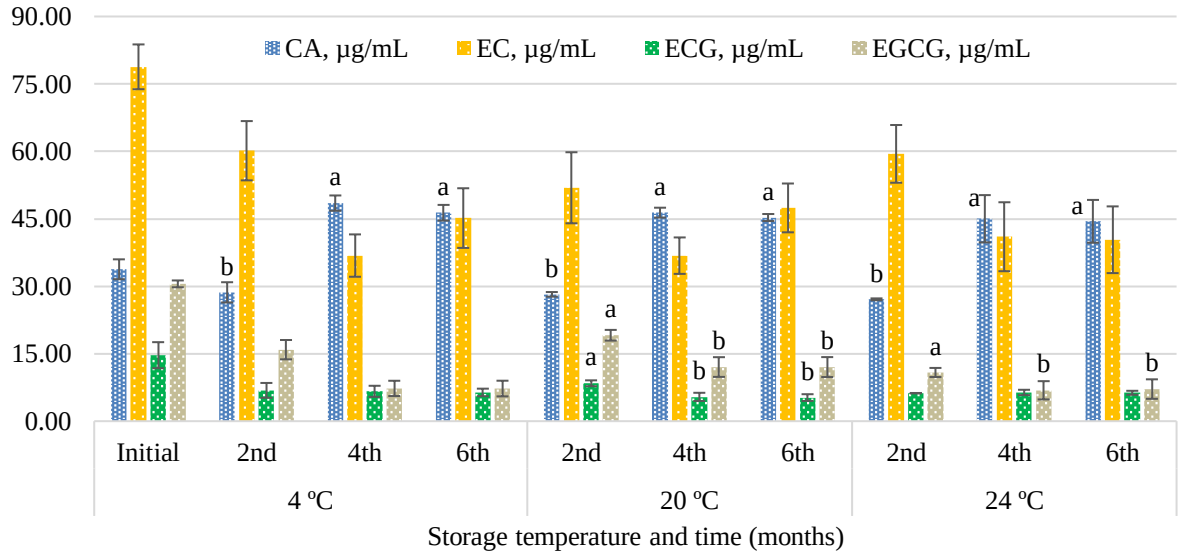

Figure 5. The changes in flavan-3-ols in the ice tea during storage (μg/mL)
*Cold storage: ~4 °C; temperature-controlled storage: 20°C; room conditions: ~24°C.

This study investigated the flavan-3-ols (CA, EC, ECG and EGCG) in ice tea samples and their changes with storage conditions. The flavan-3-ols in tea have biological benefits for human health. On average, 36 % of fresh tea leaves are polyphenols, of which approximately 90 % are catechin phenolics (Luczaj and Skrzydlewska, 2005; Skotnicka et al., 2011; Xu et al., 2023). The main flavan-3-ols in tea are CA, EC, ECG, EGCG and (-)-epigallocatechin (EGC) (Henning et al., 2003; Skotnicka et al., 2011). In a study investigating the catechin phenolics in 11 black tea samples CA was found to be between 2.7 and 15.4 mg/100 mL, EC 1.1 and 9.0 mg/100 mL, ECG 1.4 and 21.3 mg/100 mL and EGCG 3.8 and 74.5 mg/100 mL. These compounds could be undetected in the 2 ice tea samples investigated (Henning et al., 2003). EGCG and ECG concentrations in 7 different Turkish black tea grades ranged from 1.06 to 3.16 mg/g DW and 0.73 to 2.54 mg/g DW, respectively (Erol et al., 2010). In another study, the CA, EC, ECG and EGCG contents of black tea were reported to be 20 µg/mL, 37 µg/mL, 763 µg/mL and 128 µg/mL, respectively (Skotnicka et al., 2011). Serpen et al. (2012) determined the nutritional and functional properties of seven grades of black tea produced in Türkiye. They reported that CA varied from 59.3 to 98.3 mg/100 mL, EC from 61.9 to 83.9 mg/100 mL, ECG from 89.5 to 115 mg/100 mL and EGCG from 102 to 155 mg/100 mL in seven different grades of black tea. CA, EC, ECG and EGCG were found to be 0.012-0.156 µg/g, 0.579-4841 µg/g, 1087-5578 µg/g and 3.27-4376 µg/g, respectively in 79 black tea samples from different origins (Aydemir et al., 2023). These references indicated that the concentration of flavan-3-ols in tea could be varied dramatically depending on the variety, brewing conditions, production conditions and various parameters such as origin. The results of this study regarding flavan-3-ols were compatible with the results of Xu et al. (2023). They revealed that EGCG values were 4.5-182.7 mg/L, ECG values were 2.6-38.1 mg/L, EC values were 2.3-20.4 mg/L and CA were 2.0-44.9 mg/L in 18 canned or bottled tea drinks.

PCA was used to provide additional information on the influence of the storage conditions on the soft drink quality parameters and their relations with each other. Two main principal components accounted for 73.04 % of the total variance. Principal component 1 (C1) and component 2 (C2) accounted for 59.21 and 13.83 % of the total variance, respectively. C1 discriminated two months of storage from other storage times and clearly distinguished the investigated flavan-3-ols except CA. In addition, TSS and brix-acid ratio dissociated similarly to flavan-3-ols. PCA is generally used to simplify a large and complex set of data into a smaller set that is easier to understand. The variables of PCA are defined as linear combinations of the original (Jayasuriya and Edirisinghe, 2016). For this purpose, in this study, both the storage time and temperature of the investigated parameters and their relationships with each other were evaluated together. This method was used to determine multidirectional relationships between flavan-3-ols, organic acids and physico-chemical parameters in beverages depending on storage conditions. The score plot and correlation scatterplots of the variables for PCA were indicated on Figure 6. PC1 discriminated the second month of storage from the other storage durations and perfectly separated epicatechin derivatives (EC, ECG and EGCG) of ice tea samples by this storage condition. In addition, TSS, brix-acid ratio and tartaric acid were also similarly separated by PC1. PC2 was associated with the EC, EGCG, TA,

total flavonoid and acidity of soft drinks stored at 24°C for all storage times. Taking into account the results of the PCA, a strong correlation was found between the two-month storage treatment and EC, ECG, EGCG, TA, brix-acid ratio and TSS. Furthermore, four and six-month treatments at 4 and 20 °C were strongly correlated with the total flavonoids, CA, MA and acidity. These findings provided us with information about on the relationship between epicatechin derivatives and the physico-chemical properties of beverages and on how these components can be better protected during storage.

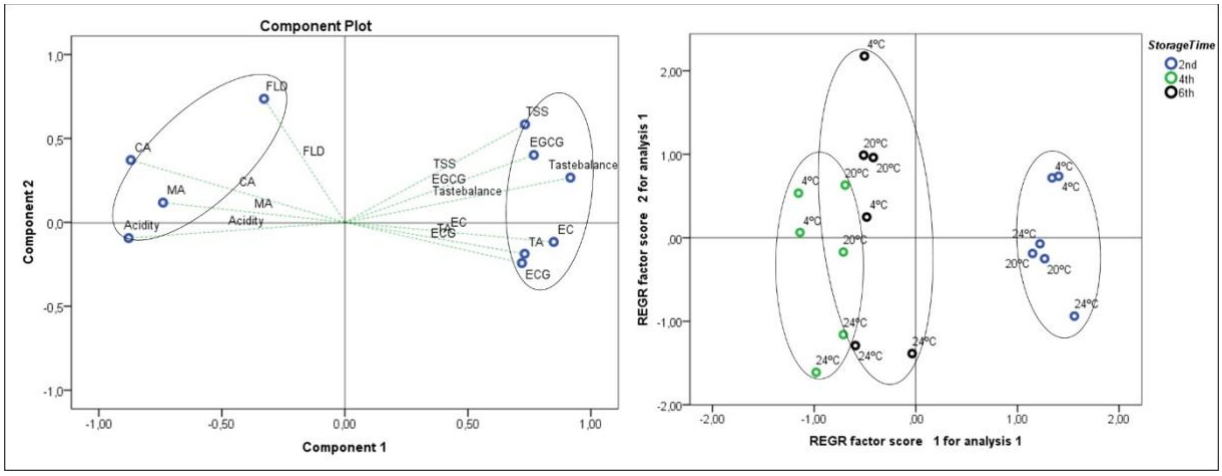

Figure 6. The score plot and correlation scatterplots of the variables for principal component analysis, with C1 and C2 as a function of quality parameters from different storage conditions. TSS: Total soluble solid, Taste balance: brix-acid ratio, FLD: Flavonoids, TA: Tartaric acid, MA: Malic acid, CA: (+)-Catechin, EC: (-)-Epicatechin, ECG: (-)-Epicatechin gallate, EGCG: (-)-Epigallocatechin gallate, Storage temperatures: 4, 20 and 24 ºC, Storage time: 2nd, 4th and 6th months.

CONCLUSION

This study revealed the effects of different storage conditions on the physico-chemical properties, flavonoid and organic acid contents and flaval-*3*-ols concentrations of beverages produced by adding sour grape concentrate. The acidity and TSS values of sherbet and ice tea samples changed less with different storage temperatures than soda samples because they were pasteurized. Tartaric acid concentrations of ice tea and soda were more stable during storage than sherbet. Malic acid was the main organic acid in the beverages. The flavonoid content of ice tea was higher than that of other soft drinks.

The concentration of flavan-*3*-ols in ice tea varied significantly with the storage conditions and duration. The levels of epicatechin, epigallocatechin and epigallocatechin gallate in ice tea samples decreased between an average of 43.72 and 71.15% at the end of six months of storage. Epigallocatechin gallate concentrations decreased by approximately 60-76% at the end of the storage compared to initial. There was a dramatic decrease in epicatechin derivatives during storage of ice tea beverages and this decrease increased from the second month of storage. Furthermore, increases in storage temperatures influenced the changes in their concentrations. Principal component analysis showed that storage conditions affected the epicatechin derivatives in ice tea and the physicochemical properties of beverages. The two-month storage was distinguished from other storage periods by principal component analysis. In future studies, it is recommended that studies be carried out on the usability of sour grape juice concentrate in different beverage and food products.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare they have no conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. The authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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The effect of biochar obtained from waste filter coffee grounds on plant germination

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DergiPark AKADEMİK

Abstract
Nowadays, coffee consumption is quite high, and the consumption of filter coffee is steadily increasing. Consequently, there is a significant increase in waste filter coffee. This study aims to evaluate waste filter coffee grounds using a zero-waste approach. In this context, the solid product of pyrolyzed waste filter coffee grounds was added to the soil in specific ratios to improve soil quality and increase yield. The effects on the root and stem development of arugula *(Eruca vesicaria)* and garden cress *(Lepidium sativum)* plants were investigated. Waste filter coffee grounds was homogeneously mixed with soil at application rates of 1, 2, and 4 tons/ha. The results of the study observed that the pyrolysis solid product positively affected plant growth. Comparing the data, the highest yield in plants was observed in soil with added biochar, while lower yields were seen in soil with added raw waste filter coffee grounds, and the lowest yield was found in soil without biochar. Among the soils with added biochar, the most significant root and stem development was observed in plants with 2 tons/ha of added biochar.
Keywords: Waste Filter Coffee, Plant Growth, Biochar, Pyrolysis, Soil Improvement

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INTRODUCTION

Biomass is a renewable, naturally occurring material that stores solar energy as chemical energy through photosynthesis. Biomass consists of polymers composed of macromolecules containing C-C bonds. The main skeleton of these polymers is formed by C-C bonds, but they may also include bonds involving C-O, C-H, C-N, C-S, or other elements. The polymers that make up biomass are generally macromolecular materials consisting of large structures formed from smaller units (Wang et al., 2017).

Biomass sources that can be used for energy production can be classified into plant-based sources, animal waste, and urban and industrial waste. These sources can be listed as plant and agricultural residues (branches, stems, straw, roots, bark, etc.), sugar and starch crops (potatoes, wheat, corn, sugar beetroot), oilseed crops (canola, sunflower, soybean, etc.), fibre plants (flax, hemp, sorghum, miscanthus, etc.), protein plants (peas, beans, etc.), and forest and wood waste (Greinert et al., 2019; Zardzewiały et al., 2023; Bijarchiyan et al., 2020).

Used coffee grounds contain a high amount of solid matter. Coffee beans include various health-related chemicals, such as phenolic compounds, melanoidins, diterpenes, xanthines, and carotenoids. Additionally, used coffee grounds contain significant amounts of organic compounds such as fatty acids, lignin, cellulose, hemicellulose, and other polysaccharides. As a solid waste, used coffee grounds, rich in both organic and inorganic compounds, are produced in substantial quantities. Therefore, the management of coffee waste, which must be handled before being released into the environment, is becoming increasingly important (Machado et al., 2011). According to the International Coffee Organization, coffee is one of the most consumed beverages in the world. According to the World Coffee Consumption Report 2018 prepared by the International Coffee Organization

(ICO); Coffee consumption in Turkey, which has increased by 13.2 percent in the last 5 years, has reached 93.9 thousand tons. The US revenue is at the top of the list with 1.5 million tons per year, followed by Brazil with 1.3 million tons and Japan with 465 thousand tons. (International Coffee Organization, 2019). Coffee consumption in Turkey increased to 1.1 kilograms in 2021 (Gastronomi Journal, 2021). During the production of espresso or instant coffee, a significant amount of solid residue known as spent coffee grounds is generated, ranging from 550-670 g/kg of coffee beans (Lane, 1983; Farah, 2009). Following the consumption of brewed coffee, approximately 6 million tons of waste from spent coffee grounds accumulate annually worldwide on filter papers (Hardgrove and Livesley, 2016).

Spent coffee grounds contain a high amount of solid matter. As with most biological raw materials, the composition of spent coffee grounds depends on various factors such as brewing method, germination conditions, and coffee type. However, most have a similar composition. The largest component of spent coffee grounds is polysaccharides, which make up about 50% of the dry mass of the spent coffee grounds. These polysaccharides include cellulose and hemicellulose. Spent coffee grounds are an organic waste material containing high amounts of sugars, fats, antioxidants, and other compounds, making them an important biodegradable component (McNutt, 2019).

The disposal of industrial and urban solid waste is a significant problem, and new methods have been developed for the reuse of raw materials to obtain useful products. Pyrolysis is one of the primary thermochemical conversion methods used to transform biomass into solid (char), liquid (bio-oil), and gaseous products (biogas), as well as valuable chemicals (Tophanecioğlu, 2009).

The use of biochar produced by the carbonization of biomass through pyrolysis in soil improves soil reclamation, carbon sequestration, and soil fertility (Akgul, 2017). The application of biochar in agriculture has become increasingly popular over the past decade, with the primary aim of enhancing soil properties and structure and contributing to the soil's carbon content. The characteristics of biochar are closely related to the quality of the biomass and the pyrolysis process used to produce it (Weber and Quicker, 2018). The porous structure of biochar contributes to sustainable soil fertility by improving the physical, chemical, and biological properties of soils (Zhang, 2021). The application of biochar to clay soils significantly increases soil aeration by enhancing macropores (Lehmann and Joseph, 2015). Additionally, the porous structure of biochar reduces nitrate leaching in sandy soils (Chen et al., 2020).

A study conducted in 2017 observed that the application of biochar to sandy soil increased total porosity and reported a significant increase in water holding capacity by 127% (Liu et al., 2017). Additionally, the highly porous structure of biochar increases the total pore number in the soil, creating a habitat for microorganisms and enabling the enzymatic functions necessary for plant growth to occur (Baiamonte et al., 2019). Studies have reported that biochar can increase the water holding capacity of soil (Villagra-Mendoza and Horn, 2018), while others have reported that it can decrease (Carvalho et al., 2016) or have no effect on water holding capacity. Biochar contains a high amount of carbon and may also include basic cations such as K, Ca, and Mg (Novak et al., 2019). Due to its basic nature, biochar acts as a liming agent by increasing the pH in acidic soils and is recognized as a soil pH regulator (Li et al., 2019).

Historically, filter coffee has been a widely consumed beverage in most countries. Its consumption has steadily increased, and the resulting coffee grounds are typically discarded directly. This practice has raised economic and environmental costs. Therefore, alternative methods for utilizing waste filter coffee grounds are necessary. This study investigates the recovery of biochar from waste filter coffee grounds through pyrolysis and its effect as a soil improvement on plant growth. In the initial stage, biochar was produced through the pyrolysis of biomass. The effects of the obtained biochar on plant growth were assessed through pot experiments.

MATERIALS AND METHODS

Preparation of Biochar

Biochar was obtained from waste filter coffee grounds by pyrolysis methods, and the properties of raw waste filter coffee and the produced biochar were examined.

Pyrolysis of Waste Filter Coffee

For the pyrolysis process, waste filter coffee samples were initially dried in an oven at 50°C. The dried samples (50 grams) were brought to a constant weight and then placed in a fixed bed steel reactor with an inner diameter of 8 cm and a volume of approximately 3 liters. The system’s heating rate was regulated via an electrical panel, and rock wool was utilized to minimize heat loss. To maintain an inert atmosphere during pyrolysis, nitrogen gas (N_2) was introduced at a flow rate of approximately 1.5 L/min. The pyrolysis was conducted for 4 hours. To prevent gas leakage from the reactor, pure graphite and graphite-lead spiral gaskets were employed between the reactor and its lid.

The reactor was equipped with a gas inlet line for nitrogen gas supply and an outlet line in the lid area to evacuate the synthesis gas produced. The internal temperature of the reactor was measured using a thermocouple. Two consecutive cooling columns were utilized to remove condensable organic volatiles from the synthesis gases, with the condensed liquids being collected in conical flasks attached to the columns. The synthesis gas passing

through the cooling columns was directed to a gas analyzer to determine its composition. To protect the analyzer from uncondensed volatile organic substances and particulate matter, one ceramic and two microfiber filters were used in the pipeline. The gas analyzer measured the volumes of CO, CO_2, H_2, CH_4, and O_2 in the released gases. Pyrolysis studies were performed at reactor temperatures of 400, 500, and 600°C under laboratory conditions with a heating rate of 5°C/min. The experimental setup used for the pyrolysis is shown in Figure 1.

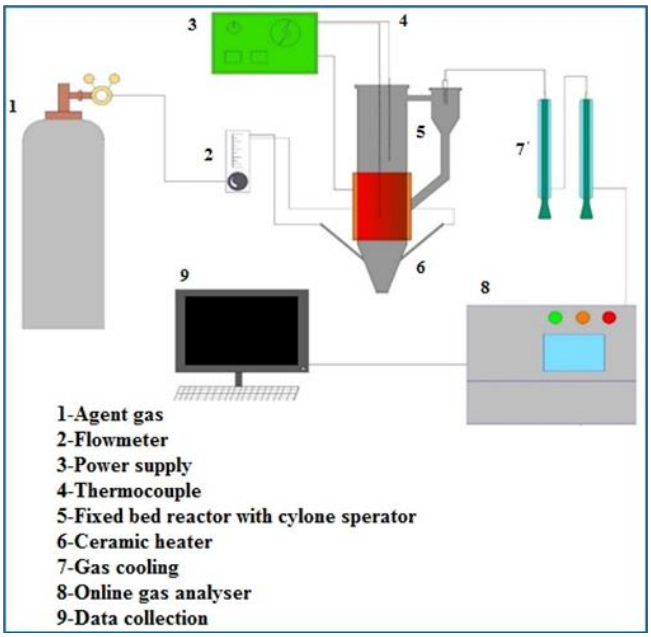

Figure 1. The experimental setup used for the pyrolysis.

Characterization Studies

Physical and chemical analyzes were carried out for biochar obtained as a result of pyrolysis and thermally activated biochar after the pyrolysis process. All analyzes were performed according to ASTM standards. The list of technical standards used for characterization is given in Table 1. Three technical replicates were performed for each analysis to minimize error.

Table 1. Technical standards for characterization.

Technical Standards	Analysis type
ASTM D-3173	Determination of Moisture Content and Solids Content
ASTM D-3174	Determination of Loss on Burning and Ash Content
ASTM D-5865	Determination of Calorific (Thermal) Value
ASTM D-5373	Elemental analysis
ATSM E-1252	Fourier Transform Infrared Spectrometer (FTIR)

Determination of Moisture Content and Solids Content

Total moisture and solids content of solid fuels are measured according to the oven drying method. This is achieved by placing the samples in a crucible and drying at 105°C for approximately 1 hour until constant mass is achieved. Then, the percent moisture content and solid content of the sample are calculated by equations (1) and (2), respectively. This analysis was carried out using a Memmert brand oven.

$$\text{Weight Moisture (\%)} = \frac{(A-B)}{A}\ x\ 100 \quad (1)$$

$$\text{Weight Solids (\%)} = \left(\frac{B}{A}\right)\ x\ 100 \quad (2)$$

Here,

A= First test sample, g

B= Dried test sample, g

Determination of Loss on Burning and Ash Content

Combustion loss analysis involves examining and evaluating the losses that occur during the combustion process in various systems such as engines, power plants, furnaces, or industrial processes. This analysis includes measuring and understanding energy losses, efficiency, and emissions related to combustion. Determining ash content is also a common analytical procedure used to measure the total mineral content in a sample. Ash is the inorganic residue remaining after the complete combustion of the sample's organic components, providing an estimate of the total mineral or inorganic content. In this analysis, combustion loss and ash content are determined using a muffle furnace. Samples are placed in a ceramic crucible and heated in an oven at 550°C for 120 minutes; then, the oven is cooled to 105°C. Finally, ash content and combustion loss are calculated using Equations (3) and (4). This analysis was conducted using a Magma Therm Primary MTP Series Model No. MTP-1000-8-P Furnace.

$$\text{Weight Loss on Combustion (\%)} = \frac{(A-B)}{A}\ x\ 100 \quad (3)$$

$$\text{Weight Ash Content (\%)} = \left(\frac{B}{A}\right) x\ 100 \quad (4)$$

Here,

A= First test sample, g

B= Dried test sample, g

Determination of Calorific Value

Calorific value analysis, also known as energy content analysis, is a process used to determine the amount of heat energy released when a substance burns. It is a crucial parameter for various applications, particularly in energy production and combustion. In this study, the process was conducted using the IKA C200 bomb calorimeter, which measures the higher heating value (HHV) of solid samples. The analysis follows the IKA guideline, describing the determination of the HHV of a solid biofuel in a bomb calorimeter at constant volume and a reference temperature of 25°C. Fuel samples are burned in a closed vessel with a pressurized (30 bar) oxygen-rich atmosphere, immersed in a known amount of water. The sample is placed in a quartz crucible within a plastic bag and ignited using a cotton thread connected to an electrode. The increase in the water temperature in the calorimeter system (ΔT) due to combustion is measured, and the HHV is evaluated on a wet basis.

Elemental Analysis

This method provides information about the elemental composition of samples, including the elements carbon (C), hydrogen (H), and nitrogen (N). The final analysis determines the percentage of these major elements (C, H, N) in the samples. The analysis is often conducted using techniques such as combustion analysis or instrumental methods like elemental analyzers. In this study, elemental analyses were conducted at the Central Research Laboratory of Bursa Technical University.

Fourier Transform Infrared Spectrometer (FTIR)

FTIR (Fourier Transform Infrared Spectroscopy) is a technique used to analyze both organic and inorganic materials by examining their chemical bonds and composition (Nandiyanto et al., 2019; Lopes et al., 2018). This method utilizes the light absorption properties of a material, leveraging how different molecular compounds react to infrared light to determine the material's structure. FTIR works by using multiple different frequencies in the beam, as each frequency interacts uniquely with the material, allowing for accurate determination of the composition of an unknown substance. In this study, FTIR analysis was employed to determine the chemical characterization of the samples. The FTIR analyses were conducted at the Central Research Laboratory of Istanbul University-Cerrahpaşa.

SEM Analysis

Scanning electron microscope analyses were performed at Recep Tayyip Erdoğan University Central Research Laboratory.

Plant Germination Experiments

Commercial soil samples were mixed with the pyrolysis product biochar and non-pyrolyzed waste filter coffe grounds in various proportions, and the prepared mixtures were placed into pots. Polyethylene-coated plastic pots with a capacity of 1.5 kg of soil were used. Before sowing the seeds (arugula and garden cress), biochar and waste filter coffe grounds were homogeneously mixed with the soil at application rates corresponding to 1, 2, and 4 tons/ha, equating to 6, 12, and 24 g/pot. Before sowing the seeds, the pots containing the mixtures were conditioned by watering daily with distilled water for one week. Subsequently, arugula and garden cress seeds were sown into each pot. At least three pots were used for each mixture to obtain a sufficient number of samples. Additionally, a control group with soil samples without the addition of biochar or waste filter coffe grounds was prepared. After 20 days, measurements were taken of the germinated plants. After harvesting, the lengths and weights of the roots and stems of the plants were measured. Throughout the experiments, the plants were irrigated with tap water and periodically rotated.

RESULTS AND DISCUSSION

Moisture, Ash Amount, Solid Matter and Burning Loss Tests

The moisture, ash, and solid matter values presented in Table 2 indicate that the waste filter coffe grounds samples used in this study are suitable for the pyrolysis process.

The literature suggests that the moisture content of raw materials for thermal processes should be in the range of 5-35% (Demirbas, 2009; Belgiorno et al., 2003). The moisture content obtained in this study falls within this range (8.28%). Additionally, the solid matter content of 91.72% further indicates that the waste filter coffe grounds samples are suitable for the pyrolysis process.

The characteristics of the raw material used in the pyrolysis process significantly affect the efficiency of the process. Materials with high carbon and hydrogen content are the most suitable for pyrolysis. Therefore, to determine the suitability of waste filter coffe grounds samples for the pyrolysis process, calorific value analyses were conducted. The calorific value results of both the raw waste filter coffe grounds and the biochar obtained from the waste filter coffe grounds are presented in Table 2.

Table 2. Results of moisture, Ash amount, solid matter and burning loss tests and energy values of biochar after pyrolysis.

Sample	Moisture (%)	Ash amount (%)	Solid matter (%)	Combustion loss (%)	Energy value (cal/g)
Raw	8.28	98.08	91.72	98.08	5121
Pyrolysis product biochar at 400°C	2.67	84.38	97.33	84.38	7259
Pyrolysis product biochar at 500°C	2.13	94.5	97.87	94.5	6970
Pyrolysis product biochar at 600°C	3.34	82.7	96.66	82.7	6358

SEM Images

In the study, SEM images were taken to investigate the pore structure of biochars obtained through the pyrolysis method. To observe the changes in the pore structure after pyrolysis, SEM images of both the raw samples and the post-pyrolysis samples are presented in Figures 2-5.

When examining the SEM image of the raw waste filter coffe grounds, it is observed that the untreated raw waste filter coffe grounds has a relatively smooth, non-porous structure (Figure 2). As the pyrolysis temperature increases and the pyrolysis products are subsequently thermally activated, an increase in porosity is observed (Figures 3-5).

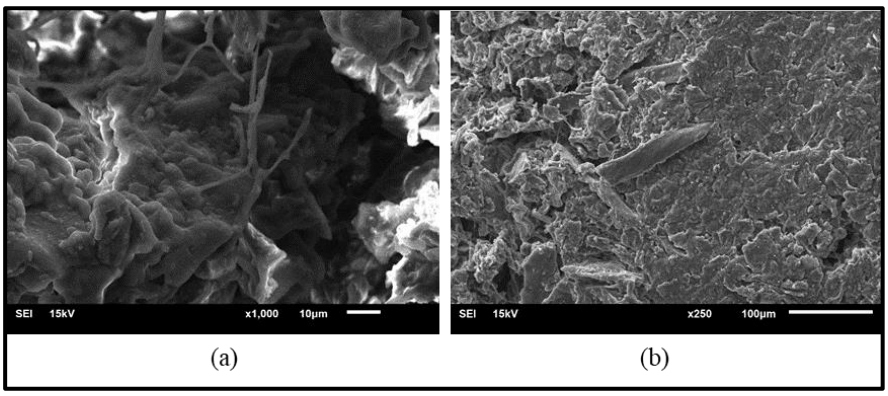

Figure 2. SEM images of raw waste filter coffe grounds a- magnified 1000 times b- magnified 250 times.

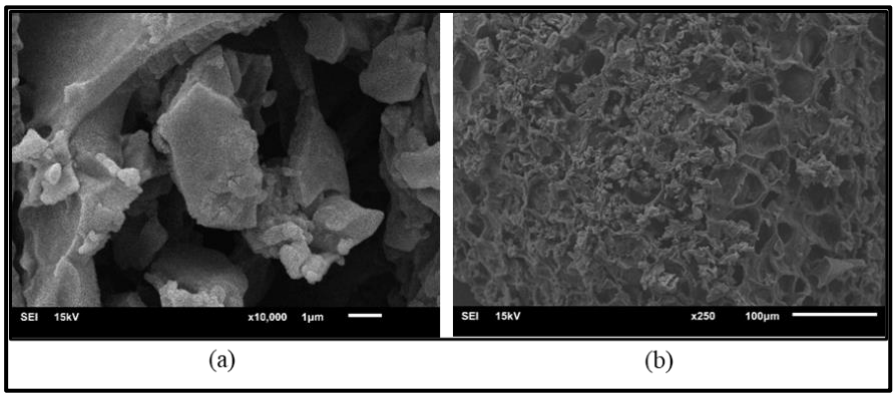

Figure 3. SEM images of biochar obtained from waste filter coffe grounds after pyrolysis at 400°C a- Magnified 10.000 times b- magnified 250 times.

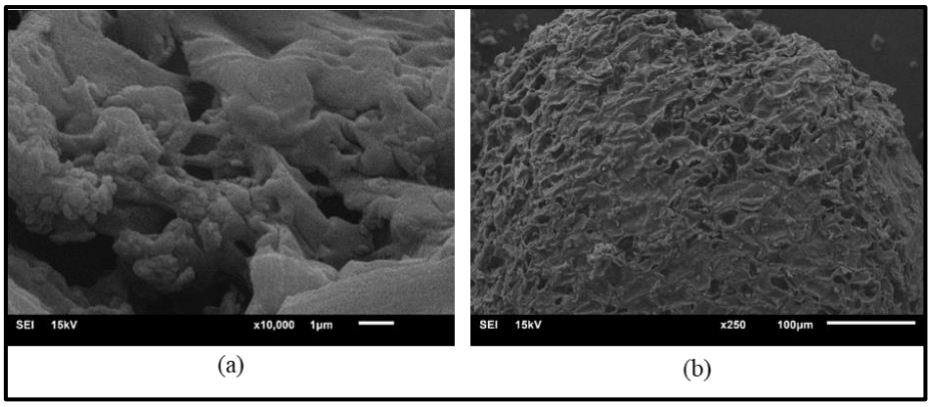

Figure 4. SEM images of biochar obtained from waste filter coffe grounds after pyrolysis at 500℃ a- Magnified 10.000 times b- Magnified 250 times.

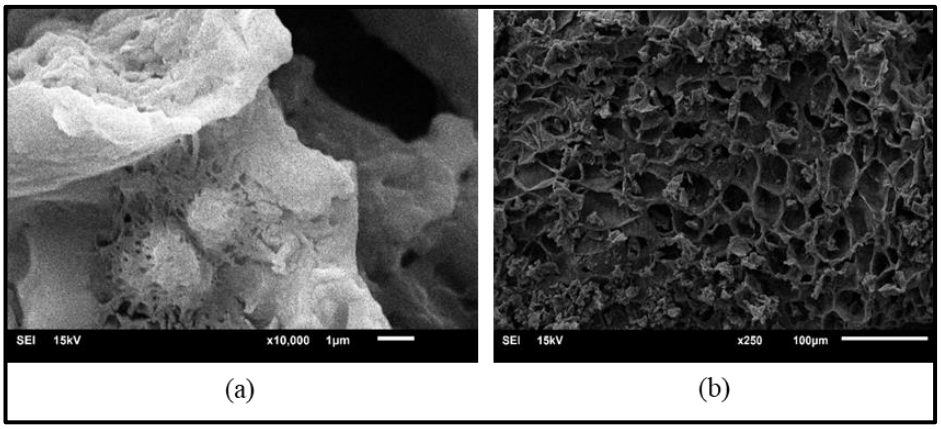

Figure 5. SEM images of biochar obtained from waste filter coffe grounds after pyrolysis at 600℃ a- Magnified 10.000 times b- magnified 250 times.

C, H, N, S Elemental Analysis Results

Materials with high carbon and hydrogen content are the most suitable for pyrolysis. Therefore, an elemental analysis was conducted to determine the suitability of waste filter coffe grounds samples for the pyrolysis process. The elemental analysis results of both the raw waste filter coffe grounds and the biochar obtained from the waste filter coffe grounds are presented in Table 3. Upon examining the elemental analysis results, it was found that the raw waste filter coffe grounds sample contains 50.84% carbon (C) and 6.87% hydrogen (H). These values indicate that the waste filter coffe grounds samples are a suitable raw material for pyrolysis methods (Tsai et al., 2012).

Table 3. C, H, N Elemental analysis results.

Sample	C%	H%	N%
Waste filter coffe grounds	50.84	6.87	2.29
Biochar obtained by pyrolysis at 400℃	66.61	4.99	1.99
Biochar obtained by pyrolysis at 500℃	74.34	2.90	2.15
Biochar obtained by pyrolysis at 600℃	68.93	2.87	2.43

FTIR Analysis Results

Approximately 1-2% of waste filter coffe grounds content consists of caffeine. When examining Figure 6, the stretching vibrations observed at 2923 cm^{-1} and 2854 cm^{-1} are attributed to the caffeine in the coffee content. Additionally, 5-10% of coffee content is composed of chlorogenic acids. The presence of ester groups in these acids is evidenced by the bands at 1704 cm^{-1} and 1635 cm^{-1} in the spectra. Furthermore, the stretching vibration of the C-N bond related to tertiary amines from the structures of caffeine, theobromine, and theophylline molecules in coffee is typically seen in the 1350 cm^{-1} to 1200 cm^{-1} region, appearing at 1247 cm^{-1} in the figure. Additionally, coffee contains 2-5% moisture-derived H_2O, depending on storage conditions. The band corresponding to the

stretching vibration of water molecules is observed at 3294 cm^{-1} in the FTIR spectra (Barrios-Rodríguez et al., 2021).

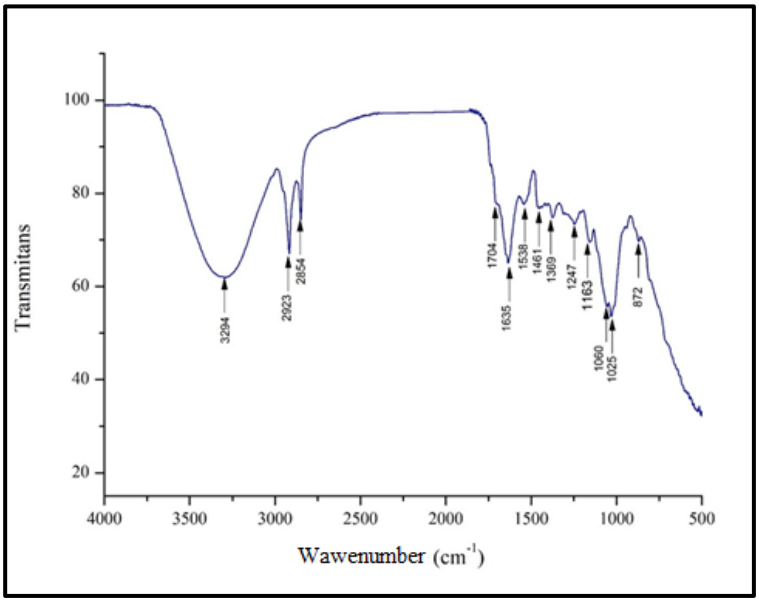

Figure 6. FTIR Spectra of Waste filter coffe grounds.

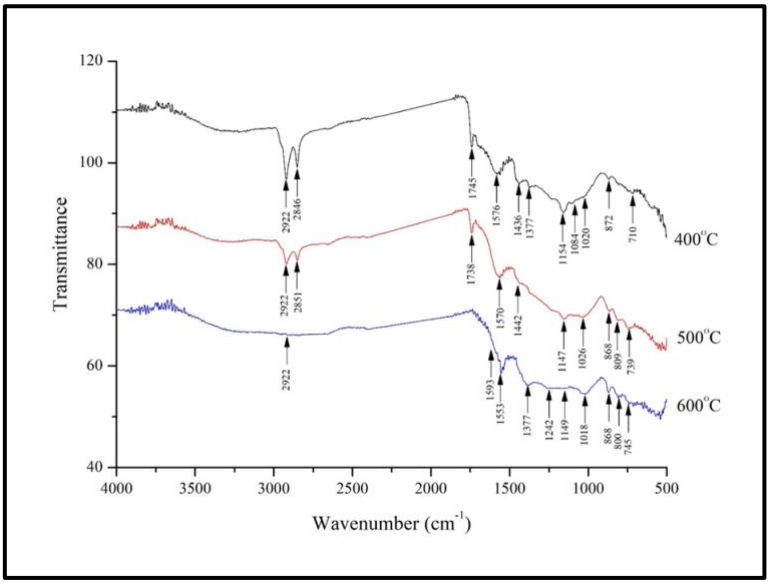

Figure 7. FTIR Spectra of the final structure at different pyrolysis temperatures.

When examining Figure 7, the stretching vibrations observed at 2922 cm^{-1} and 2846 cm^{-1} (occurring at 2831 cm^{-1} at 500ºC) are attributed to the caffeine content in the coffee. The decrease in the absorbance values of these peaks with increasing temperature, and particularly the disappearance of the corresponding band in the residue after pyrolysis at 600ºC, indicates that this molecule is removed from the environment post-pyrolysis. The presence of ester groups in the chlorogenic acids and groups originating from caffeine is evidenced by the phenomenon observed in the range of 1750 cm^{-1} to 1550 cm^{-1}, shown in Figure 9 at 1745 cm^{-1} (which occurs at 1738 cm^{-1} at 500ºC). The peak intensity of this band initially decreases with increasing temperature during pyrolysis, and at 600ºC, it creates a minimal effect, nearly disappearing from the environment (Barrios-Rodríguez et al., 2021). The bands located between 1300 cm^{-1} and 1150 cm^{-1} are also related to the chlorogenic acid structure. Pyrolysis temperature has a similar effect in this region; the intensity of the 1154 cm^{-1} peak, particularly noticeable at 400ºC, decreases at 500ºC, and shifts to 1147 cm^{-1} at 600ºC. It appears at 1149 cm^{-1}, again with significantly reduced intensity (Briandet et al., 1996). This demonstrates that the relevant molecules in the content gradually dissipate from the environment at these temperature values.

Plant Germination Experiments

As a result of the elemental analyses of the biochars, the biochar obtained through pyrolysis at 500°C, which had the highest carbon content, was used in the plant germination experiments. Figures 8-9 show the photographs of the prepared pots and the growing plants.

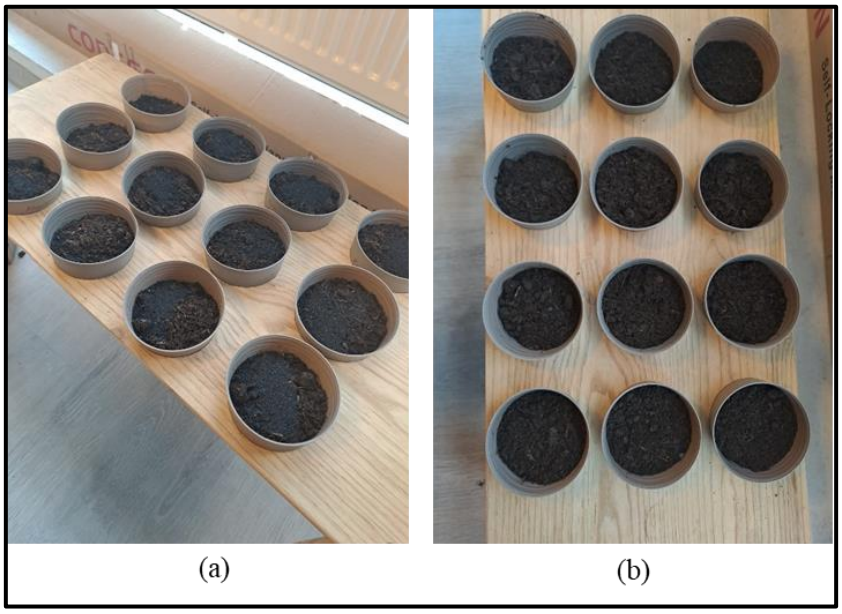

Figure 8. a- Soil not mixed with Biocar b- Soil left for conditioning.

Figure 9. Adult plants.

In this study, the effects of biochar on the root and stem length of plants were experimentally investigated. The results are presented in Table 4. Upon measuring the results, it was observed that the most suitable biochar application for arugula and garden cress plants was 2 tons/ha. The longest and heaviest plants were obtained when 2 tons/ha of biochar was used. Plants in pots with added biochar showed more growth compared to those grown in soil without biochar. Although plants in soil mixed with raw coffee developed less than those in soil with biochar, they still showed more growth compared to plants grown in just soil. The arugula plant demonstrated better development with 12 tons/ha biochar application and when grown solely in soil, having three leaves compared to the garden cress. In all other conditions, the plants had only two leaves (Table 4). The use of biochar obtained through pyrolysis in soil contributes to soil reclamation, carbon sequestration in soil, and increased soil fertility (Akgul, 2017). The porous structure of biochar enhances physical, chemical, and biological properties of soils, contributing to sustainable soil fertility (Zhang, 2021). The results obtained from this study support these positive attributes of biochar mentioned in the literature.

Table 4. Measurement results of plants after germination.

Mixtures in Pots	Root Length (cm)	Fringe Length (cm)	Plant Length of the whole (cm)	Plant Rhizome Length (cm)	Rooted Weight (g)	Rootless Weight (g)	Leaf Number (number)
Arugula (1 ton/da biochar)	1.8	0.3	7.6	5.8	0.03805	0.03735	2
Arugula (2 ton/da biochar)	3	-	10.25	7.25	0.0568	0.0534	3
Arugula (4 ton/da biochar)	1.9	1.1	7.95	6.05	0.0456	0.0438	2
Garden cress (1 ton/da biochar)	1.45	-	8.1	6.65	0.02335	0.0228	2
Garden cress (2 ton/da biochar)	1.3	0.3	9.85	8.55	0.03585	0.03505	2
Garden cress (4 ton/da biochar)	1.75	-	9.45	7.7	0.03375	0.03305	2
Arugula +soil	1.9	-	7.2	5.3	0.0379	0.0371	3
Garden cress +soil	2.8	-	7.8	5	0.0229	0.0211	2
Arugula + raw coffee	2	0.5	7.4	5.4	0.03795	0.03715	2
Garden cress + raw coffee	2.8	-	7.9	5.1	0.02314	0.02302	2

CONCLUSION

The results of this study indicate that biochar obtained from the pyrolysis of waste filter coffe grounds is suitable for use as a soil improvement to support plant germination. The findings demonstrate that when biochar derived from pyrolyzed waste filter coffe grounds is added to soil, it supports plant germination and growth.

Moreover, this study is significant as an application supporting the "zero waste" approach. The utilization of waste filter coffe grounds not only enhances environmental sustainability but also offers innovative solutions to waste management issues. It was determined that the biochar obtained after the pyrolysis of waste filter coffee grounds at 600°C contained 68.93% Carbon and 2.43% Nitrogen. It was observed that the best results were obtained in the application of 2 tons/da biochar to the soil for both aragula and garden cress plants. However, despite the promising initial results, further research is needed to enable the widespread application of this method in agricultural practices. To better understand the effects on soil and plants, field trials should be conducted in addition to laboratory-scale studies. These studies will help determine how biochar performs under different soil types and climatic conditions.

In conclusion, the pyrolysis of waste filter coffe grounds provides dual benefits in terms of waste management and soil improvement. This approach is seen as an important step towards the efficient use of resources.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare no conflict of interest.

Author contribution

Conceptualization, M.N.C., E.E.Ö., H.S., Ş.G., H.K.Ö. and A.Ö.; methodology, M.N.C., E.E.Ö. and H.K.Ö.; investigation, M.N.C., E.E.Ö., H.S., Ş.G., H.K.Ö. and A.Ö.; writing—original draft preparation, M.N.C., E.E.Ö. and H.K.Ö. All authors have read and agreed to the published version of the manuscript.

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Participation of urea application stages on flour quality in bread wheat

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Abstract
Laboratory studies, which field trial was conducted in the 2020-2021 growing season at the Eastern Mediterranean Agricultural Research Institute, were conducted at Gümüşhane University. In this study, the findings showed that urea was used as the top fertilizer in different growth stages of the 'Yakamoz' bread wheat cultivar used as material, and the effects of urea application periods and flour type, on the quality traits, such as dry matter, ash, protein, acidity, fresh and dry gluten, gluten index, sedimentation, and falling number were investigated. For this purpose, in addition to the control application, tillering, stem elongation, milky and dough stages were chosen as urea application periods. Laboratory studies were carried out in randomized plots using the split-plot design with three replications. In the present study, all quality traits showed statistically significant differences for urea application periods; these values were ranged as follows: Dry matter: 90.20-90.77%, ash: 1.037-1.213%, protein: 14.01-15.15%, acidity: 0.037-0.056%, wet gluten: 41.49-43.67%, dry gluten: 14.75%-15.46, gluten index: 69.28-80.38%, sedimentation: 20.0-21.0 mL, late sedimentation: 23.5-29.8 mL and falling number: 753.8-881.7 s. In addition, other quality parameters except dry matter, protein and sedimentation changed statistically for flour type. Accordingly, whole wheat flour for ash (1.443%), acidity (0.051%) and gluten index (82.53%); white flour showed high values for wet (44.90%) and dry (15.96%) gluten, late sedimentation (35.60 mL) and falling number (836.4 s). As a result, while applying urea as a top fertilizer, it is recommended to choose the stem elongation stage for high gluten index, delayed sedimentation and protein, and the dough maturity stage for high dry matter. It would be appropriate to represent more genotypes and different nitrogen sources in further studies to be more inclusive.

Keywords: Bread wheat, Nitrogen, Flour, Quality traits

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INTRODUCTION

Since the early ages of human history, cereals have been used as one of the basic nutritional sources of societies. Cereals commonly grown in countries around the world include wheat, paddy, corn, barley and oats. According to data from FAO, the Food and Agriculture Organization, in 2021, the most produced grains worldwide were corn, wheat and paddy rice, respectively (FAOSTAT, 2022). Wheat covers approximately 17% of the cultivated areas in the world and constitutes a large portion of the protein and carbohydrate source in nutrition. Wheat constitutes 35% of the world's food resources thanks to its ability to meet food needs, climatic adaptation, ease and efficiency in production, ease of transportation, and ease of storage and processing. It is a nutritional source of great importance in human nutrition, thanks to the rich carbohydrates, proteins, vitamins and minerals it contains (Tosun, 1980). In addition, wheat is the type of grain with the highest protein value among the grain types used for nutrition. People meet more than 20% of their daily calorie needs from wheat and products made from wheat

(Peng et al., 2011; Anonymous, 2017). Wheat grain contains approximately 65-75% starch, 8-15% protein, 1-5% fat, 1.5-3% sugar, 1-2% ash and 11-13% water (Kün, 1996; McKevith, 2004).

The lands on which crops are cultivated during crop production, including wheat, become poorer over time. Thus, essential nutritional elements, such as C, O, H, N, P, K, Ca, Mg, S, Fe, Mn, Mo, Cu, B, and Zn, which are extremely important in plant growth and significantly affect the quantity and quality of the product, must be returned to the soil for the sustainability of crop production (Kacar and Katkat, 2009). To obtain 5.5 t ha^{-1} biomass (grain + straw) from wheat, 78 kg N, 37 kg P_2O_5 and 57 kg K_2O are removed from the soil per hectare (Cooke, 1982). It can be understood that wheat removes mostly nitrogen (N) elements from the soil. According to IFA (1992), 13% of the total nitrogen required for the wheat crop is taken between germination and tillering. Nitrogen uptake increases rapidly from the beginning of tillering to the heading stage, and 55% of the total nitrogen is taken in this period. From heading to maturity, 32% of the total nitrogen is taken.

The first known synthetic nitrogenous fertilizer was calcium nitrate (Collings, 1949). Another known nitrogenous fertilizer is urea, first synthesized from ammonium carbamate in Germany in 1920. By 1970, urea had become the most used nitrogen fertilizer (Kacar and Katkat, 2009). Obtaining urea (CO(NH)2) is primarily based on the synthesis of ammonia and carbon dioxide (Kacar, 1997), and it is an odorless, white, easily soluble fertilizer containing 46% N (TSE, 1986).

Among the quality criteria in wheat, grain moisture content is a criterion that affects storage; if the amount of water in wheat is high, it will cause the dry matter to decrease. Thus, storage problems will arise due to bacterial and fungal growth (Elgün et al., 1998). The falling number is an important criterion in terms of the amount of gas to be released during the making of bread, the bread's volume and the bread's color. It is also a criterion used to determine diastatic activity in terms of flour (Ünal, 2002). The ash ratio gives information about the yield of flour. Ash content varies depending on the type of wheat and the climatic conditions during the growing process (Elgün et al., 1998). Wet gluten ratio is an important factor indicating the quality of bread wheat, and it is an elastic protein that helps to determine whether the dough is suitable for bread making (Tayyar, 2008).

In this study, urea fertilizer was applied at different development stages of bread wheat to examine the effect of grain on quality criteria, such as moisture, dry matter, falling number, ash content, acidity, wet and dry gluten, gluten index, sedimentation and late sedimentation value.

MATERIALS AND METHODS

Identification of plant material and trial site

In the trial, the 'Yakamoz' cultivar, registered by the Eastern Mediterranean Agricultural Research Institute in 2014, was used as plant material. Yakamoz *cv* has the following features: early in heading, plant height around 119 cm, resistant to lodging, grain yield of 6.5-9.7 t ha^{-1}, moderately resistant to drought and cold, moderately resistant to yellow and brown rust, resistant to septoria disease, protein content between 12.1-13.5%, 36.0-41.0 g thousand grain weight, white and awny ears, white grain, use for bread purposes and base areas, with spring nature, 75.8-81.0 kg hL^{-1} hectoliter weight, and 29-65 mL sedimentation value (Anonymous, 2024).

The experiment was established on the lands of the Eastern Mediterranean Agricultural Research Institute in Doğankent location. 'Yakamoz' bread wheat cultivar, widely planted in Çukurova Region, was used as seed material. Soil samples were taken from the trial area before planting and fertility status and micro-element contents were determined. Soil analysis results for the trial site are shown in Table 1. Thus, trial land has a slightly alkaline-saline and loamy structure, high iron and potassium, moderate copper and lime and low content of organic matter, phosphorus, manganese, and zinc content.

Table 1. Soil Analysis Results for The Trial Site.

Saturation		pH	Salt	Lime	Organic matter	P_2O_5	K_2O	Zn	Fe	Cu	Mn
%	Class		%	%	%	kg ha^{-1}		mg kg^{-1}			
53	loamy	8.05	0.02	11.46	1.64	44.3	1095.0	0.41	4.99	10.8	26.0

Climatic data

The climate characteristics of the trial area, located in the Eastern Mediterranean Region, including the wheat season, are given in Table 2. During the 2020-2021 wheat growing season, the average temperature values on a monthly basis were higher in the first four months and the last two months compared to many years; the highest temperature (26.00°C) was in June. Since the amount of precipitation during the wheat growing season in the Mediterranean climate is more than the water consumption of wheat, wheat can generally be grown without irrigation in Cukurova. However, rainfall must be sufficient and in the appropriate regime for optimum efficiency. In the 2020-2021 season, the amount of precipitation in other months except November and May was higher than in many years. When we look at the amount of precipitation in the trial location during the wheat growing season, it is seen that it is 58.79% more than the long-term average (Table 2). Approximately 34.62% of the seasonal

precipitation fell in January alone. This situation has negatively affected tillering in places where water accumulates. When we look at the relative humidity values, higher values were detected in all months except May compared to many years. Regarding the highest relative humidity (81.10%) value, it was observed that the rainfall was 83.12 mm more in December than in many years. The lowest relative humidity values were 69.90% and 62.94% in November and May.

Table 2. Climatic Data Which belong to The Trail Area.

Months	Mean temperatures (°C)		Total precipitation (mm)		Relative humidity (%)	
	Long years	2020-2021	Long years	2020-2021	Long years	2020-2021
November	14.82	15.82	75.360	45.80	65.17	69.90
December	10.43	11.50	121.48	204.6	68.67	81.10
January	9.05	9.86	109.01	306.0	67.69	78.50
February	10.15	10.78	81.860	96.60	65.68	79.70
March	13.14	13.00	63.080	104.2	66.74	76.30
April	17.27	16.00	49.670	102.2	68.02	75.54
May	21.40	22.77	42.150	6.900	68.03	62.94
June	25.17	26.00	13.970	17.50	69.01	76.96
Total			556.58	883.80		

Crop management

The experiment was carried out with four replications according to the split-plot trial design in completely randomized plots at a planting norm of 450 pieces m^{-2}. The size of the test plots was adjusted to be 1.4 m wide and 5 m long. With the planting, base fertilization was applied at the rate of 15 kg DAP da^{-1} per unit area. In top fertilization, it was applied at 30 kg UREA per dam per unit area in four different periods: tillering, stalking, milk formation and yellow maturity periods. This study, in which five subjects were examined, including negative control, was carried out under normal rainfall and no external irrigation was applied.

All necessary maintenance procedures were carried out to ensure the healthy running of the trials and wheat cultivation. As herbicide in weed control, 20 mL da^{-1} 'Terdok 240 EC' was used against narrow-leaved weeds during the tillering period. At the end of the tillering period, herbicide applications were made against broad-leaved weeds with '2-4 D Amine'. As a precaution against fungal diseases (yellow rust-brown rust-black rust-septoria) that may be seen in April and May, 'Opera-max' fungicide at a dose of 200 mL da^{-1} was applied twice with an interval of 25 days.

Evaluated quality traits and experiments

Dry matter, which expresses the remaining mass of a substance without considering the amount of water it contains, was defined by Wulyapash et al.'s (2021) method. After the wheat samples were weighed as 2.5 g, they were burned in a muffle furnace at 900°C for an hour and at 550°C for four hours. The amount of ash was determined by burning it until it reached a constant weight (Anonymous, 2007). Protein amount determination of wheat samples was made using the Kjeldahl Method according to TS 2282. Total protein was calculated by multiplying the amount of nitrogen obtained by a factor of 6.25 (Anonymous, 2014). After weighing 5 g of wheat samples into centrifuge tubes, 30 mL of ethanol was added. The prepared tubes were mixed in the mixer at 20°C for an hour and then centrifuged in the centrifuge at 2000 rpm for five minutes. After centrifugation, 20 mL of the upper liquid was taken and placed in the conical flask. The titration process was carried out after dropping phenolphthalein solution onto it (Anonymous, 2019). After weighing 10 g of wheat samples, 5.5 mL of buffer solution was added. Then, the mixture was mixed and kneaded with a spatula. The kneaded mixture was washed with a buffer solution. The wet gluten obtained was weighed, and the wet gluten was found by proportioning it to the sample amount (Anonymous, 2008a). 10 g of wheat samples were weighed and placed in the prepared wet gluten drying device, dried for four minutes and weighed (Anonymous, 2008b). The wet gluten obtained by washing was centrifuged for 60 seconds, and the gluten index was calculated by proportioning the remaining part on the cassette sieve to gluten and finding its percentage. After weighing the wheat samples into a graduated cylinder, 50 mL of bromophenol solution was added. After the resulting mixture was shaken by hand in a horizontal position 12 times, it was placed in the shaking device, and the device was turned on for five minutes. After the time was up, 25 mL of the sedimentation test reagent was added and placed back into the device. After another five minutes of shaking, the cylinder was removed from the device and kept upright for five minutes. The volume of the resulting sediment was read and recorded as Zeleny sedimentation (Anonymous, 2013a). After adding 50 mL of bromophenol solution to the weighed wheat samples, the cylinders were placed in the shaking device set for five minutes. After the time was up, the cylinders were taken from the device and kept for two hours. At the end of two hours, 25 mL of test reagent was added and placed in the shaking device set for five minutes. After the time expired, the cylinder was removed from the device, and after waiting for five minutes, the volume of the sediment obtained was read and recorded as late sedimentation (Anonymous, 2013a). The weighed 7 g flour

sample was placed in a viscometer tube, and 25 mL of pure water was added. Then, it was shaken with a mixer and placed in a water bath. The experiment was completed when the viscometer stirrer reached the bottom of the gelatin suspension. The number that appeared determined the number of falls (Anonymous, 2013b).

Statistical analysis

Laboratory studies were conducted in three replications using a split-plot trial design in completely randomized plots. Urea application periods were the main-pilot factors and flour types were the sub-pilot factors. Variance analysis (effect tests), the compares of treatment means (by LSD test), and correlation coefficients were made by the JMP (2007) packet programme.

RESULTS AND DISCUSSION

Dry matter

Urea application stages (AS) and application stages×flour type (AS×FT) interaction had statistically significant effects on the dry matter (DM) of flour; however, there was a non-significant difference between whole grain flour (WGF) and White flour (WF) for DM (Table 3). While the maximum DM mean (90.76%) was obtained from the urea applied during the dough ripeness stage of bread wheat, the lowest DM (90.20%) was obtained from the control in which urea was not applied. In bread wheat, the interaction observed between the different growth stages at which urea is applied and the flour type is due to the reactions of the fertilizer applied during the stem elongation stage to the flour type. Shahzad et al. (2019) examined the effect of different nitrogen levels on the dry matter content and other quality characteristics of wheat grains. They observed that nitrogen fertilizer application significantly affected the dry matter content of wheat grains, and that the highest dry matter content was observed in wheat plants fertilized with the highest nitrogen level. Researchers also found that improved dry matter content positively affected other quality parameters of the flour, such as protein content and baking properties.

Ash content

All variation sources, such as AS, FT and AS×FT, showed statistically significant differences at $p < 0.01$ (Table 3). When an evaluation is made according to the urea AS, while the highest ash content was obtained from the control application (no-urea) with 1.21%, the lowest ash content was from the urea application of the milky stage with 1.04%. According to FT, the highest ash content was not surprisingly obtained from WGF (1.44%), while the lowest ash content was obtained from WF (0.78%). AS×FT interaction arises from the fact that flour type showed different ash content values with urea applied at different growth stages of bread wheat (Table 3). Turan et al. (2017) evaluated the effect of nitrogen fertilization on the ash content of wheat grains and flour. Although the ash content of wheat grains and flour increases linearly with increasing nitrogen levels, it was determined in our study that the control application gave higher ash values than the others. Elgün et al. (1998) also emphasized that the ash rate can vary depending on the wheat variety, climate and soil conditions, and the ash rate decreases on dry days. It has been observed that the ash ratio decreases as the whiteness degree of the bread increases and increases as the whiteness degree of the bread decreases (Anonymous, 2012).

Table 3. Mean values for dry matter and ash content of whole grain flour (WGF) and white flour (WF) from the urea application to bread wheat at different growth stages

Application stage (AS)	Dry matter (%)			Ash content (%)		
	WGF	WF	Mean	WGF	WF	Mean
Control (no urea)	90.27^{ef*}	90.14^{f}	90.20^{c}	1.62^{a}	0.80^{d}	1.21^{a}
Tilling	90.46^{cd}	90.59^{bc}	90.52^{b}	1.45^{b}	0.80^{d}	1.12^{b}
Stem elongation	90.73^{ab}	90.37^{de}	90.55^{b}	1.35^{c}	0.79^{de}	1.07^{c}
Milky	90.52^{cd}	90.58^{bc}	90.55^{b}	1.32^{c}	0.75^{e}	1.04^{d}
Dough	90.76^{a}	90.77^{a}	90.76^{a}	1.47^{b}	0.75^{e}	1.11^{b}
Mean	90.55	90.49	90.52	1.44^{a}	0.78^{b}	1.11
Brief view from the analysis of variance (p values)						
AS	$p < 0.01$			$p < 0.01$		
Flour type (FT)	ns			$p < 0.01$		
AS×FT	$p < 0.01$			$p < 0.01$		

*There was no statistical difference between the same letter groups at the 0.05 significance level by LSD test. ns: non-significant.

Protein content

While protein content showed statistically significant differences in terms of AS at the $p<0.05$ probability level, FT had no significant effects on protein content. On the other hand, AS×FT affected protein content at a probability level of $p<0.01$ (Table 4). In this concern, the highest protein content (15.15%) was from the stem elongation stage while the lowest protein content (14.00%) was from the tilling stage for AS. Also, AS×FT interaction originated

from the fact that FTs statistically differed in terms of protein content at the tillering stage (Table 4). The protein ratio of wheat flour is an important quality parameter that affects the nutritional value and processing properties of the flour; Xu et al. (2017) evaluated the effect of nitrogen on the protein content and baking properties of wheat flour. As a result, they stated that increasing nitrogen levels significantly increased the protein content of wheat grains and flour. Abedi et al. (2011) reported that nitrogen applied in the vegetative period increased the yield, and nitrogen applied in grain filling increased the protein ratio.

Acidity

The effects of urea applied in different growth stages of bread wheat on the acidity value of flour were significant at the $p<0.01$ probability level according to AS and FT (Table 4). Thus, the highest acidity value was from the control application (0.06%), but the lowest values were from stem elongation, milky and dough stages with the value of 0.04%. Also, acidity was higher in WGF (0.05%) compared to WF (0.04%) (Table 4). The acidity content of wheat flour is an important quality parameter that affects the taste, texture and processing properties of the flour; the acidity value is higher in whole wheat flour than in white flour.

Table 4. Mean values for protein content and acidity of whole grain flour (WGF) and white flour (WF) from the urea application to bread wheat at different growth stages

Application stage (AS)	Protein (%)			Acidity (%)		
	WGF	WF	Mean	WGF	WF	Mean
Control (no urea)	14.57^{bcd*}	14.97^{ab}	14.77^{ab}	0.06	0.05	0.06^{a}
Tilling	14.53^{bcd}	13.48^{e}	14.00^{c}	0.06	0.04	0.05^{b}
Stem elongation	14.98^{ab}	15.31^{a}	15.15^{a}	0.05	0.03	0.04^{c}
Milky	14.45^{cd}	14.33^{d}	14.39^{bc}	0.05	0.03	0.04^{c}
Dough	14.43^{cd}	14.84^{abc}	14.63^{ab}	0.04	0.04	0.04^{c}
Mean	14.59	14.58	14.59	0.05^{a}	0.04^{b}	0.04
Brief view from the analysis of variance (p values)						
AS	$p < 0.05$			$p < 0.01$		
Flour type (FT)	ns			$p < 0.01$		
AS×FT	$p < 0.01$			ns		

*There was no statistical difference between the same letter groups at the 0.05 significance level by LSD test. ns: non-significant.

Wet and dry gluten

Wet and dry gluten contents were affected at the $p<0.01$ significance level in terms of all variation sources (AS, FT and AS×FT) (Table 5). In this context, mean wet gluten content varied from 41.49% (Tilling) to 43.66% (control, no urea) for AS. Also, the wet gluten content of WF (44.90%) was higher than WGF (40.27%). AS×FT interaction varied from 39.36% to 45.91%; the interaction is because the WF values were in the higher group than the WGF values in each AS. Dry gluten content changed between 14.75% and 15.46% for AS. Thus, the application stages with the lowest dry gluten values were the tillering (14.75%) and stem elongation (14.87%), while the highest dry gluten value was from the milky stage (15.46%). Additionally, the dry gluten value of WF (15.96%) was higher than WGF (14.25%). AS×FT values varied from 13.83% to 16.57%. It is thought that the interaction is because dry gluten is in a higher group in WF than in WGF for each application stage. Abedi et al. (2011) found that when nitrogen was not applied during the grain filling period, the gluten content of the grain decreased significantly; nitrogenous fertilizer given during the vegetative period (such as tillering) results in productivity; however, they reported that nitrogen given during late development periods would result in improved gluten quality. Because gluten parts, “gliadin and glutenin,” are known as proteins that build up in the grain during the grain-filling period (Shewry and Halford, 2002). In addition, Marconi et al. (1999) found dry gluten values in the range of 11.2-14.7%; these values are consistent with the findings obtained in this study.

Table 5. Mean values for wet and dry gluten content of whole grain flour (WGF) and white flour (WF) from the urea application to bread wheat at different growth stages

Application stage (AS)	Wet gluten (%)			Dry gluten (%)		
	WGF	WF	Mean	WGF	WF	Mean
Control (no urea)	41.50^{e*}	45.83^{a}	43.66^{a}	14.63^{d}	15.89^{b}	15.26^{ab}
Tilling	39.59^{g}	43.40^{d}	41.49^{d}	13.83^{f}	15.67^{bc}	14.75^{c}
Stem elongation	40.53^{f}	43.79^{c}	42.16^{cd}	14.37^{de}	15.38^{c}	14.87^{c}
Milky	40.38^{f}	45.91^{a}	43.14^{ab}	14.34^{de}	16.57^{a}	15.46^{a}
Dough	39.36^{g}	45.56^{b}	42.46^{bc}	14.07^{ef}	16.30^{a}	15.18^{b}
Mean	40.27^{b}	44.90^{a}	42.58	14.25^{b}	15.96^{a}	15.10
Brief view from the analysis of variance (p-value)						
AS	$p < 0.01$			$p < 0.01$		
Flour type (FT)	$p < 0.01$			$p < 0.01$		
AS×FT	$p < 0.01$			$p < 0.01$		

*There was no statistical difference between the same letter groups at the 0.05 significance level by LSD test.

Gluten index

Gluten index values were affected at the $p<0.01$ significance level in terms of all variation sources (AS, FT and AS×FT) (Table 6). The highest gluten index value (80.38%) was from the stem elongation stage, while the lowest value (69.28%) was from the control. In addition, the gluten index of WGF (82.53%) was higher than the value of WF (66.07%). AS×FT interaction differed between 55.86% and 80.38%; the reason for interaction is attributed to the fact that WGF values being in the higher group than WF values in each application stage (Table 6). Hao et al. (2023) stated that nitrogen applied during the flowering and grain-filling periods increased the gluten index (80.5-86.2%) and brought it almost to 100%.

Falling number

Falling number values showed statistical differences at the $p<0.05$ significance level for urea application stages, and they also differed according to flour type at the $p<0.01$ significance level. AS×FT interaction was not statistically significant (Table 6). Thus, the highest value (881.67 s) was observed from the control application, while the lowest value (753.83 s) was from the dough stage. Also, falling numbers of WF (836.40 s) were higher than the value of WGF (741.87 s) (Table 6). Al-Saadi et al. (2021) investigated the effects of nitrogen management on the falling number value of wheat flour. It has been reported that the falling number value of wheat flour decreases with increasing nitrogen application doses, indicating an increase in amylase activity and starch damage.

Table 6. Mean values for gluten index and falling number of whole grain flour (WGF) and white flour (WF) from the urea application to bread wheat at different growth stages

Application stage (AS)	Gluten index (%)			Falling number (s)		
	WGF	WF	Mean	WGF	WF	Mean
Control (no urea)	82.69^{ab*}	55.86^{h}	69.28^{c}	803.00	960.33	881.67^{a}
Tilling	83.64^{ab}	60.71^{g}	72.18^{bc}	698.33	835.00	766.67^{b}
Stem elongation	81.86^{bc}	78.90^{d}	80.38^{a}	758.33	751.67	755.00^{b}
Milky	79.90^{cd}	64.95^{f}	72.42^{bc}	763.00	814.00	788.50^{b}
Dough	84.56^{a}	69.94^{e}	77.25^{ab}	686.67	821.00	753.83^{b}
Mean	82.53^{a}	66.07^{b}	74.30	741.87^{b}	836.40^{a}	789.13
Brief view from the analysis of variance (p values)						
AS	$p < 0.01$			$p < 0.05$		
Flour type (FT)	$p < 0.01$			$p < 0.01$		
AS×FT	$p < 0.01$			ns		

*There was no statistical difference between the same letter groups at the 0.05 significance level by LSD test. ns: non-significant.

Zeleny and late sedimentation

Zeleny and late sedimentation values were affected at the $p<0.01$ significance level in terms of all variation sources (AS, FT and AS×FT) (Table 7). Thus, the highest Zeleny sedimentation values (21.0 mL) were observed from the control and dough stages while the lowest values were determined from the stem elongation stage. Also, the Zeleny sedimentation value of WF (30.53 mL) was higher than that of WGF (10.60 mL). AS×FT interaction values of Zeleny sedimentation changed between 10.00 mL and 30.67 mL. The difference in interaction values has been explained by the fact that the WF Zeleny sedimentation value was higher than the WGF value at each urea application stage (Table 7). For late sedimentation value, the highest values were from stem elongation (29.83 mL) and milky stages (29.50 mL), while the lowest value was from the control application (23.50 mL). As with the Zeleny sediment, the late sedimentation value of WF (35.60 mL) was higher than that of WGF (19.20 mL).

AS×FT interaction values of late sedimentation differed between 17.00 mL and 39.00 mL. The impact of interaction (AS×FT) originated from the late sedimentation value of WF, which was higher than the WGF value at each urea application stage (Table 7). Hao et al. (2023) reported that nitrogen given by dividing into two during the flowering and grain-filling periods increased sedimentation values both years and in two of three varieties.

Table 7. Mean values for Zeleny sedimentation and late sedimentation of whole grain flour (WGF) and white flour (WF) from the urea application to bread wheat at different growth stages

Application stage (AS)	Zeleny sedimentation (mL)			Late sedimentation (mL)		
	WGF	WF	Mean	WGF	WF	Mean
Control (no urea)	12.00^{d*}	30.00^{c}	21.00^{a}	17.00^{h}	30.00^{d}	23.50^{d}
Tilling	11.00^{e}	30.00^{c}	20.50^{b}	18.67^{g}	35.00^{c}	26.83^{c}
Stem elongation	10.00^{f}	30.00^{c}	20.00^{c}	20.67^{f}	39.00^{a}	29.83^{a}
Milky	10.00^{f}	30.67^{b}	20.33^{b}	22.00^{e}	37.00^{b}	29.50^{a}
Dough	10.00^{f}	32.00^{a}	21.00^{a}	17.67^{h}	37.00^{b}	27.33^{b}
Mean	10.60^{b}	30.53^{a}	20.57	19.20^{b}	35.60^{a}	27.40
Brief view from the analysis of variance (p values)						
AS	$p < 0.01$			$p < 0.01$		
Flour type (FT)	$p < 0.01$			$p < 0.01$		
AS×FT	$p < 0.01$			$p < 0.01$		

*There was no statistical difference between the same letter groups at the 0.05 significance level by LSD test.

Correlations between quality traits

Acidity showed a significant negative correlation (r=-0.38*) with dry matter and a significant positive correlation (r=0.68**) with ash content. Wet gluten content was significantly negative correlated with ash content (r=-0.88**) and acidity (r=-0.47**). Dry gluten content showed significant negative relationships with ash content (r=-0.0.88**) and acidity (r=-0.55**), whereas it showed significant positive correlation with wet gluten content (r=0.96**). Gluten index was significantly positively correlated with ash content (r=0.78**) and significantly negatively correlated with wet (r=-0.81**) and dry gluten (r=-0.81**) content. Zeleny sedimentation showed significant negative relationships with ash content (r=-0.96**), acidity (r=-0.60**) and gluten index (r=-0.78**), and significant positive relationships with wet (r=0.93**) and dry (r=0.92**) gluten content. Late sedimentation showed significant negative correlations with ash content (r=-0.97**), acidity (r=-0.73**) and gluten index (r=-0.65**), and significant positive correlations with wet gluten (r=0.85**), dry gluten (r=0.87**) and Zeleny sedimentation (r=0.95**). Falling number was significantly negative correlated with dry matter (r=-0.41*), ash content (r=-0.46**), and gluten index (r=-0.61**), although it was significant positive correlated with wet gluten (r=0.62**), dry gluten (r=0.59**), and Zeleny sedimentation (r=0.52**) (Table 8).

Table 8. Correlation coefficients (r) between investigated quality traits (n=30)

Traits	Dry matter	Ash	Protein	Acidity	Wet gluten	Dry gluten	Gluten index	Sedim	Late sedim
Ash	0.03								
Protein	-0.08	0.00							
Acidity	-0.38*	0.68**	0.04						
Wet gluten	-0.21	-0.88**	0.13	-0.47**					
Dry gluten	-0.07	-0.88**	-0.05	-0.55**	0.96**				
Gluten index	0.22	0.78**	0.18	0.33	-0.81**	-0.77**			
Zeleny sedim	-0.14	-0.96**	-0.01	-0.60**	0.93**	0.92**	-0.78**		
Late sedim	0.00	-0.97**	0.03	-0.73**	0.85**	0.87**	-0.65**	0.95**	
Falling	-0.41*	-0.46**	-0.05	-0.05	0.62**	0.59**	-0.61**	0.52**	0.37*

* and ** indicate probability levels of $p < 0.05$ and $p < 0.01$, respectively.

CONCLUSION

Wheat is a strategic product that is crucial to human nutrition. Both breeding research and agronomy research are continuing worldwide to produce more efficient and high quality wheat in agricultural production. Wheat quality can mean different things to the farmer who grows the wheat, the miller who mills it and the industrialist who processes it into the final product. Wheat quality may vary according to the species and variety, ecological regions and cultivation techniques applied. One of the most critical factors in yield and quality in agricultural production is balanced fertilization. The most important nutrient element affecting yield and quality in wheat is nitrogen. Generally, nitrogen applied in the top fertilization of wheat in the Çukurova region is applied during the tillering period. However, the nutrient requirements of wheat at different developmental stages may have different effects on its quality. In this study, nitrogen applications were made at tillering, stalk emergence, milk maturity

and yellow maturity stages of wheat. It was determined that nitrogen applications were more important for high gluten index, delayed sedimentation and protein at emergence and for high dry matter at yellow maturity. For high yield and quality in wheat, it will be significant to conduct research with more genotypes with different yield potentials in different regions according to climatic conditions and soil characteristics and with new-generation fertilizer sources in further studies.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of interests

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

BBK: MSc thesis and experiments; BB: Thesis supervisor and paper writing; CB: Arrangement of laboratorial experiments; SA and HB: Seed providing (field experiments).

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Investigation of the effects of psyllium powder addition on the quality of fresh and frozen gluten-free bread

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Abstract
The interest in gluten-free (GF) products has been growing since both the increase in prevalence of celiac disease and the preferences of GF diet. In this study, the contribution of psyllium powder (PP) to gluten-free (GF) bread quality, dough rheology and volatile compounds (VCs) profile was investigated throughout the frozen storage period of GF dough (-30 °C for 0, 7, 15, and 30 days). GF doughs containing 7.5% PP (PSY1) and 15% PP (PSY2) had lower tanδ value than GF control dough ($p<0.05$) according to the results obtained from fundamental rheological analysis. Frozen storage caused no effect on the tanδ value of PSY1 and PSY2 ($p \geq 0.05$). PP addition increased the specific volume (SV) of GF breads ($p<0.05$). No significant effect of frozen storage on SV was shown for PSY2 while SV values of GF control bread (GFB) and PSY1 decreased ($p<0.05$). Lower crumb hardness was shown for PSY1 and PSY2 on day 0. Significant effect of frozen storage on crumb hardness was observed for PSY1 on day 30 while harder crumb structure was shown for GFB throughout the frozen storage ($p<0.05$). Psyllium addition led to a significant reduction in both L* value of crust and crumb color ($p<0.05$). In the VCs analysis performed by HS/GC-MS, ethanol and 1-butanol, 3-methyl from alcohol group, butanal, 3-methyl- and hexanal from aldehydes were common for GFB and GF breads containing psyllium. 1-butanol, 3-methyl-, butanal, 3-methyl- and hexanal were the VCs of PSY1 and they were also shown after frozen storage. This study suggested that quality deterioration due to frozen storage was less in gluten-free breads containing psyllium.
Keywords: Frozen storage, Gluten-free dough, Fundamental rheology, Texture, Volatile compounds

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INTRODUCTION
The function of gluten in wheat based product has been known for years and excluding gluten from a bakery formulation causes a big challenge in terms of obtaining high-quality gluten-free (GF) product (Santos et al., 2020). A cohesive, extensible and elastic dough is formed thanks to the unique properties of gluten, and in turn the gas producing during fermentation is retained in the structure (Belorio & Gómez, 2020). However, the strict adherence to a GF diet is still an effective solution for people suffering from celiac disease. Also, GF diet is becoming a worldwide trend due to some claims that GF products are healthier. Therefore, the demand for gluten-free products has increased and extensive researches have been performed to improve quality and nutritional characteristics of GF products (Fratelli et al., 2021; Mezaize et al., 2010).

GF bakery products can be formulated by GF flour obtaining from cereals (mostly maize and rice), pseudo-cereals (mostly buckwheat) and legumes starch, hydrocolloids, enzyme and protein sources (Mezaize et al., 2010). Dietary fibers and minerals can also be incorporated into formulation to ameliorate the nutritional quality of GF products (Stantiall & Serventi, 2018). The use of dietary fibers in GF bakery products also provides better physical properties as well as lower glycemic response (Fratelli et al., 2018).

Recent literatures have focused on psyllium (Plantago ovata) as it is considered as a natural bioactive fiber. Addition of psyllium fiber into a wheat-based flat dough can restrict enzyme mobility behaving like a physical

barrier, thus affecting in vitro starch digestion (Güler & Sensoy, 2023). Cappa et al. (2013) investigated the effects of psyllium and sugar beet fibre in GF bread formulation and indicated that psyllium had better performance on bread development. Hydroxypropylmethylcellulose (HPMC) is commonly used hydrocolloids that improves dough development and gas retention (Ylimaki et al., 1991). In a study of Mancebo et al. (2015), HPMC, psyllium and different levels of water were optimized in GF bread formulation and no synergistic effects between both hydrocolloids observed. Fratelli et al. (2018) obtained a 33% reduction in glycemic response by using 17.14% psyllium and 117.86% water in GF bread formulation. Similar studies were also performed to examine the combination effects of chickpea and psyllium (Santos et al., 2020) and HPMC, psyllium and xanthan gum (Belorio & Gómez, 2020).

Frozen storage of bakery products has been considered as a challenge due to the disruptive effects on dough matrix and yeast activity. However, food industry uses freezing process and frozen storage for bakery products that exhibit a faster rate of staling in order to provide fresh products to consumers at any time (Leray et al., 2010; Mezaize et al., 2010). Numerous studies have been performed to reveal the effects of frozen storage on wheat dough and the improvement strategies for its quality. As for GF dough, type of hydrocolloids, freezing and frozen storage conditions were investigated in some studies.

Lorenzo et al. (2009) investigated the effect of some hydrocolloids (HPMC, xanthan and guar gums) on refrigerated and frozen non-fermented gluten-free dough, suggesting that xanthan gum exhibited the best results in terms of dough elasticity while refrigerated and frozen storage for twenty days did not affect the quality of baked dough. Leray et al. (2010) compared the different formulations of non-yeasted wheat and gluten-free bread dough and studied the effects of freezing and frozen storage conditions. This comparison study showed that wheat dough was more sensitive than gluten-free dough to frozen storage. No differences were determined in terms of elastic and viscous moduli while consistency index and flow behaviour index were reduced by the presence of a freezing step in a study of Mezaize et al. (2010) who examined the gluten-free frozen dough in terms of rheological properties. Ozkoc and Seyhun (2015) evaluated the effects of gum type and flaxseed concentration on GF breads made from frozen dough and indicated that combination of flaxseed (5%) and guar gum (1%) had better characteristics in the quality of gluten-free breads. Hayıt and Gül (2019) developed a GF formulation with quinoa for partially baked GF bread and reported that 30% quinoa flour addition led to a part-baking 45 days of frozen storage without any significant changes in sensorial properties. The using of cryoprotectants is also another way to improve the quality of frozen dough. In a recent study, 9% fructo-oligosaccharide and 31 % hydrolyzed soy protein as a potential cryoprotectant have been incorporated into GF bread and the improved specific volume and crumb texture have been obtained (de Oliveira Teotônio et al., 2021).

The use of psyllium in GF bread has been widely studied to improve the quality and nutrition content. In the present study, it was aimed to investigate the effects of psyllium in GF bread during frozen storage (-30 °C for 7, 15 and 30 days) in terms of rheological properties of doughs, quality characteristics and volatile compounds of breads.

MATERIALS AND METHODS

Materials

Gluten-free flour (Sinangil, Eksim Co., İstanbul, Türkiye), salt, sugar, compressed yeast, and oil were obtained from a local market. Gluten-free flour contains rice flour, sugar, pectin and xanthan gum as thickener, and baking powder (sodium bicarbonate and sodium acid pyrophosphate). Psyllium powder (PP) (81.1% fiber, 43% carbohydrate and 4.3% protein) was purchased from Talya Foods (Antalya, Türkiye).

Gluten-free bread making, freezing and thawing

Gluten-free bread formulation was shown in Table 1. Preliminary experiments were performed to determine the water absorption capacity in each formulation. Firstly, flour and salt were mixed in a kneader (Arzum, Türkiye) for 1 min at speed 1. Yeast and sugar were dissolved in water at room temperature, and then they were added into flour-salt mixture. After mixing for 1 min at speed 1, oil was added. Final mixing was performed for 3 min at speed 1 and 7 min at speed 2. 150 g of dough was placed on a baking pan, fermented for 1 hour at 30 °C and 85% relative humidity (Nuve TK 252, Ankara, Türkiye). Frozen storage (-30 °C for 7, 15 and 30 days) was performed for frozen dough samples. After storage, they were thawed at 30 °C for 1 hour. The baking conditions were at 190 °C bottom temperature and 230 °C top temperature for 40 min (Maksan MKF-4P, Türkiye).

Table 1. Gluten-Free Bread Formulations.

Bread Type	Gluten-Free Flour Mix (g)	Psyllium (g)	Salt; Sugar; Yeast; Liquid Oil (g)	Water (g)
GFB	300	0	4.5 ; 7.5 ; 12 ; 12	300
PSY1	277.5	22.5	4.5 ; 7.5 ; 12 ; 12	450
PSY2	255	45	4.5 ; 7.5 ; 12 ; 12	570

GFB: gluten-free control bread
PSY1: gluten-free bread containing 7.5% PP
PSY2: gluten-free bread containing 15% PP

Fundamental rheological analysis

A frequency sweep test (between 0.1 rad/s and 100 rad/s at 20 ˚C) was conducted to determine the rheological properties of the GF dough samples. A constant strain of 0.1% within linear viscoleastic region was applied. Antonpaar MCR 302 rheometer (Graz, Austria) was used with a PP50 probe by placing GF dough (~2 g) on the parallel plate with a 2 mm gap. The storage modulus (G′), loss modulus (G″) and tanδ (G″/ G′) values were obtained by triplicate measurements.

Determination of quality characteristics of gluten-free breads

The quality characteristics of samples (specific volume, texture and color) were performed after cooling for 2 h.

Specific volume

The volume of bread was determined according to the rapeseed displacement (AACC Method 10-05.01, 2000). The volume of bread (mL) was divided by the weight (g) of bread to calculate the specific volume (mL/g). Two breads from each formulation were subjected to volume measurement and the means were calculated.

Texture

The bread samples were divided into two 12.5 mm slices for texture profile analysis (Stable Micro Systems TA.XT2 Plus, UK). A 36 mm cylindrical probe was used in the analysis, and a force of 50 N was applied to the samples at a speed of 55 mm/min. Hardness, springeness, cohesiveness, chewiness, and resilience parameters were evaluated. Triplicate measurements were performed.

Color

The lightness (L*), redness (a*) and yellowness (b*) parameters of the crust and crumb were determined by using a Chromameter (CR-100 Konica Minolta, Tokyo, Japan). The following equation was used to calculate the color differences (ΔE):

$$\Delta E = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2}$$

L^*_0, a^*_0, and b^*_0 are the values of fresh breads (day 0) while L*, a*, and b* values correspond to the days of frozen storage (7th, 15th and 30th). Three measurements were performed for crust color of each bread. Crumb color was determined by two measurements for each type of bread.

Determination of volatile compounds

The GF bread crumb (~3 g for each bread type) was subjected to HS/GC-MS analyses (GCMS-QP2010, Shimadzu, Milan, Italy) to determine the profile of volatile compounds (VCs). The details of the method was previously stated in the studies of Rzepa et al. (2009), Tulukcu et al. (2019) and Ozulku (2024). The detected volatile compounds showing 70% similarities with the commercial mass spectra libraries (NIST27 and WILEY7) were evaluated to exhibit VCs profile.

Statistical Analysis

One-way analysis of variance (ANOVA) followed by post hoc Tukey test was used to determine the difference between groups at 95% significance level with IBM SPSS Statistics 25 package program (Chicago, IL, USA).

RESULTS AND DISCUSSION

Rheological properties

The content and structure of ingredients significantly affect the rheological properties of GF dough.Therefore, rheological measurements have been widely performed to design the GF formulation (Yazar & Demirkesen, 2023). The effect of psyllium addition and frozen storage on the storage modulus (G′) and loss modulus (G″) of GF dough samples were presented in Figure 1., where G′ reflects the elasticity of dough, while G″ reflects the viscosity of dough. In this study, GF control bread (GFB) dough exhibited a lower G′ than the GF dough containing 7.5% PP (PSY1) and 15.0% PP (PSY2). PP addition into GF dough led an increase in G′ and a decrease in G″ on day 0 (Figure 1). In a study of Mancebo et al. (2015), psyllium addition (2-4%) increased the G′, particularly with reduced dough water levels. This was attributed to presence of ingredients with water-binding ability (Matos & Rosell, 2013). Also, a solid elastic-like behavior was shown in all GF dough samples since G′ was greater than G″ as stated in many studies (Lorenzo et al., 2009; Mancebo et al., 2015; Mezaize et al., 2010).

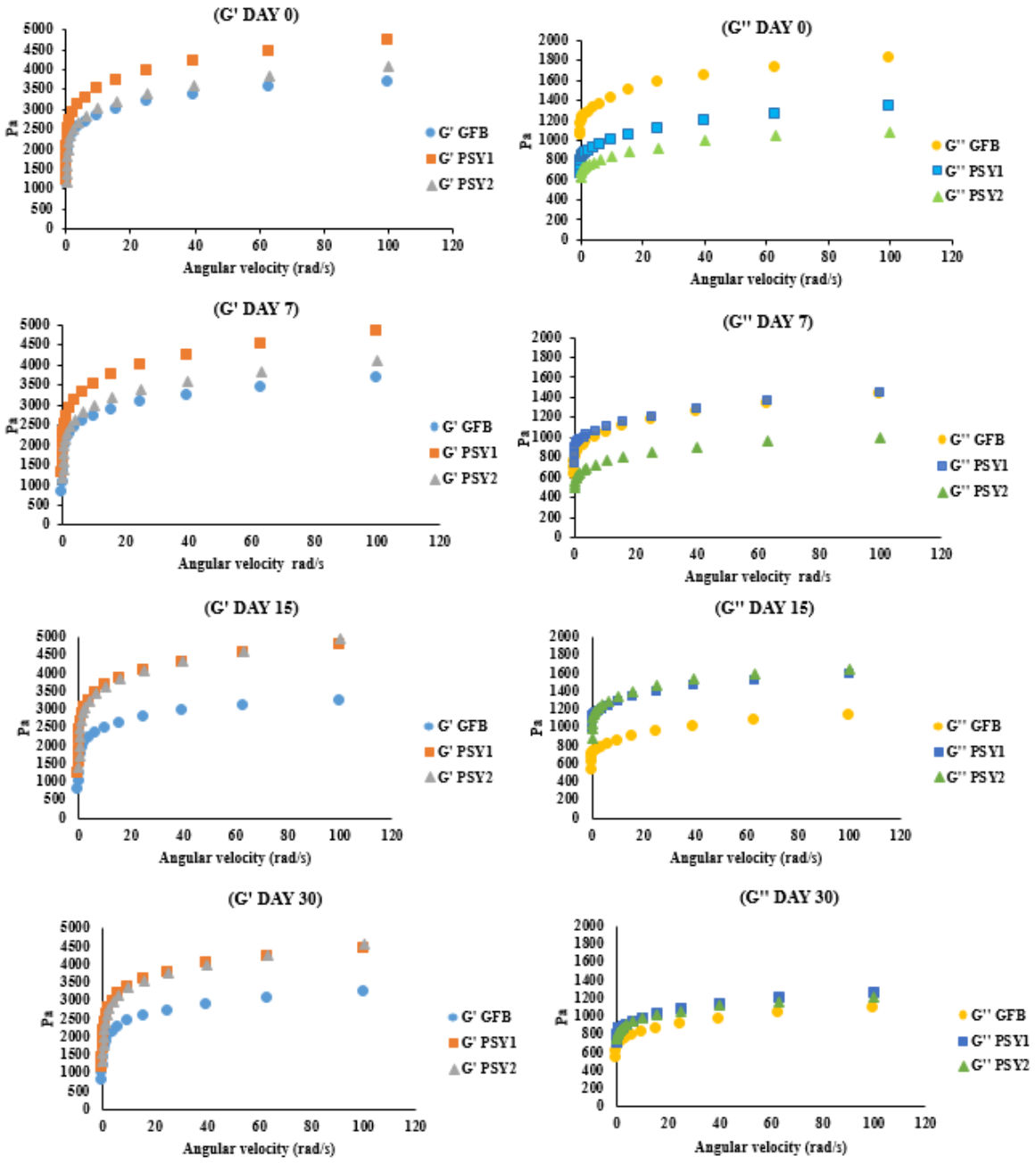

Figure 1. Rheological properties of gluten-free breads during frozen storage.
GFB : gluten-free control bread; PSY1: gluten-free bread containing 7.5% PP; PSY2: gluten-free bread containing 15% PP

The ratio of G″ to G′ (G″/G′) is corresponds to tanδ which shows the degree of dough viscoelasticity (Lu et al., 2023). Figure 2 shows the loss tangent (tanδ) value of all dough samples. The tanδ value was obtained lower than 1, suggesting that elastic behavior is more dominant than viscous behavior. PSY1 and PSY2 had lower tanδ values when compared to GFB dough ($p<0.05$, Figure 2). Mancebo et al. (2015) also reported that psyllium decreased the tanδ parameter. Frozen storage caused a significant reduction in tanδ value of GFB dough, but no significant differences were observed between 15 days and 30 days of frozen storage. Leray et al. (2010) reported that no significant effect of 28 days of frozen storage on the tanδ value of GF reference dough containing corn flour, starch, hydrocolloids (HPMC, guar gum, highly methylated pectin), etc. Frozen storage caused no effect on the tanδ value of PSY1 and PSY2 ($p>0.05$, Figure 2). Addition of psyllium decreased the impact of frozen storage when compared to GFB dough due to its high amount fiber.

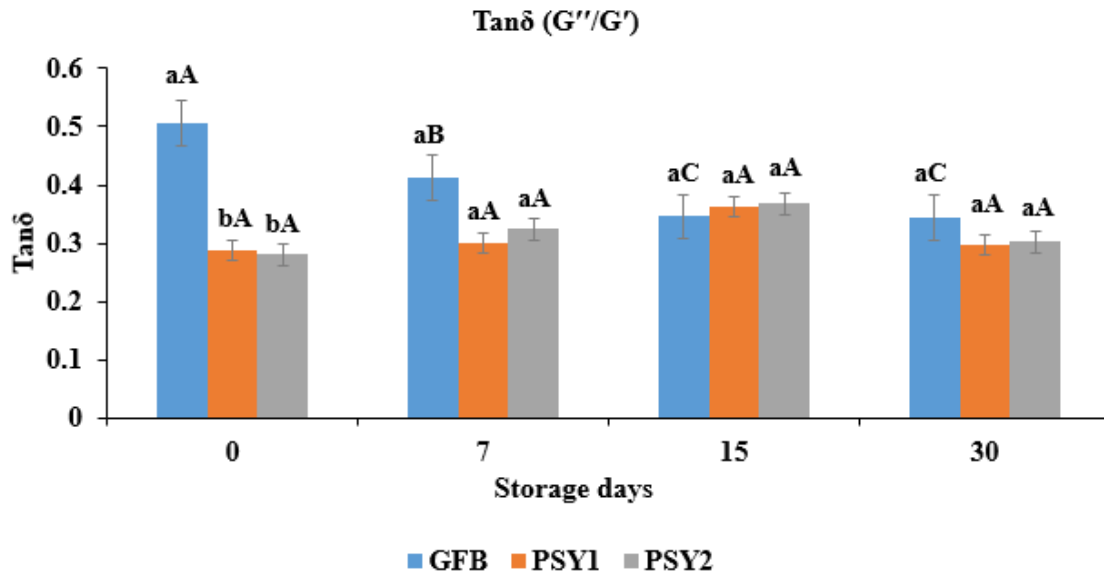

Figure 2. The changes of tanδ values of gluten-free breads during frozen storage.
Different uppercase letters show significant differences during the frozen storage of same bread type ($p < 0.05$). Different lowercase letters show significant differences between bread types for the same frozen storage time ($p < 0.05$). GFB : gluten-free control bread; PSY1: gluten-free bread containing 7.5% PP; PSY2: gluten-free bread containing 15% PP

Quality Characteristics of GF breads

Specific volume and texture

Specific volume (SV) and crumb properties are the main criteria to evaluate bread quality. Table 2 shows SVs and textural characteristics of GF bread samples. Highest SV values were obtained in PSY1 and PSY2 on day 0. Psyllium addition increased SV when compared to control formulation, similar with the study of Fratelli et al. (2021). However, some studies reported that psyllium didn't improve the volume of GF bread (Mancebo et al., 2015; Santos et al., 2020). But, it was suggested that the hydration level and amount of psyllium in the formulation were important in order to be shown the effect of psyllium on GF bread quality (Fratelli et al., 2018; Santos et al., 2020). No significant effect of frozen storage on SV was shown for PSY2 while SV values of GFB and PSY1 decreased ($p<0.05$). The bread SV is inversely correlated with crumb hardness as stated in many studies (Capriles and Arêas, 2014; Gallagher et al., 2004; Sabanis et al., 2009). Therefore, lower crumb hardness was shown for PSY1 and PSY2 on day 0. Fratelli et al. (2021) also observed lower crumb firmness value with the addition of psyllium when compared to control. This has been explained that interactions between psyllium and water enhanced the formation of gel network, and in turn improved the gas retention capacity during baking, volume and texture of GF bread (Fratelli et al., 2018). Moreover, water adjustment was recommended for fibre-enriched GF formulations to achieve optimum dough viscosity and bread characteristics (Capriles et al., 2021; Conte et al., 2019). Harder crumb structure was shown for GFB throughout the frozen storage. Significant effect of frozen storage on crumb hardness was observed for PSY1 on day 30 while it was on day 7 for PSY2. Springeness and cohesiveness values of GF breads weren't significantly different on day 0. Frozen storage had no effect on springeness, cohesiveness and chewiness values of GFB sample, but its resilience value increased on storage day 15 (Table 2). Resilience value is a kind of indicator of crumb elasticity and a reduction of this value is associated with loss of elasticity (Onyango et al., 2011). Resilience value of PSY2 remained stable throughout the frozen storage while there was a significant reduction in resilience value of PSY1 at the end of frozen storage. Chewiness value which is defined as energy to masticate a solid food was influenced by frozen storage of PSY2 (Table 2). 15 days of frozen storage was better in terms hardness, chewiness and resilience value of PSY1 and PSY2 while 7 days of frozen storage is more suitable for GFB due to its sharp increase in hardness value.

Table 2. Specific volume and textural characteristics of gluten-free breads

	Frozen Storage Times (Days)	BREAD TYPES		
		GFB	PSY1	PSY2
Specific Volume (mL/g)	0	2.46 ± 0.01^{bA}	3.28 ± 0.01^{aA}	3.10 ± 0.17^{aA}
	7	2.29 ± 0.08^{bAB}	2.93 ± 0.06^{aB}	2.83 ± 0.22^{abA}
	15	2.13 ± 0.01^{cB}	2.95 ± 0.01^{aB}	2.62 ± 0.001^{bA}
	30	2.27 ± 0.01^{bB}	2.73 ± 0.003^{aC}	2.51 ± 0.17^{abA}
Hardness (N)	0	4.34 ± 0.48^{aB}	2.57 ± 0.67^{bB}	1.78 ± 0.54^{bC}
	7	6.56 ± 1.16^{aA}	3.91 ± 0.55^{bAB}	3.17 ± 0.48^{bB}
	15	6.25 ± 0.38^{aA}	3.13 ± 0.47^{bB}	3.22 ± 0.2^{bB}
	30	6.53 ± 0.44^{aA}	5.49 ± 1.03^{aA}	5.2 ± 0.62^{aA}
Springeness	0	0.98 ± 0.05^{aA}	0.99 ± 0.01^{aA}	0.99 ± 0.02^{aA}
	7	0.97 ± 0.02^{aA}	0.98 ± 0.01^{aA}	0.98 ± 0.01^{abA}
	15	0.99 ± 0.05^{aA}	0.99 ± 0.01^{aA}	0.99 ± 0.003^{aA}
	30	0.96 ± 0.01^{aA}	0.99 ± 0.16^{aA}	0.97 ± 0.01^{aA}
Cohesiveness	0	0.8 ± 0.01^{aA}	0.85 ± 0.03^{aA}	0.84 ± 0.04^{aA}
	7	0.79 ± 0.02^{aA}	0.8 ± 0.03^{aB}	0.84 ± 0.02^{aA}
	15	0.81 ± 0.01^{aA}	0.84 ± 0.03^{aAB}	0.82 ± 0.01^{aA}
	30	0.8 ± 0.02^{aA}	0.83 ± 0.03^{aAB}	0.82 ± 0.03^{aA}
Chewiness (N)	0	3.42 ± 0.36^{aA}	2.96 ± 0.28^{aA}	1.83 ± 0.34^{bC}
	7	6.87 ± 3.71^{aA}	3.54 ± 2.06^{aA}	2.59 ± 0.37^{bB}
	15	4.98 ± 0.69^{aA}	3.25 ± 0.44^{bA}	2.93 ± 0.5^{bB}
	30	5.00 ± 0.34^{aA}	4.28 ± 0.81^{aA}	4.12 ± 0.49^{aA}
Resilience	0	0.47 ± 0.01^{bBC}	0.53 ± 0.02^{aA}	0.48 ± 0.01^{bA}
	7	0.45 ± 0.01^{cC}	0.54 ± 0.02^{aA}	0.51 ± 0.01^{bA}
	15	0.52 ± 0.01^{aA}	0.52 ± 0.01^{aA}	0.5 ± 0.01^{aA}
	30	0.49 ± 0.02^{aAB}	0.47 ± 0.02^{aB}	0.48 ± 0.03^{aA}

Different uppercase letters show significant differences during the frozen storage of same bread type ($p < 0.05$). Different lowercase letters show significant differences between bread types for the same frozen storage time ($p < 0.05$). GFB : gluten-free control bread; PSY1: gluten-free bread containing 7.5% PP; PSY2: gluten-free bread containing 15% PP

Color

The color is another criterion that affects the consumer preferences. The water content, pH and presence of reducing sugars and amino acid in dough formulation besides relative humidity, temperature, mode of heat transfer as baking conditions determine the color characteristics of bakery products. GF bakery products often present lighter color than control bread. The dietary fibers used in GF formulations generally provides better color properties (Arslan et al., 2019). The crust and crumb color characteristics of GF breads were shown in Table 3. Psyllium addition led to a significant reduction in both L* value of crust and crumb color. The increasing amount of psyllium in GF breads also caused a darkening effect on crumbs as seen in Figure 3. These darker characteristics of PSY1 and PSY2 than GFB were probably due to the brownish color of psyllium (Fratelli et al., 2018). A significant impact of frozen storage on wheat bread crust color was reported by Sharadanant and Khan (2003), which reducing the crust L* value. In the present study, frozen storage caused no regular changes in L*, a* and b* values of all GF breads (Table 3). However, total color changes (ΔE) in the crust of GFB increased significantly throughout the frozen storage ($p<0.05$). Also, PSY1 and PSY2 breads showed higher ΔE values on day 15 and 30 of frozen storage.

Table 3. Color properties of gluten free breads

Bread type	Storage time (days)	Crust				Crumb			
		L*	a*	b*	ΔE	L*	a*	b*	ΔE
GFB	0	54.81±1.59^{aB}	11.48±1.35^{aA}	29.13±2.47^{aB}		76.93±2.54^{aA}	-1.73±0.11^{bA}	11.16±1.12^{aA}	
	7	56.89±0.5^{aB}	10.44±1.04^{aA}	31.87±0.88^{aA}	3.59±1.96^{bC}	71.09±1.08^{aB}	-2.28±0.09^{cC}	8.15±0.43^{aB}	6.84±1.62^{aC}
	15	68.38±2.97^{aA}	3.68±1.04^{cB}	17.55±1.53^{aC}	19.47±1.70^{aB}	64.68±0.4^{aC}	-1.79±0.08^{cB}	6.23±0.37^{aC}	13.41±2.28^{aA}
	30	43.38±1.14^{bC}	10.37±0.47^{aA}	12.66±0.7^{aD}	20.08±2.03^{aA}	71.24±1.71^{aB}	-1.9±0.09^{bB}	7.11±0.94^{aAB}	7.29±0.85^{aB}
PSY1	0	46.52±2.17^{bA}	10.87±0.99^{aA}	17.73±1.18^{bA}		61.97±1.57^{bA}	5.63±0.29^{aA}	6.11±0.78^{bA}	
	7	43.23±1.93^{bB}	11.26±0.44^{aA}	18.27±1.48^{bA}	3.35±0.67^{cC}	56.57±0.5^{bB}	5.64±0.19^{bA}	1.97±0.26^{bB}	6.81±1.19^{aB}
	15	38.94±1.04^{cC}	8.65±0.96^{aB}	10.43±0.5^{bB}	10.76±1.32^{cA}	52.8±0.9^{bC}	4.87±0.25^{bB}	1.68±0.14^{bB}	10.21±0.92^{cA}
	30	42.7±1.07^{bC}	8.17±0.38^{bB}	9.65±0.57^{bB}	9.34±1.40^{cB}	57.67±1.76^{bB}	5.71±0.18^{aA}	1.32±0.28^{bB}	6.44±0.55^{bC}
PSY2	0	35.31±0.63^{cC}	8.25±0.37^{bB}	7.96±0.66^{cBC}		50.87±1.26^{cA}	5.7±0.37^{aB}	4.09±0.17^{cA}	
	7	43.56±0.33^{bB}	8.62±0.41^{bA}	10.07±0.31^{cA}	8.52±0.47^{aC}	49.71±1.5^{cA}	6.62±0.24^{aA}	-0.56±0.33^{cC}	4.88±0.31^{bB}
	15	47.01±0.9^{bA}	5.32±0.22^{bC}	7.24±0.27^{cC}	12.08±0.5^{bA}	41.63±0.93^{cB}	5.28±0.06^{aB}	-0.5±0.28^{cC}	10.33±0.47^{bA}
	30	45.6±2.00^{aA}	7.89±0.52^{bB}	9.10±1.66^{bAB}	10.36±0.47^{bB}	49±2.65^{cA}	5.76±0.59^{aB}	0.09±0.41^{cB}	4.41±1.42^{cC}

Different uppercase letters show significant differences during frozen storage of same bread type ($p<0.05$). Different lowercase letters show significant differences between bread types on the same frozen storage time ($p<0.05$). GFB : gluten-free control bread; PSY1: gluten-free bread containing 7.5% PP; PSY2: gluten-free bread containing 15% PP

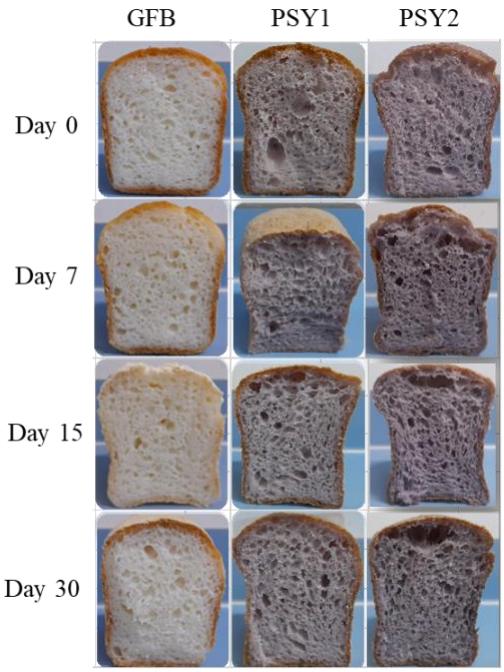

Figure 3. Images of gluten-free breads during frozen storage. GFB : gluten-free control bread; PSY1: gluten-free bread containing 7.5% PP; PSY2: gluten-free bread containing 15% PP

Volatile compounds

The final aroma of bread is also important for the choice of consumers. The type of flour or flour mixtures used in GF formulations are the primary factors that influence the aroma characteristics. Both the effects of psyllium addition and frozen storage were evaluated and presented in Table 4. GFB contained more volatile compounds (VCs) when compared other GF breads. This maybe explained by the compositional characteristics of psyllium affected the generation of VCs from Maillard reaction. The amino acid profiles and sugar types of added ingredients determine the rate of Maillard reaction (Pico et al., 2019). Psyllium is a kind of dietary fibre consisting primarily of highly branched arabinoxylans, composing of arabinose 22.6%, xylose 74.6%, only traces of other sugars with about 35% of non-reducing terminal residues. This profile can cause a lower extent of Maillard reactions and less detected VCs than GFB (Singh, 2007; Pico et al., 2019). Ethanol and 1-butanol, 3-methyl- from

alcohol group, butanal, 3-methyl- and hexanal from aldehydes were common for GFB and GF breads containing psyllium (Table 4). Butanal, 3-methyl- and hexanal have been described apple-like and fruit-like, respectively (Öncel, 2023; Pico et al., 2017). 2-Propanol, 1-methoxy-, which gives sweet ether-like aroma, was only found in GFB and not detected after frozen storage. 2,3-pentanedione, butanal, 2-methyl-, 3-hepten-2-one, 2-propen-1-one,1-cyclopropyl-, and formic acid, ethenyl ester were the VCs of GFB which were not found after frozen storage (Table 4). 2,3-pentanedione and butanal, 2-methyl- have been reported as pleasant descriptors like caramel and fruity-almond, respectively (Pico et al., 2017). 1-butanol, 3-methyl-, butanal, 3-methyl- and hexanal were the VCs of PSY1 and they were also shown after frozen storage (Table 4). 1-butanol, 3-methyl-, butanal, 3-methyl- and hexanal have been known to exhibit organoleptic characteristics such as balsamic-alcohol, apple-like and green grass, respectively (Pico et al., 2017). Butanal, 3-methyl- was the preserved VC of PSY2 after frozen storage. A change of VCs intensity during frozen storage was reported in a study of He et al. (2023). Therefore, freezing treatment can cause both decrease and increase in signal intensities of some VCs by affecting the aromatic compounds in the dough by the yeast cells and the interaction of dough components with VCs (He et al., 2023).

Table 4. Volatile compounds of gluten- free breads during frozen storage

		Bread type											
		GFB				PSY1				PSY2			
		Day 0	Day 7	Day 15	Day30	Day 0	Day 7	Day 15	Day 30	Day 0	Day 7	Day 15	Day 30
Alcohol	Ethanol	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	1-Butanol, 3-methyl-	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	
	1-Hexanol		✓	✓							✓		
	2-Propanol, 1-methoxy-	✓											
	1,3-Butanediol		✓										
Aldehydes	Butanal, 3-methyl-	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
	Butanal, 2-methyl-	✓						✓	✓	✓		✓	✓
	Hexanal	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓
	Pentanal			✓				✓					✓
	Heptanal		✓	✓	✓	✓	✓						
	Octanal				✓								
	Nonanal		✓	✓	✓								
	2-heptenal			✓									
	Valeraldehyde			✓									
Ketones	2,3-Pentanedione	✓						✓		✓			
	Acetoin	✓		✓	✓			✓					
	3-Hepten-2-one	✓											
	2-Propen-1-one,1-cyclopropyl-	✓											
Esters	Formic acid, 2-propenyl ester				✓	✓	✓						
	Formic acid, ethenyl ester	✓											
Furans	Furan, 2 pentyl-												
	Furan<2-amyl>		✓	✓	✓	✓		✓	✓				
Terpenes	dl-Limonene		✓	✓				✓	✓				

GFB : gluten-free control bread; PSY1: gluten-free bread containing 7.5% PP; PSY2: gluten-free bread containing 15% PP

CONCLUSION

In this study, the improvement effects of psyllium addition into gluten-free bread were observed as stated in previous studies. Quality deterioration in terms specific volume reduction and crumb hardness increments due to frozen storage was less in gluten-free breads containing psyllium than gluten-free control bread. Especially, the gluten-free bread with 7.5% psyllium showed no significant differences in terms of crumb hardness throughout 15

days of frozen storage. The increase in crumb hardness due to frozen storage was more notable on day 7 of frozen storage for gluten-free bread with 15% psyllium when compared to the 7.5% psyllium addition level.Therefore, 7.5% addition level and the frozen storage for 15 days were more suitable. According to the results of volatile compounds analysis, gluten-free control bread exhibited more volatile compounds than the breads containing psyllium. Nevertheless, further studies are required in order to reveal the effects of both psyllium addition and frozen storage on volatile compounds of gluten-free breads.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

This study is the part of the master thesis of SCK. She (SCK) conducted experiments, data analysis. GÖ supervised SCK and wrote the manuscript. All the authors reviewed the manuscript.

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Effects of different rootstocks on the growth and yield characteristics of Papazkarası (*Vitis vinifera* L.)

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Abstract
Rootstocks, which are becoming increasingly important in viticulture, influence the growth, yield, and grape quality of the grafted variety. Therefore, understanding the resistance characteristics of rootstocks to phylloxera, nematodes, environmental conditions, and abiotic and biotic stresses is crucial. Selecting a rootstock that is appropriate for the region where the vineyard will be established optimizes grape quality. The aim of this research is to determine the performance of the cv. Papazkarası on different rootstocks. For this purpose, an experiment was established at Irem Çamlica Viticulture and Winery Co. vineyard in Kırklareli province. Ten-year-old Papazkarası vines grafted onto 1103P, 110R, and 420A rootstocks were used as plant material. To determine the growth of the vines, parameters such as shoot elongation rate (cm/week), shoot length changings (cm), pruning wood weight (PW) (kg/vine), vigor (g), puissance, number of buds per square meter (number), balanced pruning buds number (number/vine), vegetative growth (VG), Ravaz Index (RI), Partridge Index (PI), and yield (kg/vine) were examined. Additionally, to determine cluster characteristics, cluster width and length (cm), weight (g), and the volume of gappy and spaceless clusters (cm^3) were measured. The results of the research indicated that the 1103P rootstock had the lowest yield, puissance, RI, PI, and VG values; moderate values for PW, vigor, number of shoots, number of berries per cluster, and cluster length; and the highest values for cluster weight, the volume of gappy and spaceless clusters. The 110R rootstock was found to be more balanced compared to other rootstocks, with the highest values for vigor, RI, and PI; average values for yield, puissance, and VG; and the lowest values for PW, number of shoots, number of berries per cluster, cluster length, weight, and the volume of gappy and spaceless clusters. The 420A rootstock had the highest values for yield, PW, VG, number of shoots, number of berries per cluster, and cluster width and length; average values for RI, PI, cluster weight, and the volume of gappy and spaceless clusters; and the lowest value for one-year-old cane weight. In conclusion, based on the characteristics outlined, a selection can be made from these rootstocks according to cultivation purposes, but other rootstocks should also be investigated.
Keywords: Autochthonous, Thrace, Cluster, Partridge Index, Ravaz Index

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INTRODUCTION
Rootstocks influence the vigor, yield, and quality of the grafted grapevine cultivar. They also facilitate the adaptation of the grafted variety to environmental conditions (Zombardo et al., 2020; Ferrandino et al., 2023; Bahar et al., 2024). However, viticulture is also affected by climate change, making rootstock selection crucial for adaptation (Atak, 2024). Rootstocks are becoming increasingly important in vineyard management and can alter the yield and response of the cultivar to edaphic stresses (Rodriguez et al., 1998; Stevens et al., 2008). It has been indicated that certain rootstock-cultivar combinations affect the source-sink relationship, vegetative expression, vigor, yield, and the balance of vegetative-generative growth (Mattii et al., 2005; Sampaio and Vasconcelos, 2005).

On the other hand, for success in viticulture, it is necessary to graft cultivars onto rootstocks resistant to abiotic and biotic stresses (El-Gendy, 2013; Opazo et al., 2020; Tedesco et al., 2020; Fayek et al., 2022). Tandonnet et al. (2005) reported that rootstocks can be used not only to impart resistance to certain biotic stresses but also to control vegetative expression and yield, thereby optimizing grape composition. These stresses include resistance to drought, salinity, Fe-deficiency, and diseases (Cookson et al., 2014; Corso and Bonghi, 2014; Mariani and Ferrante, 2017; Abdel-Mohsen and Rashedy, 2023). In recent years, the demand for stress-resistant cultivars has grown, leading to a significant increase in breeding studies on rootstocks tolerant to biotic and abiotic conditions (Atak, 2022). The breeding of new grape rootstocks that use water more efficiently is a very important strategy against global climate change (Atak et al., 2023). Rootstocks, mandated in 19th-century Europe due to phylloxera, are now required globally, partly due to nematodes (Whiting, 2012; Bona et al., 2007).

Yazar et al. (2023) noted that grafting different varieties onto the same rootstock yields varying results, attributing this to environmental factors and the compatibility between the scion and rootstock. An example of this is the study by Rodriguez et al. (1998), which found that the pruning wood weight of the cv. Cabernet Sauvignon grafted onto the 140Ru rootstock was equal to the pruning wood weight obtained from 1103P, SO4, and Harmony rootstocks.

The relationship between the rootstock-scion combination affects vegetative growth and yield in vines (Santarosa et al., 2015). Additionally, it should be noted that rootstocks have a significant impact on the mineral nutrition of the grafted variety, affecting the nutrient levels of the scion differently (Ibacache and Sierra, 2009). Csikász-Krizsics and Diófási (2008) reported that the absorption of minerals from the soil depends on the root system of the rootstock, the soil, and the above-ground organs of the vine. This distribution may be influenced by the genotype of the rootstock (Rizk-Alla et al., 2011). Rootstocks, likely due to their role in root density, influence numerous scion traits such as water and gas exchange, canopy growth, and yield. Rootstocks can increase the vigor of the grafted variety, potentially leading to lower phenolic maturity in grapes (Koundouras et al., 2009). Different rootstocks are reported to alter the growth, development, fruit quality, and stress resistance of the variety (Ulaş et al., 2014).

Papazkarası, an autochthonous cultivar grown primarily in the Trakya region, particularly in Edirne-Kırcasalih (Korkutal et al., 2019), and very late ripening (Çelik, 2006). Ozen et al. (1993) studied the development of Papazkarası grapevines grafted onto seven different rootstocks under the ecological conditions of Edirne in the Trakya region from 1988 to 1993. They found that the 140Ru rootstock had the most balanced values in terms of bome degree (9.54), yield (624 kg/da), and grape juice (51.3%). Karaca Sanyürek et al. (2018) reported that cv's Papazkarası and Ulaş Siyahı had the highest total phenolic compound content when they examined the content of some local wine grapes. Bozan et al. (2008) also found that the total phenolic content of Papazkarası seeds was higher than other grape varieties. Gülcü et al. (2018) stated that, in terms of suitability for grape juice, the cv's Öküzgözü and Papazkarası had the highest grape juice. Additionally, the traditional fermented drink hardaliye, which contains high levels of antioxidants, is produced from this cultivar (Faikoğlu, 2014).

The aim of this research is to determine the performance of the cv. Papazkarası on different rootstocks. Within this scope, morphological characteristics (yield, shoot elongation rate, shoot length changings, pruning wood weight, vigor, puissance, number of buds per m^2, balanced pruning buds number, number of clusters, number of shoots, vegetative growth, Ravaz Index, Partridge Index) and cluster characteristics (cluster width-length, weight, the volume of gappy and spaceless clusters) will be evaluated.

MATERIALS AND METHODS

Plant Material

The trial was conducted in a vineyard located in Poyralı Village, Kırklareli, at an altitude of 304 m. Ten-year-old cv. Papazkarası scions were used as plant material. The scions were grafted onto 1103P, 110R, and 420A vine rootstocks. The vineyard had a planting density of 2x1 m, and the vines were trained to a bilateral cordon with Royat pruning system.

Papazkarası cultivar is a wine and table grape variety grown in Uzunköprü (Kırcasalih, Yeniköy, Aslıhan) in Trakya and in Central Anatolia (Anonymous, 1990). It has been noted that Kırklareli and Üsküp are the best terroirs for this variety (Lacombe et al., 2012). It produces medium-sized, dense clusters. Another characteristic is its very late ripening. The wines with medium-low tannins, high acidity, and aromatic profiles.

1103P rootstock (Berlandieri Resseguier No.2 x Rupestris du Lot) was hybridized by Paulsen in 1892. This rootstock delays the ripening of the grafted variety and promotes the formation of numerous lateral shoots. When grafted with late-ripening varieties in northern regions, its shoots may be damaged by early autumn frosts. It is tolerant to about 16-17% active lime in the soil (Whiting, 2003; Plantgrape, 2024).

110R rootstock (Berlandieri Resseguier No.2 x Rupestris 110 Richter) is highly tolerant to the root form of phylloxera. It has moderate resistance to limestone but can withstand about 17% active lime in the soil. It is highly drought-resistant but not well-suited to excessively moist soils (Whiting, 2003; Plantgrape, 2024).

420A rootstock (Berlandieri x Riparia 420A Millardet et de Grasset) is a weak-growing rootstock. It is highly resistant to both limestone and phylloxera. It accelerates the ripening of the grafted variety. It does not thrive in dry soils; it prefers well-drained, moist, and fertile soils. Rooting of its cuttings is quite difficult (Whiting, 2003; Plantgrape, 2024).

Methods

The experiment was established in accordance with a Randomized Complete Block Design with 4 replications in Poyralı Village. A total of 144 vines were used, with 12 vines per replication, and the vines at the beginning and end of each row were excluded from the trial. For cluster measurements, 120 clusters (10 clusters per vine in each replication) were selected. In total, 1440 clusters were harvested from the vineyard and evaluated.

Statistical Analysis

The criteria examined during the development phase and the analysis results of the grape clusters harvested were evaluated using the MSTAT-C statistical software package. Differences among rootstocks were determined using the Least Significance Difference test.

Climate and Phenological Development Stage

Climate data between 2014 and 2021 were obtained from the Kırklareli Directorate of Meteorology (KDM, 2022). Phenological development stages were recorded throughout the vegetation period.

Determination the Vigor and Yield

Shoot growth rate (cm/week) was measured weekly after marking one shoot per vine (Bahar et al., 2008). Shoot length changings (cm) was determined by measuring the difference between the last and previous measurements of the same shoot (Bahar and Öner, 2016). Pruning wood weight (PW) (kg/vine) was determined by weighing the main and lateral branches obtained from pruning 12 vines per plot (Carbonneau et al., 2007). Vigor (g) was calculated by dividing the total PW values by the total number of shoots (Carbonneau et al., 1998; Smart et al., 1990). Puissance was calculated using the following formula (Carbonneau et al., 1998):

$$\text{Puissance} = \left[\left(\text{PW}\left(\frac{\text{kg}}{\text{vine}}\right) \text{x } 0{,}5\right) + \left(\text{Yield}\left(\frac{\text{kg}}{\text{vine}}\right) \text{x } 0{,}2\right)\right]$$

The number of buds per square meter (number) was determined by assuming 5 to 6 buds/m² on a vine. The area per vine (APV) was calculated, and the formula APV x (5 and 6 buds/m²) was used to determine the number of buds per unit area of soil. Calculations were made separately for 5 and 6 buds/m², and the required number of buds per vine was determined from the resulting values. Balanced pruning bud number (BPBN) was calculated based on the assumption of 20 buds for the first 0.5 kg PW, 10 buds for the next 0.5 kg PW (for wine grape varieties), and 10 buds for each remaining 0.5 kg PW (per vine) (Shaulis, 1950; Skinkis and Vance, 2013). Vegetative growth (VG) was given in the following formula:

$$\text{VG} = \text{PW}\left(\frac{\text{kg}}{\text{vine}}\right) + \text{Yield}\left(\frac{\text{kg}}{\text{vine}}\right)$$

A balance between vegetative and generative growth should be established in the vineyard. To determine this, the Ravaz Index (RI) was calculated as the ratio of yield to PW. A result of 5-10 indicates balanced vegetative-generative growth; <5 indicates more vegetative growth, and >10 indicates higher yield (Ravaz, 1903; Smart et al., 1990). The Partridge Index (PI) was determined by comparing yield (kg/vine) to the previous year's PW (kg/vine), reflecting the idea that the previous year's pruning wood weight affects the yield and quality of the following year (Partridge, 1925). Yield (kg/vine) was calculated by weights of all harvested clusters.

To determine cluster characteristics, one cluster was selected from each vine, and measurements were performed on a total of 144 clusters (OIV, 2009). Cluster width (cm) and length (cm) were measured using a ruler. Cluster weight (g) was obtained by dividing the yield per vine by the number of clusters. Cluster spaceless volume (cm³) was determined by submerging the cluster in a water-filled glass container and measuring the displaced water volume; gappy volume (cm³) was determined similarly after packaging the cluster in plastic (Bahar et al., 2023).

RESULTS AND DISCUSSION

Climate Data and Phenological Development Stages

The Kırklareli region, classified as semi-arid and low-moisture, exhibits a Black Sea climate in its northern parts and a continental climate in its interior. Over the long term (2014-2020), the annual average temperature recorded was 14.26°C, decreasing to 13.88°C in 2021. These two temperature values were found to be quite close to each other. The long-term average rainfall was 634.69 mm, whereas in 2021, it was determined to be 913.20 mm (KDM, 2022). However, it was observed that while the long-term average precipitation during the vegetation period (April-October) was 323.81 mm, in 2021, the precipitation during the vegetation period was recorded at 341.10 mm. In summary, it was noted that the rainfall in 2021 occurred largely outside the vegetation period.

When examining the phenological development stages of the cv. Papazkarası, it was observed that EL23 occurred on June 26 for the 1103P and 110R rootstocks, and on June 22 for the 420A rootstock. Veraison was determined to take place on September 10 for the 1103P rootstock, and on September 5 for the 110R and 420A rootstocks. Harvest was recorded on October 15 for the 1103P and 420A rootstocks. However, due to the maturity of grafted vines with the 110R rootstock occurring on October 10, to better assess uniformity and the impact of the rootstock on maturity, it was conducted on October 10 (Figure 1). Similar conclusions were drawn in this study as Keller et al. (2012), indicating that rootstocks did not significantly affect vine phenology. Likewise, the study corroborates the findings of Harbertson and Keller (2012), showing a minor influence of rootstocks on harvest dates.

Candar et al. (2022) recorded that cv. Papazkarası vine saplings grown under Tekirdağ conditions in pots on their own roots reached EL04 stage on April 2, 2019 and April 18, 2020, EL17-19 stage on June 24, 2019 and July 16, 2020, and EL33-35 stage on July 24, 2019 and July 17, 2020. According to the research, the EL04 stage varied depending on the rootstocks but generally occurred in the first week of May. It was observed that cv. Papazkarası grown on its own roots and in pots started bud break within the month of April. These findings contradict the researcher's own findings. However, it is believed that controlled conditions such as pots may have caused this difference. Flowering initiation was found to occur in the second week of June, which is consistent with the researcher's findings. The dates of veraison observed in the second half of July conflicted with the veraison dates obtained from the study, with the difference potentially originating from variations in climate between Kırklareli and Tekirdağ, vine age, applied cultural practices, and other factors. It should also be noted that Candar et al. (2022) grew the cv. Papazkarası on its own roots, which is an important aspect to consider.

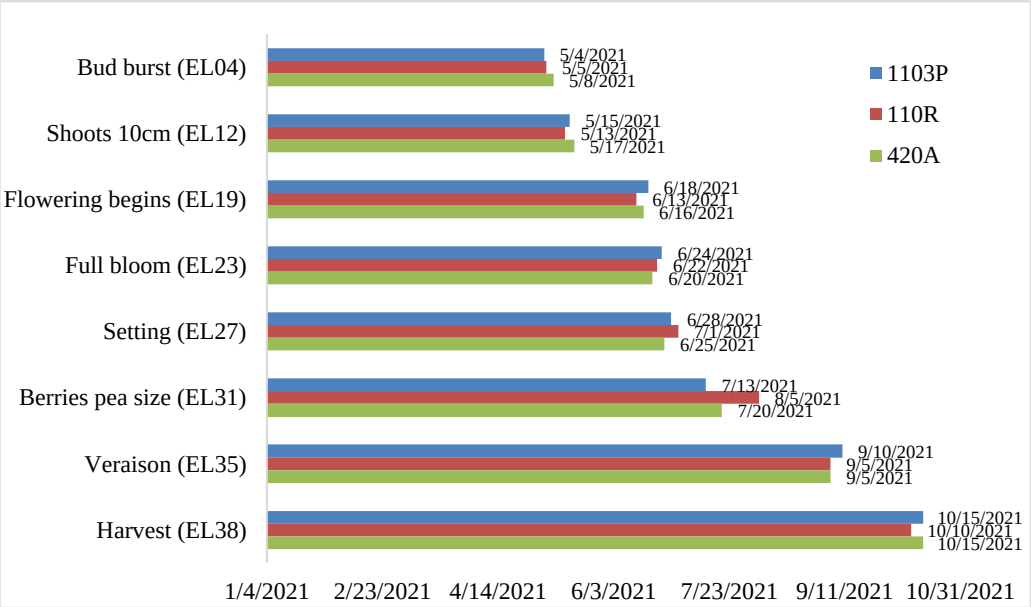

Figure 1. Phenological development stages of cv. Papazkarası grafted onto different rootstocks

Soil Analysis

Analysis was conducted using samples taken at depths of 0-30 cm and 30-60 cm. In the soil where Papazkarası vines grafted onto 1103P rootstock were located, the percentages of sand were 46.6% and 48.4%, silt 29.7% and 31.9%, clay 23.5% and 19.6%, respectively, for depths of 0-30 cm and 30-60 cm. For the 110R rootstock at both depths, the sand percentages were 40.1% and 38.1%, silt 41.4% and 45.3%, clay 18.3% and 16.5%. The soil percentages for the 420A rootstock were 36.5% and 35.1% sand, 46.8% and 44.4% silt, and 16.5% and 20.4% clay for depths of 0-30 cm and 30-60 cm, respectively. The higher sand content in the area where 1103P vines are planted suggests faster heating and cooling of the soil, leading to more rapid vine development, which is considered normal. An increase in silt values was observed from 1103P to 110R and 420A. The clay content in the soil ranged from a minimum of 16.5% to a maximum of 23.5% at both depths.

Growth and Yield

The rootstocks onto which cv. Papazkarası has been grafted have statistically influenced the number of shoots per vine. The highest number of shoots per vine, 14, was recorded in vines grafted onto the 420A rootstock. The lowest number of shoots, 11, was observed in vines grafted onto the 110R rootstock. The number of shoots per vine for the 1103P rootstock was determined to be 13, placing it between the other two rootstocks (Table 1). In cv. Malbec grafted onto the 1103P rootstock, it was found that there were 22 shoots per vine. This value was

significantly higher compared to the findings of the research, indicating that the variety grafted onto the rootstock influenced this value.

Table 1. Effects of rootstocks on yield and vine development.

Criteria	Rootstocks			
	1103P	110R	420A	Rootstock Main Effect
Number of shoots per vine	13,00 ab	11,00 b	14,00 a	12,60
Pruning wood weight (PW)	0,440 ab	0,404 b	0,477 a	0,440
Vigor	37,00	38,00	36,00	37,00
Puissance	0,527 b	0,595 ab	0,645 a	0,589
Balanced pruning bud number (BPBN) (0.5 g)	17,60	16,16	19,08	17,60
Vegetative growth (VG)	2,00 b	2,37 a	2,51 a	2,29
Ravaz Index (RI)	3,81 b	5,00 a	4,52 ab	4,40
Partridge Index (PI)	4,04 b	5,68 a	5,15 ab	4,90
Yield	1,57 b	1,96 ab	2,03 a	1,85

Number of shoot $LSD_{0,01}$=0,2103; PW $LSD_{0,01}$=0,2103; Vigor $LSD_{0,05}$=0,2802; VG $LSD_{0,05}$=1,1773; RI $LSD_{0,01}$=0,2802; PI $LSD_{0,05}$=1,1773; Yield $LSD_{0,01}$=1,3950

The seven-week shoot growth rate values are given in Figure 2. Starting from the first week, the shoot growth rate in the 1103P rootstock has noticeably stood out and has extended more each day compared to the other rootstocks. The shoot growth rates of 110R and 420A rootstocks followed a similar trend (Figure 2).

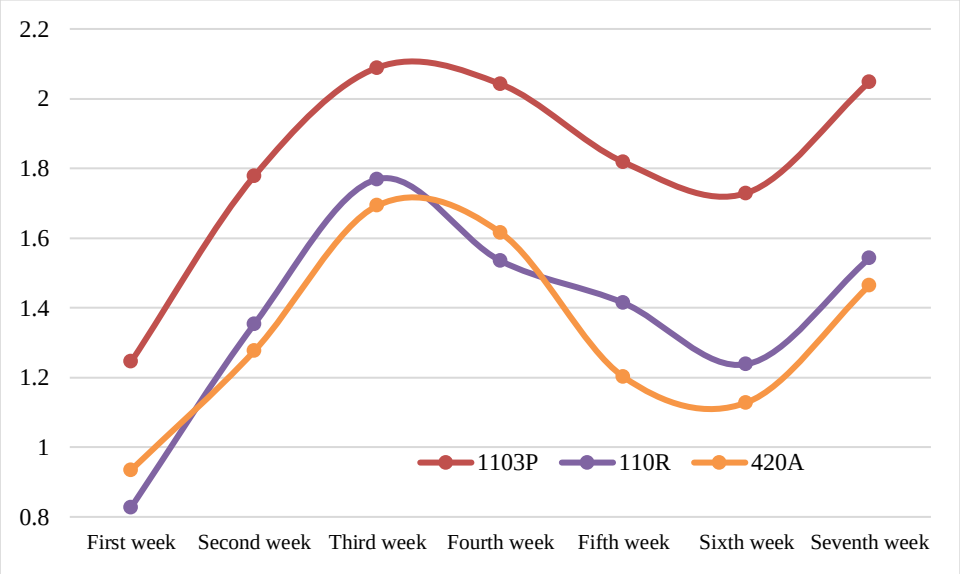

Figure 2. Shoot growth rate changings per week

The variation in shoot lengths of cv. Papazkarası according to the rootstocks has been examined based on phenological development stages (Lorenz et al., 1995). It was observed that the shoot length of the 1103P rootstock was longer than the others at every phenological stage. During the last measurement period at berry set (EL 27), the shoot length of the 1103P rootstock was 95.6 cm. On the same day, the shoot lengths of the other two rootstocks were recorded as 73.6 cm for 110R and 71.3 cm for 420A (Figure 3).

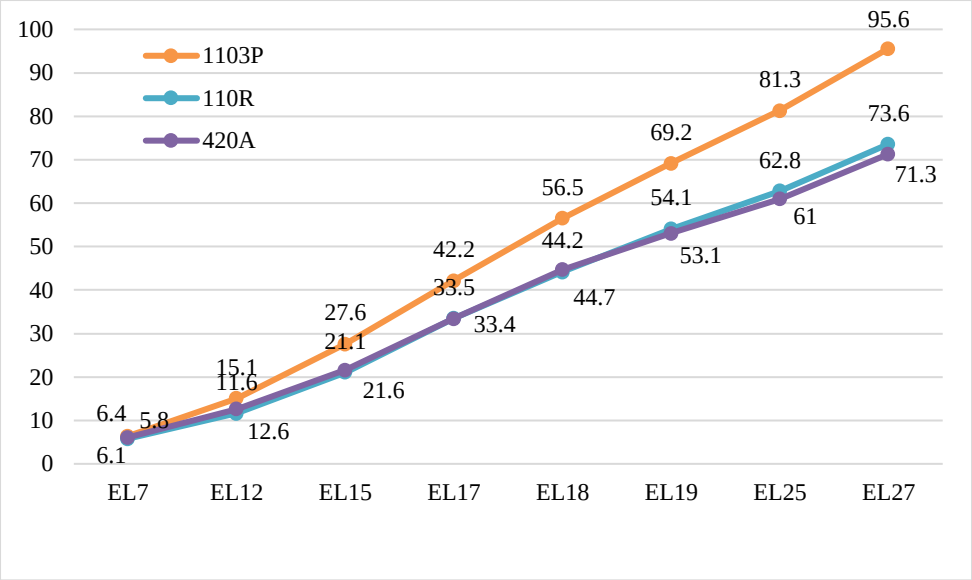

Figure 3. Variation of shoot length according to phenological development stages

The statistical analysis determined that the pruning wood weight (PW) of cv. Papazkarası varied according to the rootstocks (Table 1). The highest PW was obtained from the 420A rootstock (0.447 kg/vine), and the lowest was from the 1103P rootstock (0.440 kg/vine). It was noted that the 1103P rootstock (0.440 kg/vine) fell between these two. Contrary to the findings indicating an average PW of 1.39 kg/vine for the cv. Malbec grafted on the 1103P rootstock by Di Filippo and Vila (2011), significantly different results were obtained, which were attributed to the grafted variety.

The influence of different rootstocks on the vigor of cv. Papazkarası was found to be insignificant. However, numerical values were lined up as 38 g (110R), 37 g (1103P), and 36 g (420A) (Table 1). According to Smart et al. (1990), the evaluation based on the formula "vigor = pruning wood weight / shoot number" indicated that values between 20-40 g are among the thresholds for "moderate vigor".

The effect of rootstocks on the vigor of the cv. Papazkarası was found to be statistically significant at the $LSD_{0.05}$ level (Table 1). In terms of vigor, the highest value was recorded for the 420A rootstock (0.645 g). Following this, the 110R rootstock showed a 0.595 g. The lowest vigor was obtained from the 1103P rootstock (0.527 g). Since vine vigor between 0.5-1 g is considered "ideal" for wine grape varieties (Carbonneau et al., 1998), it was determined that all rootstocks exhibited "ideal" vigor. According to the research findings, the vigor of the 110R rootstock states between the values of the other two rootstocks. Similarly, Satisha et al. (2010) found the Thompson Seedless/110R graft combination to perform well in terms of moderate vigor, increased berry set rates, and consistently higher yields. Consistent with this research, Keller et al. (2012) also noted a tendency for the 1103P rootstock to reduce shoot vigor.

In general, the number of buds per square meter on vines is reported to be 5 to 6 buds (Carbonneau et al., 1998). In the trial vines, the area allocated per vine was calculated as 2 m². Based on this area, it was determined that there should be 10 buds/m² (2 x 5 = 10 buds/m²) or 12 buds/m² (2 x 6 = 12 buds/m²). Therefore, the number of buds per unit area was established as 10-12 buds/m². Considering the trial vines are trained in the bilateral cordon Royat System, with 3 shoots on each side (left and right) forming a total of 6 shoots per vine, the target of 10-12 buds/m² was achieved.

Shaulis (1950) explained that balanced pruning involves leaving 20 buds for the first 0.5 kg of PW, followed by 10 more buds for each additional 0.5 kg (for wine grape varieties). Using this method, leaving 20 buds per vine (Table 1) was found to be appropriate (Skinkis and Vance, 2013).

It has been determined statistically that different rootstocks affect the vegetative growth (VG) of cv. Papazkarası. The highest VG was obtained from the 420A rootstock (2.51); the lowest was from the 1103P rootstock (2.00), with the value between them from the 110R rootstock (2.37) (Table 1).

Ravaz Index (RI) varied significantly depending on the rootstocks onto which cv. Papazkarası was grafted (Table 1). The highest RI value of 5.00 was recorded from the 110R rootstock. This value, being at the lower limit, indicates "a balanced vegetative-generative development". However, the RI values of 3.81 for 1103P rootstock and 4.52 for 420A rootstock are significant in showing a tendency towards "more vegetative growth" (Ravaz, 1903; Smart et al., 1990).

Analyzing the Partridge Index (PI) for rootstock effects, the 110R rootstock achieved the highest value of 5.68 PI, while the 1103P rootstock showed the lowest value of 4.04 PI. In the case of the 110R rootstock, the previous

year's vegetative growth had a promoting effect on this year's yield, whereas for the 1103P rootstock, it reduced yield. The 420A rootstock (5.15 PI) among these two values (Table 1).

In terms of yield, statistically significant differences have been observed. The highest yield of 2.03 kg/vine was obtained from the 420A rootstock, while the lowest yield of 1.57 kg/vine from the 1103P rootstock. The value between these two was recorded from the 110R rootstock at 1.96 kg/vine (Table 1). This finding aligns with Ulaş et al. (2014), who also indicated that rootstocks significantly influence yield. Di Filippo and Vila (2011) found that the Malbec/1103P grafting combination increased yield to 2.78 kg/vine. Similarly, Rodriguez et al. (1998) reported that among different rootstocks used for Cabernet Sauvignon, the 1103P achieved the highest yield of 2.7 kg/vine. However, in this study, the 1103P rootstock yielded the lowest, contrasting with these findings. This discrepancy suggests that there may be an interaction between the scion and rootstock, as noted by Tandonnet et al. (2005). However, Striegler et al. (2002) observed minimal differences in yield when grafting Cabernet Franc onto four different rootstocks, which contradicts these research findings. It is hypothesized that this variation could be due to the genetic makeup of the variety, cultural practices applied, and soil characteristics.

Cluster Characteristics

It has been statistically determined that the number of clusters per shoot varies depending on the rootstock (Table 2). Accordingly, the rootstock with the highest number of clusters per shoot is 110R (10 cluster/shoot). The rootstock with the lowest number of clusters per shoot is 1103P (7 cluster/shoot). The 420A has an intermediate value with 8 cluster/shoot. Satisha et al. (2010) found contradictory results regarding the minimum number of clusters per shoot from the Thompson Seedless/110R grafting combination. This discrepancy is thought to come from environmental, soil, climate, and stress factors. Striegler et al. (2002) indicated that the Cabernet Franc/110R combination produced more clusters per shoot compared to the Freedom rootstock, although the clusters from the Freedom rootstock were heavier. Similar findings were obtained in this study, where the 110R rootstock produced more clusters per shoot, while the 1103P and 420A rootstocks resulted in heavier clusters.

Table 2. Some cluster characteristics of Papazkarası grape variety grafted onto different rootstocks.

Criteria	Rootstocks		
	1103P	110R	420A
Number of clusters per shoot (cluster/shoot)	7,00 b	10,00 a	8,00 ab
Berry number of cluster (number)	108,83 ab	93,52 b	120,31 a
Cluster weight (g)	278,13 a	201,07 b	263,46 ab
The volume of spaceless clusters (cm^3)	230,94 a	163,01 b	206,21 a
The volume of gappy clusters (cm^3)	380,83 a	270,29 b	346,04 a

Number of clusters per shoot $LSD_{0,01}$ =1,3950; Berry number of cluster $LSD_{0,05}$=18,93728; Cluster weight $LSD_{0,01}$=62,6115; The volume of spaceless clusters $LSD_{0,05}$=39,78276; The volume of gappy clusters $LSD_{0,05=}$ 74,41092

Berry number of cluster was found to be significantly influenced by rootstocks at the $LSD_{0.05}$ level. The rootstock with the highest number of berries per cluster was 420A (120.31 number), while the lowest number was recorded with the 110R rootstock (93.52 number). The 1103P rootstock fell between these two values (108.83 number) (Table 2). Di Filippo and Vila (2011) reported a berry number per cluster of 50.28 for the Malbec/1103P grafting combination, which contrasts with the findings of this research. This discrepancy may underscore the genetic influence of the variety on berry number within clusters. Yazar et al. (2023) obtained consistent results when grafting the Ekşi Kara grape variety onto the 110R rootstock, showing the lowest cluster berry number.

The rootstock effect on cluster width and length was found to be statistically insignificant. Cluster width values ranked highest to lowest as 420A, 110R, and 1103P. The highest width value (14.48 cm) was attributed to the 420A rootstock. Cluster length values followed the order of 420A, 1103P, and 110R (Figure 4). Yazar et al. (2023), grafting the cv. Ekşi Kara onto the 110R rootstock, reported obtaining the lowest cluster width and length values, conflicting with the cluster length findings in this study, although the cluster width findings are consistent.

Figure 4. Cluster width and length according to the rootstocks

The effect of rootstocks on cluster weight is statistically significant. The rootstock with the highest cluster weight is 1103P, with a value of 278.13 g. The lowest cluster weight, 201.07 g, is obtained from the 110R rootstock. The 420A rootstock cluster weight between these values with a of 263.46 g (Table 2). For the Malbec/1103P graft combination, Di Filippo and Vila (2011) reported a 92.63 g, which differs significantly from the findings of this study, indicating the prominent role of grape variety characteristics. However, the higher cluster weight obtained from this variety grafted onto other rootstocks supports the findings of this research. Additionally, consistent with findings from Yazar et al. (2023), the Ekşi Kara/110R graft combination yielded the lowest values. Moreover, the research findings align with Todorov (1970)'s observation of a positive relationship between cluster weight and shoot growth. Clusters from the 1103P rootstock, which also exhibited the highest shoot elongation rate and length, were found to be heavier compared to those from other rootstocks.

The volume of spaceless clusters shows a statistically significant rootstock effect. Rootstocks 1103P and 420A (230.94 cm^3 and 206.21 cm^3, respectively) are in the same significance group. The 110R rootstock, with a value of 163.01 cm^3, is in another significance group (Table 2).

The volume of gappy clusters also varied significantly by rootstocks; the highest value was obtained from rootstocks 1103P and 420A (380.83 cm^3 and 346.04 cm^3, respectively). The 110R rootstock, with a value of 270.29 cm^3, is in another group (Table 2).

CONCLUSION

Based on the research, rootstocks were evaluated, with 1103P generally showing moderate to lower results in morphological characteristics. It had the lowest values for yield, vigor, RI, PI, and VG but exhibited the highest for cluster weight and volume. The 110R rootstock provided balanced results, excelling in vigor, RI, and PI, while showing lower values for cluster characteristics. The 420A rootstock, known as the weakest, showed the highest values for yield, PW, VG, and shoot number but smaller berry size, indicating strong cluster development but smaller berries. The 420A showed high morphological development and balanced cluster development.

In conclusion, the rootstocks 1103P, 110R, and 420A used in this study are the most commonly used for the Papazkarası variety in the Thrace Region due to their suitability. However, for successful rootstock selection, the soil structure and environmental conditions of the area should be examined more carefully and in detail. For the cv. Papazkarası grown in this region, if balanced cluster development and relatively high yield (1015 kg/da) are desired, the 420A rootstock is recommended. If average development and yield (980 kg/da) are preferred, the 110R rootstock is suitable. For rapid shoot development and low yield (785 kg/da), the 1103P rootstock should be chosen. However, similar studies should be conducted with rootstocks other than these, and the results should be observed.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of Interests

The authors have no conflicts of interest to declare.

Author contribution

Concept -E.B.; Data Collection and/or Processing-S.E., E.B.; Analysis and/or Interpretation- E.B., İ.K., S.E.; Literature Search-İ.K., S.E.; Writing Manuscript-İ.K.; Critical Review-E.B.

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Separation of free bran using electrostatic field system with electrically assisted flat PVC surface

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DergiPark AKADEMİK

Abstract
The objective of this study was to investigate the possibility of separating free bran from bulgur using the triboelectric property of a flat PVC surface. The findings demonstrate that bran, which has an adverse impact on the physical appearance and quality of bulgur, can be effectively separated. It was established that the study offers a solution to the issue of bran. The use of a flat PVC surface, which can be defined as a flat inclined channel, represents a new technological development for bulgur production technology. The dimensions of the device were designed to be 4, 5 and 6 cm in width, 20, 40 and 60 cm in length, and with angles of 30, 35 and 40 degrees, respectively. A series of plates was fixed at the final point of the PVC surface in order to establish an electric field. To generate the electric field, one of the plates was subjected to a positive charge, while the other was treated with a negative charge. A variety of distances were observed between the plates, contingent upon the width of the tunnel. The flat PVC system proved to be an effective means of achieving the desired outcome. Consequently, a fine bulgur-bran mixture with an initial bran content of 5 g per 1000 g of bulgur was conducted through the system at a flow rate of 0.89 g/s. This resulted in a significant reduction in bran content from 5 g to approximately 2 g (60% reduction).

Keywords: Bulgur, Bran, Electrostatic separation, Electric field, PVC

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INTRODUCTION

Bulgur is a wheat product. The production process involves cooking, dehulling, cleaning, drying, milling, and, if necessary, polishing, before the final classification stage (Balci & Bayram, 2015). The size of bulgur can vary depending on the specific type or grade, and is typically classified into different size categories, ranging from fine to coarse. Fine bulgur is characterised by smaller grains, which are similar in size to couscous, while coarse bulgur has larger grains. Fine bulgur is a specific variety of bulgur, defined by its exceptionally small particle size, which is generally between 0.5 and 1.0 mm (Yılmaz, 2020). Due to a number of favourable characteristics, including low cost, high nutritional value, storability and ease of preparation, bulgur is an excellent food source. Additionally, it resists mould contamination, which makes it an ideal choice for those seeking a cost-effective, convenient, and nutritious food source (Bayram 2000).

Bulgur bran is a by-product of the bulgur production process. It is separated during the partial dehulling (debranning) stage and sold as animal feed at a significantly reduced price (Saka et al., 2020). Bulgur bran, a by-product of the agricultural industry, has significant potential as a renewable resource for the production of chemicals, materials, and biofuels (Kocabaş et al., 2021). Although bulgur bran is a nutritionally rich foodstuff, it is not a preferred food item for the end consumer. Bulgur bran has been the subject of various studies investigating its nutritional, chemical, and physical properties, as well as its potential applications. Research has shown that bulgur bran is a rich source of dietary fiber, bioactive compounds, and antioxidants (Saka et al., 2020; Tekin et al., 2021). It has been highlighted as a functional food due to its nutritional components, including B vitamins, minerals, and dietary fiber, as well as its low glycemic index (Tekin et al., 2021; Erbaş et al., 2016). Additionally, bulgur bran has been found to contain phytic acid, which varies based on particle size (Saka et al., 2020). Bran, a nutrient-rich cereal considered a staple food, is recognized for its many health benefits within a balanced and

healthy diet. One of the main reasons for bran positive reputation is its excellent fiber content, which helps to support digestive health and general wellbeing.

Traditional separation methods such as fine mesh sieving, density difference, gravity separation and magnetic separation require more time and effort compared to modern techniques, they are highly respected for their ability to produce the highest quality bulgur with the least damage to the grains. However, it should be noted that bran cannot be completely separated from bulgur using classical separation methods, especially for fine bulgur due to its similar size (Kayıran & Bayram, 2024). The inability of conventional techniques to separate bulgur bran leads to the product forming a cohesive bond with the internal surface of the packaging, which creates the false impression of inferior quality.

In the field of particle technology, electrostatic separation represents a highly effective method for the separation of particles from granular mixtures (Matsusaka et al., 2010). This is due to the utilization of electrical forces which, in turn, allows for the separation to be carried out in a controlled and precise manner, thereby making it an attractive technique for various industrial applications (Hou et al., 2010). The application of electrostatic separation techniques has been investigated in the food industry, with particular emphasis on the separation of wheat grain and straw particles. Additionally, the technique has been employed for the recovery of food waste, thereby demonstrating its efficacy in the management of food waste and the recycling of valuable resources (Jafari et al., 2019; Lai et al., 2016).

Using an electrostatic field to separate bran from bulgur is a new technology to increase efficiency in the bulgur production process and separate these two components that are difficult to separate by the traditional method. It is of great importance to be aware of the nature of the constituents of bran and bulgur. In addition, the characteristics of the particles in question, specifically their size and shape, may also influence the separation process (Kayıran & Bayram, 2024). The particles themselves react in different ways to the electrostatic field depending on their inherent physical properties. (Zhu et al., 2023). The electrostatic separation process employs polyvinyl chloride (PVC). As a result of the triboelectric effect, electric charge is generated by electron transfer during friction or contact between materials (Wang et al., 2014). Polyvinyl chloride (PVC) is a synthetic polymer and a widely used material in the plastics industry (Flynn et al., 2017). PVC is recognised as a material that accumulates a negative electric charge. While other materials in contact with PVC show positive charge accumulation, PVC itself accumulates negative charge (Liu et al., 2021).

The purpose of these investigations is to utilize a flat PVC surface electrostatic system and electric field for the separation of bran from bulgur (see Figures 2 and 3). Additionally, the objective is to determine the capability of the PVC surface (flat inclined channel) in generating an electrostatic field and the success of the electric field.

MATERIALS AND METHODS

Sample Preparation

The experiments were conducted using samples of fine bulgur and bulgur bran provided by a local food company, (Tiryaki Agro Gıda San. ve Tic. A.Ş., Gaziantep, Turkey, in 2021). These samples are illustrated in Figure 1. Fine (köftelik) bulgur was processed according to Antep type method (Yousif et al., 2018). A series of analyses were performed on bulgur and bran samples, including moisture, protein, ash content and colour.

Both components (bulgur and bran) were sieved before the samples were prepared to contain 5 g/1000 g bulgur bran according to industry data. While 0.5 mm bulgur was passed through the first sieve, bran was passed through a 2 mm sieve. Thus, the particle sizes for both inputs were brought to close dimensions.

Figure 1. Images of (a) microscope images of bran, (b) bran, (c) fine bulgur [3x images] and (d) fine bulgur [magnification 300 dpi, scale 1000 micrometer.

Determination of protein content

Protein analysis was conducted in accordance with the Kjeldahl method (AOAC 1990) to determine the percentage of protein in the sample, expressed in grams per gram (g/g) on a dry basis. A conversion factor of 6.25 was employed for this calculation.

Determination of colour

The colour values (L*, a*, b* and YI) were determined utilizing a Hunter Lab Colourimeter (Colourflex, USA). The Hunter scale is employed to quantify lightness (luminosity) via the L parameter, which ranges from white to black, as perceived by the human eye. The a-value quantifies the degree of redness, whereas the b-value assesses the intensity of yellowness (positive values), greyness (zero values), and blueness (negative values). The YI, or yellowness index, is a measure of the relative intensity of yellow tones in a given colour.

Determination of ash content

Ash content was measured according to AOAC (1990) methods.

Electrostatic seperation experiment with electric field

The design of the system incorporates a PVC surface that is as flat as possible to generate an electrostatic and electric field (Figures 3). To this end, fine bulgur (5 g/1000 g bulgur bran) was passed through the PVC surface at various widths (4, 5 and 6 cm), lengths (20, 40 and 60 cm) and angles (30, 35 and 40°), respectively.

As a result of trial and error tests in a laboratory environment, it was determined that Bulgur and bulgur bran exhibit positive triboelectric properties. By utilizing the negative triboelectric properties of PVC material, an electrostatic field was created by friction.

Polyvinyl chloride (PVC) is a polymer that is employed in a multitude of applications, including cables, thermoplastics, and polymerized vinyl chloride products (Thabet, 2024). The material is produced through the polymerization of vinyl chloride and is renowned for its versatility and durability. Due to its cost-effectiveness and resistance to chemicals, weathering and impact, PVC is one of the most commonly used materials (Andrady and Neal, 2009).

PVC compound is observed to accumulate a net negative electric charge through a phenomenon known as the triboelectric effect. This effect refers to an electron transfer process occurring between materials that are subjected to either contact or friction (Miura et al., 2014).

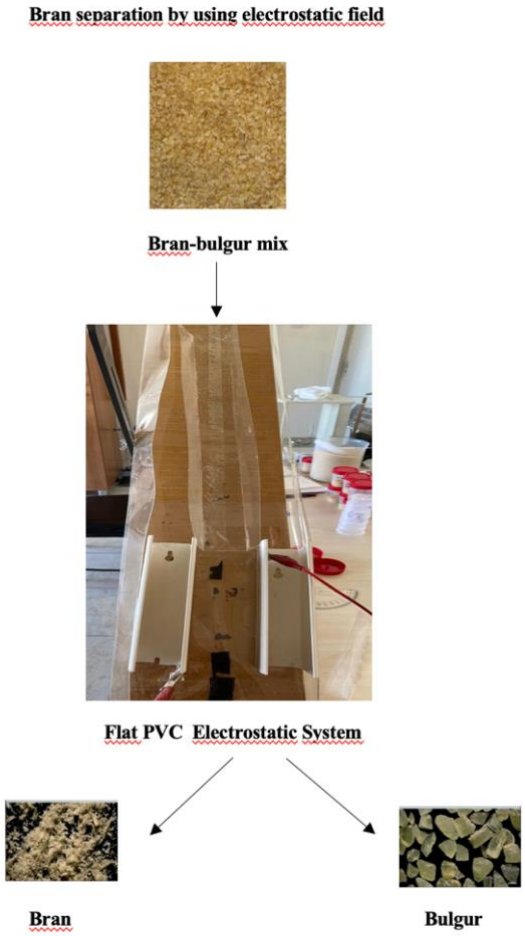

Figure 2. Schematic diagram of the electrostatic separation of system.

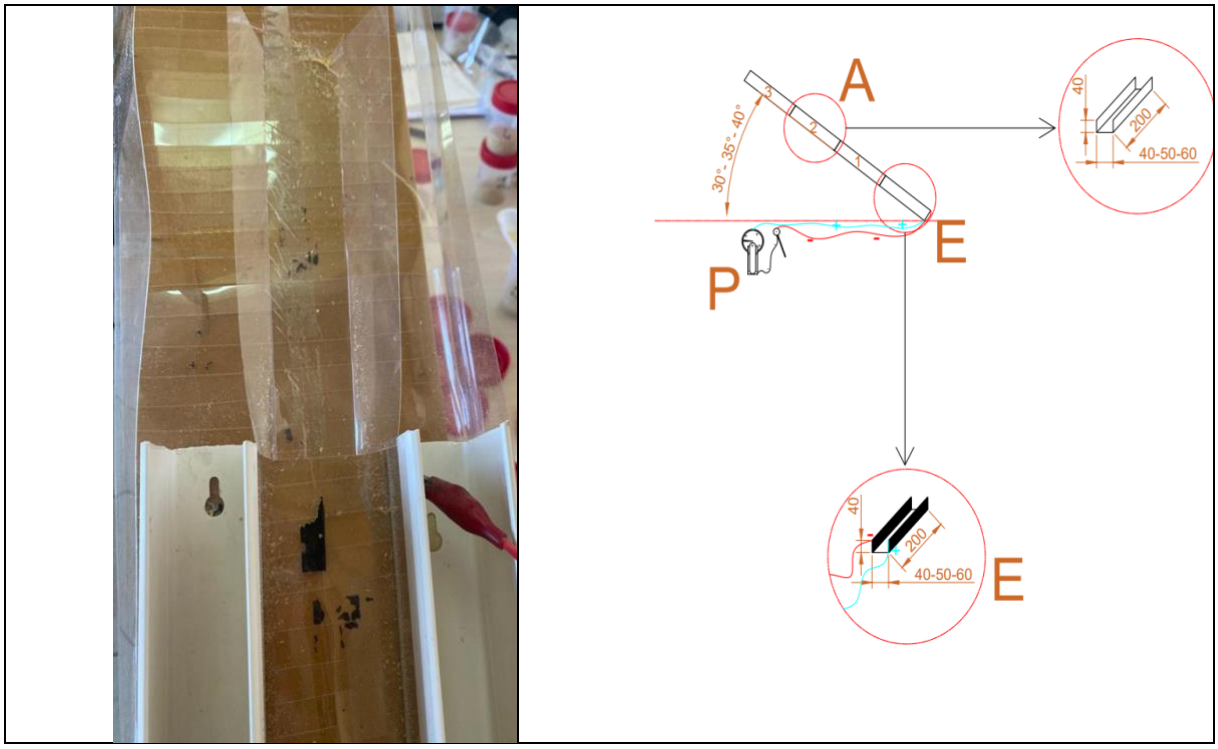

Figure 3. Flat PVC surface systems.

It was observed that how much bulgur bran could be separated by passing the bulgur through the devices prepared from PVC material and the electric field with a certain mass flow rate.

Figure 2 and figure 3 show that the system design of flat PVC surfaces and electric field. The flat system was prepared with PVC assemblies with the widths of 4, 5 and 6 cm and the lengths of 20, 40 and 60 cm, respectively. Different angles were designed therefore the angle of the system was 30, 35 and 40°. Plates are placed to create an electric field where the PVC surface ends. To create the electric field, one of the plates was charged with a positive and the other with a negative charge. The distance between the plates was varied as the tunnel distances (4, 5 and 6 cm). The electric field was calculated according to the following formulation (Fitzpatrick, 2010) (Eqn. 1).

$$E = \frac{V}{d}$$

In this formula, E (V/m) represents the electric field, V (V) refers to the potential difference between the two plates, and d (m) defines the distance between the two plates. (Fitzpatrick, 2010). According to this formula, the electric field for 4, 5 and 6 cm width is 5500 kv, 4400 kv and 3666 kv respectively.

Van de Graaff generator was also used as the power source for the electric field. The generator works on the principle of generating high voltages from a current-limited source (Lee et al., 2017). This generator consists of an isolating belt driven by an electric motor and stretched between two roller (Sessler &Wilson, 2014). The maximum voltage capacity of the generator depends on the diameter of the generator sphere. The voltage collected by the generator can be calculated approximately over the maximum value. For a 25 cm diameter sphere, the theoretical voltage collected by the generator is based on 220kv (Ege et. al., 2014). In addition, in order to accumulate sufficient potential voltage, the generator was waited for 5 minutes after the generator started and then the bulgur flow was realized.

Characterization by infrared spectroscopy (FT-IR)

The aim was to establish the impact of groupings within the chemical composition on the electrostatic field. For this purpose, Fourier Transform Infrared Spectroscopy (FT-IR) was used, utilizing a PerkinElmer Spectrum 100 FT-IR spectrometer (PerkinElmer Inc., Waltham, MA, USA). The procedure was carried out on a variety of samples, including bran, fine bulgur and bulgur–bran blends, with 5 g of bran and 1,000 g of bulgur used for each blend. Fourier transform infrared (FTIR) spectroscopy was used to obtain mid-infrared spectra with four scans, 4 cm-1 resolution and 4 scans using the Spectrum 10 Software (PerkinElmer Inc., Waltham, MA, USA). As a result, the spectra could be collected in the range of 4000-650 cm-1. Four separate transmittance spectra were obtained for each individual sample, with the ground sample positioned at the center of the diamond crystal on each occasion.

Statistical analysis

The analysis of variance (ANOVA) was conducted according to standard procedure to evaluate the impact of the system on ΔX, L, and α. The Pearson coefficient was utilized to ascertain the statistically significant correlation, with a level of significance set at $P \leq 0.05$. The statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). The measurements were conducted in duplicate, and the experiments were replicated.

RESULTS AND DISCUSSION

The moisture content of the bulgur sample was found to be 10.50% (db), and the bran sample exhibited a moisture content of 10.00% (db). Bulgur exhibited a protein content of 10.26% (dry basis), whereas bran exhibited a significantly higher protein content of 14.22% (dry basis). It was determined that the ash content of the bulgur was 0.89% (d.b.), while the ash content of the bran was 3.80% (d.b.). To ensure an accurate assessment of the system's efficiency, it is essential to consider the changes in both the ash content and the bran yield. Concurrently, the colour values were as follows: The following values were obtained for the bulgur sample: L*: 70.62, a*: 4.86, b*: 25.99 and YI: 57.56. The values for the bran sample were as follows: L*: 64.18, a*: 6.34, b*: 38.39 and YI: 81.07. The samples were subjected to a process involving a flat PVC surface. The bulgur-bran mixture displaying the identified characteristics was then subjected to the same process, with the efficiency of the system being interpreted based on the results obtained for the bran (GB). This involved weighing the amount of bran that had been separated by the electrostatic field and analysing the ash content. The term "gained bran (GB) refers to the amount of bran that was successfully removed from the system with the assistance of the PVC surface.

The impact of various variables, including surface width (ΔX), length (L), and angle (α), on the separation of bran from bulgur was examined using a PVC surface with highly negative triboelectric properties.

A statistical analysis revealed a significant difference ($P \leq 0.05$) in the amount of bran gained according to the width (ΔX) of the flat PVC with an electric field system. Understanding the efficiency of the system requires consideration of changes in both the amount of ash and gained bran. Therefore, both quatities were measured. The bran gain results showed that there were significant differences ($P \leq 0.05$) in bran separation between flat PVC surfaces. The quantity of bran obtained from PVC samples with widths of 4 cm, 5 cm, and 6 cm was found to be 0.10 ± 0.02 g, 0.09 ± 0.03 g, and 0.03 ± 0.00 g, respectively. A comparison between the groups indicated that the 4 cm width PVC samples yielded a higher quantity of bran than the other widths. The results indicated that significant differences ($P \leq 0.05$) existed between the length (L), (ΔX* L) and (ΔX* α) variables. The observed differences in the quantities of bran acquired according to the variables α, (L* α), and (ΔX* L* α) were not statistically significant, as indicated by ($P \geq 0.05$). The narrowest channel width and length proved optimal for the separation of the bran from the bulgur. The highest bran separation was observed at tunnel widths of 4 cm, lengths of 20 cm and system angles of 40 degrees. The ash values of the flat PVC system are in alignment with the bran values obtained. Duncan's Multiple test analysis, as illustrated in Table 1, revealed that the channel width and length were identified as crucial variables influencing the separation of bran from bulgur.

The structural characteristics of bulgur, bran and bulgur–bran mixtures were examined using FT-IR transmission spectroscopy, with the samples prepared by passing them through different angle assemblies. The figures demonstrate the positioning of bulgur, bran and the angle of the system, as illustrated in Figure 4. The peaks observed at approximately 3278 cm-1 are linked to the O-H stretching region of hydrogen bonds, which are formed between aliphatic and aromatic groups of alcohol and water. It may be inferred from the peak at 2925 cm^{-1}that a C-H linkage is involved in an aldehyde. It appears that these peaks have a cellulose and starch origin (Berthomieu, C., et al., 2009). The presence of a peak at 1635 cm-1, assigned to the Amide I functional group, was verified. It can be reasonably inferred that the peaks observed at 1365 and 1148 cm^{-1} are indicative of deformation of the C-H bond, thereby suggesting the presence of cellulose structures. In addition, the deformation of a range of polysaccharides is evident in the peaks observed at 1077 cm^{-1}. A maximum value of 994 cm^{-1} is suggestive of a C-OH bending mode (Amir et al., 2013; Bledzki et al., 2010). The FTIR spectra provide confirmation that no significant structural alteration took place during the separation of bulgur from bran using PVC surfaces. Additionally, the data indicate that the composition of bulgur and bran is chemically similar. The FTIR spectra confirm that no major structural changes occur during the separation of bran from bulgur. Therefore, the FTIR spectra evidence that the process of bran separation causes no structural alteration to the bulgur.

Application of the Systems in Industry

The traditional method of sieving is carried out after the milling process, where the bulgur is ground to various particle sizes. This step is very important as it allows the separation of finer bulgur particles from coarser ones that can be used for different culinary applications. In the sieving process, a series of sieves or screens with different pore sizes are typically used to achieve the desired granulation (Erbaş et al., 2016). Additionally, the sieving process can also help in the removal of any remaining bran particles, thereby enhancing the overall quality and appearance of the bulgur (Yağcı et al., 2022).

Although some traditional separation systems are used to separate the bulgur bran after the grinding process in the bulgur production, the separation of bulgur and bran is not completely efficient. Unseparated bran still creates problems in the sector, today. The bran adhering to the inner surface of the package causes the fine bulgur to look

different and creates problems for the end-consumer. The process in the present study to solve the problem in the sector was designed as an improvement. The system mentioned in the study can be added to the process before packaging.

Before packaging, the fine bulgur can be separated from the bulgur bran using a PVC surface that gives electrons by friction. The bran remaining on the system surface can be designed to be removed from the process in two ways. The system surface can be completely covered with bran after a certain amount of bulgur passage, and the PVC surface must be cleaned periodically by using moving brush and/or negative pneumatic (vacuum) for efficient operation of the system.

Table 1 The results of the flat PVC electrostatic system with using electric field.

Parameters			System Elestrostatic systems with electric field Flat PVC	
ΔX (cm)	L (cm)	α (º)	Gained bran (g)	Ash content (%, g/g, d.b.)
4	20	30	0,11±0,00 ^{h,i,j,k}	3,05±0,13 ^{b,c,d,e}
		35	0,12±0,00 ^{k}	2,99±0,09 ^{b}
		40	0,13±0,01 ^{k}	2,75±0,13 ^{a}
	40	30	0,09±0,00 ^{f,g,h,i,j}	3,23±0,17 ^{d,e,f,g}
		35	0,08±0,01 ^{e,f,g,h}	3,30±0,08 ^{e,f,g,h,i}
		40	0,09±0,02 ^{f,g,h,i,j}	3,29±0,10 ^{e,f,g,h}
	60	30	0,06±0,00 ^{b,c,d,e,f}	3,45±0,17 ^{h,i,j,k,l}
		35	0,09±0,00 ^{f,g,h,i,j}	3,20±0,22 ^{c,d,e,f,g}
		40	0,09±0,01 ^{e,f,g,h,i,}	3,23±0,17 ^{d,e,f,g}
5	20	30	0,10±0,08 ^{g,h,i,j,k}	3,09±0,09 ^{b,c,d,e}
		35	0,11±0,03 ^{i,j,k}	3,00±0,08 ^{b,c}
		40	0,09±0,01 ^{f,g,h,i,j}	3,25±0,17 ^{d,e,f,g,h}
	40	30	0,12±0,02 ^{j,k}	3,05±0,13 ^{b,c,d,}
		35	0,09±0,02 ^{f,g,h,i,j}	3,15±0,24 ^{b,c,d,e,f}
		40	0,08±0,01 ^{d,e,f,g}	3,35±0,06 ^{f,g,h,i,j}
	60	30	0,07±0,00 ^{c,d,e,f,g}	3,40±0,08 ^{g,h,i,j,k}
		35	0,06±0,00 ^{a,b,c,d,e}	3,50±0,08 ^{i,j,k,l,m}
		40	0,05±0,00 ^{a,b,c,d}	3,53±0,25 ^{i,j,k,l,m,n}
6	20	30	0,05±0,00 ^{a,b,c,d}	3,55±0,06 ^{i,j,k,l,m,n}
		35	0,05±0,00 ^{a,b,c}	3,60±0,12 ^{k,l,m,n,o}
		40	0,04±0,00 ^{a,b}	3,60±0,08 ^{k,l,m,n,o}
	40	30	0,04±0,00 ^{a,b}	3,73±0,10 ^{n,o}
		35	0,04±0,00 ^{a,b}	3,63±0,17 ^{l,m,n,o}
		40	0,03±0,01 ^{a}	3,70±0,08 ^{m,n,o}
	60	30	0,03±0,00 ^{a}	3,78±0,05 ^{o}
		35	0,03±0,00 ^{a}	3,78±0,05 ^{o}
		40	0,03±0,00 ^{a}	3,78±0,05 ^{o}

The results were also correlated using Pearson's correlation coefficient. In the flat PVC system, a significant negative correlation (-0.692) between ΔX and GB was observed and negative correlation (-0.358) between ΔX and length, as shown Table 2.

Table 2. Pearson correlation of flat PVC electrostatic system with using electric field.

		ΔX	L	α	GB	ASH%
ΔX	Pearson Correlation	1	0.000	0.000	-0.692*	0.704*
	Sig. (2-tailed)		1000	1000	.000	.000
	N	108	108	108	108	108
L	Pearson Correlation	0.000	1	0.0000	-0.358*	0.416*
	Sig. (2-tailed)	1.000		1000	0.000	0.000
	N	108	108	108	108	108
α	Pearson Correlation	0.000	0.000	1	-0.053	0.023
	Sig. (2-tailed)	1.000	1.000		0.587	0.816
	N	108	108	108	108	108
GB	Pearson Correlation	-0.692*	-0.358*	-0.053	1	-0.803*
	Sig. (2-tailed)	0.000	0.000	0.587		0.000
	N	108	108	108	108	108
ASH%	Pearson Correlation	0.704*	0.416*	0.023	-0.803*	1
	Sig. (2-tailed)	0.000	0.000	0.816	0.000	
	N	108	108	108	108	108

*: Correlation is significant at the 0.05 level (2-tailed).
GB: Gained bran (g), ΔX: width of channel (cm), L: length of channel (cm), α: angle of channel (°), Ash%: Ash content

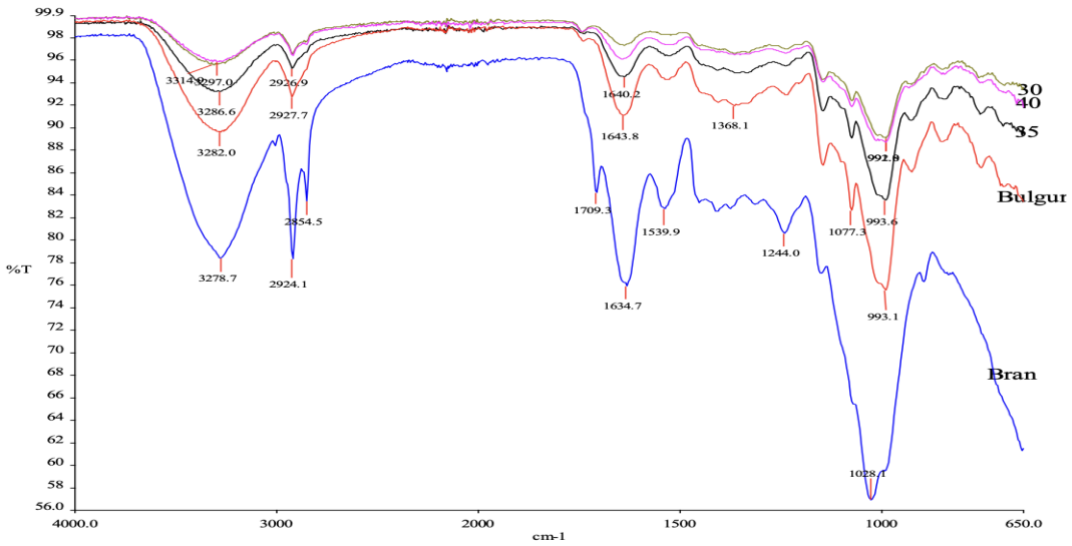

Figure 4 F-TIR Spectra of Bulgur, bran and angle of the system were shown. F-TIR Spectra of flat PVC with electric field system, ΔX 4 cm, L 20 cm.

CONCLUSION

The research has major implications for the food industry as it demonstrates the feasibility of a novel approach to cereal processing, including the production of bulgur. The implementation of sustainable practices is of critical importance for the development of optimal nutrient profiles and product quality. The use of electrostatic field and electric field separation of powder components that cannot be separated by conventional methods represents a sustainable method that can be used in this context.

Consequently, there is a clear potential for the utilization of both an electrostatic field and electric field to represent a promising methodology for the separation of the bran component of bulgur. To ensure the successful separation of the bran component of bulgur, it is of paramount importance to carefully consider and evaluate a number of factors. Furthermore, the intensity of the electrostatic field, the electric field, the properties of the particles and the precise configuration are also factors that must be taken into account. Further improvement may be achieved through experimentation and optimisation, resulting in enhanced outcomes. The findings of this study will prove invaluable in investigating the impact of the separation of cereal products using electrostatic and electric fields on product quality and production efficiency. The advancement of this and analogous methodologies will facilitate the production of superior-quality products. Furthermore, the findings of this study contribute to the scientific literature by providing data obtained from experimental studies on the use of electrostatic and electric fields in the separation of cereal products, as well as methods to improve the quality of cereal products in industry.

In addition to all the advantages, the flow must be stopped in order to remove the bran accumulated in the system. For this disadvantageous situation, alternatives to the foreseen methods can be developed. It may constitute a basis for further research.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of Interests

The authors have no conflicts of interest to declare.

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Alternative vegetable cultivation option in semi-arid conditions: the example of broccoli

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Abstract

The present study investigated broccoli cultivation under semi-arid conditions, focusing on the Parthenon F1 and Orantes F1 cultivars. Turkey has made significant advances in vegetable production, particularly in modern agricultural techniques that ensure higher yield eventually income of the growers. This study was conducted at the Adıyaman University Agricultural Practice and Land Management Research Center, with soil analyses performed before planting. Experimental plots were established with four replications, each containing ten plants. The main parameters recorded includes head diameter, head length, plant height, head weight, SPAD readings, vitamin C and nitrogen contents. The obtained results indicated that the Parthenon F1 had a higher average head diameter (11.83 cm) and head length (13.42 cm) compared to the Orantes F1 (8.46 cm and 10.95 cm, respectively). The average plant height for Parthenon F1 was 54.48 cm, while Orantes F1 had an average height of 49.39 cm. The average head weight of Parthenon F1 was significantly higher at 299.07 g compared to Orantes F1's 164.46 g. The SPAD readings were found similar for both cultivars, with Parthenon F1 at 72.86 and Orantes F1 at 72.57. Vitamin C content was higher in Parthenon F1 (111.76 mg/100 g FW) than in Orantes F1 (100.62 mg/100 g FW). However, Nitrogen content was higher in Orantes F1 (4.31%) compared to Parthenon F1 (3.64%). These findings highlight the importance of planting season and variety selection in achieving optimal broccoli production in semi-arid regions. The Parthenon F1 variety, especially when planted in autumn, demonstrated superior results in most parameters. These results suggested that adopting suitable cultivars and adjusting planting seasons can enhance broccoli yield and quality under semi-arid conditions. Further research on different cultivars and cultivation techniques is recommended to improve the sustainability and productivity of broccoli cultivation in semi-arid regions.

Keywords: Broccoli, Semi-arid, Vitamin C, Head weight, SPAD, N Content

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INTRODUCTION

Turkey made significant progress in vegetable production, and the commercially and economically active vegetable sector was established (Balkaya et al., 2023). Under current scenario, with the use of new vegetable species and varieties, as well as advancements in modern agronimical practices and techniques, high productivity per unit area and consequently, higher income is being achieved (Balkaya et al., 2016; Yanmaz et al., 2020; Balkaya et al., 2023). In Turkey, with the increase in productivity in vegetable cultivation areas, the rise in domestic and foreign demand for vegetables, and the investments in industrial vegetable growing which is a different sector a shift has been achieved in vegetable production activities from the small family business model to modern open-field and greenhouse vegetable growing (Yanmaz et al., 2015; Balkaya et al., 2016; Balkaya et al., 2023). Summer vegetables are grown more than winter vegetables. Nowadays, edible vegetables (tomatoes, peppers, eggplants, melons, watermelons, etc.) constitute the most cultivated group, accounting for 80.7% of production. This group is followed by vegetables with edible roots and tubers such as carrots, radishes etc. with 12.2%. Other types of

vegetables (arugula, cabbage, cauliflower, cress, dill, lettuce, spinach, purslane etc.), including broccoli, constitute 7.1% (TUIK, 2022; Balkaya et al., 2023).

According to Turkey's 2023 fresh fruit and vegetable sector report, the production amount of fresh vegetable products in the fresh vegetable production area decreased by 0.2% compared to the previous year, reaching 29 million tons. Vegetable production in open conditions is generally carried out by small landholding families. Depending on these factors, greenhouse vegetable cultivation has also developed in Turkey, with production occurring in many regions. Among vegetables, the significance of winter vegetable production has been better understood in the last 30 years. Broccoli, in particular, has become one of the most important vegetables for healthy diets possessing of various phytonutrients. It's production and consumption has been increasing rapidly in recent years due to its health benefits.

In Turkey, broccoli production increased considerably in 2023, reaching 104,614 tons (TUIK, 2022). It grows well in the climatic conditions of the Aegean, Mediterranean, and Eastern Marmara regions, and it could be successfully grown in semi-arid ecosystems with appropriate irrigation methods and cultivation techniques. Optimal growth occurs at temperatures between 15-20°C, but high temperature can adversely affect its growth and development. There are early, mid-season, and late-season varieties of broccoli, with edible parts available depending on the harvesting time. The vegetation period for these cultivars ranges from 70 to 160 days. Broccoli grows best with a day length of 12-16 hours and in well-drained, rich in organic matter, slightly acidic soils (pH: 6.0-7.5).

When growing broccoli in semi-arid regions, drip irrigation can be used as irrigation is of great importance. Fertilizers that generally contain nitrogen, phosphorus, and potassium are ideal for broccoli, as they complement the missing nutritional elements identified through soil analysis. To obtain healthy seedlings, these were grown under controlled conditions. Seeds are sown in trays 26-35 days before transplanting. Seedlings that have reached transplant size transplanted into the field. Generally, the distance between plants in a row was set at 30-50 cm, and the distance between rows was kept 50-80 cm. The formation of side shoots affects these distances. Broccoli heads were harvested while they are tight and green. Delayed harvesting can cause the heads to flower. Semi-arid regions are characterized by low and irregular rainfall. Producing crops in these regions presents many challenges. Factors that make crop production challenging under these conditions include the scarcity of water and water resources, soil problems, evaporation and temperature, plant protection issues (diseases, pests, and weeds), and climatic conditions.

Failure to consistently provide the water needed in agriculture will lead to water scarcity. In these conditions, plants need regular irrigation water for their growth to become regular and, most importantly, to maintain productivity. In these regions, the presence of irrigation systems to compensate for insufficient rainfall ensures efficient crop production and does not negatively affect agricultural output.

Soil structure and fertility play crucial roles in crop production. In semi-arid regions, high temperatures generally lead to increase water loss through evaporation from both soil and plants. This high evaporation rate stresses plants, negatively impacting their growth and yield. Additionally, water scarcity, compounded by its high cost, further affects productivity. Insufficient irrigation exacerbates this issue by depriving plants of the water they need, leading to decreased productivity. Moreover, soils in semi-arid regions often lack organic matter and might be nutrient-deficient, further hindering plant nutrient uptake and resulting in low productivity. Another significant challenge is wind and water erosion. The topsoil is the most productive part for plants, and its loss undesirably impacts agricultural production. Some weeds, diseases, and pests may exhibit increased resistance in semi-arid conditions. Controlling these pests and diseases requires significant effort and cost. Climate change further complicates crop production in semi-arid regions. In vegetable cultivation, the timing of seed sowing or seedling transplanting is very crucial. To address these challenges, various crop production strategies and techniques would be employed including implementing water-saving irrigation techniques, such as drip irrigation, as part of water management practices; using organic fertilizers or cultivation techniques like mulching to enhance soil fertility as part of soil management; selecting drought-resistant and low-water-consumption plant species for resilient crop choices; and employing integrated pest management strategies to reduce reliance on synthetic chemicals. Additionally, training and raising awareness among farmers about farming practices suited to semi-arid conditions are essential components of educational and awareness initiatives.

These strategies can enhance the sustainability of agricultural production in semi-arid conditions, increase crop productivity, and support the livelihoods of people living in these regions. This study focuses on how broccoli cultivation can be effectively carried out in semi-arid conditions.

MATERIALS AND METHODS

Plant Material

The plant materials used in this study were Parthenon F1 and Orantes F1 cultivars. Parthenon F1 is known for its productivity and high quality suitable for both fresh table use and industry. It features compact, dome-shaped heads weighing 400-600 g, with a dark green color, a strong root system, and a harvesting time of approximately 80-85 days after transplanting seedlings. Orantes F1, recommended for fresh consumption and industry, has an

average head weight of 500 g and matures in about 70-75 days. Seeds of the Orantes F1 variety were sown on March 3, 2022, while seeds of the Parthenon F1 variety were planted in peat-filled plastic vials on September 1, 2022. Orantes F1 seedlings were transplanted to the field on April 12, 2022, and Parthenon F1 seedlings were introduced to the field on October 11, 2022. Images of the seedlings at planting size are shown in Figure 1.

a b

Figure 1. Seedlings at planting size of Orantes F1 (a) and Parthenon F1 (b) cultivars.

Site Discription

The current research was conducted at the Adıyaman University Agricultural Application and Land Management Research Center (ADYÜTAYAM), Before planting, land preparation and soil analyses were conducted. The experimental area has a clay soil structure with a pH of 7.5, 1.38% organic matter, 2.10% calcium carbonate, and is non-saline. The experiments were set up with 4 replications, each containing 10 plants. During the trial, three hoeings were performed for earthing up and weed control purposes. No plant protection products were used throughout the trial. Fertilization included 90 g of P-Smart, 25 g of K-Smart, and 100 g of urea applied to each sub-plot after planting. The average temperature, total rainfall, and humidity values between September 2022 and February 2023 are presented in Table 1.

Table 1. Climate data of the experimental area

Year/Month 2022	Avarage Teperature ^{0}C	Average Humidity (%)	Total rainfall (mm)
September	27.67	21.21	0.4
October	21.11	30.93	8.2
November	12.96	69.20	165.0
December	9.29	73.55	24.6
2023			
January	6.69	61.95	37.6
February	5.16	52.34	88.4

Recorded Parameters

Head width (cm), head height (cm), head weight (g), plant height (cm), yield (kg/da), and SPAD values were recorded in the plants. Vitamin C (mg/100 g FW) and N (%) contents were examined in the heads. The diameter of the main head was determined by measuring with a ruler. Head width was determined by measuring the diameter of the cut heads with a ruler. The height of the head was determined by measuring the part from the cut section to the top of the head with a ruler. Plant height was determined by measuring the plant from the soil surface to its top with a tape measure. Yield was obtained by collecting all the heads harvested in each plot replication wise. The SPAD readings were taken with the Konica Minolta SPAD-502 Plus device before 11 o'clock in the morning when the heads reached maturity. Vitamin C analysis (ascorbic acid) and the amount of Vitamin C (L-ascorbic acid) were measured by the spectrophotometric method according to Pearson and Churchill (1970).

RESULTS AND DISCUSSION

The measurements in Table 2 are given as the average of the measurements made from 5 plants in the sub-plots.

The main head diameter of the Parthenon F1 variety (11.83 cm) was measured higher than that of the Orantes F1 variety (8.46 cm) (Table 2). Similarly, in other studies, main head diameter values reported for broccoli include 17.85 cm (Alan and Sönmez, 2012), 9.93 cm (Yılmaz and Şahin, 2014), 12.44-13.15 cm (Sharif, 2008), 7.50-10.30 cm (Ouda and Mahadeen, 2008), 9.30-12.40 cm (Tan et al., 1999), 11.70-15.70 cm (Aslan, 2018), and 8.3-9.7 cm (Kaymak et al., 2023). The main head height of the Parthenon F1 variety (13.42 cm) was measured higher than that of the Orantes F1 variety (10.95 cm) (Table 2). Figure 2 shows the average curd diameter and length

measurements. The averages of the Parthenon F1 variety yielded higher results due to variety characteristics. In studies, main head height values measured in broccoli range from 11.28-16.23 cm (Alan and Sönmez, 2012), 11.2-16.8 cm (Yoldaş and Eşiyok, 2004), 10.50-14.53 cm (Ece and Güler, 2017), and 6.8-10.7 cm (Kaymak et al., 2023). It is well known that ecological conditions are crucial for broccoli cultivation (Günay, 1984) and for the adaptation of different varieties to semi-arid conditions (Ece and Güler, 2017). The differences in main head diameter and height are believed to be related to the planting season, aside from variety characteristics. These studies indicated that main head measurement values vary depending on factors such as planting time, planting density, fertilization, and irrigation practices. According to various literature sources, including Ece and Güler (2017), the adaptation of different broccoli varieties to ecological factors plays a significant role in cultivation.

Table 2. Measures parameters of broccoli cultivars.

Parametres & Cultivars	Replication	Mean Head width (cm)	Mean Head length (cm)	Mean Plant Heigh t (cm)	Mean Head Weight (g)	Mean SPAD reading	Mean Vitamin C	N Rate (%)	Yield (kg/da)
Orantes F1	1	8.32	10.79	48.64	166.920	73.56	100.16	3.80	968.225
	2	8.80	11.36	50.62	163.610	71.38	102.54	4.62	958.313
	3	8.28	10.70	48.90	162.840	72.78	99.15	4.52	955.424
	Mean Values	8.46	10.95	49.39	164.455	72.57	100.62	4.31	960.654
Parthenon F1	1	11.44	13.18	53.36	294.700	74.14	117.36	3.80	1.708,17
	2	11.88	13.54	55.02	299.590	70.32	108.22	3.54	1.731,33
	3	12.16	13.54	55.07	302.920	74.12	103.86	3.40	1.749,38
	Mean Values	11.83	13.42	54.48	299.070	72.86	111.76	3.64	1.729.63

In the trial, measurements of plant height parameters showed that Parthenon F1 resulted in higher measurements compared to Orantes F1, with average heights of 54.48 cm and 49.39 cm, respectively (Table 2). Plant height is influenced by factors such as climate, soil properties, planting density, transplanting date, and fertilization (Francescangeli et al., 2006; Singh et al., 2017; Solunke et al., 2011; Singhal et al., 2009). In various studies, broccoli plant heights have been measured as 54.80 cm, 53.70 cm, 59.87 cm, 71.52 cm, and 61.4 cm (Hafiz et al., 2015; Singhal et al., 2009; Meena et al., 2023; Yılmaz and Şahin, 2014; Yıldırım and Geyik, 2019). These variations are largely attributed to planting time and fertilization factors. In this study, autumn planting has been observed to yield better results.

The main head weight measured 166.640 g in initial spring cultivation in Adıyaman, whereas it measured 299.070 g in autumn cultivation (Table 2). Despite differences in variety characteristics, planting season has proven to be a crucial parameter in semi-arid conditions. Considering these results, trials with different varieties suitable for autumn planting have been established. In various studies, average head weights of different broccoli varieties have been measured as 547-678 g (Alan and Sönmez, 2012), 204-389 g in the Aegean region (Eşiyok, 1996), 536-729 g in Konya conditions (Karakaya, 2006), 380-560 g (Bozokalfa et al., 2009), 81-295 g (Kaymak et al., 2023), and 273.32-350.97 g (Moniruzzaman et al., 2007). The distribution of average curd weight (g) values in the trial is shown in Figure 4. Furthermore, the average head weight was measured in the Parthenon F1 variety, influenced by both variety characteristics and autumn planting.

When examining SPAD measurements (Table 2), it is evident that measurements for both varieties are similar, as shown in Figure 3. The SPAD readings in broccoli plants grown under semi-arid conditions were recorded as 72.56 and 72.86 (Table 2 and Figure 3). Kaymak et al. (2023) recorded a SPAD reading value of 87.6. SPAD readings underscore the importance of fertilization. In another study (Taşcı and Kuzucu, 2023), SPAD readings were measured at 70.20 in broccoli plants treated with chemical fertilizer. Vidigal et al. (2021) recorded SPAD reading values ranging from 64.28 to 71.33, emphasizing the impact of fertilizer applications. Kaymak et al. (2023) highlighted nitrogen's role as a constituent of amino acids and the chlorophyll molecule, explaining why SPAD readings are lower in control applications in various studies (Sattel et al., 1998; Taşcı and Kuzucu, 2023; Kaymak et al., 2023).

Toivonen et al. (1994) reported that climatic conditions strongly influence the vitamin C content in broccoli heads. Koh et al. (2009) found that vitamin C levels in broccoli range between 57.35–131.35 mg/100 g FW. In this study, the average vitamin C content was measured as 100.62 mg/100 g FW for the Orantes F1 variety and 111.76 mg/100 g FW for the Parthenon F1 variety (Table 2). The data obtained generally fall within the upper range of these reported values, likely due to variety characteristics and favorable post-harvest storage conditions. Kurilich et al. (1999) reported that vitamin C levels in 50 broccoli subspecies varied depending on the variety, ranging from 54.0 to 119.8 mg/100 g FW. Vitamin C content is an important parameter that changes with storage duration. Analyses of vitamin C at different storage periods (Carvalho and Clemente, 2004) found that levels in broccoli stored at 1°C decreased within 15 days, with the most significant decline occurring between days 12 and 15.

In the experiment, nitrogen content (% N) measured was 4.31 for the Orantes variety and 3.61 for the Parthenon

variety. Yılmaz and Şahin (2017) measured an average % N content of 2.27 in broccoli heads. Yoldaş et al. (2008) reported % N values ranging from 3.06 to 5.08 in broccoli heads also they reported the variation is attributed to fertilizer applications; the lowest measurement was obtained from the control treatment, while the maximum N content in the heads was measured with the application of 450 kg N ha−1. In another study, % N values in broccoli leaves ranged from 3.18 to 3.87 (Ouda and Mahadeen, 2008). The effect of organic fertilizers on % N content is reported to improve both the mineralization of these fertilizers and the physical and chemical properties of the soil (Ouda and Mahadeen, 2008).

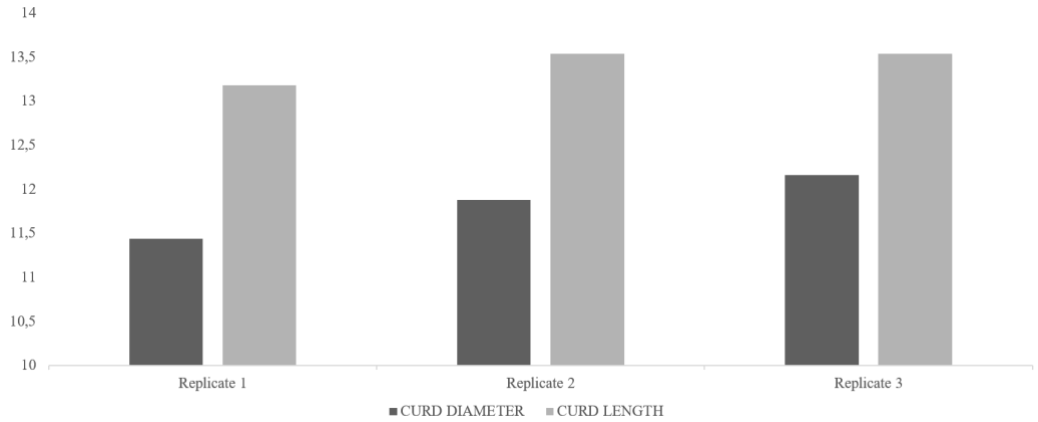

Figure 2. Average curd diameter and curd length values of broccoli cultivars.

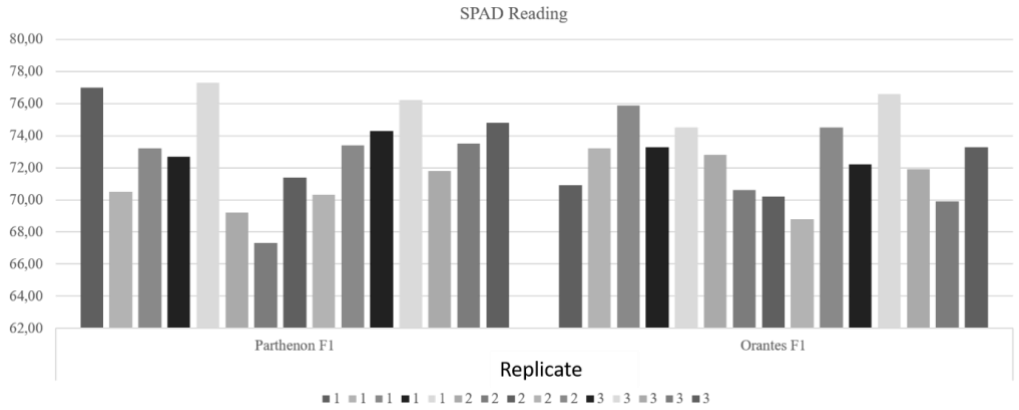

Figure 3. SPAD Reading Parameters measured in Parthenon F1 and Orantes F1.

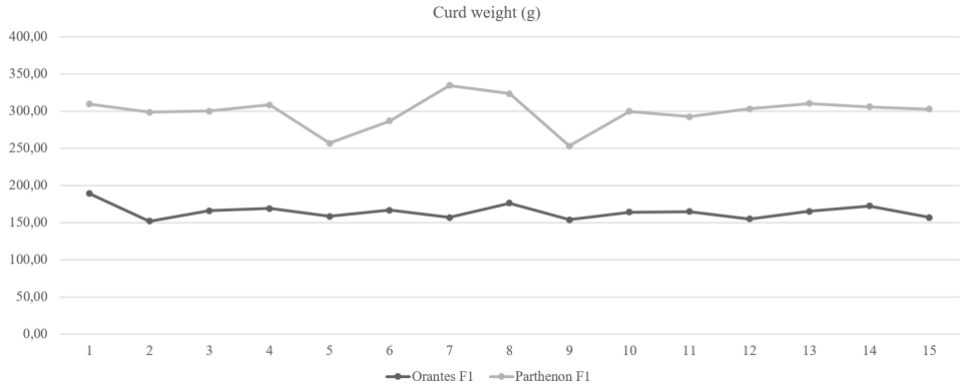

Figure 4. Head weights of broccoli cultivars in semi-arid conditions.

CONCLUSION

Broccoli, which is important for human health, is not very selective in terms of soil requirements. Depending on the variety characteristics, broccoli plants can be grown in high quality in areas where soil organic matter is high or where plant nutrition is provided. Broccoli is sensitive to drought, loses its crown quality in case of sudden

heat increase and forms scattered crowns, but it has been observed that it can adapt to the environment as well as cauliflower and can be resistant to temperature changes. It has been observed that broccoli can be economically cultivated in Adıyaman when planted in the autumn season. It is believed that promoting alternative vegetable cultivation such as broccoli in Adıyaman, among the GAP (Southeastern Anatolia Project) provinces, could address the deficiency in seedling production facilities in the region. Broccoli is grown in a very small portion of home gardening in Adıyaman during the winter season. The limited growth and yield of broccoli are thought to result from inadequate application of fertilizers, lack of appropriate cultural control methods, and management practices. The expansion of broccoli cultivation and analysis of its growth parameters are of great importance. Harvest strategies, optimal number for selective hand harvesting per date, and the harvestable head count per date (biological aspects) impact both current market supply and price conditions (economic aspects). Price fluctuations during harvesting are also crucial for economic viability and therefore need to be analyzed economically (Lindemann-Zutz et al., 2016). Vegetable cultivation can enhance productivity in semi-arid conditions through techniques such as drip irrigation, mulching, and appropriate fertilization methods. Additionally, sustainable agricultural practices can be supported through integrated pest management techniques. Experiments should be conducted on fertilizer types and dosages in semi-arid conditions, leveraging the significant benefits of nitrogen on plant measurement parameters such as head diameter, head weight, and above ground biomass. In semi-arid regions, these methods can make broccoli cultivation economically and ecologically advantageous. These studies demonstrate that with irrigation programs, particularly during periods of low rainfall, and using fertilizers high in nitrogen content, alternative vegetable cultivation such as broccoli can be successfully conducted while mitigating water stress.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors state there is no competing interest.

Author contribution

Designed and performed the experiment: Bayram C.A., Formal data analysis and writing: Bayram C.A.

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Characterization of F_2 generation tomato plants and marker assisted selection against tomato spotted wilt virus (tswv) and tomato yellow leaf curl virus (tylcv)

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Abstract
Identifying the morphological characteristics of genetic material such as leaf, flower, yield, and fruit shape is crucial to genetic diversity assessment. Agronomic and morphological traits of 47 tomato plants in F_2 generation were assessed, as well as their resistance to Tomato Spotted Wilt Virus (TSWV) and Tomato Yellow Leaf Curl Virus (TYLCV). The highest average fruit weight of the tomato lines in F_2 generation was measured in the plants of the line with pink beef fruit type (G300), while the lowest was measured in the plants of the lines with round (cocktail) (S15) and ovate (V30, V31 and V32) fruit types. The highest fruit flesh firmness was measured as 2.74 kg/cm^2 in F_2 plants of line S230 with single red fruit type. The highest SSC (soluble solids content) was measured in F_2 plants of line V31 and S230 with 6.93% and 6.73%, respectively. The longest internode was determined in F_2 plants of the line with single red (S230) fruit type, while the highest stem diameter was measured in plants of the line with pink (G300) fruit type. Despite the variation in leaf color, G300 and S230 plants have potato-shaped leaves, while the other lines have tomato-shaped leaves. There were 2 homozygote resistant plants and 8 heterozygote resistant plants among the F_2 plants. Among the F_2 plants, 2 plants were homozygote resistant and 8 plants were heterozygote resistant to TYLCV. Heterozygote resistance to TSWV was detected only in 6 plants of line V30 and no resistance to TSWV was detected in plants of other lines. The F_3 lines obtained by selfing because of the study can be the material of the breeding programmes in the coming years and testing studies against biotic and abiotic factors should be carried out. The results obtained here should be reinforced with further studies such as the determination of post-harvest preservation storage and shelf-life potentials.

Keywords: Fruit type, Selection, Susceptible, Resistant

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INTRODUCTION
Tomato (*Solanum lycopersicum*) is an annual vegetable species belonging to the *Solanaceae* family, which is widely cultivated in the world. In the world, a total of 1.173.069.683.43 tonnes of vegetables were produced in 2022, with tomatoes (186.107.972 tonnes) accounting for approximately 16% of this vegetable production. While China ranks first in world tomato production, Türkiye ranks third after India (Fao, 2023). Due to the commercial importance of tomatoes, there is a great need for the development of new varieties with higher yield and disease resistance characteristics. In order to achieve this, plant breeders need to characterise well the properties of the genetic material they use. For a breeding programme to be established using a gene pool, a good understanding of genetic diversity is essential. Moreover, analysing the interrelationship between characters helps to select important traits that contribute to yield (Kouam et al., 2018; Grozeva et al., 2021). Therefore, the information in a collection of tomato genotypes can contribute to the formulation of the new breeding plan (Mitra et al., 2023).

Tanksley & McCouch, (1997) reported that without genetic diversity, breeding efforts will result in failure and plants may lack important traits such as resistance.

Morphological and agronomic traits are widely used in the study of genetic diversity in plants (Tecirli et al., 2018; Athinodorou et al., 2021; Morilipınar et al., 2021; Yaman, 2022; Coşkun, 2023; Khan et al., 2024). Morphological characterization studies and determination of traits such as leaf, flower, yield and fruit shape of genetic material are critical in determining genetic diversity (Svetlana et al., 2012; Türkmen et al., 2022). In tomato breeding, factors during intensive selection and cultivation have led to a narrowing of genetic diversity. For these reasons, tomato is more prone to high disease incidence. From sowing to post-harvest, tomatoes worldwide can be affected by more than 200 diseases caused by different pathogens (Bai et al., 2007; Williams & St. Clair, 2011). The most important of these pathogens are tomato spotted wilt virus (TSWV) and Tomato Yellow Leaf Curl Virus (TYLCV). These viruses are extremely damaging in agricultural production and can cause serious economic losses and even devastating consequences in terms of yield in tomato production in some countries and regions (Qiao et al., 2023).

Various strategies can be applied to prevent economic damage caused by these viruses. These methods include cultural, physical, biological, plant-based, chemical and resistance mechanisms. However, the use of each method may depend on their suitability for the planting system and the scale of farmers' activities. Also, some methods may not be compatible when applied together to manage virus populations in the field. Therefore, to control pathogens, it is most practical to use resistant cultivars because of the reduced cost of production and because they are environmentally friendly and compatible with other control methods. Molecular techniques are very sensitive and versatile for the diagnosis of viruses and the selection of resistant plants (Noris & Miozzi, 2015). The aim of this study was to characterise tomato plants in F_2 generation in terms of some agronomic and morphological characteristics and to determine their resistance status to TSWV and TYLCV.

MATERIALS AND METHODS

Plant Material

In the experiment, F_2 plants obtained by selfing of different types of tomato varieties widely used in greenhouse cultivation in Turkey in 2022-2023 were used as plant material. The nematode resistance status of 47 F_2 generation tomato plants used in the study was determined by using MI-23 SCAR marker in the study conducted by Başak et. al., (2024) (Table 1).

Method

Morphological and pomological characterization study was carried out in 2024 in the geothermally heated, venlo type, glass and fully automated R&D greenhouse of Kırşehir Ahi Evran University. Seed sowing was carried out in 128-well trays consisting of peat:perlite mixture at a ratio of 3:1. Plants were grown in the greenhouse by irrigation and fertilisation until the first true leaf stage. When the seedlings reached planting size (seedlings at the true 2-4 leaf stage), they were planted in cocopeat medium with a distance of 25 cm between rows and 100 cm between rows. In the experiment, the number of plants specified in Table 1 was planted from each F_2 line. Irrigation, fertigation and acclimatization processes (the amount of water and fertilizer was adjusted depending on the plant growth stage and greenhouse temperature) were carried out with an automation system. Since the plants were in F_2 generation, the experiment was not set up with replicates. The averages of the measurements and observations were determined according to the number of F_2 plants within the lines.

Examined Parameters

In the experiment, morphological and pomological characterization was carried out according to UPOV criteria in terms of plant and fruit characteristics in the parameters specified in Table 2. Fruit measurements were completed when the first fruits ripened on the plants. Fruits were harvested 60 days after flowering. Observations, measurements and analyses were carried out on 3 fruits selected from each plant. The data obtained were averaged. During the observation and measurement period, the traits to be analysed were measured with a ruler for length and callipers for diameter. Fruit juice EC and pH values were measured with Extech device. Fruit flesh firmness was measured with PCEPTR 200 penetrometer. SSC (soluble solids content) was measured with Hanna HI96801 digital refractometer.

Table 1. Resistance of 47 tomato plants in F_2 generation against nematode races (*M. incognita*, *M. javanica*, *M. arenaria*)

DNA Code	F_2 Code	Nematode Resistance (Mi 1.2 gene)	DNA Code	F_2 Code	Nematode Resistance (Mi 1.2 gene)
1	V30-1	aa	25	S15-1	Aa
2	V30-2	aa	26	S15-2	Aa
3	V30-3	aa	27	S15-3	Aa
4	V30-4	aa	28	S15-4	Aa
5	V30-5	aa	29	S15-5	AA
6	V30-6	aa	30	S15-6	AA
7	V30-7	aa	31	S15-7	AA
8	V30-8	aa	32	V31-1	aa
9	G300-1	aa	33	V31-2	aa
10	G300-2	aa	34	V31-3	aa
11	G300-3	aa	35	V31-4	-
12	G300-4	aa	36	V31-5	-
13	G300-5	aa	37	V31-6	-
14	G300-6	aa	38	V31-7	aa
15	G300-7	aa	39	V31-8	aa
16	G300-8	aa	40	V-32-1	aa
17	S230-1	Aa	41	V-32-2	aa
18	S230-2	AA	42	V-32-3	aa
19	S230-3	AA	43	V-32-4	aa
20	S230-4	Aa	44	V-32-5	aa
21	S230-5	-	45	V-32-6	aa
22	S230-6	-	46	V-32-7	-
23	S230-7	AA	47	V-32-8	aa
24	S230-8	aa			

(-) Band was unable to be obtained, aa: Susceptible, Aa: Heterozygote Resistant, AA: Homozygote Resistant

Table 2. Examined morphological parameters

No	Observed parameters	Scale
1	Plant Strength	Weak , Medium, Strong
2	Leaf Attitude	Semi-Erect, Horizontal, Semi-Drooping
3	Leaf Type	Tomato Leaf , Potato Leaf
4	Leaf, Intensity of Green Color	Light, Medium, Dark

DNA Isolation and PCR Analysis

DNA isolation was performed by modification of the CTAB method developed according to Doyle and Doyle (1990). Fresh leaves were shredded in porcelain mortars using liquid nitrogen, then 100-200 mg aliquots were taken into a 1.5 ml eppendorf centrifuge tube and 250 µl of extraction solution [100 mM trisHC1, pH 8.0; 1.4 M NaC1; 20 mM EDTA; 2% hexadecyl-trimethyl-ammonium bromide (CTAB, Sigma Chemical Co, MO, USA); 0.4% β-mercaptoethanol] were added, mixed thoroughly and incubated in a water bath at 65 ºC for 30 min. 100 µl chloroform/isoamyl alcohol (24/1) was added, mixed well, centrifuged at full power for 3 min and the upper liquid phase was transferred to a new eppendorf tube. In order to precipitate DNA, 500 µl ethanol-acetate solution (96 mL EtOH, 4 mL 3 M NaAc, pH 5.2) was added and mixed gently and centrifuged at full power for 3 min. After the application, the liquid part was poured and left to dry at room temperature for 1 hour. At the end of the observations, 200 µl sterile distilled water was added and the DNA pellet was dissolved. Co-dominant SCAR (SW5-2F 5‘- AATTAGGTTCTTGAAGCCCATCT -3’ and SW5-2R 5‘- TTCCGCATCAGCCAATAGTGT 3’) markers developed in previous studies were used for selection of Sw-5 gene which provides resistance against

TSWV in tomato (Dianese et al., 2010). P6-25 co-dominate SCAR (P6-25F 5‘-GGTAGTGGAAATGATGCTGCTC -3’ and P6-25R 5‘- GCTCTGCCT ATTGTCCCATATATAACC 3’) marker specific for Ty-3 gene was used against TYLCV (Jensen et al., 2007). PCR reactions were performed in a volume of 15 µl. Reactions were performed using 2.0 µl DNA (20 ng), 1.5 µl 10X PCR buffer, 1.0 µl dNTP (200 µM of each dNTP), 1.5 µl MgCl2 (25 mM), 0.2 µl Taq DNA polymerase (0.5 U/µl), 0.5 µl forward and reverse primers (0.3 µM of each primer) and 7.8 µl ddH2O. The PCR products were run on a 2% agarose gel at 115 V in 1x TBE buffer for 3 hours, photographed under UV light and scored to determine disease resistance.

Statistical Analysis

The data obtained in the studies were analysed by one-way analysis of variance (ANOVA) using SPSS 18.0 statistical software (IBM, Chicago, IL, USA) at 5% significance level and the difference between the means was determined by Duncan’s multiple comparison test.

RESULTS

When the plants in the F_2 generation obtained by selfing 6 hybrid tomato varieties of different types, which are widely used in greenhouse cultivation, were classified according to fruit type, it was determined that line G300 had single-pink beef, line S15 had cluster-round (cocktail), line S230 had single-red, lines V30, V31 and V32 had single-ovate fruit type. Among 6 tomato lines in F_2 generation, the highest average fruit weight was determined in line G300 with pink beef fruit type (219.81 g) and in line S230 with single red fruit type (159.47 g). Lines V30 (32.19 g), V32 (20.35 g), V31 (18.10 g) with single ovate fruit type and line S15 (16.64 g) with round (cocktail) fruit type had the lowest average fruit weight. Fruit length and diameter parameters were generally parallel with the average fruit weight. When the lines were evaluated in terms of fruit flesh firmness, the highest fruit flesh firmness was measured in line S230 (2.74 kg/cm^2), which has single red fruit type, while there was no statistical difference between the other lines. The highest SSC was measured in line V31 (6.93%) and S230 (6.73%), while the lowest was measured in line V30 (5.53%). While there was no statistical difference in juice pH content of the lines, the highest fruit juice Ec was measured in line G300 (5.39 dS/m) and the lowest in line V31 (3.31 dS/m) (Table 3).

Table 3. Mean fruit weight, fruit length, fruit diameter, fruit flesh firmness, fruit juice SSC (soluble solids content), Ec and pH of tomato lines in F_2 generation with different fruit types

F_2 Code	Fruit Type	Fruit Weight (g)	Fruit Length (cm)	Fruit Diameter (cm)	Fruit Flesh Firmness (kg/cm^2)	SSC (%)	pH	Ec (dS/m)
G300	Single-Pink Beef	219.81a	5.25a	8.45a	1.60b	6.70ab	4.46	5.39a
S230	Single-Red	159.47b	4.00b	6.40b	2.74a	6.73a	4.36	5.18ab
S15	Cluster-Round	16.64c	3.43b	3.37cd	1.67b	5.87ab	4.39	4.72ab
V30	Single-Ovate	32.19c	3.53b	3.67c	1.89b	5.53b	4.31	3.96a-c
V31	Single-Ovate	18.10c	3.57b	2.50d	1.53b	6.93a	4.21	3.31c
V32	Single-Ovate	20.35c	3.30b	2.80cd	1.80b	6.23ab	4.35	3.87bc
p		***	***	***	***	**	n.s	***

Different letters in the same column indicate that the difference between groups is significant $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

The fruit weights of 8 F_2 plants of line G300 obtained by selfing of pink beef hybrid tomato cultivar varied between 216.50 g and 223 g, the fruit weights of plants of line S230 with single red fruit type varied between 103.50 g and 185.25 g, while the fruit weight of line S15 with cluster-round (cocktail) fruit type varied between 11.50 g and 20.33 g. The average internode length of line G300 was 7.05 cm, line S230 was 9.75 cm and line S15 was 7.00 cm. Among the lines, the thickest stem was measured in F_2 plants of line G300 (11.58 mm), while the thinnest stem average was measured in F_2 plants of line S15 (10.09 mm). Twenty-three F_2 plants of lines G300, S230 and S15 had medium plant strength and potato leaf type. The F_2 plants of lines G300 and S230 had semi-erect leaf attitude, while the F_2 plants of line S15 had horizontal leaf attitude. F_2 plants of line S15 had light green leaf color, while F_2 plants of lines G300 and S230 had medium green leaf color (Table 4).

Table 4. Average fruit weight, internode length, stem diameter, plant strength, leaf attitude, leaf type and leaf intensity of green color of single pink beef (G300), single red (S230), and round cluster (cocktail) (S15) type F_2 plants

F_2 Code	Fruit Weight (g)	Internode Length (cm)	Stem Diameter (mm)	Plant Strength (Weak, Medium, Strong)	Leaf Attitude (Semi-Erect, Horizontal, Semi Drooping)	Leaf Type (Tomato Leaf, Potato Leaf)	Leaf Intensity of Green Color (Light, Medium, Dark)
G300-1	223.00	7.17	12.48	Medium	Semi-Erect	Potato Leaf	Medium
G300-2	221.00	6.00	11.82	Medium	Semi-Erect	Potato Leaf	Medium
G300-3	220.00	6.83	12.67	Medium	Semi-Erect	Potato Leaf	Medium
G300-4	224.00	7.50	10.74	Medium	Semi-Erect	Potato Leaf	Medium
G300-5	219.00	5.50	8.95	Medium	Semi-Erect	Potato Leaf	Medium
G300-6	218.00	8.83	12.70	Medium	Semi-Erect	Potato Leaf	Medium
G300-7	216.50	5.00	12.90	Medium	Semi-Erect	Potato Leaf	Medium
G300-8	217.00	9.60	10.41	Medium	Semi-Erect	Potato Leaf	Medium
Average	219.81	7.05	11.58				
S230-1	165.00	9.73	9.38	Medium	Semi-Erect	Potato Leaf	Medium
S230-2	158.50	8.07	11.24	Medium	Semi-Erect	Potato Leaf	Medium
S230-3	150.00	9.07	10.93	Medium	Semi-Erect	Potato Leaf	Medium
S230-4	165.00	16.67	10.50	Medium	Semi-Erect	Potato Leaf	Medium
S230-5	103.50	9.80	10.32	Medium	Semi-Erect	Potato Leaf	Medium
S230-6	168.00	6.27	9.98	Medium	Semi-Erect	Potato Leaf	Medium
S230-7	185.25	6.77	9.95	Medium	Semi-Erect	Potato Leaf	Medium
S230-8	180.50	11.67	10.75	Medium	Semi-Erect	Potato Leaf	Medium
Average	159.47	9.75	10.38				
S15-1	20.33	6.53	9.63	Medium	Horizontal	Tomato Leaf	Light
S15-2	11.50	6.27	10.66	Medium	Horizontal	Tomato Leaf	Light
S15-3	16.00	7.67	11.32	Medium	Horizontal	Tomato Leaf	Light
S15-4	15.00	6.93	9.28	Medium	Horizontal	Tomato Leaf	Light
S15-5	15.67	6.17	9.58	Medium	Horizontal	Tomato Leaf	Light
S15-6	17.67	8.10	10.81	Medium	Horizontal	Tomato Leaf	Light
S15-7	20.33	7.37	9.35	Medium	Horizontal	Tomato Leaf	Light
Average	16.64	7.00	10.09				

Among the F_2 plants of lines V30, V31 and V32 obtained by selfing 3 hybrid tomato varieties with ovate fruit type, the highest fruit weight was measured in V30-1 plant with 48.28 g and the lowest was measured in F_2 plant of V31-4 with 15.00 g. Regarding the internode length of the lines, the highest average internode length was obtained in the plants of line V32 (9.66 cm), while the lowest was measured in the plants of line V30 (7.58 cm). The average stem diameter of F_2 plants of line V30 was 10.40 mm, of line V31 was 10.29 mm and of line V32 was 8.77 mm. The plant strength of the 3 lines with ovate type fruit type was found to be medium, leaf attitude was semi-erect, leaf type, tomato leaf type and leaf color were found to be dark green (Table 5).

Table 5. Average fruit weight, internode length, stem diameter, plant strength, leaf attitude, leaf type and leaf intensity of green color of F_2 plants with ovate fruit type (V30, V31 and V32)

F_2 Code	Fruit Weight (g)	Internode Length (cm)	Stem Diameter (mm)	Plant Strength Weak, Medium, Strong)	Leaf Attitude (Semi-Erect, Horizontal, Semi Drooping)	Leaf Type (Tomato Leaf, Potato Leaf)	Leaf, Intensity of Green Color (Light, Medium, Dark)
V30-1	48.28	7.67	9.37	Medium	Semi-Erect	Tomato Leaf	Koyu yeşil
V30-2	25.00	7.67	10.90	Medium	Semi-Erect	Tomato Leaf	Dark
V30-3	27.32	6.50	12.82	Medium	Semi-Erect	Tomato Leaf	Dark
V30-4	27.64	6.00	9.50	Medium	Semi-Erect	Tomato Leaf	Dark
V30-5	32.55	8.67	12.91	Medium	Semi-Erect	Tomato Leaf	Dark
V30-6	41.25	8.00	10.60	Medium	Semi-Erect	Tomato Leaf	Dark
V30-7	24.20	7.33	8.57	Medium	Semi-Erect	Tomato Leaf	Dark
V30-8	31.28	8.83	8.55	Medium	Semi-Erect	Tomato Leaf	Dark
Average	32.19	7.58	10.40				
V31-1	20.00	8.17	9.02	Medium	Semi-Erect	Tomato Leaf	Dark
V31-2	20.50	11.50	9.04	Medium	Semi-Erect	Tomato Leaf	Dark
V31-3	17.33	7.90	10.89	Medium	Semi-Erect	Tomato Leaf	Dark
V31-4	15.00	6.50	12.81	Medium	Semi-Erect	Tomato Leaf	Dark
V31-5	17.67	6.50	9.95	Medium	Semi-Erect	Tomato Leaf	Dark
V31-6	17.67	8.17	10.80	Medium	Semi-Erect	Tomato Leaf	Dark
V31-7	18.33	8.40	10.32	Medium	Semi-Erect	Tomato Leaf	Dark
V31-8	18.33	9.93	9.47	Medium	Semi-Erect	Tomato Leaf	Dark
Average	18.10	8.38	10.29				
V32-1	25.00	7.93	8.91	Medium	Semi-Erect	Tomato Leaf	Dark
V32-2	16.33	10.60	8.07	Medium	Semi-Erect	Tomato Leaf	Dark
V32-3	23.00	9.73	11.03	Medium	Semi-Erect	Tomato Leaf	Dark
V32-4	19.67	9.53	9.95	Medium	Semi-Erect	Tomato Leaf	Dark
V32-5	22.00	10.47	8.49	Medium	Semi-Erect	Tomato Leaf	Dark
V32-6	16.33	10.43	7.79	Medium	Semi-Erect	Tomato Leaf	Dark
V32-7	22.00	9.73	7.54	Medium	Semi-Erect	Tomato Leaf	Dark
V32-8	18.50	8.83	8.35	Medium	Semi-Erect	Tomato Leaf	Dark
Average	20.35	9.66	8.77				

The resistance of plants to tomato yellow leaf curl virus (TYLCV) and tomato spotted wilt virus (TSWV) was evaluated. 2 plants were homozygote resistant and 8 plants were heterozygote resistant to TYLCV among tomato plants of different types. Only 6 plants of line V30 showed heterozygote resistance to TSWV, while no resistance was detected in plants of other lines (Table 6).

Table 6. Resistance to TYLCV (Tomato Yellow Leaf Curl Virus) and TSWV (Tomato Spotted Wilt Virus) of tomato plants in F_2 generation with different fruit types

F_2 Code	TYLCV	TSWV
V30-1	aa	Aa
V30-2	-	Aa
V30-3	aa	aa
V30-4	Aa	Aa
V30-5	aa	Aa
V30-6	aa	Aa
V30-7	aa	-
V30-8	aa	Aa
G300-1	Aa	aa
G300-2	AA	aa
G300-3	aa	-
G300-4	Aa	aa
G300-5	Aa	aa
G300-6	Aa	aa
G300-7	aa	aa
G300-8	AA	aa
S230-1	Aa	aa
S230-2	aa	aa
S230-3	aa	aa
S230-4	-	aa
S230-5	-	aa
S230-6	-	aa
S230-7	aa	aa
S230-8	aa	aa
S15-1	Aa	aa
S15-2	Aa	aa
S15-3	aa	aa
S15-4	aa	aa
S15-5	aa	aa
S15-6	aa	-
S15-7	aa	aa
V31-1	aa	aa
V31-2	aa	aa
V31-3	aa	aa
V31-4	aa	aa
V31-5	aa	-
V31-6	aa	-
V31-7	aa	aa
V31-8	aa	aa
V-32-1	aa	aa
V-32-2	Aa	aa
V-32-3	aa	-
V-32-4	aa	aa
V-32-5	aa	aa
V-32-6	aa	aa
V-32-7	-	aa
V-32-8	Aa	aa

-: Band was unable to be obtained, aa: Susceptible, Aa: Heterozygote Resistant, AA: Homozygote Resistant

DISCUSSION

As a result of the characterization study carried out on the plants of the lines with different fruit types, the plants of the line with single pink beef fruit type had the highest fruit weight, while the lowest fruit weight was determined in the plants of the lines with ovate and cocktail fruit type. Fruit length and fruit diameter parameters were in parallel with fruit weight. Pradeepkumar et al. (2001) found that the average fruit weight of tomato fruits varied between 1.40-115.0 g. Turhan et al. (2022) reported that the fruit width of tomato genotypes varied between 33.0-93.0 mm and the average fruit weight varied between 18.18-332.45 g. Bernousi et al. (2011) determined the fruit heights of tomato genotypes in a study in which they determined tomato fruit height and found that fruit height of genotypes varied between 26.8-74.1 mm. The results obtained from the study are in parallel with the results of fruit heights, fruit diameter and average fruit weight in the studies conducted by previous researchers.

In the study, the highest fruit flesh firmness and SSC were determined in the plants of the line with single red fruit type, while the highest fruit juice Ec value was determined in the plants of the line with pink beef fruit type. Güngör et al. (2023) reported that the fruit flesh firmness of 14 different tomato genotypes of different types varied between 0.56 and 2.61 kg/cm^2. Periago et al. (2002) reported that the average amount of SSC varied between 4.0-7.50%, Ziaf et al. (2016) reported the amount of SSC in tomato genotypes between 8.38-13.85%, Lázaro, (2018) reported an average of 6.73% in indigenous tomato genotypes in Madrid Region of Spain, Salim et al. (2020) between 2.97-5.51%, Bakir et al. (2020) between 5.5-9.42% and Athinodorou et al. (2021) between 3.20-5.07%. The reason for the difference in SSC in all studies is that the variety and climatic conditions have an effect on SSC. The longest internode was determined in the plants of the line with single red fruit type, while the highest stem diameter was measured in the plants of the line with pink fruit type. Leaf color varied between light green and dark green, and in terms of leaf type, plants belonging to line G300 and line S230 had potato leaf type, while plants belonging to other lines had tomato leaf type. Demir and Ünlü, (2023) reported that leaf color varied between light green and dark green in tomato genotypes.

The leaf attitude of the plants varies between horizontal and semi-pendent. Yana and Rahima, (2023) reported that stem diameter varied between 1.09 cm and 1.17 cm at the end of 60 days in three different tomato varieties Demir and Ünlü, (2023) reported that the leaf attitude of 24 tomato genotypes had horizontal, semi drooping or semi erect posture and the stem diameter varied between 13.06 mm and 20.99 mm.

An effective measure to manage TYLCV and TSWV does not exist, except for the cultivation of resistant crops. A small number of whiteflies can spread begomoviruses throughout a large area. (Horowitz et al., 2005; Schuster et al., 2010; Elbaz et al., 2016). For the management of TYLCV and TSWV, host plant resistance is the most effective, environmentally friendly and durable approach. Host plant resistance can be achieved by breeding or selection. Over the last few decades, breeding disease-resistant tomato cultivars has become increasingly important for introducing resistance genes. These resistant cultivars can reduce the need for chemical treatments and reduce environmental impact. Furthermore, they can help to reduce the amount of money spent on agricultural inputs. In regions and seasons prone to TYLCV and TSWV, resistant varieties/hybrids have significantly stabilized tomato production (Dhaliwal et al., 2020). The use of molecular markers in plant breeding has a wide range of applications. These markers can be used to select desirable traits, determine genetic diversity, and track genetic traits in plants. They can also be used to track the impact of agricultural activities such as pesticide use and climate change on plant populations. Several resistance genes discovered in wild tomato species have been transferred to cultivated tomatoes. This has resulted in varieties that are more resistant to diseases and pests.

Furthermore, the gene pyramid, which combines multiple resistance genes from various species through molecular markers, has been an important component of the modern tomato breeding program. Tomato is rich in the number of molecular markers available (Foolad, 2007). Marker-assisted selection (MAS) has been effectively used in tomato breeding program to transfer many disease resistance genes. Some of the resistance genes have been shown to be linked to other genes with epistatic traits (Consuegra et al., 2015; Gómez et al., 2004; Mejía et al., 2005; Rani et al., 2008). In our study, the co-dominant SCAR SW5-2 marker developed in previous studies was used for the selection of the Sw-5 gene that provides resistance to TSWV in tomato. P6-25 co-dominant SCAR P6-25 marker, which is specific to Ty-3 gene, was used against TYLCV.

In a previous study using 14 tomato cultivars with the SW5-2 marker, 4 cultivars were resistant with a 574 bp band, 2 cultivars were susceptible with a 510 bp DNA band and 8 cultivars were susceptible with a 464 bp band. It was reported that the SW5-2 primer set efficiently determines tospovirus resistance under greenhouse and field conditions and is a good marker for marker-assisted selection (Dianese et al., 2010). P6-25 marker was used for TYLCV resistance in different tomato varieties and it was reported that P6-25 marker was accurate and reliable in selection studies (Caro et al., 2015; Kim et al., 2020; Aktaş & Aydın, 2022). The P6-25 molecular DNA marker used was found to be helpful in determining the resistance responses of pink-fleshed tomato to TYLCV and the findings were rapid, accurate and reproducible. Due to the availability of this information and the fact that some tomatoes showed disease resistance, it was determined that the primers could be used in future breeding trials (Tekin et al., 2024).

CONCLUSION

TYLCV and TSWV are a serious constraint in tomato cultivation worldwide. The identification of sources of resistance and their transfer to commercial varieties has stabilized tomato production in disease-prone regions and seasons. The late emergence of the disease does not threaten tomato production. Genetic diversity is necessary for the development of a new variety. The present study, the results of morphological and pomological traits obtained from F_2 generation plants and plants showing resistance to TSWV and TYLCV can be the material for future breeding programs and will help in the management, classification and conservation of germplasm.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors state there is no competing interest.

Author contribution

Authors' individual contributions to the article are equal.

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Development of low oil emulsion gels by solidification of oil droplets and determination of their rheological properties

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Abstract

This study aims to develop low-fat emulsion gels by physically solidifying oil droplets using a combination of pectin, soy protein, and bovine gelatin, and to investigate the rheological properties of these emulsion gels. The emulsion gels were formulated with different combinations of these biopolymers [PSG30 (pectin + soy protein + gelatin + 30% oil), PS30 (pectin + soy protein + 30% oil), P30 (pectin + 30% oil), G30 (gelatin + 30% oil)] and compared with commercially available low-fat mayonnaise (DYM40, 40% oil), mayonnaise (TM80, 80% oil), and spreadable fat (SY59, 59% oil) samples. The consistency index (K, Pa.s^n) of the emulsion gels ranged from 1.903 to 150.739 Pa.s^n, with PSG30 and PS30 formulations exhibiting higher K values than the commercial samples. The highest structural recovery percentage was observed in the SY59 sample at 114.91%. Thermal stability tests demonstrated that PSG30 and PS30 maintained their viscosity and storage modulus (G') values over a wide temperature range. Fourier Transform Infrared Spectroscopy (FTIR) analysis revealed significant hydrogen bonding and cross-linking interactions between pectin, soy protein, and gelatin. Microstructural imaging showed that PSG30 had the most homogeneous structure, consistent with its superior rheological performance. Molecular docking analysis determined the binding energy between gelatin and pectin to be -6.40 kcal/mol. Interaction between pectin (Arg-522 residue) and soy protein (11S globulin TGT) was facilitated by salt bridge formation. The developed formulations of pectin, soy protein, and gelatin demonstrate potential for producing low-fat emulsion gels with acceptable texture and stability properties for various food applications.

Keywords Low-fat, Emulsion gels , Pectin, Soy protein, Gelatin

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INTRODUCTION

There is a growing interest in developing emulsion gels with low oil content. However, there is limited literature on how to obtain such emulsions by changing the oil phase and solidifying the oil droplets by physical methods. Emulsion gels have attracted attention with their properties such as reducing trans fat and saturated fat (Montes de Oca-Ávalos et al., 2016), improving food structures (Foegeding et al., 2017) and increasing bioavailability (Lee et al., 2019) by exhibiting both emulsion and gel properties.

Emulsion gels are complex colloidal systems that combine the properties of emulsions and gels, characterized by a three-dimensional network structure that incorporates dispersed lipid droplets within a continuous gel matrix (Fontes-Candia et al., 2020). These emulsions develop the characteristics of soft solid materials with remarkable physical stability and viscoelastic capabilities due to their network topologies, which contribute to their high mechanical properties (Chen et al., 2022). In particular, cold-setting emulsion gels have outstanding potential for food applications in reducing fat content in foods and encapsulating bioactive compounds (Souza Paglarini et al., 2021; Muñoz-González et al., 2021). Fat-reduced products are of considerable interest in the food industry due to the increasing consumer demand for healthier options. Emulsion gels play an important role in the development

of reduced fat products as effective fat replacers while maintaining the desired sensory properties and structural integrity of foodstuffs (Dickinson, 2012). By incorporating emulsion gels into fat-reduced formulations, the total fat content and energy density of products can be reduced, thereby improving their nutritional profile (Ren et al., 2022). Emulsion gels, when used as fat substitutes in meat products, effectively reduce product fat content while maintaining desirable textural and sensory properties (Kim et al., 2018). Emulsion gels prepared with ingredients such as chia mucilage and olive oil have been used in the development of reduced fat burger formulations and have been shown to be an alternative for healthier food options (Liu et al., 2022). Emulsion gels have outstanding potential as fat substitutes to achieve desired structural properties in low-fat products without compromising taste or quality (Silva, Ferdaus, Foguel, & da Silva, 2023).

Pectin and gelatin are two popular biopolymers that are used in the food industry. Pectin, a natural polysaccharide that is predominantly derived from the cell walls of fruits, particularly citrus fruits and apples, is known for its gelatinization, thickening, and stabilizing properties. It is particularly advantageous in the production of jams, jellies, and other structured food products due to its capacity to form gels in the presence of sugar and acid (Robledo & Vázquez, 2019). In addition, pectin is acknowledged as a source of dietary fiber, which has been associated with potential cholesterol-lowering effects and enhanced digestive health (Blanco-Pérez et al., 2021). Gelatin possesses a distinct amino acid content and is a naturally occurring hydrocolloid that is produced by the hydrolytic breakdown of collagen (Alipal et al., 2021). In applications of oil-in-water emulsions such as confectionary, low-fat margarine, and milk cream, gelatin serves as an emulsifying agent due to its amphoteric properties and hydrophobic regions in the peptide chain (Aewsiri et al., 2009).

Proteins and polysaccharides are safe additives that can improve the stability of emulsion gels while producing physically stable emulsions (Chityala et al., 2016). The characterization, mechanical properties, and applicability of emulsion gels stabilized with soy protein-xanthan gum complexes in plant-based processed meat products were investigated (Funami et al., 2023). Ren et al. (2022) used gelatin and pectin in their study to formulate emulsion gels for three-dimensional food printing using a 3D printer. Li et al. (2020) created food-grade emulsions and emulsion gels using soy protein-pectin complex nanoparticles and glycyrrhizic acid nanofibrils, demonstrating the interaction between soy protein isolate and pectin. Souza et al. (2021) used an emulsion gel high in fiber that had inulin, soy protein isolate, and soybean oil instead of animal fat in bologna-style sausages with less salt and fat. This was done to make healthier meat products using emulsion gels. No study revealed the interaction of pectin, soy protein, and gelatin to determine a fat-reduced emulsion gel formulation with strong rheological properties.

The aim of this study is to develop emulsion gels with reduced fat content by combining pectin, soy protein, and gelatin, and to investigate the interactions between these components to obtain strong rheological properties. Within the scope of the study, low-fat emulsion gel structures will be developed in different combinations of pectin, soy protein, and gelatin, and their structures will be examined by detailed rheological analyses. The emulsion gels obtained will be compared rheologically with the target products of commercial spreadable oil, mayonnaise, and reduced-fat mayonnaise products. In addition, pectin-gelatin and pectin-soy protein interactions will be revealed by Fourier transform infrared (FTIR) spectroscopy and molecular docking. This research aims to provide value-added products to industries producing healthy food products by developing fat-reduced, strong emulsion structures and low-calorie alternatives to fats and oils.

MATERIALS AND METHODS

Materials

Pectin (E440, apple pectin with medium methoxyl), soy protein (81.7%), and bovine gelatin (220 Bloom, E441) Kimbiotek Kimyevi Maddeler San. Tic. A.Ş. (Istanbul, Turkey). Sunflower oil, low-fat mayonnaise (40% fat), commercial mayonnaise (80% fat), and spreadable oil (59% fat) were purchased from a national market in Turkey.

Preparation of Emulsion Gels

Emulsion gels were formed by combining pectin (P), soy protein (SP), gelatin (G), and sunflower oil (O) in varying proportions (Table 1). The ratios of P, SP, G, and O were determined as a result of preliminary trials.

According to the % ratios specified in the formulation, gelatin was first completely dissolved in pure water at 50–55ºC with a magnetic stirrer (Heidolph Instruments GmbH & Co., P/N: 506-11100-00, Germany). Then pectin was added, and the dissolution process was continued until a homogeneous mixture was obtained. After obtaining a homogeneous solution, soy protein was added and stirred for another 10 minutes. The mixture was cooled to 25ºC and then kept at 4ºC refrigerator temperature for 20 hours. Low-oil emulsions were obtained by adding 30% oil phase to the solution phase containing the P-SP-G mixture to form emulsions. A homogenizer (Ultraturrax T18, IKA, Germany) was used at 13,500 rpm for 70 seconds to solidify the oil droplets. The prepared emulsions were rested at 4 °C for 24 hours and then analyzed (Kamer, 2024).

Table 1. Emulsion gel formulations

Sample Code	Pectin (%)	Soy Protein (%)	Gelatin (%)	Sunflower oil (%)
PSG30	5	1	0.5	30
PS30	5	1	0	30
P30	5	0	0	30
G30	0	0	0.5	30

* Commercial control samples were coded with the abbreviations DYM40 for low fat mayonnaise (40% fat), TM80 for commercial mayonnaise (80% fat), and SY59 for spreadable oil (59% fat).

Figure 1. Emulsion gels with low oil content produced in different formulations

Rheological Characterisation of Emulsion Gels

The rheological properties of low oil content emulsion gels and commercial products were carried out using two different deformation tests, constant shear and dynamic shear, using a temperature controlled (peltier system) Discovery Hybrid Rheometer-2 (TA Instruments New Castle, USA). TRIOS Software (V3.0) was used for equipment control and obtaining rheological parameters. The data obtained were visualised using OriginPro 2016 software (OriginLab Corporation, USA).

Steady shear flow behaviour measurements

During the flow behavior analysis of emulsion gels, the samples were placed between parallel plates (measurement range 1 mm; diameter 40 mm), and a total of 100 data points were collected at 10-second intervals. The constant shear test was performed at 20 °C with a shear rate of 0.1–300 s^{-1}. Data collected included shear rate, shear stress, and apparent viscosity (Kamer, 2024). Flow behavior, coefficient of consistency (K, Pa.s^n.), and flow behavior index (n) values were determined using the power-law model with the highest coefficient of determination (R^2).

Power law (Ostwald de -Waele equation) model: $\sigma=K(\gamma)^n$ (Eq. 1)

For the Power law model, σ is the shear stress (Pa) and γ is the shear rate (s^{-1}). K is the consistency index (Pa. s^n) and n is the flow behaviour index.

Structural recovery - thixotropy test

To determine the structural recovery, or thixotropy, an oscillation time scan was performed at 1 Hz with three intervals. In the first stage, the specimens were subjected to a 0.1% strain for 120 s. In the second stage, 20% strain (above the LVR) was applied for 300 s to simulate structural failure conditions. In the third stage, the strain was restored to the first stage (0.1% strain) and reapplied for 120 s. The recovery was calculated as a percentage by dividing the storage modulus at the beginning of the thixotropy (G'^1) by the storage modulus at the end of the thixotropy (G'^3) to obtain three thixotropy ranges (3-ITT) (Abdolmaleki et al., 2020).

The effect of temperature on the rheological parameters of emulsion gels

The effect of temperature on the apparent viscosity of emulsion gels was determined by a heating (5 °C/min, 1 Hz) test between 5 and 60 °C. Temperature screening for thermo-viscoelasticity properties was carried out by heating from 5 to 45 °C under constant shear (f = 1 and y = 1%) (Rao, 2010).

Dynamic shear rheological measurements

The linear viscoelastic region (LVR) of emulsion gels and commercial samples was determined by a scanning test (0.1%–100%, 1 Hz, 20 °C) using a parallel plate geometry with a measurement range of 1 mm and a diameter of 40 mm. The modulus of accumulation (G′) and loss (G″) were recorded as a function of frequency through a

dynamic oscillation measurement at 0.1% strain, with a frequency sweep ranging from 0.1 to 100 rad/s. Using the Bohlin model, the frequency dependence of G′ and G″ was investigated (Abdolmaleki et al., 2020).

$G' = A'(\omega')^{b'}$ (Eq. 2)

The dynamic rheology parameters G′ and G″ determined against angular frequency values were subjected to linear regression analysis and intercept values (A′), slope values (b′) and R^2 values were calculated using the above equations. Here ω is the angular frequency (rad/s).

Fourier transform infrared spectrometry (FTIR) analysis

The molecular behavior of the emulsion gels was analyzed using ATR-FTIR spectroscopy (Pintado et al., 2015). For this purpose, a Nicolet iN5 infrared microscope (Thermo Fisher Scientific, Waltham, MA, USA) was used with a 500–4000 cm^{-1} survey range, transmission mode (2 cm^{-1} resolution by accumulating 128 scans), and post-processing of spectra in Omnic 9 software (Thermo Fisher Scientific, Waltham, MA, USA). The data obtained were visualized with OriginPro 2016 software (OriginLab Corporation, USA).

Investigation of Pectin-Soy Protein and Pectin-Jelatin Interaction by Molecular Docking Method

AutoDock Vina, Pyrex, Discovery Studio, and the molecular graphics program UCSF Chimera (University of California, San Francisco) were used as virtual screening software for docking simulation in this study. In this study, 7S (PDB ID: 3AUP) and 11S globulin (PDB ID: 1OD5) (Zhang et al., 2023), which are the main components of soya protein, and type I collagen (PDB ID: 5CTI) protein models with heterotrimer structure for bovine gelatin were used. These proteins were obtained from the Protein Data Bank (https://www.rcsb.org) of the Research Collaboratory for Structural Bioinformatics (Fu et al., 2023). Galacturonan (tetragalacturonic acid, CID 5459352) (Wang et al., 2024) was chosen as a representative ligand because the pectin molecule was too large to simulate docking. PyMol software performed the visualization of the binding sites. The 2D diagram was generated by Proteins Plus (DoG Site Scorer) (https://proteins.plus/) (Schöning-Stierand et al., 2020).

Statistics

The statistical significance of the proposed models was evaluated using a one-way analysis of variance (ANOVA). Experiments were carried out in three replicates, and differences between samples were evaluated using Duncan's multiple range tests with SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The statistical significance level was 95%.

RESULTS AND DISCUSSION

Rheological Properties of Emulsion Gels

In order to reveal the rheological characterization of the emulsion gels, the viscosity and shear stress values in steady shear were first investigated at 20 °C. Figure 2A shows the change in shear stress as a function of shear rate for various formulations. In all samples, the apparent viscosity decreases with an increase in shear rate. This indicates that the emulsions exhibit shear-thinning behavior. As the shear rate increased, PSG30 and PS30 formulations exhibited the highest shear stress values. This indicates that the combination of pectin, soy protein, and gelatin leads to a significant increase in viscosity and provides a strong and homogeneous texture. The P30 formulation exhibited high shear stress values but was lower compared to PSG30 and PS30. This indicates that pectin alone can generate significant viscosity even in the absence of soy protein and gelatin. The G30 formulation showed significantly lower shear stress values compared to the other formulations. This indicates that gelatine-based emulsion alone is insufficient to increase viscosity. Commercial mayonnaise samples, DYM40 and TM80, exhibited comparable patterns in terms of shear rate, but PSG30 showed lower shear stress values compared to PS30 and P30 formulations. The rheological properties of emulsion gels, such as consistency and viscosity, are critical to determining their sensory qualities, flow behavior during processing, structural and physical stability, and optimizing formulation and processing conditions (Patel et al., 2014). Xu et al., (2021) demonstrated the versatility of pectin in food formulations by detailing the preparation of a fat substitute based on high-methoxyl pectin from citrus and its application to croissant crust. Wang et al. (2014) and Albano & Nicoletti (2018) investigated the use of gelatin as a fat substitute in various food products. Gelatin's ability to provide sensory properties similar to those of fat makes it an ideal ingredient for low-fat formulations.

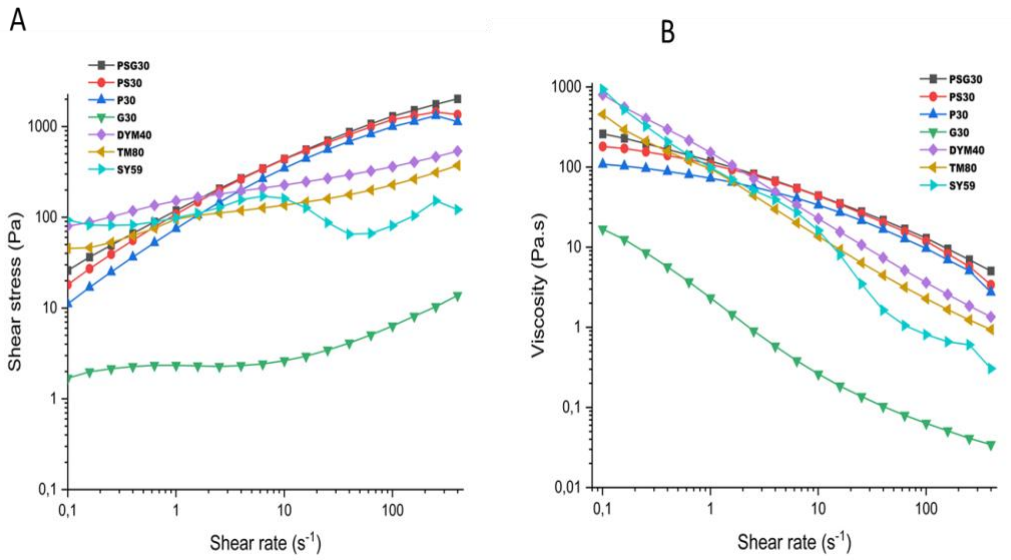

Figure 2. Shear stress and viscosity rheograms of emulsions in the range of 1-100 s^{-1}

The coefficient of consistency and flow behavior index values for the Power-Law (PL) model parameters of the emulsions are given in Table 2. The consistency index K (Pa.s^n) values ranged from 1.903 to 150.739 Pa.s^n, with the PS30 formulation achieving the highest consistency and the G30 formulation demonstrating the lowest consistency. The consistency index values of the samples differed significantly ($p<0.05$). The PSG30 and PS30 emulsion gel formulations yielded more viscous emulsions in comparison to commercial products. This indicates that the developed oil-reduced formulations may have potential for use in different food formulations, such as mayonnaise and spreadable oil. The flow behavior index (n) values of the samples were in the range of 0.019–0.466, and the difference between the samples was found to be statistically significant ($p<0.05$). All samples showed a flow behavior index below 1, and an increase in shear rate resulted in a decrease in the viscosity value. This indicates that emulsions have pseudoplastic and time-independent non-Newtonian behavior. Nasrolahi et al. (2023) obtained similar findings in a study on the rheological properties of emulsions prepared with modified starch, corn oil, and resin gum. Although the PSG30 formulation has n <1, it appears to be the sample with the highest n value. This can be attributed to the presence of gelatin in combination with pectin and soy protein, forming a more complex network with additional cross-linking points and providing additional flexibility. Gelatin interacted with both pectin and soy protein, resulting in a potentially stronger but more shear-thinning network. Hydrocolloids that exhibit shear thinning are widely used to improve or modify food texture. A lower solution viscosity makes it easier to process during high shear processes like pumping and filling. On the other hand, a high apparent viscosity makes the product feel good in the mouth (Marcotte et al., 2001). The flow curves show similar behavior, in agreement with other studies on mayonnaise rheology (Moros et al., 2002; Peressini et al., 1998). As the shear rate increases, the agglomerated droplets deform and distort, leading to the shear-thinning effect (McClements, 2004).

Table 2. Power-Law (PL) model parameters of emulsion gels

Sample codes	*K* (Pa.s^n)	*n*	R^2	Recovery(%)
PSG30	147.483±1.55^a	0.466±0.08^a	0.98±0.005^a	63.51
PS30	150.739±2.55^a	0.441±0.04^b	0.98±0.01^a	87.69
P30	112.731±1.08^c	0.464±0.05^a	0.98±0.01^a	63.19
G30	1.903±0.05^f	0.249±0.05^c	0.84±0.005^b	58.84
DYM40	142.742±0.55^b	0.202±0.01^e	0.99±0.005^a	94.09
TM80	82.650±0.61^e	0.219±0.01^d	0.98±0.01^a	83.88
SY59	104.165±0.99^d	0.019±0.02^f	0.72±0.01^c	114.91

* PSG; pectin + soy protein + gelatin + 30% fat, PS30; pectin + soy protein + 30% fat, P30; pectin + 30% fat, G30; gelatin + 30% fat, DYM40; low fat commercial mayonnaise (40% fat), TM80; commercial mayonnaise (80% fat), SY59; spreadable oil (59% fat). a, b, c : Means with different letters in the same column are different from each other ($p<0.05$)

Structural recovery and thixotropy properties of emulsion gels

A thixotropy test was carried out to determine the ability of emulsion gels to be exposed to a low rotational force followed by a high force and to recover when returned to a low force. Especially in squeezed products such as mayonnaise, the ability to recover is very important. The structural recovery values of the emulsion gels given in Table 2 are generally lower than those of the commercial samples. The lower structural recovery values exhibited in the emulsion gels, as compared to commercial samples, may be attributed to the inclusion of various gums or thickeners in commercial mayonnaise, particularly those with low fat content. Commercial formulations often incorporate a blend of hydrocolloids, including xanthan gum, guar gum, or modified starches, with a specific objective of increasing viscosity, stabilizing emulsions, and enhancing the textural characteristics of the product (Blok et al., 2023). The presence of these components enhances the thixotropic behavior by promoting faster recovery following the removal of shear pressures, therefore enabling the product to regain its structure and consistency with greater efficiency. However, it is seen that the PS30 sample with a recovery of 87.69% is higher than the commercial mayonnaise (TM80) sample with a very high oil content. The fact that the thixotropic behavior, which is defined as the process of reconstruction of the molecular structure, is the lowest in the G30 sample shows that gelatin alone cannot provide sufficient elasticity at low concentrations. This can be explained by the poor ability of the gel to trap water at low gelatin concentrations and, consequently, the weak gel network structure of the emulsion (Zeng et al., 2023).

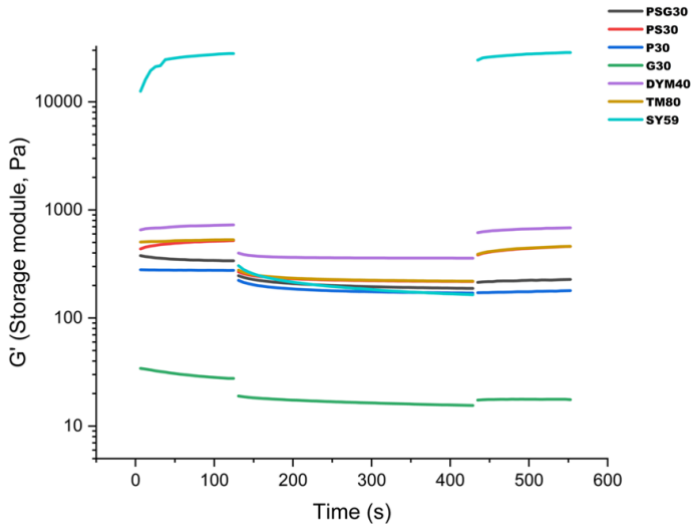

Figure 3. Structural recovery behaviour of emulsion gels

When the structural recovery behaviors of commercial samples and emulsion gels are compared in Figure 3, it is seen that the modulus of deposition is highest in spreadable oil, followed by low-fat mayonnaise, full-fat mayonnaise, and PS30 samples. The significant decrease in the deposition modulus with increasing shear rate in the SY59 sample indicates the pseudoplastic behavior of the sample (Mohammadi et al., 2021). The recovery value of PS30 was found to be 25% higher than that of P30. This is thought to be due to the synergistic effect between soy protein and pectin. In addition, the negative charge of pectin and the positive charge of soy protein may have contributed to the development of stronger emulsion structures by forming electrostatic complexes (Albano and Nicoletti, 2018). Emulsion gels that exhibit shear-thinning behavior, have sufficient elastoplastic behavioral ability to flow, and have a sufficiently high modulus of deposition to maintain their shape during flow show excellent performance in terms of both fluidity and structure stability in the 3D printing process (Feng et al., 2016). The PS30 formulation exhibits the characteristics of an emulsion gel with these qualities.

Thermorheological Properties of Emulsion Gels

The viscosity of different emulsion gel formulations is shown in Figure 4, with temperature being the independent variable. The viscosity properties of the samples provide important information about their capacity to maintain their structural integrity and thermal stability at different temperature settings. Both PSG30 and PS30 formulations showed a consistent viscosity over a wide temperature range, demonstrating a strong and stable structure. In these samples, the viscosity tended to decrease slightly as the temperature increased (above 30 °C), indicating a slight shear thinning behavior. However, the overall viscosity remained high, indicating strong thermal stability. The P30 formulation also exhibited a consistent viscosity at low temperatures. However, there was a gradual decrease in viscosity as the temperature increased. The lack of soy protein and gelatin may have resulted in a slightly less stable network compared to PSG30 and PS30. However, it still has a considerable viscosity, emphasizing the effectiveness of pectin in forming a stable gel matrix. The G30 formulation showed a significant decrease in viscosity with increasing temperature, indicating poorer thermal stability compared to the pectin-

containing formulations. This indicates that gelatin alone is not as effective in maintaining viscosity at high temperatures. Gelatin derived from bovine skin is known to exhibit melting temperatures ranging from 33.07 °C to 34.51 °C (Samatra, Noor, Razali, Bakar, & Shaarani, 2022). As expected, the gelatin-based emulsion formulation (G30) also showed a melting tendency in the range of 25–30 °C (Figure 4A). DYM40 and TM80 commercial mayonnaise samples showed a more stable structure as the temperature increased. The specific stabilizers used in their commercial production likely created these formulations to withstand moderate temperature changes. The viscosity of the SY59 spreadable oil sample decreased significantly as temperature increased, particularly when compared to the other samples. This significant decrease indicates that the formulation may not be able to withstand higher temperatures and is not stable. The temperature stability of emulsion-type food products such as mayonnaise is critical for both storage and shelf life, as well as for use in various dishes such as hamburgers, burgers, and sandwiches. These applications often expose the delicate emulsion to high temperatures, such as in meatloaf. In addition, it is important to consider the stability of the emulsion to ensure that it can withstand temperature fluctuations during transport and retail storage, especially in tropical climates. This is crucial for the commercial success of the product (Rudra et al., 2020). This supports the high commercial utilization potential of the PSG30 and PS30 formulations. These formulations maintain high G' values throughout the temperature range and show only a slight decrease as the temperature increases. This indicates that the gels have strong elastic properties and are able to maintain their structure under thermal stress, suggesting a synergistic effect of pectin, soy protein, and gelatin in increasing gel strength. The viscoelastic properties of the emulsions were analyzed at temperatures ranging from 5 to 60 °C at a heating rate of 5 °C per minute. The results are shown in Figure 4B. At temperatures above 40 °C, an increase in the modulus of accumulation of PSG30 and DYM40 samples was observed, which can be attributed to the interaction between soya proteins and other components in the emulsion at high temperatures (Rudra et al., 2020). Chang et al. (2017) observed similar results and attributed this behavior to the enhanced interaction between protein granules, pectin molecules (the hydrocolloid they focus on), and oil droplets. This interaction leads to a small increase in elasticity.

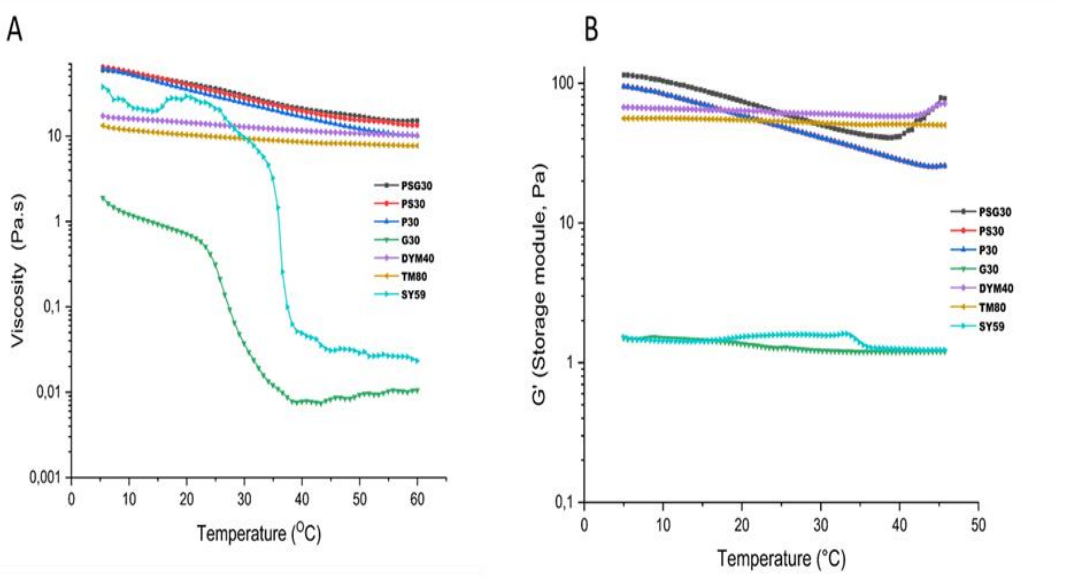

Figure 4. Temperature dependent changes in viscosity and storage modulus of emulsion gels.

Viscoeleastic properties of emulsion gels

The frequency dependence of the G' (storage modulus) and G" (loss modulus) values of the emulsions is given in Figure 5, and their viscoelastic rheological properties according to the Bohlin model are given in Table 3. A dynamic frequency sweep revealed the elasticity and viscosity of the emulsions (Figures 5A–B). G′ (storage modulus) shows the elastic properties of the emulsions, and G″ (loss modulus) shows the viscous properties of the emulsions. In all of the emulsions, the storage modulus was clearly greater than the loss modulus. This indicates that the emulsions exhibit solid-like behavior. As the angular frequency increased to 10 rad/s, the commercial samples showed stable behavior, while the accumulation modulus of the emulsion gels increased. When the angular frequency increased above 10 rad/s, all samples showed an increase in mechanical strength. This shows the tendency toward solid-like behavior of the samples (Han et al., 2024; Ma et al., 2023).

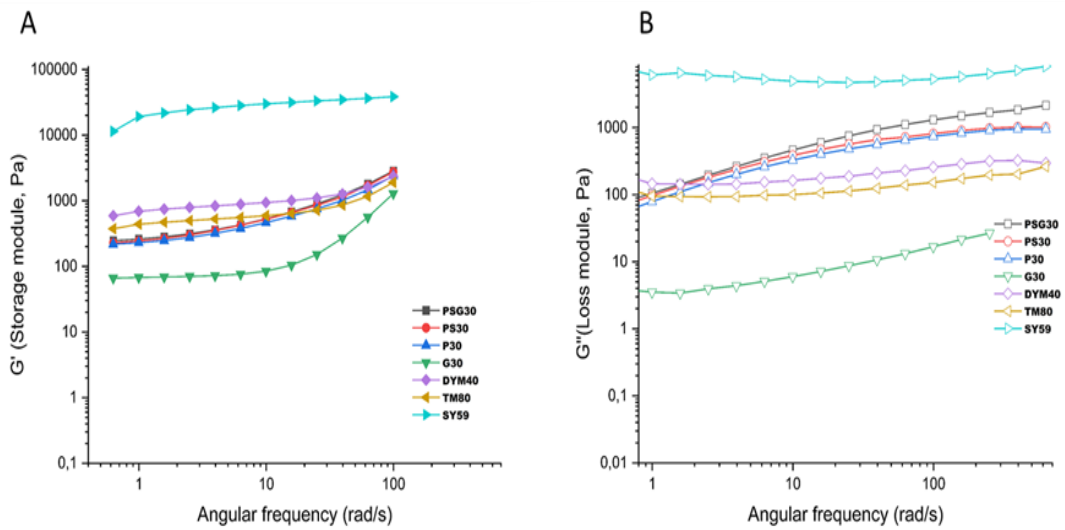

Figure 5. Storage modulus (G') and loss modulus (G'') rheograms of emulsion gels in the range of 1-100 rad/s angular velocity

The highest storage modulus was found in commercial spreadable oil, while the lowest was found in emulsion gel containing 0.5% gelatin. The structural firmness and stability of the emulsion gels were compared by calculating the A' and b' values with the Bohlin model over the storage modulus. Here, the A' value is a coefficient indicating the strength of the bonds in the emulsion, while the b value provides information about their interactions (Xu et al., 2021). The b' values of the samples vary between 0.16 and 1.61. It was seen that the frequency-dependent changes of emulsion gels other than G30 were less. PSG30 emulsion gel has the highest A' value. Pectin has a greater effect on the A' value than soy protein and gelatin. There was a statistical increase in both the A' and b' values in the PSG30 sample compared to the P30 sample. The increase in the b' value indicates an increase in the interaction sites of the complexes between the oil droplet surface and the aqueous phase, while the increase in the A' value indicates a significant increase in the interactions between the sites (Wei et al., 2017). The more complex structure of the PSG30 sample suggests a higher number of interaction sites between oil droplets and complexes.

Table 3. Bohlin model fitting of storage and loss modulus of emulsion gels and equation parameters.

Samples	G' (Storage Modulus)		
	A' (intercept)	*b'(slope)*	R^2
PSG30	96.90±21.28^{b}	0.521±0.05^{b}	0.99
PS30	90.63±21.40^{b}	0.527±0.03^{b}	0.99
P30	85.39±20.69^{b}	0.509±0.05^{b}	0.99
G30	0.767±0.481^{b}	1.61±0.13^{a}	0.99
DYM40	535.611±72.87^{b}	0.281±0.03^{cd}	0.99
TM80	273.458±60.38^{b}	0.376±0.05^{bc}	0.99
SY59	19684±1105^{a}	0.156±0.01^{d}	0.99

a, b, c : Means with different letters in the same column are different from each other ($p<0.05$)

Fourier transform infrared spectrometer (FTIR) Analysis Results

The FTIR spectra of the emulsions are shown in Figure 6. All of the samples showed a strong absorption band around ≈3000-3340 cm^{-1} resulting from the stretching vibrations of the O-H bond. This phenomenon is caused by intramolecular and intermolecular hydrogen bonding. This broad peak that was seen in all the samples is caused by O-H stretching vibrations, which show that hydrogen bonds are present and there is water in the gels (Singh et al., 2013).The peak intensity is highest in sample G30, indicating a higher degree of hydrogen bonding or water content in this formulation. The peaks observed around 2925-2853 cm^{-1} are indicative of the presence of lipid components in the samples. These peaks are associated with C-H stretching vibrations of methyl and methylene groups commonly found in lipids and fatty acids (Forfang et al., 2017). The band near 2907 cm^{-1} corresponds to symmetric stretching of methylene chains in membrane lipids or proteins, while the peak near 2957 cm^{-1} is attributed to asymmetric stretching of methylene groups, further supporting the presence of lipids or proteins (Calabrò et al., 2013). In addition, the high-intensity bands at 2957 and 2907 cm^{-1} are characteristic of the C-H stretching vibrations of lipids and correspond specifically to the methyl and methylene groups of fatty acid chains

(Pereira et al., 2019). The shift of the band from 2108 cm^{-1} to 2173 cm^{-1} in the PSG30 sample indicates a change in the molecular interactions within the emulsion gel. The shift to a higher wavenumber (2173 cm^{-1}) suggests a change in the hydrogen bonding environment. This could mean stronger hydrogen bonds or altered molecular interactions between pectin, soy protein, and gelatin in the PSG30 sample compared to other formulations in emulsion gels. The band around 2108 cm^{-1} is usually associated with C≡C or N≡C stretching vibrations in proteins or lipids. Stronger interactions or changes in the conformation of protein-lipid complexes in the gel can explain the shift to a higher wavenumber. This may be due to the specific combination of pectin, soya protein and gelatin in the PSG30 formulation. Peaks around 1235-1105 cm^{-1} are probably related to C-O stretching vibrations arising from pectin components (Makebe et al., 2020). PSG30 and PS30 samples show different peaks in this region, indicating the presence of pectin and its interaction with proteins. The micrographs on the right side of Figure 6 show the microstructures of samples G30, P30, PS30, and PSG30. The G30 micrograph showed a relatively coarse and less homogeneous structure, which is consistent with the weaker hydrogen bonding and less stable gel network shown by FTIR analysis. P30 similarly showed a weaker structure. PS30 exhibited a more uniform and fine structure, indicating a well-formed gel matrix with strong interactions between pectin and soy protein. This is consistent with the distinct peaks observed in the FTIR spectrum, reflecting strong molecular interactions. PSG30 showed the most uniform and smooth structure, resulting in the most stable and well-integrated gel network among the formulations. The combination of pectin, soy protein, and gelatin showed a robust gel structure, as supported by the FTIR spectrum showing significant peaks for the O-H, C-H, and amide I bands.

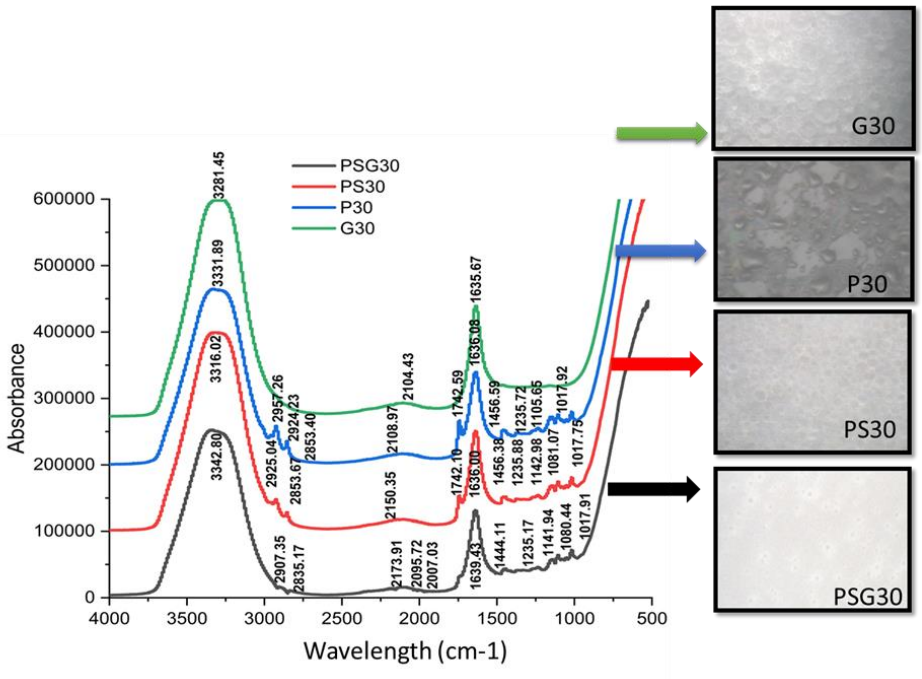

Figure 6. FTIR spectra of emulsion gels.

Molecular Docking Results

Molecular docking is applied to determine the appropriate binding sites between the target protein molecule and the ligand and to obtain information about the binding affinities of the molecules (Kınaytürk, 2023). Accordingly, the binding energies between 7S and 11S globulin, which are the main components of soya protein, and pectin were determined to be -8.97 and -7.74 kcal/mol, respectively. 2D and 3D images of these bonds are given in Figures 7B and C. The repeating heterotrimer structures of glycine (Gly), proline (Pro), and alanine (Ala) amino acids responsible for the triple helix structure of gelatin were simulated with type I collagen, and the binding energy with tetra-galacturonic acid (TGT), the main component of pectin, was determined to be -6.40 kcal/mol. The binding energies of all proteins that interacted with pectin were lower than -5.00 kcal/mol. This indicates that protein binding with pectin is relatively stable (Zhang et al., 2023). It can be suggested that the most stable binding is with 7S globulin. Tetra-galacturonic acid with 7S was linked via hydrogen bonding with the amino acid and bond position: Leu-956, Gln-957, Asp-1157, Asn-1159, Asn-1161, Asn-1161, Thr-1191, His-1192, Gln-1193, Gly-1211, Met-1213, Thr-1215, Thr-1215, Gln-1220, and Arg-1441.The bonding distances of these hydrogen bonds were determined as 2.84, 2.76, 2.94, 2.35, 2.62, 2.44, 2.26, 3.24, 3.34, 2.72, 3.14, 3.53, 3.42, 3.33, and 2.88 Å, respectively. In addition, His-1162 (5.20 Å), Arg-1141 (4.22 Å), and Arg-1441 (5.15 Å) amino acid and binding positions were determined in electrostatic interactions. Arginine (Arg) may contribute to emulsion stability by exhibiting high binding affinity through both electrostatic and hydrogen bonds (Cao et al., 2022). 11S globulin

TGT and residual amino acids Tyr-438, Val-523, Glu-533, His-534, Thr-537, and Gly-542 and their binding sites were hydrogen bonded with a distance of 2.26, 2.15, 2.37, 2.78, 1.82, and 2.99 Å, respectively. It was observed to have a more basic structure compared to 7S, with a closer bonding distance. A salt bridge represents non-covalent interactions combining electrostatic attraction and hydrogen bonding. They are stronger than hydrogen bonds and play an important role in maintaining protein stability (Spassov et al., 2023). 11S globulin also binds with TGT via a salt bridge with the residual amino acid Arg-522 at a distance of 5.12 Å. We examined TGT binding with gelatin (Type 1 collagen) (Figure 7A) and found nine amino acid residues in the hydrogen bond interaction. These are Glu-120 (2.98 Å), Gln-121 (2.27 Å), Gln-121 (3.10 Å), Ser-189 (2.28 Å), Ser-189 (2.98 Å), Glu-190 (3.66 Å), Glu-190 (3.14 Å), Ser-207 (1.99 Å), and Ser-207 (2.24 Å). A salt bridge was also detected between the carboxyl group of amino acid Arg-181 and TGT at a distance of 5.31 Å. The binding between gelatin and TGT may be suppressed by the interaction between 7S and 11S and TGT. This may explain the lower stability of sample PSG30 (Zhang et al., 2023). Furthermore, these findings are in agreement with the results obtained from FTIR analysis.

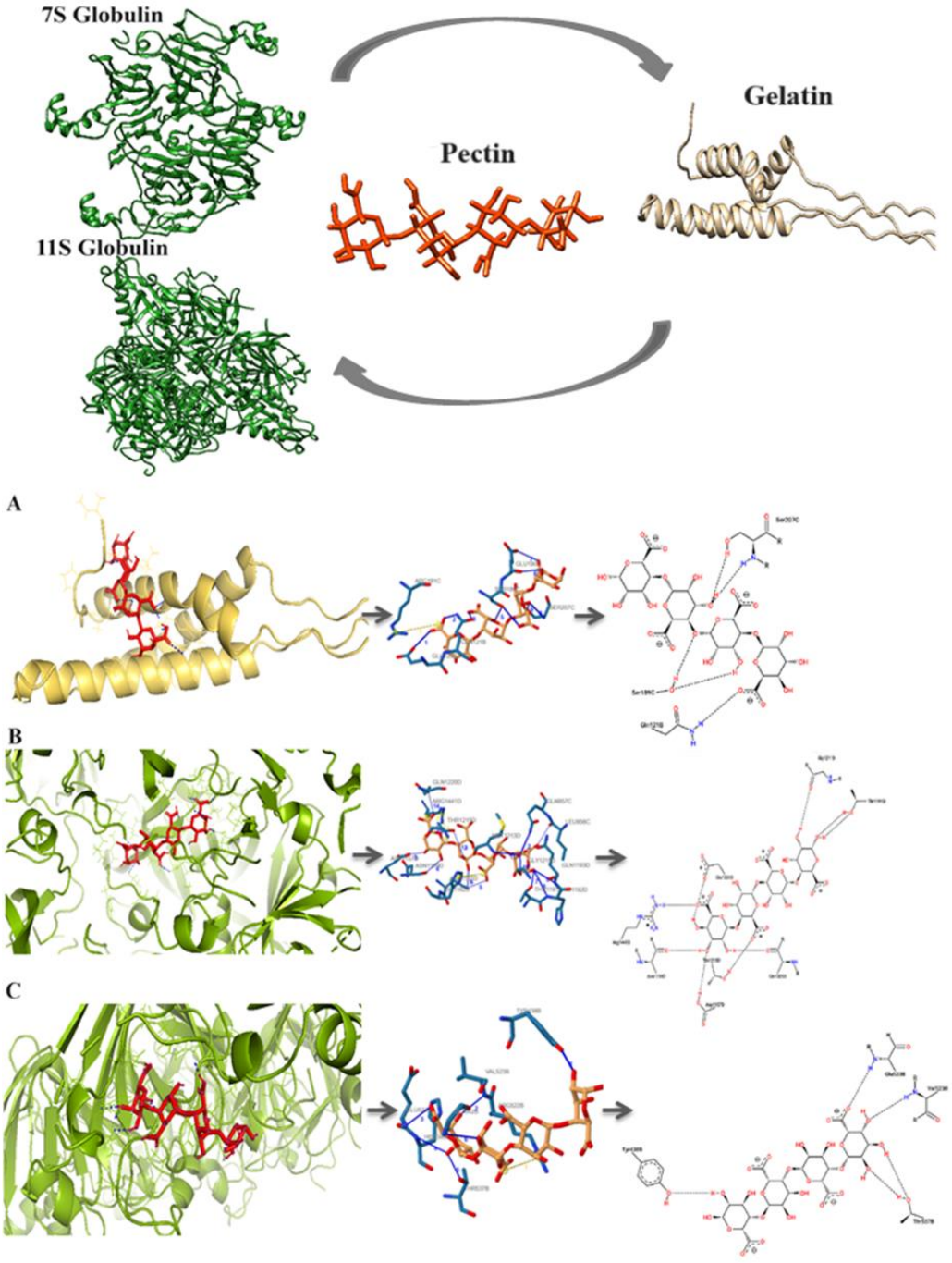

Figure 7. 2D and 3D molecular simulation of the interaction of soya proteins (7S and 11S globulin), gelatin and pectin. A: Gelatin and pectin, B: 7S globulin and pectin, C: 11S globulin and pectin.

CONCLUSION

This study investigated the effects of the combination of pectin, soy protein, and gelatin on consistency and viscosity properties in developing low-fat gel emulsions. PSG30 and PS30 formulations formed thicker emulsions compared to commercial products. Thermorheological analyses revealed that PSG30 and PS30 formulations demonstrated consistent viscosity across a wide temperature range and high thermal stability. The gel structure in PSG30 was the most stable. It had stronger hydrogen bonds or different molecular interactions between pectin, soy protein, and gelatin. It was found that the binding energy between soy protein and pectin was more stable than that of gelatin. This study reveals the synergistic effects of pectin, soy protein, and gelatin in the development of low-fat and high-viscosity food products. The findings provide an important basis for the development and optimization of low-fat emulsion products, especially in the food industry. These formulations have a lot of potential for commercial use. Especially for its high thermal stability and desirable rheological properties.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Determination of energy usage and greenhouse gas (GHG) emissions in artichoke production

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DergiPark AKADEMİK

Abstract
This study has been conducted with the purpose of determining the energy usage (EU) and greenhouse gas (GHG) emissions of artichoke production. It has been conducted in Efeler district of Aydın province of Türkiye during the 2022 production period. According to the results of the study, total input energy (EI) was calculated to be 32 211.48 MJ/ha and output energy (OE) was calculated to be 5 460 MJ/ha. EI in artichoke production were 15 718.20 MJ/ha (48.80%) chemical fertilizers energy, 8 896.98 (27.62%) diesel fuel energy, 3 832.27 (11.90%) machinery energy, 1 958.40 (6.08%) electricity energy, 1 036.35 (3.22%) irrigation water energy, 329.55 (1.02%) human labour energy, 294 MJ/ha (0.91%) plant energy and 145.73 (0.45%) chemicals energy, respectively. Energy use efficiency (EUE), specific energy (SE), energy productivity (EP) and net energy (NE) values were found as 0.17, 4.72 MJ/kg, 0.21 kg/MJ and -26 751.48 MJ/ha, respectively. The total energy inputs that were involved in artichoke production were classified as: 37.94% (12 221.28 MJ/ha) direct (IE), 62.06% (19 990.20 MJ/ha) indirect (IDE), 5.15% (1 659.90 MJ/ha) renewable (RE) and 94.85% (30 551.58 MJ/ha) non-renewable (NRE). Total GHG emission was calculated as 1 401.64 kgCO_{2eq}/hafor artichoke production with the greatest share for diesel fuel (31.11%). GHG ratio value was calculated as 0.21 kgCO_{2eq}/kg in artichoke production.

Keywords: Artichoke, Energy usage, Net energy, Specific energy

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INTRODUCTION

The ancient perennial plant species Artichoke (Cynara scolymus L.) is native to the Mediterranean Basin and has been known since the first century AD. It was widely spred in the southern Mediterranean area during the Middle Ages (Sgroi et al., 2015) by Arabs.Artichoke is particularly widespread in the Mediterranean Basin, where the climate is characterized by warm summers and mild winters (D'Asaro and Grillone, 2012; Leskovar et al., 2013; Sgroi et al., 2015).Artichoke, which has various benefits for human health, is also widely used in the pharmaceutical industry. Artichoke has a diuretic effect. In addition, it is known to be used for purposes such as shedding kidney stones, treating jaundice, increasing bile perception, protecting against arteriosclerosis, and reducing blood fat levels (Eser, 2002; Kenenoğlu Bektaş and Saner, 2013).

Artichoke is a significant vegetable of economic importance in the Asteraceae family. In 2018, a total of 1,680,992 tons of artichoke was produced in 127,472 hectares of land in the world. The countries that produce the most artichokes are Italy, Egypt and Spain. In Turkey, 39,477 tons of artichoke was produced in 3,065 hectares of land in 2018 (FAO, 2018; TÜİK, 2019; Duman and Nas, 2020). İzmir, Aydın, Bursa and Sakarya stand out as the provinces with the highest production in our country. The share of these four provinces constitutes 82% of the total production in Turkey (TÜİK, 2019; Duman and Nas, 2020).

The energy balance sheet to be made in terms of agricultural production is an important approach in defining and grouping agricultural systems in terms of energy consumption. The ratio between the energy equivalent of the product per unit area in any agricultural production branch and the energy equivalent spent for production can be used as an indicator and a benchmark for successful and profitable production. In addition, it also constitutes an important value in terms of the efficient use of energy in today's world where environmental sensitivity is rapidly increasing (Topdemir, 2018; Candemir, 2020).

Energy consumption per unit area in agriculture is directly related to the available technological level and production. The inputs such as fuel, electricity, machinery, seed, fertilizer and chemical take significant share of the energy supplies to the production system in modern agriculture. The use of intensive inputs in agriculture and access to plentiful fossil energy has provided an increase for standards of living and food production. However, some problems in agricultural production have been faced mainly due to high level dependency on fossil energy. In recent years, energy use and associated greenhouse gas emissions and their potential impacts on the global climate change have become worldwide concerns. Improving the end-use energy efficiency is one of the most effective ways to reduce energy consumption in the industrial, commercial, transportation, utility, residential and agricultural sectors and their associated pollutant emissions (Dyer and Desjardins, 2003; Oren and Ozturk, 2006).

Carbon dioxide gas has a significant share in the warming of the world and 80% of this gas originates from fossil fuels. In this sense, reducing our dependence on fossil fuels and increasing the share of renewable energy are very important in the fight against global warming. In our age, fossil fuel prices have increased significantly and, together with concerns arising from climate change, have brought about a series of innovations in the energy sector in terms of both supply and demand. In this context, developments aimed at expanding the use of renewable energy sources are also gaining momentum on a global scale. Carbon dioxide emission is defined as the emission of carbon dioxide resulting from the combustion of carbon-containing fuels (fossil fuels: oil, natural gas, coal, etc.) into the atmosphere. In addition to negatively affecting air quality, the formation of greenhouse gases is also an important problem. Carbon dioxide is not the only gas that causes the temperature of the earth to increase. Various gases such as methane, carbon monoxide, and nitrogen oxides also cause a similar effect (Çelen, 2016).

Several studies have been conducted on EUE and GHG of agricultural production, for instance;on vegetable (Ozkan et al., 2004), on carrot (Celik et al., 2010),on tomato (Ozkan et al., 2011), on lettuce (Kamburoğlu Çebi et al., 2017), on onion (Ozbek et al., 2021), pepper (Baran et al., 2022), garlic (Baran et al., 2023), etc. A review on the literature has been performed and it concluded that no studies were conducted on the EUE and GHG emission of artichoke in the area and therefore the significance of this current study is important.

MATERIALS AND METHODS

The district's altitude above sea level is 40 meters and its surface area is 631 km^2. Efeler district covers the Büyük Menderes Valley, which narrows from west to east. Its elevation is 130 meters in the middle parts and 30 meters in front of Gümüş Mountain. The plain is surrounded by the Aydın Mountains to the north and the northern part of the mountainous Menteşe region to the south. Summers are very hot and winters are mild in the plain parts of the district. The average temperature in summer is 28.3 °C and in winter is 8.1 °C (Anonymous, 2024a). The district is located at 37.8402 latitude and 27.8379 longitude (Anoymous, 2024b).

This current study has been conducted in Efeler district of Aydınprovince in Türkiye during the 2022 production period. The area that was studied spanned over a 0.10 ha artichoke production area. Randomized complete-block design with three replications has been performed. The amount of fuel usage wascalculated and full-tank method wasusaged to achieve this. The amount of fuel used per unit area was determined to measure the trial area and the amount of fuel that was placed in the tank (Göktürk, 1999; El Saleh, 2000; Sonmete and Demir, 2007).

The work productivity for the area wascalculated and it was deemed to be an effective productivity. Work productivity in (ha/h) was achieved by calculating the effective working time (t_{ef}) (Güzel, 1986; Özcan, 1986; Sonmete, 2006).

Time durations weredetermined in the study with the help of a chronometer (Sonmete, 2006).The energy equivalents and GHG equivalents of inputs in artichoke productionwere shown in Table 1 and Table 2, respectively. According to Mohammadi et al. (2010); EUE, SE, EP and NE were calculated by using the formulates (Mandal et al., 2002; Mohammadi et al., 2008).

$$\text{Energy use efficiency} = \frac{\text{Energy output } (\frac{\text{MJ}}{\text{ha}})}{\text{Energy input } (\frac{\text{MJ}}{\text{ha}})} \quad (1)$$

$$\text{Specific energy} = \frac{\text{Energy input } (\frac{\text{MJ}}{\text{ha}})}{\text{Product output } (\frac{\text{kg}}{\text{ha}})} \quad (2)$$

$$\text{Energy productivity} = \frac{\text{Product output } (\frac{\text{kg}}{\text{ha}})}{\text{Energy input } (\frac{\text{MJ}}{\text{ha}})} \quad (3)$$

$$\text{Net energy} = \text{Energy output (MJ/ha)} - \text{Energy input (MJ/ha)} \quad (4)$$

Table 1. Energy equivalents in artichoke production.

Inputs	Unit	Energy Equivalent (MJ/unit)	References
Human labour	h	1.96	Mani et al. 2007; Karaağaç et al. 2011
Machinery	h	64.80	Singh,2002; Kizilaslan, 2009
Chemical fertilizers			
N	kg	60.60	Singh, 2002; Ekinci et al., 2020
P	kg	11.10	Singh, 2002; Ekinci et al., 2020
K	kg	6.70	Singh, 2002; Demircan et al., 2006
Chemicals	kg	101.20	Yaldız et al., 1993; Demircan et al., 2006
Diesel fuel	L	56.31	Singh 2002; Demircan et al., 2006
Irrigation water	m^3	0.63	Yaldız et al., 1993
Electricity	kWh	3.60	Ozkan et al., 2004
Plant	Unit	0.28	Canakci and Akinci, 2006
Output	kg	0.80	Ozkan et al., 2004

Table 2. GHG emissions coefficients in artichoke production.

Inputs	Unit	GHG Equivalent ($kgCO_{2eq}$/unit)	References
Machinery	MJ	0.071	Dyer, J.A. and Desjardins, 2006; Ekinci et al., 2020
N	kg	1.300	Lal, 2004;Ozalp et al., 2018
P	kg	0.200	Lal, 2004;Ozalp et al., 2018
K	kg	0.200	Taghavifar and Mardani 2015; Ozalp et al., 2018
Chemicals	kg	13.900	Biograce, 2015; Eren et al., 2019
Diesel fuel	L	2.760	Clark et al., 2016; Eren et al., 2019
Electricity	kWh	0.608	Khoshnevisan et al., 2013; Ozalp et al., 2018

Eren et al. (2019) concluded that the GHG emissions ($kgCO_{2eq}$/ha) that take place through the inputs usaged to grow 1 ha of fruit werecomputed as follows, as adapted by Hughes et al. (2011).

$$GHG_{ha} = \sum_{i=1}^{n} R(i)\, x\, EF(i) \quad (5)$$

Eren et al. (2019) stated as follows, $\sum$ where *R(i)* is the application rate of input *i* ($unit_{input}$/ha) and EF(*i*) is the GHG emission coefficient of input *i* ($kgCO_{2\text{-}eq}/unit_{input}$). However, an index is defined to evaluate the amount of emitted $kgCO_{2\text{-}eq}$ per kg yield. This is indicated in the following formula adapted Houshyar et al. (2015) and Khoshnevisan et al. (2014), where I_{GHG} is GHG ratio and Y is the yield as kg per ha.

$$I_{GHG} = \frac{GHG_{ha}}{Y} \quad (6)$$

The input energy can be categorized into D, IDE, RE and NRE forms(Mandal et al., 2002; Singh et al., 2003; Koctürk and Engindeniz, 2009). Energy balance, energy utilization efficiency computations, energy inputs types, GHG emissions of inputs related to artichokeproduction are presented in Tables 3 to 6, respectively.

RESULTS AND DISCUSSION

In this study, the average amount of artichoke produced per hectare was calculated as 6 825 kg for the 2022 production season. As indicated in Table 3, EI in artichoke production, 15 718.20 MJ/ha (48.80%) chemical fertilizers energy, 8 896.98 (27.62%) diesel fuel energy, 3 832.27 (11.90%) machinery energy, 1 958.40 (6.08%) electricity energy, 1 036.35 (3.22%) irrigation water energy, 329.55 (1.02%) human labour energy, 294 MJ/ha (0.91%) plant energy and 145.73 (0.45%) chemicals energy, respectively. Total EIwascalculated as 32 211.48 MJ/ha,OEwas calculated as 5 460 MJ/ha.

In previous studies, Celik et al. (2010) determined fertilizer energy had the biggest share by33.19% in carrot (conventional)production, Ozkan et al. (2004) determinedfertilizer energy had the biggest share by 29.42% in

pepper production, Ozkan et al. (2011) determined fertilizer energy had the biggest share by 38.22% in tomato production,etc.Yield, EI, EO, EUE, SE, EP and NE in artichoke production were calculated as 6 825 kg/ha, 32 211.48 MJ/ha, 5 460 MJ/ha, 0.17, 4.72 MJ/kg, 0.21 kg/MJ and -26 751.48 MJ/ha, respectively (Table 4). In previous studies, Celik et al. (2010) determined (conventional carrot) EUE as 1.30, Ozkan et al. (2004) determined (pepper) EUE as 0.99, Ozkan et al. (2011) determined (tomato) EUE as 0.80.

Table 3. Energy usagein artichoke production.

Inputs	Unit	Energy Equivalent (MJ/unit)	Input Per Hectare (Unit/ha)	Energy Value (MJ/ha)	Ratio (%)
Human labour	h	1.96	168.14	329.55	1.02
Machinery	h	64.80	59.14	3832.27	11.90
Chemical fertilizers				15 718.20	48.80
N	kg	60.60	207	12544.20	38.94
P	kg	11.10	161	1787.10	5.55
K	kg	6.70	207	1386.90	4.31
Chemicals	kg	101.20	1.44	145.73	0.45
Diesel fuel	L	56.31	158	8896.98	27.62
Irrigation water	m^3	0.63	1645	1036.35	3.22
Electricity	kWh	3.60	544	1958.40	6.08
Plant	Number	0.28	1050	294	0.91
Total inputs	-	-	-	32 211.48	100
Output	Unit	Energy equivalent (MJ/unit)	Output per hectare (unit/ha)	Energy value (MJ/ha)	Ratio (%)
Product	kg	0.80	6 825	5 460	100
Total output	-	-	-	5 460	100

Table 4. EUE computations in artichoke production.

Calcutations	Unit	Values
Product	kg/ha	6 825
EI	MJ/ha	32 211.48
EO	MJ/ha	5 460
EUE	-	0.17
SE	MJ/kg	4.72
EP	kg/MJ	0.21
NE	MJ/ha	-26 751.48

As seen in Table 5, the total EIusaged in artichoke production could be classified as 37.94% (12 221.28 MJ/ha) DE, 62.06% (19 990.20 MJ/ha) IDE, 5.15% (1 659.90 MJ/ha) RE and 94.85% (30 551.58 MJ/ha) NRE. NRE was bigger than the ratio of RE in EI of artichoke production. Similarly, in previous studies on tomato (Ozkan et al., 2011), on onion (Ozbek et al., 2021), on pepper (Baran et al., 2022), among others, yielded results where the ratio of NRE was higher than the ratio of RE.

Table 5. EIin the forms of energy for artichoke production.

Energy Types	EI (MJ/Ha)	Ratio (%)
DE	12 221.28	37.94
IDE	19 990.20	62.06
Total	32 211.48	100
RE	1 659.90	5.15
NRE	30 551.58	94.85
Total	32 211.48	100

The results of GHG emissions of artichoke production are given in Table 6. The total GHG emission was calculated as 1 401.64 $kgCO_{2eq}$/ha (0.21 $tonCO_{2eq}$/ha). The results of the study given to the fact that the share of diesel in total GHG emissions had the highest value 31.11%, machinery 19.41, N (nitrogene) 19.20% held the second and third place. GHG ratio (per kg) wascalculated as 0.21. In previous studies on the subject, Ozbek et al. (2021) calculated the total GHG emission of artichoke production as 2.92 $tonCO_{2eq}$/ha, Baran et al. (2022)

calculated the total GHG emission of pepper production as 3.70 tonCO_{2eq}/ha, Baran et al. (2023) calculated the total GHG emission of garlic production as 8.63 tonCO_{2eq}/ha.

Table 6. GHGEmissions in artichoke production.

Inputs	Unit	GHG Coefficient (kgCO_{2eq}/unit)	Input usaged per area (unit/ha)	GHG Emissions (kgCO_{2eq}/ha)	Ratio (%)
Machinery	MJ	0.071	3 832.27	272.09	19.41
N	kg	1.300	207	269.10	19.20
P	kg	0.200	161	32.20	2.30
K	kg	0.200	207	41.40	2.95
Chemicals	kg	13.900	1.44	20.02	1.43
Diesel fuel	L	2.760	158	436.08	31.11
Electricity	kWh	0.608	544	330.75	23.60
Total	-	-	-	1401.64	100.00
GHG ration (per kg)	-	-	-	0.21	-

CONCLUSION

This current study aimed todeterminethe energy balance and GHG emissions in artichoke production. Study results are summarized below. EUE, SE, EP and NE in artichoke production werecalculated as 0.17, 4.72 MJ/kg, 0.21 kg/MJ and -26 751.48MJ/ha, respectively.

The highest energy input in artichoke production was determined to be chemical fertilizers energy by 48.80% (12 544.20 MJ/ha). The total energy inputs usage in artichoke production could be classified as 5.15% RE and 94.85% NRE. Usage of chemical fertilizers usage can be deemed and usage of farm fertilizers should be increased in order to riseEUE. The total GHG emissions werecalculated as 1 401.64 kgCO_{2eq}/ha (1.40 tonCO_{2eq}/ha) and GHG rate (per kg) as 0.21.

The findings of thestudyshowed that the ratio (NRE) of diesel fuel in total GHG emissions had the highest value by 31.11%.

According to Akbolat et al. (2014), artichoke production is not a profitable production activity in terms of EUE (0.17). Machinery-use related fuel expenses could be deemed by using RE terms (Yıldız, 2023).

The conscious use of fertilizers and chemical inputs will ensure more efficient use of energy. According to the results of the energy production function estimation, machinery and diesel use showed negative impacts on energy production. These results are likely related to the excessive use of inputs. Consequently,machinery and diesel inputs should be used more carefully to increase energy productivity and efficiency in the research area. The variability in input use among pomegranate producing farmers was relatively high, determining the need to improve individual farm management abilities (Ozalp et al., 2018).

By taking the above recommendations into consideration, EUE in artichoke production can be increased, production can be made economical in terms of energy use and GHG emissions can be reduced.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declared that there is no conflict

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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Effects of classical and organomineral fertilizer applications on pollen quality and quantity in gemlik olive cultivar

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Abstract
Olive is one of the important plants worldwide in terms of economic and cultural conditions. It is used in fresh consumption, olive oil, cosmetic and pharmaceutic fields and unique numinous plants. Olive can maintain its life for many years even in barren conditions but for an economic cultivation, fertile and preferred cultivars should be used in orchards and optimum maintenance conditions should be provided. At the same time the climate change is also convert the usual behavior of the plants. This study aims to determine the effects of classic and organomineral fertilizers on pollen quality and quantity parameters. In this study, classical fertilizers like Urea+$MgSO_4$ and KNO_3+H_3BO_3+$ZnSO_4$ with Raykat Start, Raykat Growth and Fitomare organomineral fertilizers were sprayed foliarly before flowering on Gemlik olive cultivar and pollen studies were conducted on the flowers formed at full flowering. Within the study, effects of the treatments were evaluated on pollen viability and germination levels, the amount of pollen produced in one flower and the normally developed pollen ratio. As a result of this study, fertilization treatments positively affected pollen quality and quantity with regard to control treatment. In this context, the pollen viability level differed between 74.15% and 89.92%, the pollen germination level between 45.48% and 70.35% and pollen per flower was between 307.238 and 446.761. The lowest data were obtained from control treatments, while the highest were from Raykat group fertilizers. Especially, the 54% increase in pollen germination level with Raykat growth fertilization was one of the most important results of this study. In conclusion, it was determined that the foliar spray of organomineral fertilizers enhanced the pollen properties. Raykat Start, Raykat Growth and Fitomare organomineral fertilizers used in this study significantly increased pollen quality and quantity.

Keywords: *Olea europea*, Fertilization, Pollen, Climate change, Fruit set

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INTRODUCTION

Olive cultivation in the World is economically carried out in countries bordering the Mediterranean or in the microclimate regions of continents and countries with a Mediterranean climate (Polat and Tunalıoğlu, 2012). Olive (*Olea europea* L.) is a multifunctional Mediterranean plant with an extraordinary history and tradition that can live for many years and has an important role in table and oil production (Sales et al., 2020). Although the olive tree is drought tolerant, it is one of the agricultural products that will be significantly affected by global climate changes. It is necessary to determine the precautions to take for olive plantations in order not to be affected by increasing climate change problems (Sevim et al., 2022). Within the scope of these precautions; determining the effects of maintenance conditions, the plant's water use efficiency and effective fertilization methods on vegetative and generative production are among the primary factors.

In recent years, problems related to fruit set problems in olive varieties have become widespread. Since low yield is mostly due to pistil and stamen development problems, it is necessary to know the obstacles that may be

experienced in the development of pollen characteristics and ovule and to develop solutions for these problems (Ateyyeh et al., 2000). Within the scope of researching these problems pollen viability of olive varieties (Mete et al., 2015), pollen germination (Atteyeh et al., 2000; Acarsoy et al., 2011; Dölek-Gencer et al., 2023), pollen tube development (Selak et al., 2013), studies on pistil and ovule situations (Rapaport et al., 2022) were increased in recent years.

Nutrition in the olive plant enables the plant to have a better physiology and therefore improves the pollen properties by allowing the plant to produce stronger pollen and pollinate better (Sharafi and Raina, 2021). Although pollen is an anatomically simpler structure compared to other differentiated tissues and plant organs, it is responsible for carrying the male gamete to the pistil of the flower (Patel and Mankad, 2014). For this reason, pollen is a structure that must have high performance to ensure a successful fruit set in species that do not form fruit parthenocarpically.

Determining the effects of different treatments on olive pollen quality and quantity in Mediterranean climate conditions, where the effects of climate change are seen at high levels, is of great importance in protecting fruit sets against climate change. In this study, it was aimed to determine the effect of recently produced organomineral fertilizers and classical fertilizers on pollen viability and germination levels and pollen production amount in the Gemlik olive variety.

MATERIALS AND METHODS

Materials

This study was conducted in an olive orchard at 400 m above sea level in the Düziçi district of Osmaniye province during the 2019-2020 growing season. In the experiment, 13-year-old Gemlik olive variety was used, which was planted at 6x6 m intervals. Routine maintenance conditions were carried out in optimum and disease and pest treatments were made with the instructions so that the pollen was not affected.

In the study, 5 different fertilizer applications consisting of classical and new organomineral fertilizers with a control treatment were applied foliarly before flowering. The applications are arranged as follows.

1. Urea+$MgSO_4$ (Urea+Mg): 0.5% urea and 0.5% magnesium sulfate were mixed.
2. KNO_3+H_3BO_3+$ZnSO_4$ (K+B+Zn): 0.5% doses of all three fertilizers were mixed.
3. Raykat Start (R-Start): 300cc Raykat Start was added to 100lt of water.
4. Raykat Growth (R-Growth): 300 cc Raykart Growth was added to 100 lt of water.
5. Fitomare: 300 cc Fitomare was added to 100 lt of water.
6. Control: Water application was made simultaneously with other applications.

Methods

Treatments were arranged in 3 replications with one tree in each replication. Ferti-Vant organic content surfactant was used with the treatments. All treatments were made in the morning before flowering as a single dose by using an automatic sprayer.

As a result of the treatments made in the experiment, pollen viability and germination levels and pollen production were determined. In this context, in order to obtain pollen, branches containing buds that had not yet opened but were about to bloom were cut and placed in water under laboratory conditions. In this way, the flowers were opened and released their pollens. Obtained pollen was used in pollen viability and germination tests. Flower buds that will open within one day were used for pollen production.

In vitro pollen viability tests: Pollen viability tests were made by 1% 2,3,5 TTC (Triphenyl Tetrazolium Chloride) solution prepared according to Norton (1966). In this context, 3 slides were prepared for each treatment and at least 100 pollen counts were made on each slide. An Olympus BX 51 light microscope was used for counts. During counting, pollens stained dark red were considered "viable" light red or pinkies were considered "semi-viable," and colorless ones were considered "non-viable." During calculation, 50% of the semi-viable pollen was assumed to be viable, along with the separate rates of each group and this value was added to the viable pollen and the "viability rate" was determined by calculation.

In vitro pollen germination tests: For pollen germination experiments, the “agar in petri method was used” with a germination medium containing 1% agar + 15% sucrose added to 100 ppm Boric acid solution (Ilgın et al., 2007; Dölek-Gencer et al., 2023). Counts were made under an Olympus BX51 light microscope. During counting, the pollens with pollen tubes longer than their own diameter were considered as germinated and the effect of the treatments on the pollen germination rate was determined. In pollen germination experiments, 3 petri dishes were prepared for each application and at least 100 pollen counts were counted in each petri dish.

Determination of Pollen Production: The amount of pollen production in the flowers obtained as a result of the applications was determined by the "hemocytometric method". For this purpose, thirty flowers were taken from the branches taken at full bloom and brought to the laboratory, from flower buds that had not yet opened but were about to open. Three replicates were formed with 10 flowers for each. Individual anthers of flowers taken from each replicate were separated from their filaments and placed in small plastic boxes. The prepared boxes with anthers were kept for 15 days for the anthers to dry. In order to perform pollen counts, pollens were prepared as explained by Eti (1990) and Eti et al. (1996). The pollen counts were made under an Olympus BX51 microscope.

The amount of pollen in a flower was calculated using the calculation as stated by Eti (1990). During the counting, normally developed pollen rates were also determined by counting the amount of pollen that did not show normal development or malformed shape and the amount of pollen that developed normally (Anvari, 1977).

Analysis of variance was applied to the data obtained in the study using the JMP statistical package program and the differences were determined using the LSD test. The arc-sin transformation was applied to the calculated percentage values.

RESULTS AND DISCUSSION

In this study, pollen viability and germination levels, pollen production amount and normally developed pollen rates were determined in the flowers obtained from 5 different classical and organomineral fertilizer treatments on the Gemlik olive variety.

Pollen Viability Rate

The viable, semi-viable and non-viable pollen ratios with pollen viability rates determined from 6 different treatments are given in Table 1. The table shows that the effect of the treatments on the pollen viability levels was statistically significant. In terms of viable pollen rates; R-Start, K+B+Zn and R-Growth fertilizers had the highest viability levels (85.71%, 78.84% and 77.81%, respectively), while Fitomare and Control applications had the lowest rates (60.90% and 64.35%, respectively).

In terms of semi-viable pollen, it was noted that Fitomare treatment had the highest semi-viable pollen rate. In the study, it was determined that the non-viable pollen rates indicating the amount of poor-quality pollen, varied between 3.59% (K+B+Zn) and 16.05% (Control).

The pollen viability levels, which were theoretically determined considering that half of the semi-viable pollen was alive, in parallel with the viable pollen rates. In this context, it was determined that the highest pollen viability rates were obtained from R-Start (89.92%) and K+B+Zn (87.63%) treatments. The lowest viability level was seen in the control application with a rate of 74.15%.

In a study made by Acarsoy et al. (2011) on Domat olive variety, it was reported that as a result of 9 different fertilizer applications also containing Boron and Potassium, the combined application of Liquid Boron + Urea + KNO3 showed the highest values in terms of pollen vitality levels (92.20%). In another study, the pollen viability level of the Gemlik olive variety in Tarsus conditions was 81.51% (Dölek Gencer et al., 2023). In this study, it was determined that pollen viability levels were sufficient in both fertilization and control treatments. Studies have shown that pollen viability levels may change depending on genetics, ecology and years (Ferri et al., 2008; Mete et al., 2015). At the same time, nutrition and maintenance conditions are also important in this change (Acarsoy et al., 2011; Karataş, 2022).

Table 1. Effects of foliar fertilizers on pollen viability levels in Gemlik olive cultivar (%)[1]

Treatments	Viable (A)	Semi-viable (B)	Non-viable (C)	Viability (A+B/2)
Urea+Mg	76.95 ab[2]	14.79 bc	8.26 abc	84.35 ab
K+B+Zn	78.84 a	17.57 b	3.59 c	87.63 a
Raykat Start	85.71 a	8.41 c	5.88 bc	89.92 a
Raykat Growth	77.81 a	12.05 bc	10.14 ab	83.84 abc
Fitomare	60.90 c	31.19 a	7.91 bc	76.50 bc
Control	64.35 bc	19.59 b	16.05 a	74.15 c
P	**	**	*	*

(1): Data were analyzed after arc-sin transformation
(2): Different letters in the same column are statistically important *means P<0.05; ** means P<0.01

Pollen Germination Level

The effect of fertilizers applied in the experiment on the pollen germination level is given in Table 2. It was determined that pollen germination levels were statistically affected by the treatments at a level of 1%. In this context, it was determined that R-Growth significantly affected pollen germination compared to other treatments as 70.35%, followed by Fitomare (59.42%), Urea+Mg (57.89%), K+B+Zn (52.25%) and R-Start (50.67%) treatments, respectively. The lowest germination level was obtained from the Control application at 45.48%.

Pollen germination and pollen tube development on stigma in fruit trees are the most important features in terms of pollen quality. A high level of pollen germination and rapid pollen tube development are required for effective pollination. Otherwise, there may be problems in pollen tubes reaching the end of the ovule's lifespan (Sharafi., 2011).

In a study conducted under Izmir conditions, it was reported that the application of Liquid Boron + Urea + KNO_3 through 9 different fertilizers on the Domat olive variety showed the highest values (52.80%) in terms of pollen germination level (Acarsoy et al., 2011). In another study, Spinardi and Bassi (2012) stated that the use of Boron in olives could increase germination by up to 6.3 times, reaching 48% in varieties with low pollen germination levels.

Studies showed that fertilizer treatments positively affect fruit set (Gündeşli and Nikpeyma, 2016), and boron-containing fertilizers are especially important in terms of pollen (Nyamora et al., 2000; Acarsoy et al., 2011;

Sarıdaş et al., 2021). Dell and Huang (1997) reported that the amount of boron contained in the plant is closely related to events such as fertilization, seed set and germination, while the low levels of boron in flowers show their primary effects on fertilization by reducing microspore formation and pollen tube development. In this context, it is thought that fertilizing the plant with boron-containing fertilizers will directly affect fruit set by increasing pollen germination level. Considering that Raykat group fertilizers, among the boron-containing organomineral fertilizers used in the study, contain 0.03% Boron and Fitomare contains 0.35% Boron, which directly shows the reason for the increase in pollen germination with the use of these fertilizers.

Table 2. Effects of foliar fertilizers on pollen germination levels in Gemlik olive cultivar (%)[1]

Treatments	Pollen germination
Urea+Mg	57.89 b[2]
K+B+Zn	52.25 bc
Raykat Start	50.67 bc
Raykat Growth	70.35 a
Fitomare	59.42 b
Control	45.48 c
P	**

(1): Data were analyzed after arc-sin transformation
(2): Different letters in the same column are statistically important ** means P<0.01

Amount of Pollen Production

As a result of fertilization, the amount of pollen in a flower and the values of the normally developed pollen ratio are given in Table 3. Although the differences between the treatments in terms of the amount of pollen in a flower were not statistically significant, it was determined that higher values were obtained in all fertilization applications compared to the Control application. In this context, the highest pollen production was determined to be in the R-Start (446.761 number), followed by the Fitomare (379.458 number). In control, it was determined that 307.238 pollen were produced in one flower which was noted that this value was the lowest amount of pollen production among the treatments.

It was determined that the values in terms of normally developed pollen rate were very close, ranging between 97.08% and 99.12% and the differences between the treatments were not statistically significant.

In a study conducted in Tarsus conditions, it was reported that the amount of pollen production in the Gemlik variety was 306.385 number and the amount of pollen production was found to be higher in the full-flowering period compared to the first-flowering and end-blooming periods (Dölek-Gencer et al., 2023). Rojo et al. (2015) determined that the amount of pollen in one olive flower was around 1.10x105. Researchers also pointed out that the amount of pollen production varies according to inflorescence structure, flowering and alternate bearing features. Although previous studies have not determined the effect of fertilization on the amount of pollen production in olives, in strawberries (Sarıdaş et al., 2021) and lemons (Karataş, 2022), the increase in pollen production was proved by using boron-containing fertilizers.

In addition to the amount of pollen produced in the flowers of a variety, the high rate of normally developed pollen is also of great importance. Eti (1992) reported that having a normally developed pollen ratio above 80-90% is important for pollen quality. Karataş (2022) reported that pruning, irrigation and Phosphorus, Zinc and Boron fertilization in lemon plants increased pollen production and the amount of normally developed pollen.

Previous studies have shown that boron fertilization treatments increase the quality and quantity of pollen, and at the same time it positively affects fruit set (Nyomora et al., 2000; Spinardi and Bassi, 2012; Sarıdaş et al., 2021; Karataş, 2022). In this study, it was determined that fertilization generally increased the quality rates and pollen production. Organomineral fertilizers, in particular, enable the production of higher quality and greater quantity of pollen compared to other treatments. In addition, in another part of the same project it has been proven that Raykat Growth organomineral fertilizer treatment stands out in terms of yield per tree, fruit quality and olive oil content (Çelik et al., 2023a; Çelik et al., 2023b).

Table 3. Effects of foliar fertilizers on pollen production per flower and normally developed pollen ratio in Gemlik olive cultivar

Treatments	Pollen Production Per Flower (number)	Normally Developed Pollen Ratio (%)[1]
Urea+Mg	354 698	98.65
K+B+Zn	345 391	98.27
Raykat Start	446 761	99.12
Raykat Growth	347 514	98.45
Fitomare	379 458	97.08
Control	307 238	97.78
P	N.S.[2]	N.S.

(1): Data were analyzed after arc-sin transformation
(2): N.S. means non-significant

CONCLUSION

Olive cultivation has been greatly affected by climate change in recent years. Climate change affects the reproductive organs the most and this directly causes a decrease in fruit set. Considering that drought and heat stress will increase in the future, it is thought that additional precautions must be taken immediately for plants to have a stronger structure, so that plant reproductive organs do not succumb to these disadvantages and complete fruit set in a healthy way. In this study which was planned based on this idea, it was determined that different organomineral and classical fertilizer treatments positively affected the quality and quantity of pollen in Gemlik olive variety compared to the control. Among the fertilizers used, Raykat Growth, Raykat Start and Fitomare organomineral fertilizers during the flowering period significantly affected the pollen properties.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

All authors declare that they have no conflicts of interest.

Author contribution

Şenay Karabıyık (Ş.K), Olcay Çelik (O.Ç), Mehmet Ali Sarıdaş (M.A.S) and Sevgi Paydaş Kargı (S.P.K) carried out experimental part of the study. Ş.K. done laboratory tests, statistical analysis and wrote the paper. O.Ç. carried out the field studies, M.A.S. and S.P.K. reviewed the manuscript.

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Trend analysis of cotton production and trade

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Abstract
The aim of this study is to analyse the cultivation areas, production amounts and foreign trade data of cotton plant in the world and Turkey from an economic point of view. The data on Turkey's cotton cultivation area, cotton production and fiber cotton import and export data for the 20-year period 2003-2022 were obtained from the Food Agricultural Organization (FAO). The data set was transferred to MS Excel spreadsheets and the trend analysis technique was used in MS Excel programme to obtain the trends in cotton cultivation area, cotton production amount and near future forecast values for eight years between 2023-2030. In addition, for the foreign trade balance data obtained from fiber cotton import and export data for the period 2000-2022, the change trends of the 23-year period and the eight-year near future forecast values between 2023-2030 were calculated. Cotton cultivation area has a decreasing trend between 2023-2030. While the forecast value of cotton cultivation area for 2023 is 417 thousand hectares, this value is predicted to decrease to 373 thousand 722 hectares in 2030. The trend in the amount of cotton production has a decreasing trend after 2022 and it is predicted that there will be no break in the amount of production between 2023-2030. The estimated foreign trade deficit in fiber cotton for 2023 is 964,516 tonnes and for 2030 is 1,106,672 tonnes. The main reasons for the decrease in cotton cultivation areas are the increases in input prices such as fertilisers, pesticides and seeds. In order to eliminate the foreign trade deficit in cotton, studies should be carried out to enter new markets and increase the share in existing markets. In addition, it should be aimed to create a sustainable cotton sector by investing in R&D activities and entering new markets.

Keywords: Cotton Production, Trend Analysis, Export, Import

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INTRODUCTION

Cotton is an important source of fiber, providing raw materials for a range of industries, not only textiles (Yılmaz et al., 2005). Cotton has a variety of uses, but its most prominent feature worldwide is that it provides natural fiber. Among the valuable agricultural products, cotton's unique fibers are the driving force of the textile industry. Today, the increasing use of synthetic fibers in textiles and apparel has reduced cotton's share of the global fiber market by up to 35%, but cotton is still the most important source, used as the main natural fiber in textile and medical applications. As a renewable resource, cotton makes a significant contribution to the economy by providing employment of economic factors, natural resources and labor force with this feature and irreplaceability (Tokel, 2021).

The oil obtained from the cotton seed, called cottonseed, is used in a variety of fields ranging from human nutrition to the cosmetics industry, while the pulp obtained after oil extraction is utilized as an important feed source in animal nutrition.

Cotton is an industrial plant that directly concerns many countries around the world in terms of production and consumption. Nearly all of global cotton production (99.5%) is concentrated in a select group of countries that rank among the top ten cotton producers worldwide. These nations include China, India, the United States, Brazil, Australia, Turkey, Pakistan, Uzbekistan, Argentina, and Greece.

In our country, cotton cultivation areas, which had a downward trend due to the unfavorable conditions in the cotton balance sheet, was 359 thousand hectares in the 2020/21 season, the lowest value of the last forty years,

increased to 432 thousand hectares in the 2021/22 season with the improvement in the balance sheet, and as a result of the improvement in the balance sheet in the same season, the cultivation areas increased and increased to 550 thousand hectares in the 2022/23 season (ICAC, 2022).

According to the Food and Agriculture Organization of the United Nations (FAO) data for 2022, cotton is cultivated on a total of 32 million hectares in 84 different countries around the world. India, the USA and China, which rank in the top three in terms of the size of agricultural areas allocated for cotton production, are home to approximately 59% of the total cotton production areas in the world (Table 1).

Table 1. Cotton cultivation areas in the world by country (ha) (FAO, 2024)

Countries	2018	2019	2020	2021	2022	Average	%
India	12.586.000	12.614.000	13.477.000	13.285.890	12.371.520	12.866.882	39,48
USA	4.130.190	4.699.460	3.348.610	4.157.100	3.011.180	3.869.308	11,87
China	3.354.410	3.450.000	3.250.000	3.028.170	3.000.000	3.216.516	9,87
Pakistan	2.372.968	2.517.287	2.078.899	1.936.969	2.143.605	2.209.946	6,78
Brazil	1.150.014	1.627.163	1.633.091	1.369.430	1.648.836	1.485.707	4,56
Uzbekistan	1.108.246	1.050.631	1.057.794	1.022.448	1.026.858	1.053.195	3,23
Burkina Faso	473.375	590.999	566.635	611.325	692.036	586.874	1,80
Mali	698.184	738.193	164.833	720.093	596.093	583.479	1,79
Benin	640.000	670.000	620.000	640.000	580.000	630.000	1,93
Turkmenistan	546.351	551.061	620.797	620.000	580.000	583.642	1,79
Turkey	518.634	477.807	359.220	432.279	573.223	472.233	1,45
Other countries	5.182.585	4.990.256	4.959.107	4.819.255	5.203.383	5.030.917	15,44
World	32.760.957	33.976.857	32.135.986	32.642.959	31.426.734	32.588.699	100,00

Almost all cotton cultivation in Turkey is carried out in the Aegean Region, Southeastern Anatolia Region, Çukurova and Antalya regions (Anonymous, 2018). Cotton cultivation areas by region are given in Table 2. Accordingly, it is seen that the region with the highest cotton cultivation area in 2023 is the Southeastern Anatolia Region with an area of 2 million 998 thousand decares and a share of 60.27% (TUIK, 2024) (Table 2).

Table 2. Cotton cultivation areas by regions (decares) (TUİK, 2024)

Regions	2020	2021	2022	2023	Average	%
Mediterranean	679.991	722.016	929.841	680.658	753.127	16,35
Aegean	1.011.626	979.762	1.211.686	1.093.400	1.074.119	23,32
Southeast Anatolia	1.895.537	2.619.897	3.587.358	2.998.800	2.775.398	60,27
Other regions	5.046	1.115	2.728	1.526	2.604	0,06
Turkey	3.592.200	4.322.790	5.731.613	4.774.384	4.605.247	100,00

In 2022, total world cotton production amounted to 69 million 668 thousand tons, with China, India and the USA accounting for approximately 62% of the total production. Turkey's cotton production amount in 2022 is 2 million 750 thousand tons and ranks seventh in the world in terms of production amount (Table 3).

Table 3. Amounts of cotton production in the world by countries (tonnes) (FAO, 2024).

Countries	2018	2019	2020	2021	2022	Average	%
China	18.493.333	23.504.576	17.910.606	17.366.363	18.121.818	19.079.339	25,69
India	14.657.000	18.558.000	17.731.050	17.204.000	14.990.000	16.628.010	22,39
USA	11.098.490	12.765.628	9.204.679	11.559.278	8.468.691	10.619.353	14,30
Brazil	4.956.125	6.893.340	7.070.136	5.711.692	6.422.030	6.210.665	8,36
Uzbekistan	2.285.560	2.691.698	3.063.998	3.372.924	3.500.680	2.982.972	4,02
Australia	2.450.000	1.150.000	290.000	1.450.000	2.800.000	1.628.000	2,19
Turkey	2.570.000	2.200.000	1.773.646	2.250.000	2.750.000	2.308.729	3,11
Pakistan	4.828.439	4.480.230	3.454.334	4.096.106	2.409.642	3.853.750	5,19
Turkmenistan	1.101.073	1.110.050	1.280.220	1.280.512	1.201.421	1.194.655	1,61
Argentina	813.692	872.721	1.046.043	1.040.334	1.115.510	977.660	1,32
Other countries	9.031.547	9.455.027	8.634.641	8.966.635	7.888.351	8.795.240	11,84
World	72.285.259	83.681.270	71.459.353	74.297.844	69.668.143	74.278.374	100,00

When cotton production amounts by regions are analyzed, the cotton production amount of the Southeastern Anatolia Region in 2023 is 1 million 293 thousand tons and ranks first in terms of production amount. In the average of 2020-2023, the Southeastern Anatolia Region (58.38%) is followed by the Aegean Region (24.53%) and the Mediterranean Region (17.05%) (Table 4).

Table 4. Amount of cotton production by regions (tonnes) (TUİK, 2024)

Regions	2020	2021	2022	2023	Average	%
Mediterranean	356.311	382.648	460.539	313.407	378.226	17,05
Aegean	527.244	542.832	613.729	492.581	544.097	24,53
Southeast Anatolia	888.035	1.324.004	1.674.630	1.293.458	1.295.032	58,38
Other regions	2.056	516	1.102	554	1.057	0,05
Turkey	1.773.646	2.250.000	2.750.000	2.100.000	2.218.412	100,00

As seen in Table 5, which includes the ten countries with the highest cotton exports in the world, the average world fiber cotton exports between 2018 and 2022 were 9 million 144 thousand tons. In 2022, the USA ranks first in world exports with a share of approximately 3.4 million tons and 38.02%. The USA is followed by Brazil (18.54%), Australia (6.61%) and India (9.70%). Approximately 72.87% of world exports are realized by these four countries, while the remaining 13.13% is accounted for by other countries. Turkey's fiber cotton exports account for approximately 1.31% of world fiber cotton exports (Table 5).

Table 5. World fiber cotton exporting countries (tonnes) (FAO, 2024)

Countries	2018	2019	2020	2021	2022	Average	%
USA	3.574.865	3.562.772	3.822.303	2.976.628	3.445.820	3.476.478	38,02
Brazil	915.542	1.613.670	2.125.418	2.016.572	1.803.737	1.694.988	18,54
Australia	477.534	541.447	170.282	716.854	1.116.123	604.448	6,61
India	1.137.357	615.816	965.240	1.289.837	427.486	887.147	9,70
Benin	259.577	269.717	280.064	359.022	320.540	297.784	3,26
Greece	214.684	360.266	289.294	381.990	268.300	302.907	3,31
Burkina Faso	197.687	218.401	166.652	258.200	262.973	220.783	2,41
Ivory Coast	165.433	211.566	187.124	258.249	220.430	208.560	2,28
Sudan	91.229	204.550	98.324	75.414	185.705	131.044	1,43
Turkey	95.404	131.371	86.899	135.690	147.871	119.447	1,31
Other countries	1.219.106	1.403.690	1.205.494	1.273.596	901.414	1.200.660	13,13
World	8.348.418	9.133.266	9.397.094	9.742.052	9.100.399	9.144.246	100,00

According to FAO data, world fiber cotton imports averaged 8.7 million tons between 2018 and 2022. In 2022, China has the largest share in world fiber cotton imports with 22.14%. China is followed by Vietnam (15.83%), Bangladesh (14.65%) and Turkey (11.71%). In general, approximately 64.34% of world imports are realized by these four countries, including Turkey (Table 6).

Table 6. World fiber cotton importing countries (tonnes) (FAO, 2024)

Countries	2018	2019	2020	2021	2022	Average	%
China	1.572.760	1.849.186	2.158.095	2.142.264	1.927.386	1.929.938	22,14
Vietnam	1.405.665	1.340.652	1.388.528	1.512.475	1.252.438	1.379.952	15,83
Bangladesh	1.051.620	1.215.222	1.323.245	1.579.631	1.216.127	1.277.169	14,65
Turkey	751.703	946.099	1.064.782	1.191.084	1.148.397	1.020.413	11,71
Pakistan	605.984	399.428	818.737	903.459	782.921	702.106	8,06
Indonesia	762.949	653.435	486.258	561.788	490.958	591.078	6,78
India	270.126	686.815	174.121	193.021	389.687	342.754	3,93
Egypt	123.820	239.482	189.497	177.106	186.048	183.191	2,10
Thailand	258.912	205.258	133.589	156.314	171.424	185.099	2,12
Malaysia	170.290	205.354	121.620	114.081	135.543	149.378	1,71
Other countries	1.172.696	1.026.034	765.250	900.052	910.231	954.853	10,96
World	8.146.525	8.766.965	8.623.722	9.431.275	8.611.160	8.715.929	100,00

The aim of this study is to analyse the cultivation areas, production amounts and foreign trade data of cotton plant in the world and Turkey from an economic point of view. World cotton production has an important place in the agricultural sector and textile industry and Turkey's situation in this field is analysed in detail in this study. The changes in Turkey's cotton cultivation areas and production amounts over the years have been evaluated in comparison with the world. In addition, the analysis of Turkey's cotton import and export data will shed light on economic trends. In this context, the causes and consequences of the changes observed in cotton production and trade will be discussed and predictions will be made about the future trends of the sector. By providing information for cotton producers, exporters, importers and policy makers, the research aims to increase the competitiveness of Turkey's cotton industry in the global market and to provide the necessary data to make strategic decisions.

MATERIALS AND METHODS

The data on Turkey's cotton cultivation area, cotton production and fiber cotton import and export data for the 20-year period 2003-2022 were obtained from the Food Agricultural Organization (FAO). The data set was transferred to MS Excel spreadsheets and the trend analysis technique was used in MS Excel programme to obtain the trends in cotton cultivation area, cotton production amount and near future forecast values for eight years between 2023-2030. In addition, for the foreign trade balance data obtained from the fiber cotton import and export data for the period 2000-2022, the change trends of the 23-year period and the eight-year near future forecast values between 2023-2030 were calculated. In this study, sector reports and other researches on the subject were also utilised.

RESULTS

The 20-year change course of the cultivation areas allocated for cotton production in Turkey for the period 2003-2022 and the eight-year future forecasts for the period 2023-2030 calculated by the Trend Analysis Technique are shown in Figure 1. The trend equation is estimated as y = -7213x + 2E+07. According to this, cotton cultivation area has a decreasing trend between 2023-2030. While the estimated value of cotton cultivation area for 2023 is 417,000 hectares, it is predicted that this value will decrease to 373,722 hectares in 2030 (Figure 1).

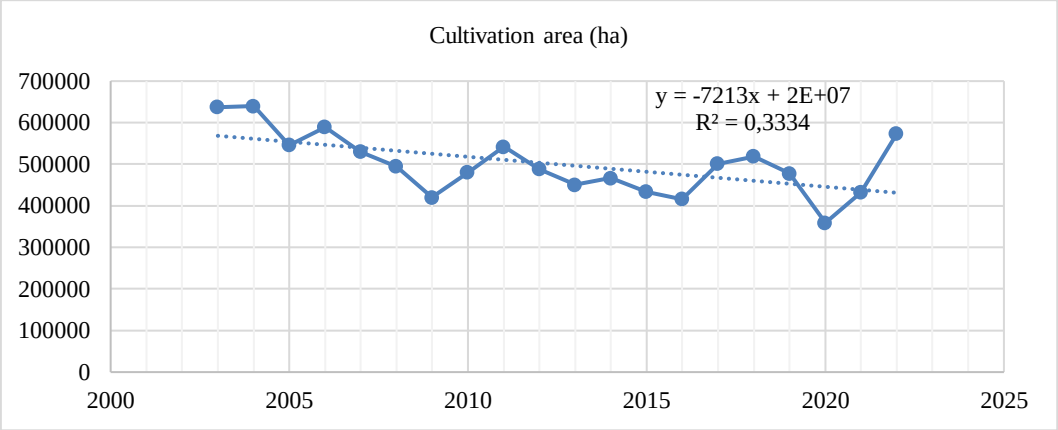

Figure 1. 2003-2022 Turkey cotton cultivation area trend analysis

The 20-year trend of change in the amount of cotton production in Turkey for the period 2003-2022 is presented in Figure 2. The trend equation calculated for the amount of cotton production is estimated as y = 2030,6x + 2E+06. The estimated cotton production amount was calculated as 2,044,673 tons in 2024 and 2,056,857 tons in 2030. The trend in the amount of cotton production has a decreasing trend after 2022 and it is predicted that there will be no break in the amount of production between 2023-2030 (Figure 2).

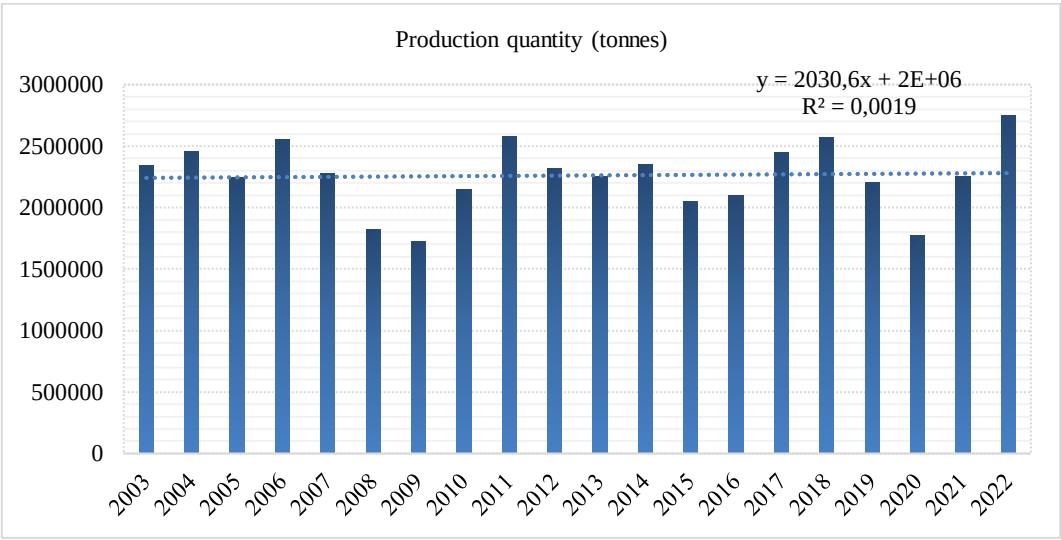

Figure 2. 2003-2022 Turkey cotton production amount trend analysis

For the 20-year period 2003-2022, Turkey's fiber cotton exports averaged 70,228 tonnes. The ten-year trend of Turkey's fiber cotton exports is shown in Figure 3. The amount of exports followed a decreasing fluctuating course between 2003-2015. After 2015, there was an increase in exports, but it started to decrease again in 2020 due to the pandemic and started to increase again after 2021. The trend equation of fiber cotton exports was estimated as y = 3710x + 31274. According to this equation, the amount of exports estimated for 2023 is 109 thousand 184 tonnes and 135 thousand 154 tonnes for 2030. It is predicted that the increasing trend will continue in the eight-year future period of 2023-2030 and the regression coefficient (R^2) for this analysis is determined as 41% (Figure 3).

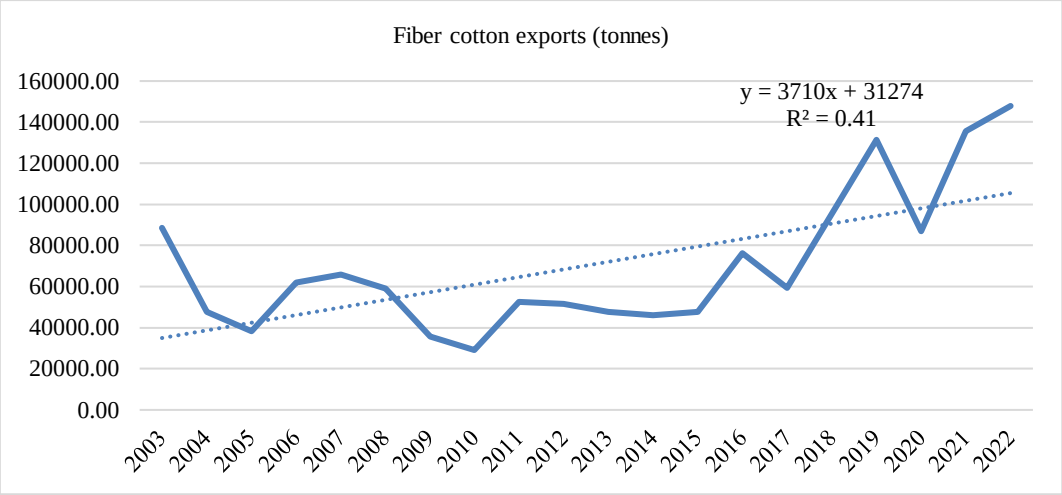

Figure 3. 2003-2022 Turkey's fiber cotton exports change course

In the twenty-year period between 2003 and 2022, Turkey imported an average of 825,622 tonnes of fiber cotton. The trend of change in this period is generally increasing. The trend equation of fiber cotton imports was estimated as y=22705x+587223. According to this equation, the amount of imports estimated for 2023 is estimated to be 1 million 064 thousand tonnes and 1 million 222 thousand tonnes for 2030. It is predicted that the increasing trend will continue in the eight-year period between 2023-2030 and the average import amount will be 1 million 143 thousand tonnes (Figure 4).

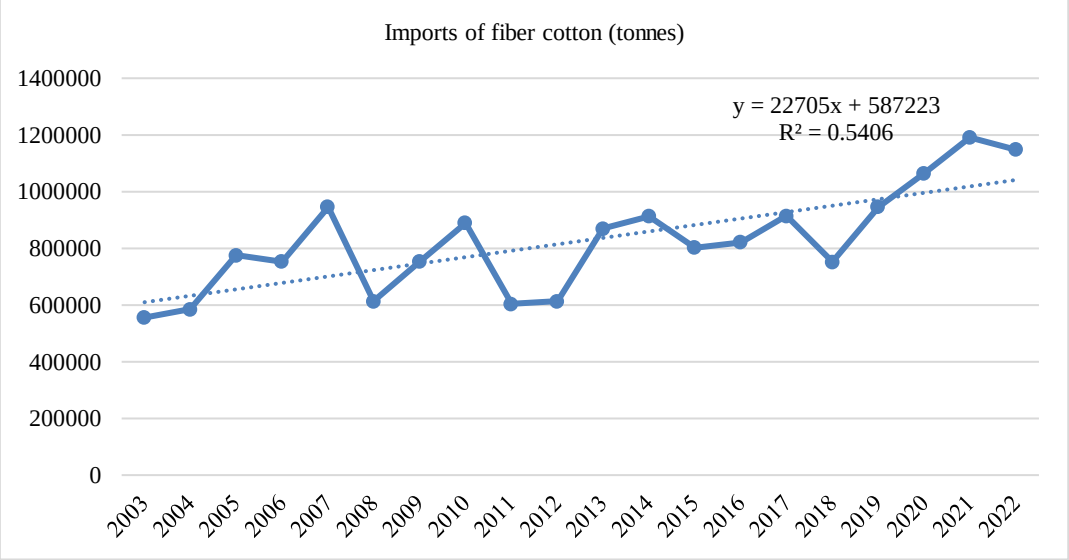

Figure 4. 2003-2022 Turkey's fiber cotton imports change course

Trends of the 23-year period for the foreign trade balance data obtained from fiber cotton import and export data for the period 2000-2022 are shown in Figure 5. Accordingly, Turkey's fiber cotton imports exceed its exports in the same period and Turkey has a foreign trade deficit. Between 2000 and 2022, the foreign trade deficit was 720,820 tons on average. The trend equation for the foreign trade balance is estimated as y = -20308x - 477124. According to this equation, the foreign trade deficit for 2023 is estimated to be 964,516 tons and for 2030 it is estimated to be 1,106,672 tons (Figure 5).

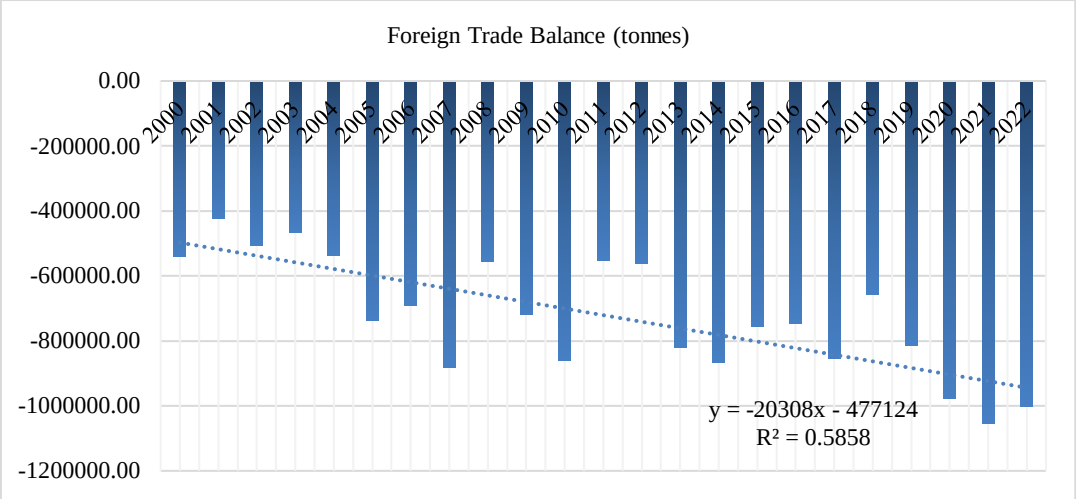

Figure 5. Trend analysis of foreign trade balance in fiber cotton

DISCUSSION AND CONCLUSION

In this study, it is predicted that cotton cultivation area will decrease to 373,722 hectares in 2030. The main reasons for the decrease in cotton cultivation areas can be listed as increases in input prices such as fertilisers, pesticides and seeds, increases in energy and irrigation costs, increase in Turkey's cotton imports as a result of the increase in cotton production in the world, and decrease in cotton yield as a result of drought and climate change in recent years. Krieg (1997) and Başal et al. (2016), stated that although cotton is tolerant to drought compared to other cultivated plants, the rate of decrease in cotton yield can reach up to 70-80% depending on the duration of drought and the growing period in which drought occurs. At the same time, high-temperature stress leads to the shedding of squares, flowers, and bolls in cotton plants, causing significant yield losses (Gören, 2017). In addition, producers' orientation towards alternative crops (maize, grapes, etc.) that bring more income also leads to a decrease in cotton supply. In addition to the change in land use pattern, the use of some cotton production areas for housing, industry and other purposes also causes a decrease in cultivation areas. Similarly, Kaya et al. (2015), stated that the increasing housing need as a result of rapidly increasing urbanisation is met from agricultural areas, and the motorways, airports, hotels and dams built lead to a decrease in agricultural areas. In terms of subsidies, the fact that the current cotton subsidy policies are inadequate for producers leads to a decrease in cultivation areas and cotton supply. Adjustments to be made in support policies and additional incentives for producers may contribute positively to the increase in cotton production. Uzmay (2009), stated that it is necessary to increase the amount of support applied in order to prevent the decrease in cotton cultivation areas and the shift of producers to other alternative products. On the other hand, uncertainties in the world economy and fluctuations in cotton prices may cause producers to hesitate and lead to the shrinkage of cultivation areas. Okumuş (2012), stated that the cotton price in the previous year emerged as the determining factor in cotton production, therefore, input prices should be provided at world standards within agricultural policies. The amount of cotton production was 1,773,646 tonnes in 2020 and was at the lowest level. After 2020, it showed an increasing trend. In 2030, it is calculated to be approximately 2,056,857 tonnes. However, it is predicted that there will not be a significant break in the production amount between 2023-2030. Özüdoğru (2021), stated that despite the limited increase in cotton cultivation areas, production increased more, and the reason for the increase in production was that innovations in areas such as mechanisation and irrigation led to an increase in cotton fiber yield. As a result of trend calculations, the foreign trade deficit of cotton is estimated to be 1 million 106 thousand tonnes in 2030. Accordingly, it is thought that Turkey will continue to be a net importer of cotton. Paksoy and Şahin (2023), stated that Turkey's competitiveness in cotton exports has been in a systematic downward trend since 2016. In order to eliminate the foreign trade deficit in cotton, efforts should be made to enter new markets and increase shares in existing markets. In the short term, practices such as developing drought-resistant cotton varieties, using modern irrigation systems and adopting new technologies in agriculture, reviewing and increasing support policies for producers, and orientation towards value-added products (yarn, fabric, etc.) will be effective in eliminating the foreign trade deficit. In the long term, it should be aimed to create a sustainable cotton sector by increasing competitiveness in the global market, investing in R&D activities and entering new markets.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that there is no conflict of interest between them.

Author contribution

The authors declare that they have contributed equally to the article and have not plagiarised.

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Characterization of flax genetic resources in Türkiye through variance analysis of antioxidant, phenolic compound and fatty acid contents

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Abstract
This study was conducted in 2021 using seeds from 10 different flax cultivars (Sarı 85, Cill 1351, Cill 1370, Cill 1400, Cill 1412, Larnaka, Milas, Newtürk, and Dilman) in the Ankara ecology. The study investigated the oil content, protein content, fatty acid composition, antioxidant capacities, and specific phenolic compound contents of these seeds. Correlations among the bioactive compound contents of flax seeds were elucidated using Principal Component Analysis (PCA) and Heatmap analysis. As a result of the research, statistically significant differences were found among the seed properties of the varieties. According to the PCA method, the correlation among fatty acids was determined as 71.2% (PC1+PC2), while the correlation between phenolic compounds and antioxidants was determined as 60.4% (PC1+PC2). In the study, the highest oil content was obtained in the Newtürk variety with 35.3%, while the lowest oil content was obtained in the Larnaka variety with 32.2%. The highest α-linolenic acid (C18:3) ratio of 53.9% was detected in the Newtürk variety, while the lowest α-linolenic acid (C18:3) ratio of 46.8% was obtained from the Cill1423 variety. Ferulic (Cill 1351: 18.51 µg/g) and protocatechuic (Cill 1423: 20.83 µg/g) acids were found to be the most abundant compounds in flax seeds. In the research, it was determined that the Cill 1351 (4.08 mg trolox/g) and Dilman (4.16 mg trolox/g) varieties had higher antioxidant capacities than the other varieties.

Keywords: Flax, Antioxidant, Phenolic compounds, Fatty acids, Protein

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INTRODUCTION
Today, people attribute increasing health problems to their dietary habits. Despite the growing human population and the increasing demand for staple food products, the reduction in available agricultural land day by day, as a result of which agricultural activities shift predominantly towards the production of staple food products, and the consideration of yield and main quality criteria in breeding efforts, have led to a decrease in food diversity and the nutritional content of consumed foods. As a result, cases of hidden hunger are observed in people. Those who want to eat healthily believe that returning to traditional dietary methods and adding new plant species to their diets will solve nutritional problems. Individuals who are conscious about nutrition and have access to the food they desire are aware that food is not only essential for satisfying hunger and meeting basic nutritional needs but also a crucial factor in preventing nutrition-related diseases and improving mental well-being. Recent studies have shown that many diseases are caused by improper or inadequate nutrition. Studies in this regard indicate that omega-3 fatty acids are effective in anti-ulcer activity, anti-secretory effect, renoprotection in lupus nephritis, anti-

atherogenic effect, CVD prevention, and decreasing blood pressure (Harper et al., 2006; Dupasquier et al., 2007; Dugani et al., 2008; Rodriguez-Leyva et al., 2010; Caligiuri et al., 2014); plant proteins are effective in neurodegenerative disease prevention, controlling blood pressure, influencing hypertriglyceridemia, influencing diabetes mellitus, and having anti-hypertensive properties (Velasquez et al., 2003; Omoni and Aluko, 2006; Oomah et al., 2007); dietary fibers are effective in hunger suppression and reducing total cholesterol in the blood (Kristensen et al., 2012); and lignans are effective in controlling hypertension, protecting against cancer and diabetes, controlling dyslipidemia, reducing breast cancer growth, and affecting postmenopausal women's symptoms (Nowak et al., 2007; Adolphe et al., 2010; Simbalista et al., 2010; Dew and Williamson, 2013; Flower et al., 2014). The flax plant, which has recently become in demand for both healthy nutrition and dietary consumption, is a member of the Linaceae family and is an annual herbaceous plant. Its gene center is Ethiopia, Central Asia and India. (Habibollahi et al., 2016; Choudhary et al., 2017; Singh et al., 2017; Goudenhooft et al., 2018; Tchoumtchoua et al., 2019; Landoni et al., 2020; Nag et al., 2020; Xie et al., 2020; Talebi and Matsyura, 2021). It is reported that human beings began to benefit from flax as far back as 30,000 years ago (Balter, 2009; Kvavadze, 2009; Fu, 2011). Flax is a plant cultivated for many years in many countries of the world because of its fibers obtained from its stems and its oils obtained its seeds (Katar, et al, 2023). It is probable that humankind first used the seeds of flax and then began to benefit from its fiber.

The area under flax cultivation for seed production worldwide is 3,540,139 hectares, with a total production of 3.367.380 tons. The highest seed production is carried out by Kazakhstan with 1,058,247 tons, followed by Russia with 787,923 tons and Canada with 578,000 tons (FAO, 2020). People's pursuit of natural and healthy nutrition has led to the resurgence of highnutrient plants like flax. The high content of polyunsaturated and monounsaturated essential fatty acids, phenolic compounds, proteins, and minerals in flax seeds has led to its inclusion in diets. The fact that 30 grams of flax seeds have the potential to meet 7% to 30% of the daily intake of elements such as calcium, magnesium, and phosphorus has increased its importance in diets (Singh et al., 2011). Furthermore, due to its rich content, it has become a preferred ingredient in bird feed mixes. The seeds contain, varying from genotype to genotype, approximately 41% oil, 28% fiber, and 20% protein (Oomah, 2001; Pengilly, 2003; Flaxcouncil, 2022). Its oil is rich in alpha-linolenic acid, an important fatty acid for health (Sargi et al., 2013). Additionally, due to its content of polyunsaturated linolenic and linoleic fatty acids, it tends to oxidize quickly, so it should be consumed fresh. If consumed directly, it should be ground daily whenever possible (Sargi et al., 2013).

Phenolic compounds are substances found in plants that influence many quality criteria such as color, taste, and aroma (Dong et al., 2001; Cemeroğlu, 2007; Predieri et al., 2006; Gundogdu et al., 2021). Due to their antioxidant properties, these compounds are effective in plants' defense systems against environmental stress factors and play a role in many physiological processes (Colaric et al., 2005; Gündoğdu, 2019). Nowadays, there is a growing demand for products with high antioxidant content in human nutrition. Particularly, foods rich in phenolic compounds with anticancer properties are in the spotlight (Scalbert et al., 2005). Studies have shown that flax seeds contain a high amount of phenolic compounds. Phenolic compounds have anticancer and antioxidant properties. It is reported that flax seeds have three different types of phenolic compounds, namely phenolic acids, flavonoids, and lignans (Kajla et al., 2015). The major phenolic acids in flaxseed meal are ferulic acid (10.9 mg/g), chlorogenic acid (7.5 mg/g), and gallic acid (2.8 mg/g), along with other phenolic acids, low amounts of pcoumaric acid glucosides, hydroxycinnamic acid glucosides, and 4-hydroxybenzoic acid. The major flavonoids in flax seeds are Flavone C- and Flavone O-glycosides (Beejmohun et al., 2007; Mazza, 2008).

Flax is produced and consumed for its oil and fiber, and intensively used in several sectors. It is important industrial plants with several uses (Zuk et al., 2015; Yaşar, 2023). Flax is a versatile plant species widely used in various industries such as oil production, cosmetics, pharmaceuticals, and more. In oil industry production, the residue from seeds after oil extraction, known as flaxseed meal, is a valuable byproduct rich in protein and mineral content. Flaxseed meal has qualities suitable for both human and animal nutrition. This research has identified the bioactive compound contents of seeds from flax varieties commonly grown in our country and statistically defined the correlations among these compounds.

MATERIALS AND METHODS

Plant material, fatty acid, and protein analyses

In the study, various sources provided Sarı 85, Cill 1351, Cill 1370, Cill 1400, Cill 1412, Larnaka, Milas, Newtürk, and Dilman flax varieties (Table 1).

To obtain the seeds used in the research, a trial was established on April 5, 2021, under Ankara ecological conditions, in a Randomized Complete Block Design with 3 replications. Plots, each with an area of 4 x 1.2 = 4.8 m2, were established with 6 rows, a row length of 4 m, and a row spacing of 20 cm. Manual seeding was performed for the trial, and organic-mineral fertilization was applied at a rate of 10 kg per hectare. Weed control was carried out, and two irrigations were performed, one at the emergence stage and the other at the pre-flowering stage. The trial was manually harvested in the yellow ripening stage on August 20th. The total annual precipitation at the trial location was 297 mm, with the lowest average temperature during the vegetation period (9.3°C) occurring in April and the highest average temperature (16.5°C) in June. The annual precipitation was lower than the long-term

average precipitation (318 mm). The soil at the trial site was determined to be clayey-loamy, low in organic matter (1.63%), with available phosphorus content at 9.7%, slightly alkaline (pH=7.7-7.8), lime-rich (28% lime), and low in salt content (1.18 dS/m). Fixed oil content analyses were conducted using a Soxhlet apparatus, fatty acid composition analyses were performed using the method recommended by Gölükcü et al. (2016), and protein content analyses were carried out using the Kjeldahl method (Balkan, 1978).

Table 1. Some information about the varieties.

Genotype name	1000 seeds Weight (g)	Flower Color	Seed Color	Oil Rate (%)	Country of Orjin	Growing Type
Sarı 85*	5.40	white	yellow	37.7	Türkiye	Spring
Larnaka*	5.40	blue	darkbrown	37.3	Pakistan	Winter
Milas*	6.20	blue	lightbrown	36.2	Türkiye	Winter
Newtürk*	5.40	blue	darkbrown	37.1	U.S.A	Spring
Dillman*	5.20	lightblue	brown	32.3	U.S.A	Winter
Clli-1351*	6.18	blue	lightbrown	34.8	Türkiye	Winter
Clli-1400*	6.18	blue	brown	38.2	Türkiye	Spring
Clli-1412*	6.15	lightblue	brown	35.3	Türkiye	Spring
Clli-1370*	5.69	blue	brown	35.0	Türkie	Spring
Clli-1423*	5.76	blue	brown	34.6	Türkiye	Spring

**The seeds used in the experiment were supplied by the United States Department of Agriculture (USDA). Yaşar,2023 and Yaşar ve Yetişsin, 2023.*

Phenolic compound analysis

Phenolics were extracted using a modification of the methods developed by Kosar et al. [1] and Trandafir et al. [2]. The seed samples were mixed with acetone and water (1: 4) and vortexmixed for 1 min. Trifluoroacetic acid (0.100 ml) was then added to the mixture followed by vortexmixing for 1 min and by incubation in a hot water bath at 60 °C for 60 min. After cooling, the extracts were filtered through a nylon membrane (pore size 0.45 µm, Merck). Extracts were analysed by HPLC with ultraviolet spectrophotometric detection using LC-20A system (Shimadzu, Tokyo, Japan). A reverse phase column Nucleosil C18 (25 cm × 3.2 mm, particle size 5 µm; Supelco) and a twosolvent system (A: formic acid-water, 2.5: 97.5, v/v and B: acetonitrile-water, 2.5: 97.5, v/v) were used. Detection was accomplished at 280–360 nm. Content of phenolics was expressed as milligrams per kilogram.

Antioxidant analysis

Determination of ABTS cation radical scavenging activity Determination of ABTS cation radical scavenging activity of methanolic extracts of seeds and sprouts was carried out according to methods of Pająk et al. (2017). ABTS cation radical was obtained in the reaction of 2mM phospate-buffered stock (PBS) solution of 2,2′-azinobis (3-ethylbenothiazoline-6sulfonic acid) diammonium salt (ABTS) with potassium persulphate. The mixture was left to stand for 24 h, until the reaction was completed and then ABTS solution was diluted by PBS to obtain the absorbance of 0.800 ± 0.03 at λ=734 nm. Fifty microliters of appropriately diluted methanolic extract of seeds or sprouts was mixed with 6 mL of the ABTS%+ solution and the absorbance of the resulting solution was measured after 15 min at λ=734 nm. Antioxidant activity (AA) was expressed as mg of Trolox equivalents per g of d.m. of seeds and sprouts.

Statistical analysis

The statistical analyses of the obtained values were conducted using the 'JUMP' statistical software program, and the differences between the means were tested using the Duncan multiple comparison method. One way analysis of variance – ANOVA and Tukey's HSD comparison of means of samples were used for analyzing variations. Correlations among studied traits were determined by Pearson's pairwise correlations using the "corrplot" package of R software. Interrelations of factors (storage periods and spermidine doses) and traits were determined by principal component analysis (PCA)with the "ggplot2" package of R software (Wickham, 2011). Heatmap analysis was performed with the R package "bioconductor" (Gentleman et al., 2004).

RESULTS AND DISCUSSION

Total fat and protein contents

In this study, it was observed that there were two distinct groups in terms of oil content values, with the highest oil content being 35.3% in the Newtürk variety and the lowest oil content being 32.2% in the Larnaka variety. When the variance analysis values of the trial are examined, it is observed that the difference in oil content values among the varieties is statistically significant at $p \leq 0.05$, while the difference in protein content is not statistically significant (Table 1). Čolovic et al. (2016) reported that in their study on the nutritional properties of 18 local flax

varieties in Serbia, the oil content ranged from 34% to 40%, and the difference among varieties was significant, while the protein content ranged from 19% to 27%, and the difference among varieties was also significant. Similarly, Sauvant et al. (2004) and Sargi et al. (2013) found in their studies on different flaxseed genotypes that the protein content ranged from 21.8% to 22.6% and 23.2% to 24.4%, respectively, and the crude oil content ranged from 32.7% to 37.91% and 37.8% to 38.1%, respectively. This indicates that the differences in protein and oil content among flax varieties can vary depending on the variety and environmental conditions. There are some studies conducted to observe the health effects of flaxseed oil and protein. Dugani et al. (2008) reported in their study with rats that oil and mucilage obtained from flaxseed reduced the number and length of ethanol-induced gastric ulcers. Kaithwas & Majumdar (2010) also reported that flaxseed oil showed a significant inhibitory effect on gastric secretion/total acidity and aspirin-induced gastric ulceration in rats. Clark et al. (2001) investigated the effect of flaxseed oil acids on lupus nephritis disease and found that plasma lipids and serum viscosity did not change, but serum creatinine decreased in some patients. Dupasquier et al. (2007) conducted a study to see the anti-atherogenic effect of flaxseed in rabbits and reported that rabbits fed with ground flaxseed supplementation on a cholesterol-enriched diet had reduced plasma cholesterol and saturated fatty acids, increased ALA plasma content, inhibited plaque formation in the aorta and aortic sinuses, reduced circulating cholesterol levels, and inhibited atherosclerosis at the cellular level through antiproliferative and anti-inflammatory effects.

Table 2. The average values of protein and oil contents in the varieties, the formed groups, and the variance analysis

Varieties	Oil Content (%)	Protein Content (%)
Cill 1351	33.33 ± 0.75 ab	24.32 ± 0.66 a
Cill 1370	33.50 ± 0.61 ab	19.16 ± 2.73 a
Cill 1400	33.17 ± 0.67 ab	21.14 ± 3.67 a
Cill 1412	33.23 ± 0.29 ab	23.71 ± 0.14 a
Cill 1423	32.93 ± 1.22 ab	21.39 ± 2.27 a
Dilman	34.03 ± 0.50 ab	23.09 ± 2.82 a
Larnaka	32.20 ± 1.30 b	23.77 ± 1.96 a
Milas	34.07 ± 0.68 ab	23.69 ± 1.55 a
Newtürk	35.30 ± 0.46 a	23.28 ± 2.83 a
Sarı 85	33.53 ± 1.20 ab	23.20 ± 2.36 a
$HSD_{0.05}$		6,74
Varyans analizi		n.s

**indicates p≤0.05. ns: not significant.*

Like other plant products, flaxseed proteins also interact with other components and possess techno-functional properties in the food system. The amino acid distribution of protein in flaxseed is reported to be similar to that of soybean protein (Rabetafika et al., 2011). Flaxseed, like many other plant protein sources, is not recommended as a sufficient protein source because it lacks some essential amino acids. However, it can be recommended as a supplementary protein source. Flax seeds and flaxseed meal contain approximately 21% and 34% protein, respectively. It is reported that the protein content can vary depending on genetic and environmental factors (Chung et al., 2005).

Since there was no statistically significant difference in protein content among the varieties evaluated in this study, it can be said that variety preference will not lead to differences in the amount of protein intake. However, it may lead to differences in oil content. It can be suggested that consuming the seeds of flax varieties with higher oil content would be a more suitable option to derive greater biobenefits from flaxseed oil's beneficial effects (Table 1).

Fat acids contents

When the obtained findings are examined, it is observed that there is a high degree of variation among varieties in terms of fatty acid composition. Due to the difference in α-linolenic acid (C18:3) content, which stands out for its health effects, four different groups have been formed among the varieties. The highest α-linolenic acid (C18:3) content was found to be 53.9% in the Newtürk variety, while the lowest α-linolenic acid (C18:3) content was obtained from the Cill1423 variety with 46.8%. In light of the findings obtained in this study, it can be seen that variety preference is important to derive greater biobenefits from flaxseed oil's beneficial effects (Table 3). Čolovic et al. (2016) reported in their study with 18 local flax varieties in Serbia that they found α-linolenic acid (C18:3) content in the range of 42.9% to 61.0%. In other similar studies, Bean & Leeson (2002) found α-linolenic acid (C18:3) content in the range of 51.5% to 59.3% in a study with 23 flax genotypes, and El-Beltagi et al. (2007) reported α-linolenic acid (C18:3) content in the range of 46.0% to 50.7% in a study with five flax genotypes. The values obtained in this study fall within the range of values found by researchers, and it is also observed that there are genotypes with higher α-linolenic acid (C18:3) content among the genotypes examined.

When evaluated together with other foods containing α-linolenic acid (C18:3), which is important for human and animal health, such as fish and walnuts, it can be said that flaxseed would be a cheaper option. This is because fish and walnuts are not accessible in many countries, and they are also more expensive compared to other foods. Flax is a field crop that can be grown in almost every part of the world. In a study conducted by Bağcı et al. (2023) in the ecological conditions of Ankara (318 mm of rainfall), it is reported that the Cill 1412 variety yielded 180 kg/ha of seeds, 59 kg/ha of oil, and 42.9 kg/ha of protein. Since the αlinolenic acid (C18:3) content in the oil of the Cill 1412 variety is 47.3%, the amount of αlinolenic acid (C18:3) that can be obtained per hectare will be around 27 kg. The total omega3 fatty acid content in fish oil, which has an omega-3 fatty acid content of approximately 3366%, is around 1.95% for α-linolenic acid (C18:3) (Mattos et al., 2004; Malayoğlu et al., 2009). When the same amount of α-linolenic acid (C18:3) is desired to be obtained from fish oil, it is seen that 14 times more fish oil needs to be consumed. However, it is necessary to continue consuming fish to obtain other omega-3 fatty acids found in fish oil, such as stearidonic acid, eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid. The daily requirement for α-linolenic acid (C18:3) for an adult woman is 1.1 g, while this amount is around 1.6 g for adult men (Pandohee, 2022). Taking these amounts into account, consuming 7 g/day for women and 10 g/day for men from flaxseed, which contains 30% oil, will meet their α-linolenic acid requirements.

Table 3. Average values, formed groups, and variance analysis for fatty acid compositions (%)

Varieties	α-linolenic acid (C18:3)	Linoleic acid (C18:2)	Oleic acid (C18:1)	Palmitic acid (C16:0)	Stearic acid (C18:0)
Cill 1351	49.20 ± 0.60 bcd	13.50 ± 0.10 f	25.20 ± 0.50 bcd	6.53 ± 0.15 a	5.60 ± 0.10 cd
Cill 1370	46.00 ± 0.00 d	15.53 ± 0.06 b	25.93 ± 0.06 ab	6.30 ± 0.00 abc	6.20 ± 0.00 a
Cill 1400	47.90 ± 0.50 bcd	11.60 ± 0.10 h	28.50 ± 0.60 a	5.97 ± 0.06 cd	6.00 ± 0.00 abc
Cill 1412	47.33 ± 0.45 cd	16.13 ± 0.35 a	25.43 ± 0.55 bc	6.20 ± 0.10 abc	4.90 ± 0.20 e
Cill 1423	46.80 ± 0.30 cd	14.80 ± 0.20 cd	26.70 ± 0.10 ab	6.37 ± 0.15 ab	5.33 ± 0.06 d
Dilman	51.03 ± 0.85 ab	15.30 ± 0.10 bc	22.83 ± 0.65 cd	6.03 ± 0.06 bcd	4.80 ± 0.10 e
Larnaka	49.13 ± 0.15 bcd	14.03 ± 0.06 ef	24.80 ± 0.20 bcd	6.30 ± 0.00 abc	5.70 ± 0.00 bcd
Milas	49.70 ± 0.20 bc	12.67 ± 0.06 g	25.27 ± 0.35 bcd	6.33 ± 0.06 ab	6.03 ± 0.06 ab
Newtürk	53.90 ± 3.80 a	12.13 ± 0.35 gh	22.53 ± 2.85 d	5.83 ± 0.25 d	5.63 ± 0.35 bcd
Sarı 85	48.07 ± 0.25 bcd	14.33 ± 0.15 de	25.43 ± 0.06 bc	6.43 ± 0.06 a	5.70 ± 0.10 bcd
$HSD_{0.05}$	3.68	0.54	2.84	0.33	0.41
Varyans analizi	**	**	**	**	**

***indicates $p \leq 0.001$.*

Antioxidant and phenolic compound contents

In this study, statistically significant differences were observed among varieties in terms of phenolic compound and total antioxidant contents ($p \leq 0.001$). According to the research, Cill 1351 (4.08 mg trolox/g) and Dilman (4.16 mg trolox/g) varieties had higher antioxidant capacities compared to other varieties. Among flaxseed varieties grown under the same ecological conditions and cultural practices, Çili 1370 was found to have the lowest antioxidant capacity (Table 3). Pająk et al. (2019) reported antioxidant activities of flaxseeds ranging from 0.70 to 6.79 mg trolox/g. The same researchers emphasized that flaxseeds have high antioxidant activities and stand out in this regard compared to many other plants. Similar findings have been reported by other researchers as well (Wang et al., 2017; Kosiorowska et al., 2022). The findings obtained in these studies are consistent with our data, and it is evident that antioxidant values vary among varieties. When the phenolic compound contents of flaxseed varieties were examined, ferulic and protocatechuic acids were found to be the most abundant compounds in flaxseeds. In light of the results, the highest ferulic acid content was determined in Çili 1351 variety at 18.51 µg/g. Protocatechuic acid was detected as the highest in Çili 1423 variety at 20.83 µg/g (Table 3). Generally, caffeic acid was lower than other phenolic compounds. In a study by Wang et al. (2017), they reported that the ferulic acid content of Zhongya2 flaxseed variety was 21.13 µg/g, and the p-coumaric acid content was 9.29 µg/g. In another similar study, the ferulic acid content in flaxseeds was determined as 0.11 mg/100g (Pajak et al., 2019). Phenolic compounds are chemical compounds that perform many important biological functions in plants, such as growth, development, defense, and protection against environmental stresses. Phenolic compounds are an important source of antioxidants for plants (Kaviarasan et al., 2007; Pandey & Rizvi, 2009; Randhir et al., 2004). In this study, it was determined that flaxseed varieties commonly cultivated in Turkey are rich in phenolic compounds, and these varieties are genetically valuable materials in terms of biochemical content compared to the literature.

Table 4. Average values of phenolic compound (µg/g) and antioxidant (mg/ trolox/g dry weight) contents, groups formed and analysis of variance.

Cultivars	Caffeic	Ferulic	Gallic	P-Coumaric	Protocatechuic	Quercetin	Total Antioxidant
Cill 1351	1.56 ± 0.09f g	18.51 ± 0.70 a	6.14 ± 0.06 a	2.20 ± 0.13 d	3.26 ± 0.08 ı	3.11 ± 0.06 e	4.08 ± 0.11 a
Cill 1370	2.72 ± 0.15 e	15.11 ± 0.62 b	5.49 ± 0.09 b	5.15 ± 0.10 c	5.80 ± 0.14 h	2.53 ± 0.06 f	2.22 ± 0.08 d
Cill 1400	1.14 ± 0.05 g	9.69 ± 0.40 d	4.86 ± 0.10 c	6.48 ± 0.19 c	12.42 ± 0.32 c	6.07 ± 0.06 a	3.50 ± 0.08 b
Cill 1412	5.58 ± 0.12 b	12.01 ± 1.34 c	3.57 ± 0.09 de	13.96 ± 1.13 ab	7.45 ± 0.64 fg	4.84 ± 0.06 b	2.84 ± 0.11 c
Cill 1423	1.87 ± 0.16f	9.54 ± 0.13 d	2.84 ± 0.15f g	1.78 ± 0.06 d	20.83 ± 0.75 a	4.65 ± 0.09 bc	3.62 ± 0.06 b
Dilman	6.40 ± 0.11 a	11.60 ± 0.44c d	3.21 ± 0.12 ef	15.88 ± 0.90 a	15.28 ± 0.45 b	4.06 ± 0.10 d	4.16 ± 0.08 a
Larnaka	2.57 ± 0.18 e	6.48 ± 0.11 e	2.51 ± 0.11 g	6.11 ± 0.08 c	8.56 ± 0.21 ef	2.17 ± 0.07 g	3.67 ± 0.09 b
Milas	4.79 ± 0.23 c	5.82 ± 0.21 e	1.53 ± 0.06 h	12.84 ± 0.70 b	6.43 ± 0.18 gh	1.72 ± 0.08 h	2.89 ± 0.06 c
Newtürk	1.80 ± 0.13 f	12.40 ± 0.35 c	3.80 ± 0.14 d	12.01 ± 0.65 b	9.62 ± 0.16 de	4.43 ± 0.08 c	2.31 ± 0.08 d
Sarı 85	3.80 ± 0.11 d	11.78 ± 0.40 cd	3.50 ± 0.12 de	12.92 ± 0.35 b	10.61 ± 0.56 d	6.25 ± 0.18 a	2.79 ± 0.11 c
$HSD_{0.05}$	-0.55	-2.29	0.42	-2.23	-1.63	-0.36	-0.35
Varyans analysis	***	***	***	***	***	***	***

*** *indicates p≤0.001.*

Determination of interrelationships between bioactive compounds and flax cultivars by PCA and Hatmap

In the statistical analysis conducted to determine the correlation between the biochemical compound contents of flaxseed varieties, a positive correlation was found between total oil content and α-linolenic acid (r=0.72, p≤0.05). However, a negative correlation was observed between the dominant α-linolenic acid and oleic acid in flaxseeds (r=0.78, p≤0.01). Among the fatty acids, a parallel relationship was found between palmitic acid and stearic acid, oleic acid, and linoleic acid, while generally, a negative correlation was found among the other fatty acids (Figure 1). When phenolic compounds were examined, the highest positive correlation was determined between gallic acid and ferulic acid (r=0.87, p≤0.001). A statistically significant positive relationship was observed between caffeic acid and p-coumaric acid at the r=0.80 level. A positive relationship was found between total antioxidant and protein, while a negative correlation was found between total oil.

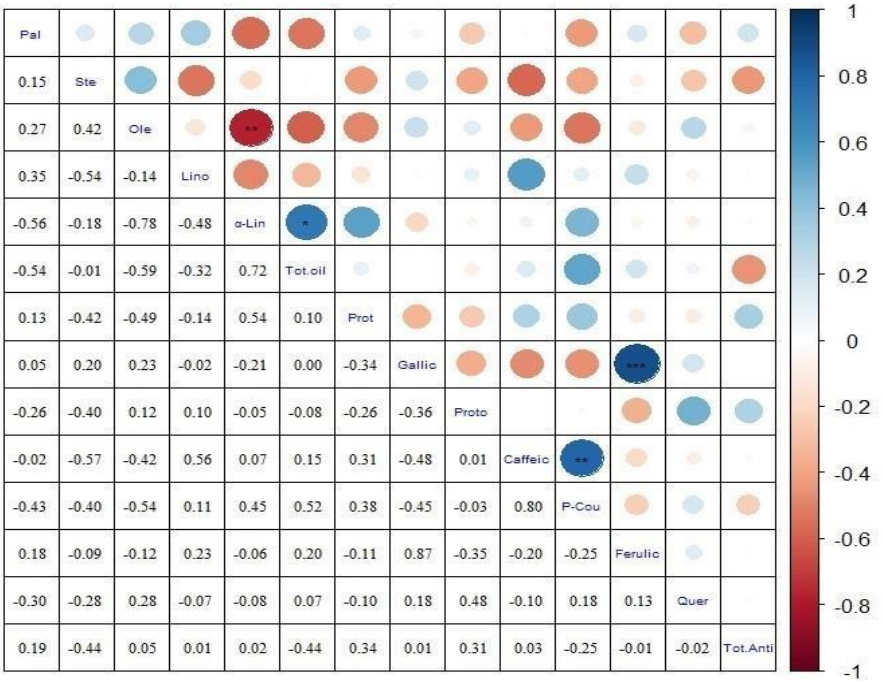

Figure 1. Variation among oil acids, protein, antioxidant and phenolic compounds. The color scale fading from red to blue indicates correlation values from -1 to +1, and the circle size illustrates the redundancy of the correlation. *,**, and *** indicates significance at p≤0.05, p≤0.01, and p≤0.001, respectively. Pal: palmitic, Ste: stearic, Lino: linoleic, α-Lin: α-linolenic acid, Tot.Oil: total oil, Prot: protein, Proto: protocatechuic acid, P-Cou: p-coumaric acid, Quer: quercetin, Tot.Anti: total antioxidant.

Principal Component Analysis (PCA) was conducted in this study to reveal the distribution of biochemical compounds according to flaxseed varieties (Figure 2). PCA is a statistical analysis method that reveals the statistical significance levels of study data and enables the evaluation of results from a scientific perspective. According to the PCA method, the correlation among fatty acids was measured as 71.2% (PC1+PC2). The correlation between phenolic compounds and antioxidants was determined as 60.4% (PC1+PC2). Newtürk and Dilman varieties stood out in terms of total fatty acid and total antioxidant content, respectively. In terms of protein content, Çilli 1351 variety exhibited superior characteristics compared to other varieties. The study found that the ferulic acid content of flaxseed varieties was higher than other phenolics, with Çilli 1351 and Çilli 1370 varieties standing out in terms of ferulic acid content.

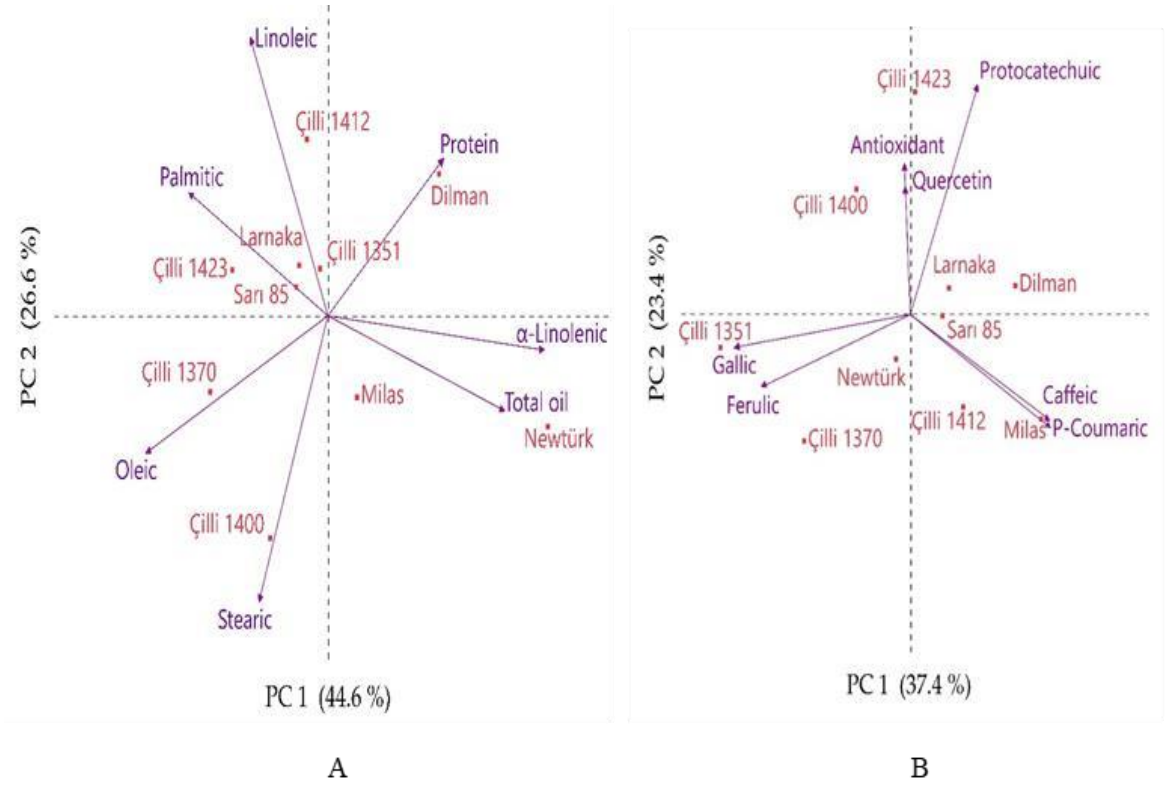

Figure 2. Characterization of the distribution of fatty acids, protein (A) and antioxidants, phenolic compounds (B) in different cultivars of flax using PCA.

According to the Hatmap analysis, in the cluster analysis of biochemical compounds and flaxseed varieties, the Newtürk variety formed a separate group from the other varieties. Çilli 1370 and Çilli 1351 were clustered together, while the other varieties formed a separate group (Figure 3). When looking at the distribution of biochemical compounds according to flaxseed varieties, it can be seen that essentially two groups have formed. Gallic acid and ferulic acid were classified in the group containing fatty acids, while total antioxidants and proteins were classified in the group containing phenolic compounds. In terms of total fatty acids and α-linolenic acid content, the Newtürk variety stood out. Regarding total antioxidant content, the Dilaman and Çilli 1351 varieties exhibited superior characteristics compared to the other varieties.

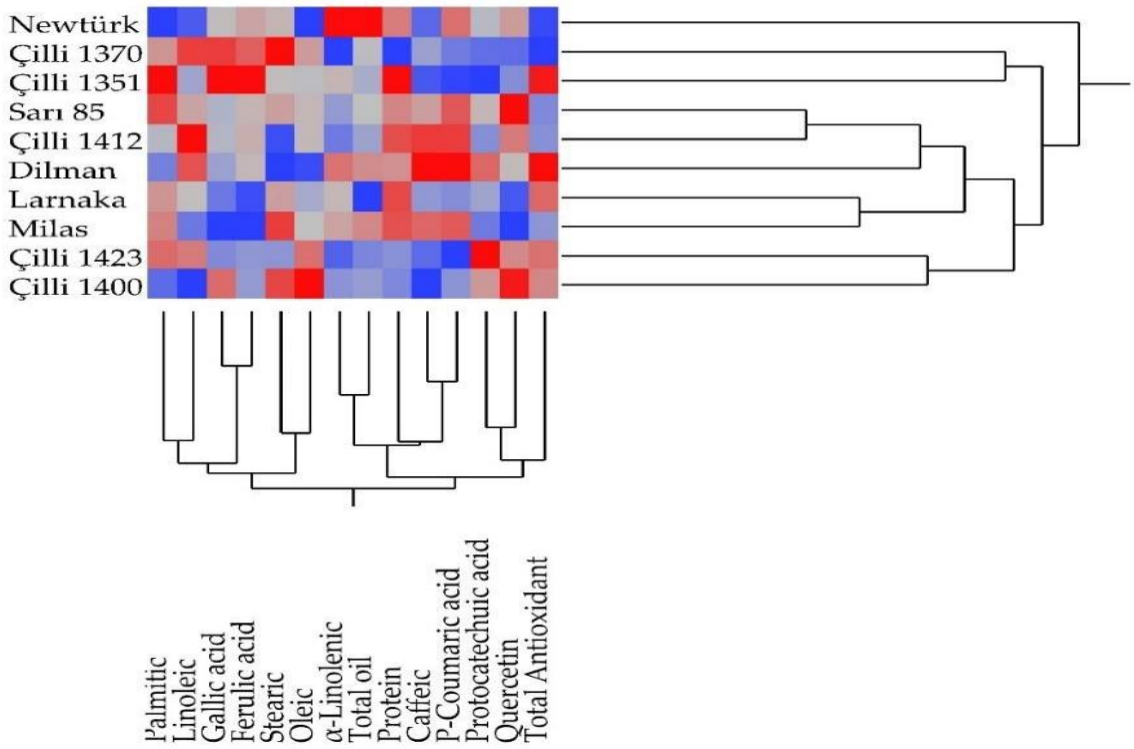

Figure 3. Correlations of biochemical compounds in different cultivars of flax using Hatmap analysis.

CONCLUSION

In this research where the biochemical compounds of flaxseeds, commonly used in various applications, were determined, Çilli 1351 variety showed promise in terms of phenolic compounds, total antioxidants, and protein content. The Newtürk variety stood out in terms of α-linolenic acid content, one of the most abundant fatty acids in flaxseeds. While no significant difference was found among varieties in terms of protein content according to the statistical analysis, it was determined that other biochemical contents showed significant variations among varieties. Ferulic acid and gallic acid, which are among the most abundant phenolics in flaxseeds, were classified in the group formed by fatty acids according to the Hatmap analysis. In conclusion, in this research conducted in the same ecological conditions and under the same cultural practices, the classification of flaxseed varieties was made in terms of biochemical contents. In this regard, especially the Newtürk and Çilli 1351 varieties showed superior performance.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that there is no conflict of interest between them.

Author contribution

The authors declare that they have contributed equally to the article and have not plagiarised.

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Nematicidal effect of powder extractions of different coloured radish seeds against *Meloidogyne incognita* on tomato

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Abstract
The study aimed to evaluate the nematicidal effect of powder extracts of different coloured radish seeds against *Meloidogyne incognita* on tomato (Gülizar F1, susceptibile to root-knot nematode) under controlled conditions. Extractions were obtained from radish seeds of different colours (white, black, red, yellow) by using ethanol and acetone solvents. The experiment was carried out using 2, 4 and 6 g powder/plant application of the extracts in the pot. The experiment was set up in a random plot design with 5 replication for each radish seed extract and concentration. Radish seed powder was applied one week after nematode inoculation (1000 *M. incognita* eggs). After 50 days, the number of galls and egg masses on the roots were counted. It was determined that radish colour, extraction solvents and concentrations of extracts differed significantly for their nematicidal effects. The mean number of galls and egg masses was found to be 56 units in the negative control. Compared to the negative control, all treatments and concentrations decreased the number of galls and egg masses. The number of galls and egg masses was lower in acetone extract than in ethanole extract. The nematicidal effect was higher in yellow and red radish seeds powder application. The highest nematicidal effect was determined at 6 g powder/plant application. While the mean number of galls was 1.4 unit in the yellow seed powder application at a concentration of 6 g/plant of the extract prepared with acetone solvent, it was found to be 3.0 units in the red seed powder application at 6 g/plant of the acetone extract. The number of egg masses was 1.0 unit in the yellow seed powder application, while it was 2.8 units in the red seed powder application at 6 g/plant of the acetone extract. The acetone extract of radish seed powder can be used as an alternative to chemicals in the root-knot nematodes control.
Keywords: Radish, Seed powder, Acetone extract, Ethanol extract, Nematicidal effect, Root-knot nematode

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INTRODUCTION
Root-knot nematodes are among the most destructive nematode groups. The galls formed as a result of feeding on the roots of the root-knot nematodes, prevent the plant roots from absorbing nutrients and water from the soil. As a result of this, yellowing, wilting and stunted growth occur in the plant. In addition, root-knot nematodes suppress the host plant's defences, making the plant more susceptible to attacks by other plant pathogens (Goverse and Smant, 2014). *Meloidogyne incognita*, the most common root-knot nematode species in vegetable fields worldwide, can attack the roots of more than 3000 agricultural crops (Sahebani et al., 2011). Synthetic nematicides have been used to protect moderate-to-high-value crops in intensive production systems throughout most of the twentieth century. In the last decades, environmental and human health concerns have steadily reduced the availability of efficient commercial nematicides (Nyczepir and Thomas 2009; Sorribas and Ornat, 2011). Recently, plant extracts have become an environmentally friendly alternative to chemical pesticides in pest control (Aji,

2024). Most plants produce various secondary metabolites during their development. These compounds are generally antioxidant phenolic compounds with redox properties (El-Abbassi et al., 2012). Brassicaceae plants have been used to reduce plant parasitic nematode population levels for biological fumigation, cover and/or poor host characteristics (Fourie et al., 2016). When Brassicaceae seeds and green parts were compared together, they were found to have higher glucosinolate levels and were reported to be more advantageous due to lower loss of glucosinolate degradation products (Lazzeri et al., 2004). Brassicaceae seed treatments with a 100% herbal product that provides significant environmental benefits as an alternative to chemicals, as well as higher renewability, biodegradability, positive impact on atmospheric CO2 levels and overall potential on nematodes, could open a new perspective in the control of plant parasitic nematodes (Lazzeri et al., 2009).

Radish is a member of the Brassicaceae (Cruciferae) family and its scientific name is *Raphanus sativus* L. Radish plant contains glycocinolate compounds in cells as a result of decomposition of plant cells and hydrolysis of glycocinolates, toxic isothiocyanates are formed (Vallejo et al., 2004; Sandler et al., 2015). Isothiocyanates have a lethal effect on nematodes (Zasada and Ferris 2004). Radish is reported to be resistant to nematode reproduction and the formation of root galls (Pattison et al., 2006). It is also reported to be a very good trap plant for root-knot nematodes and a biofumigant when applied to soil as a green manure (Melakeberhan et al. 2008). However, the consistent suppressive effect of allelopathic extracts or ground material on nematode populations has also been attributed to the nematostatic effect of released ammonia, hidrolysis products of glycosinolate content (Mazzola et al., 2007, 2009). Aydınlı and Mennan (2018) found that in biofumigation plots treated with radish and arugula, *M. arenaria* infections in tomatoes were significantly reduced. Radwan et al. (2012) reported that the application of powdered *R. sativus* seeds suppressed root galling in tomatoes by 78.3%. Shalaby et al. (2021) determined that the application of *R. sativus* seeds significantly suppressed nematode development in *M. incognita*-infected pepper compared to the control. In addition, Zasada et al. (2009) reported that seed particle size altered the nematoxic effect. They found that ground *Sinapis alba* seeds had a higher suppressive effect on *Pratylenchus penetrans* compared to larger particles. This indicates that smaller particles are evenly distributed in the soil profile, whereas larger particles create pockets of toxicity where not all nematodes are exposed, in comparison, *Brassica juncea* seeds were found to have greater nematode toxicity than *S. alba*. The 2.5% and 10% *S. alba* were required for 100% suppression of *M. incognita* and *P. penetrans*, respectively, whereas 0.5% would be sufficient for *B. juncea*.

In Türkiye, there is a limited number of studies on seed applications of nematicidal plants on soil. However, it is known that different radish varieties are cultivated in Türkiye. It has been determined that these varieties are named according to their seed colours. Whether this colour difference will make a difference in the nematicidal effect has been the subject of research. In this study, it was aimed to determine the nematicidal effect of acetone and ethanole extracts obtained from the powder of different coloured radish seeds (yellow, black, white and red) on the root knot nematode, *M. incognita* under controlled conditions.

MATERIALS AND METHODS

Material

In this study, 4 different radish seeds of local varieties with white, yellow, red and black colours were used as material. Within the scope of this study, seeds were purchased commercially from Intfa Agricultural Shopping Center (Konya, Türkiye). The ISP isolate, which continues mass production under climate chamber conditions (24±1 °C, 60%±5% RH), was used as the population of the root-knot nematode, *M. incognita* (Göze et al., 2022). As tomato material, nematode susceptible Gülizar F1 variety was used which was purchased commercially from Olympos Seedling (Kumluca, Antalya, Türkiye).

Methods

Preparation of *Meloidogyne incognita* inoculum

Eggs of *M. incognita* were extracted from infected tomato roots using 1.5% sodium hypochlorite (NaOCl) as described by Hussey and Barker (1973). Eggs were poured into a 75 µm sieve and collected on in a 5 µm sieve. At this stage, they were washed with tap water to remove the sodium hypochlorite and the number of eggs was counted under a binocular microscope at 40x magnification, appropriate dilution was made with distilled water and nematode suspensions were prepared according to the study. They were kept in the refrigerator (+4ºC) until the experiment was established.

Preparation of seed extracts from radish seeds

Ethanol and acetone solvents were used in the study. Twenty g of each radish seed was separated and crushed in a spice grinder until it was powdered. Then 200 ml of different solvents (acetone (99%) or ethanol (96%) were added to the powder samples and kept at room temperature for 24 hours. At the end of this period, the samples were filtered using filter papers. The solvents in the filtrate were evaporated by drying in an evaporator (Kabil and Adam, 2020).

In pot experiment

The study was carried out under controlled conditions (24±1°C, 60%±5% RH) in the climate chamber of Isparta University of Applied Sciences, Faculty of Agriculture, Department of Plant Protection. The tomato seedlings

were transplanted into 250 ml plastic pots containing 300 g of sterile soil mixture (68% sand, 21% silt and 11% clay). The study was established in a randomized plots experimental design with 5 replicates for each radish seed extracts (acetone and ethanol) and concentrations (2, 4 and 6 g/plant). One week after transplanted, 1000 *M. incognita* eggs with 1 ml of water were inoculated into holes drilled near the root. One week after nematode inoculation, radish seed powder extracts according to the experiment were applied at doses of 2, 4 and 6 g/plant in pot (Shalaby et al., 2021). It was then allowed to mix thoroughly with the soil. While only nematode-inoculated plants were used as a negative control, nematicide with the active ingredient Fosthiazate (Nemathorin, Sygenta) was used as a positive control at a dose of 0.3 ml/plant. The experiment was terminated 50 days after inoculation. Afterwards, the plants were uprooted and washed with clean water to remove the soil from the roots and the number of gall and egg mass in the roots were counted.

The data obtained as a result of the study were analyzed in the standard analysis of variance technique (ANOVA) using the GLM procedure in the SAS (2009) statistical package program, and the differences between the means were determined according to the LSD multiple comparison test.

RESULTS AND DISCUSSION

In this study, it was determined that radish seed variety (V), extracts (E) and concentrations (C) differed significantly ($p<0.01$) on the galls caused by *M.incognita* on tomato roots. It was also found that E X V, E X C, V X C and E X V X C interactions were significant ($p<0.01$) (Table 1).

Table 1. The variance analysis of interaction on galling of tomato roots

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value
Extraction(E)	1	42.320	42.320	22.39**
Seed variety (V)	3	499.220	166.40667	88.05**
Concentration (C)	4	78139.680	19534.920	10335.9**
E X V	3	174.280	58.09333	30.74**
V X C	4	118.280	29.570	15.65**
V X C	12	578.08	48.17333	25.49**
E X V X C	12	872.52	72.71	38.47**
Error	160	302.40	1.89	
General	199	80726.780		

In the present study, compared to the negative control, all treatments and concentrations decreased the number of galls. The difference between acetone and ethanol extracts was found to be significant in terms of gall number. The number of galls was lower in acetone extract than in ethanol. The suppressiveness of gall number increased with high concentrations. The highest suppressiveness on the number of galls was found in 6 g/plant in a soil application. When the general mean was evaluated, when 4 g/plant was applied to the soil in terms of gall number, a similar effect was observed as the positive control. However, the mean number of galls in the 6 g/plant in soil application (2.4 galls/ plant) was found less than the positive control (6.2 galls/ plant). The lowest number of galls was determined in the yellow radish seed powder extractions. The highest number of galls was found in white and black seed powder extractions (Table 2).

It was determined that radish seed variety (V), extracts (E) and concentrations (C) differed significantly ($p<0.01$) on the number of egg masses in tomato. It was also found that E X V, E X C, V X K and E X V X C interactions were significant ($p<0.01$) (Table 3).

The mean number of egg masses was lower significantly in acetone extract (16.17 units) than in ethanol (17.42). It was determined that the suppressiveness on the number of egg masses was higher in 6 g/plant in a soil application. When compared with the negative control, all treatments and concentrations decreased the number of egg masses. When the general mean was evaluated, the egg mass number in 6 g/plant application (1.8 unit) was found less than the positive control (4.4 units). The lowest mean number of egg masses in tomatoes was obtained from yellow and red radish seed powder extraction which had similar suppressive effects. The highest number of egg mass was found in black radish powder extraction (Table 4).

The nematicidal effect of different coloured radish seed powder ethanol and acetone extracts on root-knot nematode were evaluated and significant suppression was determined in all extracts compared to the negative control. The nematicidal properties of radish have been reported in different previous studies. *Raphanus sativus* has a high ability to control nematodes present in the soil, such as *Meloidogyne hapla,* it is a biosynthesis plant for toxic compounds of nematodes (Jaafar et al., 2020). Shalaby et al. (2021) reported that *Brassica rapa, Eruca sativa, Juniperus communis, Lepidium sativum, R. sativus* and *Sinapis alba* seed powders caused a significant reduction in nematode population in pepper infected with *M. incognita* under greenhouse conditions, but *S. alba* was the most effective. El-Shaefeey et al. (2023) reported that mixing radish seed extract into the soil before nematode inoculation in eggplant reduced the number of *M. javanica* galls on the roots.

Table 2. Effect of different coloured radish seed powder extractions on the number of galls in tomato roots

Application	Seed variety	Number of galls						Mean
		Concentration g/plant in soil						
		2	4	6	Negative Control	Positive Control	ExV	
Acetone	White	18.2	7.4	3.8	56.0	6.2	18.32	17.23 b
	Black	25.4	10.2	1.8	56.0	6.2	19.92	
	Yellow	4.2	1.0	1.4	56.0	6.2	13.76	
	Red	12.8	6.6	3.0	56.0	6.2	16.92	
Ethanol	White	23.4	11.8	1.2	56.0	6.2	19.70	18.15 a
	Black	16.2	10.6	5.6	56.0	6.2	18.92	
	Yellow	22.0	2.2	1.2	56.0	6.2	17.52	
	Red	14.6	4.2	1.2	56.0	6.2	16.44	
Mean		17.1 B	6.75 C	2.40 D	56.0 A	6.2 C		

Lowercase letters indicate differences between extractions in the same column, and uppercase letters indicate differences between concentrations in the same row.

Table 3. The variance analysis of interaction on egg masses in tomato roots

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value
Extraction (E)	1	78.125	78.125	27.80**
Variety (V)	3	305.775	101.925	36.27**
Concentration (C)	4	80889.27	20222.3175	7196.55**
E X V	3	191.815	63.93833	22.75**
E X C	4	198.15	49.5375	17.63**
V X C	12	425.25	35.4375	12.61**
E X V X C	12	668.61	55.7175	19.83**
Error	160	449.6	2.81	
General	199	83206.595		

Table 4. Effect of different coloured radish seed powder extractions on the number of egg masses in tomato roots

Application	Seed variety	Concentration g/plant in soil						Mean
		2	4	6	Negative Control	Positive Control	ExV	
Acetone	White	13.2	5.2	2.0	56.0	4.4	16.16	16.17 b
	Black	20.8	11.4	1.8	56.0	4.4	18.88	
	Yellow	4.2	2.0	1.0	56.0	4.4	13.56	
	Red	12.6	4.6	2.8	56.0	4.4	16.08	
Ethanol	White	22.2	11.1	1.2	56.0	4.4	18.96	17.42 a
	Black	14.2	11.0	4.0	56.0	4.4	17.92	
	Yellow	21.0	2.8	1.4	56.0	4.4	17.12	
	Red	14.0	2.6	1.4	56.0	4.4	15.68	
Mean		15.28 B	6.33 C	1.98 E	56.0 A	4.4 D		

Lowercase letters indicate differences between extractions in the same column, and uppercase letters indicate differences between concentrations in the same row.

The study revealed that extraction method and concentration were important depending on seed colour. The nematicidal effect of acetone extract was higher than ethanol. In addition, The nematicidal effect of yellow and red radish seed extracts was higher than white and black. To determine the differences in these nematicidal properties, their compounds need to be identified. Flavonoids, saponins, and tannins present in radish have also demonstrated antioxidant, antimicrobial and antibacterial activity (Ahmad et al., 2012; Lim et al., 2019; Muthusamy & Shanmugam, 2020). Goyeneche et al. (2015) reported that the most abundant free and bound phenolic compounds in the roots and leaves of red radish are pyrogallol and vanillic acid; and epicatechin and coumaric acid, respectively. Radish produces isothiocyanate that is break down product of glicosinolates and this eliminates pathogens in the soil including fungi (Melakeberhan et al., 2008). The α-amylase inhibition activity as well as antibacterial activity of radish seed and rapeseed were also significantly high (Khatiwada et al., 2018).

In our study, although the nematicidal effect of ethanol extract was found to be lower than acetone, it was observed that significantly suppressed galls and egg masses compared to the negative control. Ahmad et al. (2012) reported that ethanolic and methanolic extracts of *R. sativus* seeds were effective against the bacterial species they used. Zaidat et al. (2020) reported that *Peganum harmala* L., *Raphanus raphanistrum* L., *Taxus baccata* L., *Sinapis arvensis* L., and *Ricinus communis* had high nematicidal potential on *M. incognita* when applied in a methanolic solvent. Aissani and Sebai (2022) found that radish methanol extract was rich in 4-methylthio-3-butenyl isothiocyanate and had high nematicidal activity on *M. incognita.* Törün et al. (2017) determined that the antimicrobial activity of methanol extract of *Echinophora tenuifolia* L. and boiled water extract of *R. sativus* was more effective than ethyl acetate extract. Göze Özdemir (2024) investigated the nematicidal effect of milk thistle leaves and seeds prepared with different solvents on *M. incognita*. No statistical difference could be determined between the solvents (acetone, ethanol, distilled water) in the number of galls and egg masses in seed extraction. On the contrary, a difference was found between acetone and ethanole extract in this study.

It was observed that the nematicidal effect increased as the concentration increased and it was determined that 6 g/soil concentration was more effective and a suppression above 60% was determined. Radwan et al. (2012) reported that 5 g/kg radish seed powder treatment reduced root galling in tomatoes by 78%. Göze Özdemir (2022) found that the control effect of 6 g/plant radish seed powder alone on *M. incognita* gall and egg masses in tomato and cucumber roots was similar to the control effect of arugula (2 g/plant) + radish (2 g/plant) and cress (2 g/plant) + radish (2 g/plant). Ibrahim et al. (2007) reported that fenugreek and lupin seed powder caused reduction (92.2–98.6%) in root galls and egg masses of *M. incognita*, while treatments of acacia seed powder and camphor dried leaves induced 54.6–66.3% reduction in root galls and egg masses on infected sunflower plants. Incorporated powder seeds of pig bean (*Canavalia ensiformis*) into the soil reduced galls and egg mass of *M. incognita* on tomato plants by 48% and 64%, respectively, with the application of 10 g/kg soil (Silva et al. 2002).

CONCLUSION

From this study, it is concluded that the extracts of radish shows promising nematicidal activity and offer possibilities as non-chemical alternatives for the management of *M. incognita*. It is envisaged that yellow and red seed extracts of radish can be used as an alternative to chemicals in the control of root-knot nematodes. Yellow and red radish nematicidal compound(s) are unknown. Therefore, We should be determined and purified them. Once identified, they or their derivatives can be artificially synthesized which as a source of nematicidal agents in future pesticide design and development. Additionally, microplots and field studies are required.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors state there is no competing interest.

Author contribution

Conceived & designed the experiment, Fatma Gül Göze Özdemir & Fadimana Maril; Performed experiment, Fadimana Maril & Harun Çimenkaya; Formal data analysis & Visualization of the data Bekir Tosun, Writing-original draft and data curation, Fatma Gül Göze Özdemir, Fadimana Maril, Bekir Tosun & Harun Çimenkaya.

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Effects of different doses of zeatin, kinetin, and gibberellic acid biostimulants on growth and biochemical parameters during the seedling development stage of Istanbul Oregano (*Origanum vulgare* L. ssp. *hirtum*)

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DergiPark AKADEMİK

Abstract
This research was conducted to determine the effects of different doses of zeatin, kinetin, and gibberellic acid biostimulants, which are plant growth and development regulators, on the growth parameters and some biochemical parameters of Istanbul oregano (*Origanum vulgare* L. ssp. *hirtum*). The experiment was carried out in a greenhouse setting according to the "Completely Randomized Experimental Design" with three replications. In the study, seedling and root lengths, seedling and root fresh weights, seedling and root dry weights, chlorophyll a, chlorophyll b, total carotenoid content, total phenolic content, and antioxidant activity (CUPRAC and FRAP) parameters were examined. The results of the study showed that all biostimulants increased the growth and biochemical parameters compared to the control, except for seedling dry weight. The highest plant height was obtained from the 200 ppm dose of gibberellic acid, while the highest values in growth parameters, except for root dry weight, were obtained from the 50 and 100 ppm doses of kinetin. The highest values for chlorophyll a, total chlorophyll, total carotenoid, and FRAP antioxidant activity were found at the 40 ppm dose of zeatin, while the highest values for total phenolics and CUPRAC antioxidant activity were observed at the 100 ppm dose of kinetin.
Keywords: Antioxidant, Biostimulants, *Origanum*, Seedling Development, Total Phenolic Contents

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INTRODUCTION

The genus *Origanum*, belonging to the Lamiaceae family, is represented by 39 species, predominantly spread across the Mediterranean region (Sozmen et al., 2012). In Turkey, there are 23 species of this genus, 14 of which are endemic (Duman, 2000). In Turkey, there are four subspecies of *Origanum vulgare*. These are: O. vulgare subsp. gracile, O. vulgare subsp. hirtum, O. vulgare subsp. viride, and O. vulgare subsp. vulgare (Sarikurkcu et al., 2015).

The subspecies *O. vulgare* ssp. hirtum, known as Istanbul oregano in Türkiye, is known to be among the best oreganos in terms of essential oil quality and concentration, and its popularity is increasing (Skoufogianni et al., 2019).

Turkey holds a leading position in the global oregano market, with oregano being one of the country's significant export items. Turkey provides approximately 80% of the world's oregano trade, showcasing its effectiveness in this field. Around 90% of the country's oregano exports come from species belonging to the genus *Origanum*. Among these species, *Origanum onites* L. and *Origanum vulgare* subsp. *hirtum* are particularly the most cultivated and harvested (Bayram and Arabacı, 2021). It has been reported that the essential oil content of Istanbul oregano in the wild ranges from 1% to 6.1%, whereas in cultivated conditions, it ranges from 1.2% to 5.7% (Tınmaz et al., 2009).

Carvacrol and thymol are reported to be the main components of the essential oil in plants belonging to the genus *Origanum*, followed by components such as γ-terpinene, p-cymene, linalool, terpinen-4-ol, and sabinene hydrate (Azizi et al., 2009).

Istanbul oregano, used as a spice from its herb and leaves, and its oil is utilized in numerous industries. Oregano is known as a common spice in Mediterranean cuisine and stands out for its versatile uses. Particularly, its essential oil is used across various sectors due to its strong antioxidant, antibacterial, and antifungal properties. In the food and beverage industry, it is preferred for preserving products and keeping them fresh for a longer duration, while it also holds significant importance in the cleaning, cosmetics, and pharmaceutical fields. Chosen in complementary medicine, oregano is used as a nutritional supplement in aquaculture and as a nectar source in beekeeping, making it a highly functional plant. (Beltrán et al., 2018; Dutra et al., 2019; Guan et al., 2019).

Plant biostimulants are generally any stimulant substance (synthetic or natural) or microorganisms applied to plants in different forms and times with the aim of enhancing nutrient content, improving abiotic stress tolerance, and/or improving crop quality characteristics (Patrick 2015).

Zeatin, a member of the cytokinin biostimulant group, is a biostimulant that plays critical roles in plant growth and development, regulating various processes such as the separation of buds from the apical tip, expansion of leaves, formation of chloroplasts, slowing down the aging process, promoting the germination of seeds, and regulating the cell cycle (Havlicek et al., 1997; Mok and Mok 2001).

Kinetin, another member of the cytokinin biostimulant group, is reported to be a biostimulant that delays aging by affecting ethylene synthesis, contributes to growth and development by increasing cell division, and is effective in increasing chlorophyll synthesis (Toprak 2019). Gibberellic acid (GA), produced by plants, serves as a signaling molecule in processes such as germination, water uptake, initiation of flowering, fruit development, shoot elongation, and regulation of various metabolic events, working in conjunction with other phytobiostimulants responsible for these processes (Zhu et al., 2019; Khan et al., 2020).

This study has been conducted to determine the effects of foliar applications of zeatin, kinetin, and gibberellic acid biostimulants at different doses on the growth and biochemical parameters of the Istanbul oregano plant during the seedling development phase.

MATERIALS AND METHODS

Material

The study was conducted in the greenhouse of the Agricultural Sciences and Technologies Training, Application, and Research Center belonging to the Faculty of Agriculture, Sakarya University of Applied Sciences. The oregano seedlings used in the research were obtained from a commercial company.

Method

The experiment was conducted following a Completely Randomized Experimental Design with three replications. In the study, oregano seedlings known as "Istanbul oregano" were treated with biostimulants known to affect plant growth and development: zeatin (20, 40 mg/L), kinetin (50, 100 mg/L), and gibberellic acid (100, 200 mg/L). A total of 21 pots, each with a capacity of 2 liters, were used. These pots were filled with a homogeneous mixture prepared from finely sieved garden soil (3 parts) and Klassman TS1 brand peat (1 part). The ground in the greenhouse, where the study was to be conducted, was leveled with a rake and then rolled before placing the pots at intervals of 20 cm within rows and 30 cm between row space.

After placing the pots in the greenhouse, five randomly selected pots were each watered with 500 ml of water. Plates were then placed under the bottoms of the pots to collect the water that drained through. After the drainage process was complete, an average of 215 ml of water accumulated in the plates from each pot. The water retention capacity of the pots was calculated by subtracting the drained water from the 500 ml of water added to each pot.their water-holding capacities were measured to be 285 ml. Subsequently, the seedlings were planted in the pots at an approximate depth of 3 cm on November 1, 2023. During planting, the lower two leaves of the plants were removed by hand if they touched the soil to prevent fungal disease. Throughout the experiment, each pot was watered with approximately 100 ml of water once a week.

Kinetin and gibberellic acid hormones were dissolved in 96% ethanol, while zeatin hormone was dissolved in NaOH and completed to 1 liter with distilled water. The prepared biostimulant solutions were filled into 1-liter spray bottles, wrapped in aluminum foil to protect from light, and stored in a refrigerator. The initial foliar applications of the biostimulants were made on January 2, 2024, approximately two months after planting. Due to the experiment being conducted during the winter months, growth and development were slower compared to the summer months; therefore, the application of biostimulants was delayed. Foliar-applied biostimulants have been shown to produce effects such as improved leaf color, increased growth rate, and enhanced stress tolerance within a few days, as demonstrated in various studies on different plant species. For example, in a study by Khallouf et al. (2017), biostimulants were applied every two days and concluded on day 15. In another study by Shahzad et al. (2015), applications were made on days 5, 6, and 9, with observed positive effects. The trial lasted for an average of 2.5 months. During the period from the setup to the conclusion of the experiment, it was determined that the average daytime temperature was 15°C, while the nighttime temperature was 4°C (Anonymous 2024).

The roots of the plants were softened with water and separated from the soil. Subsequently, root lengths were measured and recorded with the help of a ruler. The fresh weights of the seedlings and roots were measured on a precision scale. The aerial parts and roots of the seedlings were placed in drying paper and then put in an oven at 35°C for 108 hours to dry. Afterwards, the dry weights of the seedlings and roots were measured.

Total Phenolic Content Analysis

The total phenolic content was assessed using the Folin–Ciocalteu method according to Waterhouse (2002). Initially, 250 µL of Folin–Ciocalteu reagent and 50 µL of the extract solution were added to a tube, with the total volume adjusted to 3 mL using distilled water. After a 5-minute incubation period, 750 µL of a 20% (w/v) Na_2CO_3 solution was added, and the tubes were mixed. The resulting solution was kept in the dark at room temperature for 90 minutes. The absorbance was then measured at 765 nm using a UV-Vis spectrophotometer (Agilent Cary-60, Santa Clara, CA, USA). A gallic acid standard curve was generated by repeating the procedure with concentrations of 50, 100, 150, 200, and 300 µg/mL. The total phenolic content was expressed as gallic acid equivalents using the standard curve (mg GAE/100 g of dry weight thyme).

Determination of FRAP Reducing Capacity

Initially, 0.3 M sodium acetate buffer (pH 3.6), 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution, 20 mM FeCl3, and 2 mM FeSO4 solutions were prepared. The working solution was obtained by mixing the buffer solution, TPTZ, and FeCl3 solutions in a 10:1:1 ratio. Absorbance measurements were taken at 593 nm using a 2 mM FeSO4 solution to create the standard curve, followed by measuring the samples at a minimum of three different concentrations. The results were reported as mg extract/µmol Fe2+ equivalents (Sachett et al. 2021).

Determination of CUPRAC Reducing Capacity

This method was based on a partially modified version of a previously reported procedure. Plant extracts were taken in different concentrations (10, 20, 40 µg) into tubes. Then, 0.25 mL of CuCl2 solution (0.01 M), 0.25 mL of ethanolic neocuproine solution, and 0.25 mL of CH3COONH4 buffer solution (1 M) were added. After incubating the mixtures in the dark for 30 minutes, absorbance values were measured at 450 nm against a blank (Ak and Gülçin 2008). The measurement results were evaluated by comparing them to trolox equivalents.

Photosynthetic Pigments (chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a/chlorophyll b ratio, and total carotenoid content)

The analysis of photosynthetic pigments was conducted according to Lichtenthaler (1987). A 0.2 g (200 mg) fresh plant sample was extracted with 10 mL of 80% acetone and centrifuged at 4600 rpm for 15 minutes. The absorbance values of the aliquots taken after centrifugation were measured at wavelengths of 663, 645, and 470 nm using a spectrophotometer (PG T60 UV-VIS) and recorded. The calculations were made using the following formulas:

Chlorophyll a (µg g−1 FW) = 11.75 × A662 - 2.350 × A645

Chlorophyll b (µg g−1 FW) = 18.61 × A645 - 3.960 × A662

Total chlorophyll (µg g−1 FW) = chlorophyll a + chlorophyll b

Total carotenoid (µg g−1 FW) = (1000 × A470 - 2.270 × chlorophyll a) - (81.4 × chlorophyll b / 227)

where A is the absorbance value, and FW is the fresh weight.

Statistical Analysis

Statistical analyses of the data obtained were performed using the COSTAT (version 6.03) package program, and multiple comparison tests were performed according to the Least Significant Difference (LSD = 0.05) test.

RESULTS AND DISCUSSION

The effect of synthetic biostimulants on all growth parameters except root length in Istanbul oregano was found to be statistically significant at the 5% level. The highest value for seedling length was observed in the GA200 treatment with 12.16 cm, while the kinetin100 treatment yielded the best results for seedling fresh weight with 2.96 g, as seen in Table 1. In terms of root fresh and dry weights, the kinetin50 treatments produced higher results compared to other treatments, with 4.89 g and 0.70 g, respectively. For seedling dry weight, the control treatments performed relatively better with 0.72 g (Table 1).

Foliar applications of kinetin on *Ervatamia coronaria* plants increased the seedling fresh and dry weights, root fresh and dry weights, and root length compared to the control (Ashour et al. 2023). The kinetin biostimulant is reported to enhance chlorophyll content in plants, promoting the production of photosynthetic proteins, accelerating cell division, and breaking apical dominance in plants. This results in increased lateral branch and root formation, leading to increases in both fresh and dry weights of above-ground and below-ground parts (Lazar et al. 2003; Bielach et al. 2017).

Table 1. Effects of certain synthetic biostimulants on the growth parameters of İstanbul oregano plant

Biostimulants	Seedling length (cm)	Seedling fresh weight (g)	Root length (cm)	Root fresh weight (g)	Seedling dry weight (g)	Root dry weight (g)
Control	9.53 b	2.90 a	25.60	4.75 a	0.72 a	0.66 a
Zeatin20	8.47 b	2.30 ab	26.75	2.85 c	0.55 ab	0.43 bc
Zeatin40	8.33 b	2.78 a	28.75	3.73 b	0.61 a	0.50 b
Kinetin50	10.23 ab	2.71 a	29.00	4.89 a	0.62 a	0.70 a
Kinetin100	9.40 b	2.96 a	29.00	4.50 ab	0.66 a	0.65 a
GA100	10.00 ab	1.98 b	28.50	2.30 c	0.39 b	0.39 c
GA200	12.16 a	2.67 ab	29.00	4.47 ab	0.58 ab	0.67 ab
LSD(0.05)	2.48	0.73	ns	0.8	0.19	0.14
CV(%)	14.58	16.08	7.29	11.74	19.13	14.4

ns=not significant

Foliar applications of GA3 have been reported to increase the height and quality of *Araucaria heterophylla* plants (Gul et al. 2006), and spraying *Dahlia pinnata* plants with 100 or 200 ppm GA3 has been shown to enhance plant growth parameters (Yousef and Gomma 2008). Similar studies have demonstrated that GA3 foliar applications increase seedling length in *Hibiscus sabdariffa* L. (Alharby 2021), and wheat (Mirheidari et al. 2022), with comparable findings by Santos et al. (1998) and Srivastava and Srivastava (2007). Gibberellic acid applications in thyme have been shown to increase growth parameters, chlorophyll pigments, and essential oil content (Dadkhah et al. 2016), and gibberellic acid applications are reported to have positive physiological, morphological, and biochemical effects on plants (Taiz and Zeiger 2010). The increases in growth parameters due to gibberellic acid applications are attributed to the enhanced activity of enzymes such as carbonic anhydrase, nitrate reductase, and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) (Yuan and Xu 2001; Afroz et al. 2005; Aftab et al. 2010). Additionally, GA3 is reported to promote growth and development by stimulating cell growth and cell division (Taiz and Zeiger 2010). The findings of previous studies support our results.

Table 2. Effects of some synthetic biostimulants on chlorophyll pigments in İstanbul oregano plant

Biostimulants	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophylls (mg/g)
Control	98.69 b	116.13 bc	214.82 bc
Zeatin20	87.46 c	115.26 bc	202.71 cd
Zeatin40	112.55 a	130.51 a	243.06 a
Kinetin50	103.07 b	126.92 ab	229.99 ab
Kinetin100	84.46 c	101.93 d	186.40 d
Gibberellic acid100	84.42 c	103.03 cd	187.44 d
Gibberellic acid200	76.34 d	131.71 a	208.05 c
LSD (0.05)	7.47	13.11	19.77
CV (%)	4.61	6.35	5.36

When evaluating Table 2, it is evident that the applied hormones had a statistically significant effect on chlorophyll pigments at the 5% level. The highest values for chlorophyll a and total chlorophyll, were 112.55, 243.06, and mg/g, respectively, measured in the 40 ppm foliar applications of Zeatin hormone. For chlorophyll b pigment, the highest value was 131.71 mg/g, found in the 200 ppm applications of gibberellic acid.

Exogenous cytokinins have been reported to enhance growth, water status, chlorophyll accumulation, antioxidant status, stomatal opening, and the functioning of the photosynthetic apparatus in Ricinus communis plants exposed to copper (Cu) heavy metal (Sameena et al. 2021). Similarly, cytokinins such as Zeatin, kinetin, benzyladenine, and thidiazuron have been reported to increase biomass production, chlorophyll pigments, and carotenoid content (Yu et al. 2024). Zeatin hormone applications in wheat have been shown to increase chlorophyll pigments, carotenoids, and non-enzymatic antioxidant substances (Ali et al. 2022). Many studies indicate that applications of Zeatin and other cytokinin group hormones increase chlorophyll pigments and carotenoid content in various plants compared to the control (Emami et al. 2011; Faraji et al. 2011; Ali et al. 2023). Cytokinin group hormones, including Zeatin, are reported to directly or indirectly influence the production of chlorophyll and other plastid pigments by re-regulating the gene regions encoding these pigments (Cortleven and Schmülling 2015). Gibberellic acid applications have been shown to increase chlorophyll pigments in stevia plants (Modi et al. 2011), and foliar applications of GA3 hormone have been reported to increase chlorophyll pigments in *Ficus benjamina* L. and *Spathiphyllum wallisii Regel* plants compared to the control (Salehi et al. 2014; Rahbarian et al. 2014).

Table 3. Effects of some synthetic biostimulants on biochemical parameters of İstanbul oregano plant

Biostimulants	Total Phenolics (mg/g GAE)	Total Carotenoids (mg/g)	CUPRAC (mM/g TE)	FRAP (mM/g AAE)
Control	0.35 bc	0.81 ab	4.73 a	0.80 b
Zeatin20	0.35 bc	0.70 ab	5.05 a	0.78 b
Zeatin40	0.43 ab	0.93 a	5.48 a	1.24 a
Kinetin50	0.38 ab	0.83 ab	5.85 a	1.15 a
Kinetin100	0.49 a	0.65 b	6.36 a	1.15 a
Gibberellic acid100	0.40 ab	0.69 ab	5.71 a	1.16 a
Gibberellic acid200	0.25 c	0.65 b	3.01 b	1.20 a
LSD (0.05)	0.12	0.25	1.68	0.29
CV (%)	18.49	19.52	18.56	15.9

Table 3 shows that the hormones applied during the seedling development stage of Istanbul oregano have a statistically significant effect at the 5% level on antioxidant activities determined by the CUPRAC and FRAP methods, as well as on the total phenolic content and total carotenoid levels. According to the CUPRAC method, which measures free radical scavenging activity, the total phenolic content was higher in the Kinetin100 treatments with 6.36 mM/g TE and 0.49 mg/g GAE compared to other treatments. Similarly, the FRAP method, which determines antioxidant activity, showed relatively better results in total carotenoid levels with 1.24 mM/g AAE and 0.93 mg/g in the Zeatin40 treatments (Table 3).

Kinetin has been reported to increase the content of phenolic compounds such as trigonelline, caffeine, and the main chlorogenic acid, 5-caffeoylquinic acid, in coffee plants (Campa et al. 2012). The current study is consistent with several reports indicating that kinetin enhances the production of phenolic and alkaloid compounds in explants of various species under different abiotic stress conditions (Steinhart et al. 1964; Angelova et al. 2001; Siahpoush et al. 2011). While the mechanism through which exogenous kinetin affects the metabolic pathways of these compounds is not yet fully understood, it has been suggested that kinetin positively regulates relevant transcription factors (Barciszewski et al. 1999) and directly enhances the activities of phenolic and alkaloid biosynthesis enzymes (Steinhart et al. 1964; Angelova et al. 2001).

CONCLUSION

In this study, the effects of synthetic biostimulants on Istanbul oregano were investigated, revealing significant effects on all growth parameters except root length. GA200 application was found to be the most effective in increasing seedling length, kinetin100 in seedling fresh weight, and kinetin50 in root weights. These findings indicate that biostimulants positively affect plant growth. Hormone applications were found to have significant effects on the increase of chlorophyll pigments, suggesting that considering the use of Zeatin and gibberellic acid to optimize plant growth could be beneficial. For Istanbul oregano seedlings during the development stage, it is recommended to use kinetin100 applications to increase antioxidant activity and total phenolic content, while Zeatin40 applications may be preferred to maximize total carotenoid levels.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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The effects of different cooking methods on the physicochemical properties of potatoes, carrots, and cultivated mushrooms

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DergiPark AKADEMİK

Abstract
This study investigated the effects of different cooking techniques (hot-air baking and deep frying) on digestibility, thermal properties, and functional compounds of potatoes, carrots, and cultivated mushrooms. Color values (Hunter *L*, *a*, *b*, and ΔE), thermal properties (To and ΔH values), total phenolic content, antioxidant activity, estimated glycemic index (eGI), and sensory properties analyses were carried out on the obtained products. According to the results, a statistically significant ($p<0.05$) effect of different cooking techniques on the physicochemical and sensorial properties of cooked potato, carrot, and cultivated mushroom samples was found. The eGI values of the samples were ranged in 42.82-68.50 and had low (<55) glycemic indexes, with the exception of deep-fried carrot samples. With the cooking process, a decrease was observed in the antioxidant activity and total phenolic content of the samples. The sensory analysis results determined that the panelists gave higher scores to the deep-fried samples than the baked samples. In addition, the general acceptance scores of deep-fried products were higher. As a result, the baking process is recommended for the preservation of physicochemical properties of the samples, although deep frying provided higher scores for sensorial properties.

Keywords: Cooking, Thermal properties, Glycemic index, Functional properties, Vegetables

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INTRODUCTION

Heat treatment is one of the methods commonly used in the food industry and is generally preferred to extend the shelf life of food or to produce a new product. With the heat treatment, several complex reactions (i.e. Maillard reaction) occur that change the quality parameters such as storage stability, sensory properties, nutritional properties of the food (Choe and Min, 2007). Heat treatments can also reduce food quality. It is known that most bioactive compounds are relatively unstable to heating (Roncero-Ramos et al., 2017). There are many suggestions regarding the healthy consumption of different vegetables. In particular, the boiling process is preferred in terms of health in consuming these products. However, the sensory properties of boiled products are significantly lower than those cooked by hot-air baking and deep/shallow frying techniques. For this reason, consumers prefer hot-air baking and frying processes more widely to provide the desired sensory quality, especially in terms of taste and smell (Tuta and Palazoglu, 2017).

Hot-air baking is a thermal process performed at high temperatures and a complex process in which some chemical and physical changes co-occur. It is essential not only for the shelf-life stability of the product but also for food quality, taste and texture. In addition to the desired quality properties, compounds formed during heat treatment, such as acrylamide and HMF, are also formed by baking (Mogol and Gökmen, 2014).

The frying process is generally divided into deep and shallow (contact) frying. In the deep-frying process, the heat transfer is equal at every point of the food, as the oil surrounds the food surface. For this reason, frying is

uniform. The deep-frying process is more preferred than shallow frying due to the higher quality of the product obtained after frying in terms of desired colour, texture and flavour characteristics (Devseren et al., 2021).

After the food is consumed, the starch molecules in the composition of the food are broken down into branched α-limit dextrins, maltose and linear oligomers of glucose by pancreatic α-amylases. The released monosaccharides are absorbed into the bloodstream via glucose transporters and used as energy in the body (Shin et al., 2019).The ability of food containing carbohydrates to increase blood sugar after consumption is defined by the concepts of glycemic index or glycemic load (Pi-Sunyer, 2002).It is known that the glycemic index value of food, which is considered very important in terms of digestibility of foods, is also significantly affected by different cooking techniques (Allen et al., 2012).

The present study aims to determine the effects of hot-air baking and deep-frying on some physicochemical properties of potatoes, carrots and cultivated mushrooms. The main reason for choosing potato, carrot and cultivated mushroom in the study is that all three vegetables are rich in different components. Studies have reported that potatoes, carrots and mushrooms are rich in starch-based (Lachman et al., 2013), pectin-based (Sharma et al., 2012) and protein-based (Manzi et al., 2004) components, respectively. Therefore, this study was carried out to measure the reactions of foods rich in different ingredients to different cooking methods and examine the effects of cooking techniques on digestibility, thermal properties and functional compounds of these foods.

MATERIALS AND METHODS

Materials

The apparatus used for slicing within the scope of the present study was procured from a local firm. Fresh potatoes, carrots and cultivated mushrooms samples were obtained from the same batch from a greengrocer in Aydın, Turkey. The same batch production of refined sunflower oil was used for frying experiments.

Methods

Preparation and cooking methods of potato, carrot and cultivated mushroom slices

Fresh potato, carrot and cultivated mushroom samples were peeled (except mushroom), washed and dried with the help of paper towels. The samples were then cut with a slicer in 12x12x10 mm dimensions and immediately taken into the cooking process. The cooking processes were carried out under the conditions determined according to the Central Composite Rotatable Design in the Design Expert package program using the maximum and minimum temperature and time values determined by preliminary trials for each method.

Deep frying (DF) was carried out using an industrial fryer at a temperature of 180-200 °C and a time interval of 3-8 minutes. Previously prepared sliced samples were fried in 200 g portions in 2000 mL refined sunflower oil under the conditions specified according to the central composite design given in Table 1.

The hot-air baking (HAB) process was carried out in a domestic hot air oven (Arçelik, KMF833I, Turkey) at a temperature of 180-220 °C and 5-30 minutes. Sliced samples were spread in 200 g portions on the baking paper in a single layer and cooked according to the composite design given in Table 1.

Table 1. Central composite design (CCRD) for deep frying and hot air baking processes

Exp. No	Frying temperature (°C)	Frying time (min)	Baking temperature (°C)	Baking time (min)
1	180	3	180	5
2	200	3	220	5
3	180	8	180	30
4	200	8	220	30
5	180	5.5	180	17.5
6	200	5.5	220	17.5
7	190	3	180	5
8	190	8	180	30
9	190	5.5	180	17.5
10	190	5.5	180	17.5
11	190	5.5	180	17.5
12	190	5.5	180	17.5
13	190	5.5	180	17.5

Analysis of color changes

Color changes of the samples were determined by using a colorimeter (PCE-CSM-5, Deutschland). Color parameters were expressed as Hunter *L* [(0) dark - (100) light], *a* [(+) red – (-) green] and *b* [(+) yellow – (-) blue]. The total color difference (ΔE) was calculated using Equation 1 (Meena et al., 2021).

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (1)$$

Where L0, a0 and b0 are the color values of the fresh potatoes, carrots and cultivated mushrooms samples.

Determination of gelatinization temperature and gelatinization enthalpy

DSC (Perkin-Elmer, DSC 6000, Massachusetts, USA) analyzes were carried out to determine the thermal behaviour of potato, carrot and cultivated mushroom samples prepared with different cooking techniques. Approximately 5-8 mg of homogeneous sample was weighed into the aluminum sample cup. Inside, the DSC oven was conditioned with nitrogen gas with a flow rate of 50 ml/min and samples were heated from 25 °C to 90 °C with a temperature increase rate of 10 °C/min. By analyzing the thermograms obtained with the software of the device (Pyris Manager Software, Perkin-Elmer, Massachusetts, USA), the phase change starting temperature (T0) of the polymer compounds (i.e. starch, pectin, and protein) present in the samples and the required enthalpy change (ΔH) for gelatinization were calculated (Chuang et al., 2016).

Determination of antioxidant activity

The stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl radical) was used to determine the free radical-scavenging activity of the samples by using the DPPH method (Anticona et al., 2021). The results calculated according to the calibration curve obtained using the Trolox standard were indicated as µmol Trolox equivalent antioxidant capacity (TEAC)/gram dry sample. The % inhibition rate was calculated according to Equation 2;

$$\%Inhibition = \frac{(A_C - A_S)}{A_C} X100 \quad (2)$$

where Ac and As are the absorbance values of the control and cooked samples, respectively.

Determination of total phenolic content

The total phenolic content of the samples was determined by using the Folin-Ciocalteu method (Şahin et al., 2009). According to the method, 100 µL of diluted sample was mixed with 900 µL of ultra-pure water and 5 mL of 0.2 N Folin-Ciocalteu reagent and 4 mL of Na_2CO_3 (7.5% in water, w/w) was added to this mixture. The final mixture was incubated at room temperature for 1 hour in a dark place. The absorption of the samples was measured at 760 nm with a spectrophotometer (Shimadzu UV-1601, Shimadzu Scientific Instruments, Inc., Tokyo, Japan). The total phenolic content of the samples was expressed as gallic acid equivalents (GAE) in mg/100 g dry matter.

Estimated glycemic index

The estimated glycemic index (eGI) values of the samples were determined using the glucose oxidase kit (GOPOD-Megazyme, Ireland). For this purpose, samples were primarily subjected to in vitro digestion with some modifications (van Kempen et al., 2010). For this purpose, 20 mL sodium acetate buffer (0.1 M, pH 5.2) and 5 mL enzyme mix (0.7 g pancreatin, 0.05 mL amyloglucosidase and 3 mg invertase in water) were added to 0.8 g of cooked sample. The mixture was incubated at 39 °C under horizontal agitation. During the incubation period, 0.5 mL of the sample was taken from this mixture at 0, 20, 60, 120 and 180 minutes and 2 mL 96% ethanol was added to stop the enzymatic reaction. Before the analysis, the sample and ethanol mixture were centrifuged at 1790 g for 5 min. The glucose content of the supernatant was measured using a glucose oxidase–peroxidase (GOPOD-Megazyme) kit. The hydrolysis index (HI) was determined by dividing the area under the samples' curve by the area obtained for white bread. The estimated glycemic index (eGI) was calculated by using Equation (3) described by (Saraswat et al., 2020).

$$eGI = 39.71 + 0.549\ (HI) \quad (3)$$

Sensory analysis

The sensory characteristics of potato, carrot and cultivated mushroom samples prepared with different cooking techniques were evaluated by a panel of 10 people, consisting of graduate students of Aydın Adnan Menderes University Food Engineering Department, who had knowledge and experience about the sensory panel. Five randomly coded samples were evaluated in each panel, and analyses were carried out in 2 replications. It was ensured that the panellists consumed water before and after the evaluation. The samples presented in the sensory panel were evaluated with a hedonic scale scored between 1 and 5 points (1-not like and 5-extremely like) in terms of appearance, color, odor, taste, texture and global preference (Gomes et al., 2013).

Statistical analysis

The effect of different cooking techniques on digestibility, thermal properties and functional compounds of the samples was determined according to the Central Composite design using the response surface method with Design Expert 10.01 (Stat.-Ease Co., Mineapolis, USA) package program. The results were statistically evaluated by Variance analysis and Duncan's multiple range tests using the Statistical Analysis System software (SAS system for Windows V7 prepared by SAS Institute (Cary, NC, ABD)).

RESULTS AND DISCUSSION

Effects of different cooking methods on the color changes of the samples

The results for color changes of potatoes are given in Table 2. According to the results, it was observed that the Hunter *L, a, b* color values of the potatoes vary between 19.12-60.55, -4.93-23.85 and 5.46-37.20, respectively. Different cooking methods affected significantly ($p<0.05$) the color properties of the potato samples (Table 4).

Similar studies in the literature on the subject also support the results. In one study, it has been observed that the cooking process has a significant effect on the color change. At the same time, high temperature causes undesirable color, and the color change in the food gives information about the product quality and the applied process (Palazoglu et al., 2010).

Table 2. CCRD experimental data for the color values of cooked potatoes

Cooking Method	Exp. No	*L*	*a*	*b*	ΔE
Deep Frying	1	30.34±0.55	20.13±0.95	32.47±0.26	37.75±0.22
	2	30.41±0.34	20.26±0.33	31.92±0.30	37.66±0.15
	3	35.33±0.91	18.32±0.51	27.70±0.15	32.01±0.33
	4	32.20±0.56	19.36±0.11	31.92±0.54	35.70±0.15
	5	44.80±0.81	7.78±0.14	25.37±0.64	18.50±0.31
	6	27.79±0.73	21.60±0.34	34.59±0.55	41.06±0.55
	7	30.17±0.54	20.44±0.23	32.34±0.34	38.03±0.26
	8	25.99±0.66	22.58±0.66	35.89±0.25	43.35±0.55
	9	22.94±0.25	23.85±0.50	37.20±0.15	46.84±0.14
	10	30.19±0.60	20.56±1.23	32.42±0.11	38.10±0.33
	11	29.96±0.33	19.90±1.54	30.47±0.36	37.63±0.52
	12	44.29±0.15	15.58±1.66	26.10±0.15	23.16±0.14
	13	30.47±0.22	20.46±0.25	32.54±0.60	37.83±0.25
Hot air Baking	1	47.57±0.33	-0.96±0.55	17.88±0.61	16.15±0.26
	2	45.28±0.74	2.37±0.14	16.40±0.54	18.93±0.96
	3	45.29±0.88	0.95±0.36	21.23±0.34	17.01±0.36
	4	46.43±0.33	0.49±0.60	24.60±0.51	15.34±1.61
	5	45.58±0.84	-0.04±0.22	21.33±0.50	16.70±0.54
	6	40.76±0.64	0.74±0.57	21.34±0.31	21.40±1.45
	7	57.17±0.44	1.47±0.51	22.82±0.25	15.42±0.22
	8	43.85±0.20	0.62±0.33	18.53±0.24	19.21±1.22
	9	43.17±0.55	0.45±0.12	22.42±0.64	18.83±0.34
	10	42.42±0.54	0.64±0.44	22.49±0.11	19.56±1.35
	11	42.69±0.64	0.49±0.22	22.50±0.25	19.29±0.55
	12	42.55±0.84	0.72±0.34	22.76±0.34	19.39±0.15
	13	42.53±0.14	0.66±0.11	22.57±0.15	19.44±0.15

Color is the first physical quality criterion evaluated by consumers in fried potatoes. Color parameters differed significantly ($p < 0.05$) depending on the temperature of the frying potatoes. While no trend could be determined for the Hunter *L* value, the redness (Hunter *a*) values showed an increasing trend due to the temperature increase. It is stated that this change is caused by the color compounds released due to non-enzymatic browning reactions that occur during the frying process (Pedreschi, 2012). Similarly, in a study by Pedreschi and Moyano (2005), a darker red color was determined as a result of non-enzymatic browning reactions in potatoes due to an increase in frying temperature from 120 °C to 180 °C.

Color parameters were affected significantly ($p<0.05$) in hot-air baked potatoes depending on the temperature. In the study, the total color change was 15.34-21.40 in baked potatoes. In a study conducted by Tuta and Palazoğlu (2017), the color values of potato chips were defined as orange-yellow in baked samples, bright yellow in fried samples, and the total color change was found to range from 16.7-22.3 for fried products and 21.7-37.4 for baked samples.

Table 3. CCRD experimental data for the thermal and chemical properties of cooked potatoes

Cooking Method	Exp. No	To (°C)	ΔH (J/g)	GI	AA (µmol trolox/g)	TPC (mg GA/g dm)
Deep Frying	1	31.70±0.22	37.01±0.26	48.83±0.41	198.28±0.78	15.97±0.48
	2	37.09±0.15	30.24±1.52	51.23±1.12	197.17±0.55	14.54±0.19
	3	32.86±0.35	46.55±1.30	50.99±1.24	197.67±1.35	13.32±0.19
	4	36.32±0.34	42.60±0.26	49.67±0.15	195.45±1.22	9.66±0.38
	5	32.07±0.64	35.10±2.13	50.05±0.42	197.83±0.95	14.75±0.48
	6	36.93±0.51	36.10±2.30	52.38±0.56	196.69±1.12	11.84±0.38
	7	37.28±0.94	28.04±1.54	50.18±0.05	197.82±0.55	15.63±0.19
	8	36.37±0.67	31.87±1.56	53.65±0.15	196.60±0.75	12.04±0.29
	9	30.39±0.60	24.54±1.47	51.10±0.31	197.29±0.22	13.46±0.19
	10	29.02±0.90	24.44±0.25	50.77±0.14	197.05±0.96	13.60±0.38
	11	29.19±0.55	23.80±0.33	50.69±0.11	196.93±0.36	13.73±0.77
	12	28.97±0.24	24.65±0.32	50.53±0.10	196.40±0.14	13.87±0.19
	13	29.32±0.28	24.49±0.40	50.38±0.30	197.29±0.22	13.46±0.00
Hot air Baking	1	29.51±0.22	27.77±0.25	52.08±0.25	196.31±.55	28.38±0.38
	2	30.15±0.15	42.21±0.36	49.53±0.15	195.57±0.99	29.19±0.38
	3	33.23±0.35	32.86±0.15	57.18±0.34	195.75±0.41	25.53±0.19
	4	32.95±0.12	28.41±0.51	56.48±0.41	194.95±0.84	30.07±0.29
	5	33.12±0.31	38.98±0.44	52.80±075	195.94±0.55	26.28±0.29
	6	32.07±0.22	36.64±0.15	51.10±0.66	195.11±0.39	29.87±0.38
	7	34.89±0.35	47.14±0.11	49.14±0.10	195.89±0.44	27.36±0.29
	8	29.06±0.14	27.17±0.40	59.11±1.10	195.35±0.14	29.12±0.29
	9	33.28±0.36	74.84±0.36	51.58±1.35	195.73±0.55	29.12±0.48
	10	31.85±0.25	67.36±0.50	51.16±1.45	195.73±0.14	28.85±0.48
	11	32.14±0.11	65.17±0.22	50.93±0.25	195.67±0.51	29.12±0.29
	12	34.30±0.25	71.20±0.39	51.12±1.14	195.85±0.12	29.67±0.29
	13	31.36±0.22	73.18±0.45	51.16±0.88	195.85±0.23	29.67±0.38

GI: Glycemic index, AA: Antioxidant activity, TPC: Total phenolic content

The Hunter *L*, *a*, and *b* values of the carrot varied between 13.34-50.75, 3.28-30.64 and 2.11-48.28, respectively (Table 5). Red, yellow and orange-colored fruits and vegetables are rich in carotenoids such as β-carotene, α-carotene and lycopene. The composition and concentration of carotenoids in foods vary due to variety, maturity, climate or growing region, and accordingly, different color values are observed. According to the results, significant losses ($p<0.05$) occurred in the color values of carrots with the cooking process (Table 7). When carrot tissue is exposed to cooking processes, color loss occurs due to the oxidation of polyunsaturated molecules (Karaaslan, 2010). When compared with other studies in the literature, it was observed that the color values of carrots using different cooking techniques were similar. As a result, as the cooking temperature and duration increase, the color values of Hunter *L*, *a*, *b*, and ΔE also change. The difference in color values is that water leaks through the pores between the tissues during cooking of the carrots, which changes the wavelength of the light reflected from the carrot surface (Koca et al., 2007; Alibas, 2007).

The analytical results of the mushroom samples cooked with different cooking techniques are given in Table 8. According to the results, it is seen that the Hunter *L*, *a*, and *b* values of the cultivated mushroom samples varied between 17.28-74.38, 0.52-13.52 and 1.62-22.49, respectively. Different cooking techniques significantly ($p<0.05$) affected the color properties of the cultivated mushroom samples (Table 10). Color variables are associated with several factors, such as the type and amount of color compounds present in foods, and the moisture content of the samples (Stich, 2016). The color values of the cooked cultivated mushroom samples decreased significantly compared to the fresh ones. When the data obtained after the cooking process were examined, it was seen that both methods were influential on the total color change.

Table 4. ANOVA evaluation of linear, quadratic and interaction terms for each response variables for potatoes

Variation Coefficients	*p*-Values for deep frying								
	L	*a*	*b*	ΔE	To (°C)	ΔH (J/g)	GI	AA (µmol trolox/g)	TPC (mg GA/g dm)
Model	<0.0001	0.1161	0.0016	<0.0001	0.0594	0.2619	< 0.0001	0.0001	< 0.0001
β_1	<0.0001	0.1448	0.0003	<0.0001	0.4172	0.5319	< 0.0001	<0.0001	< 0.0001
β_2	<0.0001	0.6248	0.2874	<0.0001	0.0140	0.9556	< 0.0001	<0.0001	0.0002
$\beta_1\beta_2$	0.0015	0.0208	0.3121	0.0009	0.8862	0.4116	0.0348	0.0199	0.0635
β_1^2	<0.0001	0.2617	0.0033	0.0002	0.0618	0.2104	0.0109	0.0197	0.0028
β_2^2	0.9224	0.4764	0.8868	0.1300	0.6272	0.0338	0.0005	0.2055	0.6699
Lack of Fit	0.0006	<0.0001	0.0001	0.0011	0.4744	0.0004	0.5226	0.0180	0.0524
R^2	0.9900	0.6564	0.9084	0.9874	0.7246	0.5422	0.9927	0.9586	0.9875
Adj-R^2	0.9829	0.4109	0.8430	0.9784	0.5278	0.2152	0.9875	0.9290	0.9786
	p-Values for hot air baking								
Model	< 0.0001	< 0.0001	<0.0001	< 0.0001	0.1675	0.7885	<0.0001	< 0.0001	< 0.0001
β_1	< 0.0001	0.0001	<0.0001	< 0.0001	0.6508	0.4135	<0.0001	< 0.0001	< 0.0001
β_2	0.0003	< 0.0001	<0.0001	0.0004	0.8594	0.5916	<0.0001	< 0.0001	< 0.0001
$\beta_1\beta_2$	0.0214	0.0062	0.0461	0.0319	0.6064	0.4165	0.0251	0.5355	0.5892
β_1^2	< 0.0001	< 0.0001	0.1812	< 0.0001	0.0156	0.6529	<0.0001	0.0106	0.0115
β_2^2	0.1892	0.8247	0.0994	0.2121	0.1239	0.5091	0.0083	0.4795	0.5014
Lack of Fit	< 0.0001	0.1973	0.0039	0.0001	0.0003	<0.0001	0.0523	0.7179	0.7021
R^2	0.9550	0.9923	0.9768	0.9731	0.6102	0.2513	0.9945	0.9790	0.9787
Adj-R^2	0.9550	0.9868	0.9603	0.9539	0.3318	-0.2834	0.9906	0.9640	0.9635

β_1: Cooking temperature, β_2: Cooking time, GI: Glycemic index, AA: Antioxidant activity, TPC: Total phenolic content

Effects of different cooking methods on the gelatinization temperature and enthalpy of the samples

The gelatinization enthalpy (ΔH) of potato varies between 60.74-23.69 J/g and the gelatinization temperature (T_0) varies between 37.25-28.95 J/g (Table 3). While the hot-air baking process did not make a statistically significant ($p>0.05$) difference in ΔH and T0values of potato samples, the deep-frying process significantly ($p<0.05$) affected ΔH values (Table 4). Semi-crystalline polymers, generally found in foods, are proteins and polysaccharides. Starch stands out as a gelatinizing polymer in potatoes. For this reason, gelatinization occurs before melting, not at the melting temperature (Chung and Lim, 2006).

According to the results in Table 6, it is seen that ΔH values of the cooked carrot samples vary between 74.62-24.11 J/g while To values vary between 41.50-27.97 J/g. Pectin is a crucial polysaccharide that shows gelation in carrots. When the results of the DSC analysis were examined, the effect of different cooking techniques showed a significant ($p<0.05$) difference in the thermal properties of the cooked carrots. The To values of the samples were not affected ($p>0.05$) by temperature and time, while the ΔH values were significantly ($p<0.05$) affected by the temperature and interaction of temperature and time (Table 7).

Table 5. CCRD experimental data for the color values of cooked carrots

Cooking Method	Exp. No	*L*	*a*	*b*	ΔE
Deep Frying	1	41.33±0.77	20.19±0.15	32.37±0.34	10.47±0.26
	2	25.08±0.64	11.72±0.61	14.39±0.12	36.13±0.33
	3	18.65±0.21	6.05±0.50	2.11±0.30	50.76±0.25
	4	15.19±0.20	7.09±0.34	8.42±1.66	48.26±050
	5	19.85±0.52	11.39±0.15	5.49±1.25	45.89±0.22
	6	19.37±0.60	8.27±0.13	10.20±1.61	43.87±0.35
	7	31.87±0.34	14.25±0.62	22.34±0.35	25.46±0.35
	8	27.14±0.44	9.41±0.51	11.88±0.25	37.50±0.68
	9	30.12±0.54	10.23±0.35	13.89±0.25	34.05±0.68
	10	30.22±0.51	10.85±0.22	13.26±0.24	34.24±0.34
	11	31.15±0.33	10.22±0.85	14.11±0.25	33.36±0.26
	12	30.14±0.41	10.96±0.44	13.82±0.38	33.81±0.22
	13	30.12±0.50	11.05±0.46	13.85±0.56	33.77±0.82
Hot air Baking	1	50.33±0.22	28.95±1.33	40.07±0.81	5.57±0.33
	2	35.34±0.14	17.50±0.25	34.61±0.26	15.16±0.22
	3	45.36±0.51	23.69±1.54	33.43±0.53	6.55±0.11
	4	28.52±0.32	13.36±0.26	25.31±0.56	26.38±0.36
	5	45.50±0.25	30.64±0.25	43.06±0.84	8.28±0.22
	6	33.25±0.14	16.44±0.33	29.47±0.64	19.43±0.36
	7	44.90±0.35	24.33±0.54	38.28±0.13	3.58±0.22
	8	36.11±0.22	15.36±0.64	39.26±0.15	14.84±0.10
	9	47.21±0.50	20.35±0.34	33.38±0.22	6.93±0.25
	10	47.17±0.61	20.25±0.84	33.13±0.25	7.20±0.15
	11	47.41±0.34	20.14±0.34	33.37±0.32	7.03±0.35
	12	47.29±0.22	20.06±0.22	33.13±0.22	7.28±0.22
	13	47.32±0.51	20.40±0.14	32.54±0.21	7.63±0.56

T_0 and ΔH results of the cultivated mushroom samples cooked by different cooking techniques are given in Table 9. It is seen that the ΔH of the cultivated mushroom samples varies between 68.39-24.42 J/g and the T_0 value varies between 39.01-27.76 J/g. T_0 and ΔH values of fried samples were significantly ($p<0.05$) affected by temperature and temperature x temperature interaction. In contrast, only T0 values of hot-air baked samples were significantly ($p<0.05$) affected by temperature x temperature interaction (Table 10). Mushrooms are known to be rich in protein. Heat treatment damages the natural structure of proteins. As a result, the protein structure is changed. When conditions are suitable, degraded polypeptides form a three-dimensional gel structure. These gels increase both texture and nutritional properties. Such denaturation due to heat effect is determined by thermal analysis. While these processes provide information about the techniques applied to foods, they also calculate the thermal energy required for protein denaturation (Özdalyan and Karaali, 2002).

Table 6. CCRD experimental data for the thermal and chemical properties of cooked carrots

Cooking Method	Exp. No	To (°C)	ΔH (J/g)	GI	AA (µmol trolox/g)	TPC (mg GA/g dm)
Deep Frying	1	32.04±0.15	36.41±1.15	52.86±0.35	788.69±0.93	149.25±0.67
	2	40.29±0.23	44.19±1.35	56.22±0.55	1521.82±0.55	131.76±1.63
	3	30.25±0.51	39.41±1.64	62.79±0.61	1248.12±0.47	133.38±0.67
	4	30.29±0.33	29.48±1.92	68.50±0.81	2079.00±0.96	116.10±0.19
	5	39.01±0.11	26.43±1.08	52.99±0.24	909.249±1.32	140.43±0.21
	6	38.76±0.82	29.42±1.23	56.30±0.51	1580.47±0.88	126.47±0.67
	7	34.66±0.77	45.06±0.55	55.43±0.41	554.089±0.47	140.30±1.44
	8	40.65±0.61	28.08±1.30	66.47±0.34	1150.37±0.64	115.08±0.86
	9	40.59±0.66	26.40±1.64	55.54±0.24	648.581±0.54	132.30±1.63
	10	41.50±0.22	35.87±0.55	55.02±0.15	664.873±0.33	131.96±2.68
	11	37.41±0.35	35.71±0.12	55.48±0.41	622.514±0.14	130.81±1.82
	12	41.09±0.38	34.67±0.33	54.69±0.44	648.581±0.21	126.20±1.25
	13	40.33±0.46	35.68±0.14	55.24±0.51	703.973±0.33	131.15±0.00
Hot air Baking	1	30.60±1.64	32.57±1.20	49.77±0.27	1124.30±0.50	203.75±0.29
	2	33.33±0.55	30.90±1.52	51.06±0.22	1407.78±0.44	200.02±0.58
	3	34.46±0.60	74.62±1.34	50.73±0.41	730.04±0.14	198.39±1.15
	4	33.43±0.16	23.79±0.66	52.41±0.13	1899.79±0.33	196.63±0.58
	5	32.83±0.92	41.09±0.61	50.23±0.21	560.60±0.51	201.92±0.38
	6	35.11±0.33	38.33±0.45	51.60±0.22	1782.49±0.12	199.07±0.19
	7	32.62±0.15	42.45±0.84	50.25±0.25	1169.92±0.95	202.80±0.29
	8	34.90±1.34	27.59±0.64	52.41±0.31	1463.17±0.22	196.70±1.44
	9	32.54±1.54	42.94±0.45	50.90±0.30	1238.34±0.21	201.38±0.00
	10	32.61±1.37	43.28±0.51	51.01±0.84	1248.12±0.14	201.17±0.67
	11	29.91±1.25	42.81±0.22	51.43±0.51	1241.60±0.13	201.31±1.05
	12	32.97±1.55	43.30±0.39	51.58±0.55	1267.67±0.32	200.77±0.67
	13	32.32±0.52	42.97±0.54	51.60±0.43	1280.7±0.54	200.50±0.86

GI: Glycemic index, AA: Antioxidant activity, TPC: Total phenolic content

Effects of different cooking methods on the antioxidant activity and total phenolic content of the samples

Antioxidant activity and total phenolic content results of potato samples cooked by hot-air baking and deep frying are given in Table 3. According to the results, it was observed that the antioxidant activity values of the cooked potato samples varied between 194.95 and 198.28 µmol trolox/g, while the total phenolic contents varied between 9.66 and 30.07 mg GA/g dry matter. It was determined that temperature and time variation in both deep frying and hot-air baking processes significantly ($p<0.05$) affected the antioxidant activity and total phenolic contents of cooked potato samples (Table 4). An increase in cooking temperature and time decreased antioxidant activity and total phenolic content of the cooked potato samples.

Table 7. ANOVA evaluation of linear, quadratic and interaction terms for each response variables for carrots

Variation Coefficients	*L*	*a*	*b*	ΔE	To (°C)	ΔH (J/g)	GI	AA (µmol trolox/g)	TPC (mg GA/g dm)
	p-Values for deep frying								
Model	0.0121	0.0002	0.0019	<0.0001	0.1088	0.0859	<0.0001	< 0.0001	0.0004
β_1	0.0677	0.0045	0.3782	0.0551	0.3362	0.9419	< 0.0001	< 0.0001	0.0003
β_2	0.0053	<0.0001	0.0004	<0.0001	0.4806	0.0359	< 0.0001	< 0.0001	0.0001
$\beta_1\beta_2$	0.1375	0.0027	0.0051	0.0148	0.2376	0.0910	0.0190	0.2552	0.9734
β_1^2	0.0122	0.3002	0.0452	<0.0001	0.1847	0.3746	0.0202	< 0.0001	0.0629
β_2^2	0.3710	0.0792	0.0321	0.0003	0.0724	0.0612	< 0.0001	0.0001	0.3325
Lack of Fit	0.0001	0.0133	<0.0001	0.0610	0.0387	0.3388	0.3311	0.1805	0.2580
R^2	0.8324	0.9475	0.9045	0.9962	0.6638	0.6893	0.9966	0.9960	0.9414
Adj-R^2	0.7127	0.9099	0.8363	0.9934	0.4236	0.4673	0.9941	0.9931	0.8995
	p-Values for hot air baking								
Model	0.0009	0.0001	0.1281	< 0.0001	0.2397	0.0976	0.0022	0.0006	< 0.0001
β_1	0.0002	< 0.0001	0.0188	< 0.0001	0.2511	0.0431	0.0011	< 0.0001	0.0002
β_2	0.0122	0.0010	0.1379	< 0.0001	0.0906	0.4000	0.0009	0.2343	< 0.0001
$\beta_1\beta_2$	0.7228	0.6984	0.7255	0.0001	0.1910	0.0312	0.5717	0.0086	0.0886
β_1^2	0.0226	0.0329	0.6681	< 0.0001	0.4719	0.8750	0.1093	0.4735	0.3523
β_2^2	0.0674	0.1162	0.5097	0.4712	0.6381	0.5133	0.8117	0.2665	0.0101
Lack of Fit	<0.0001	< 0.0001	< 0.0001	0.0211	0.3978	<0.0001	0.4553	0.0003	0.1815
R^2	0.9231	0.9556	0.6447	0.9944	0.5569	0.6758	0.9001	0.9318	0.9689
Adj-R^2	0.8682	0.9239	0.3909	0.9904	0.2405	0.4442	0.8287	0.8830	0.9466

β_1: Cooking temperature, β_2: Cooking time, GI: Glycemic index, AA: Antioxidant activity, TPC: Total phenolic content

The antioxidant activity and phenolic content of the cooked carrot samples varied between 397.68-2079.00 µmol trolox/g and 115.08-218.39 mgGAE/g dry matter, respectively (Table 6). Different cooking techniques affected significantly ($p<0.05$) antioxidant activity and total phenolic content of the cooked carrot samples (Table 7). While the antioxidant activity increased with increasing temperature and time during the cooking process, the total phenolic content decreased. It has been observed that the results are generally consistent with the studies in the literature. A study reported that the antioxidant levels of pureed carrots are higher than fresh ones. The antioxidant capacity of carrots was increased by heat treatment at 34.3%. In addition, it has been reported that processing the peeled carrot puree increases the antioxidant level by approximately 8.19% (Talcott et al. 2000). It is stated that the antioxidant activity of different vegetable juices also increases after heat treatment (Gazzani et al. 1998). The previous study has shown that carrot samples' carotenoid content increases with the chopping and heat treatment (Fabbri and Crosby, 2016). It is also known that carotenoids have high antioxidant activity (El-Agamey et al., 2004); therefore, it was thought that antioxidant activity increased with heat treatment in carrot samples.

Table 8. CCRD experimental data for the color values of cooked mushrooms

Cooking Method	Exp. No	*L*	*a*	*b*	ΔE
Deep Frying	1	46.37±0.30	8.34±0.25	20.35±0.22	23.73±0.12
	2	22.62±0.22	11.65±0.35	11.56±0.15	46.14±0.15
	3	30.21±0.52	13.52±0.50	22.49±1.32	40.49±0.66
	4	17.28±0.23	8.42±0.61	8.40±0.35	51.13±0.45
	5	38.55±0.64	14.25±0.22	27.34±0.64	34.91±0.31
	6	20.23±0.84	10.36±0.35	10.15±0.22	48.33±0.22
	7	25.47±0.95	12.48±0.24	15.07±0.15	43.60±0.15
	8	19.58±1.51	8.77±0.35	9.24±0.36	48.83±0.36
	9	22.61±1.65	10.00±0.10	10.00±0.15	45.93±0.22
	10	22.13±1.54	10.36±0.33	9.56±0.33	46.48±0.50
	11	22.30±1.12	10.26±0.51	9.35±0.26	46.31±0.36
	12	22.54±0.12	10.02±0.15	10.02±1.38	46.00±0.40
	13	22.40±0.35	10.16±0.22	9.73±1.92	46.18±0.33
Hot air Baking	1	49.22±0.22	8.03±0.20	17.63±0.25	20.16±0.23
	2	25.41±0.14	7.20±0.30	13.11±0.15	42.82±0.15
	3	32.32±0.2·	6.71±0.52	14.64±0.51	35.98±0.22
	4	19.35±0.34	3.43±0.64	17.67±0.33	48.92±0.36
	5	35.59±0.51	7.59±0.35	17.34±0.25	33.16±0.11·
	6	24.32±0.60	5.63±0.54	11.91±0.12	43.80±0.35
	7	45.39±0.44	2.74±0.55	16.31±0.31	23.14±0.25
	8	38.35±0.51	0.98±0.25	12.68±0.35	29.88±0.55·
	9	40.59±0.61	1.74±0.23	14.19±0.61	27.66±0.45·
	10	40.31±0.34	1.85±0.51	14.19±0.82	27.93±0.55
	11	40.19±0.52	1.34±0.15	14.45±0.60	28.11±0.45·
	12	40.06±0.23	1.37±0.22	14.44±1.66	28.23±0.35
	13	40.23±0.15	1.34±0.14	14.47±1.45	28.08±0.54

The chemical analysis results of the cooked cultivated mushroom samples with different cooking techniques are shown in Table 9, and the ANOVA results showing the effects of the selected independent variables on the chemical properties of the cooked cultivated mushroom samples are in Table 10. According to the results, the total phenolic content and antioxidant activity values of the hot-air baked mushroom samples were higher than the deep-fried mushroom samples. The total phenolic contents and antioxidant activity of cooked mushroom samples were similar to the literature. In a study by Vivar-Quintana (1999), a decrease in the total amount of phenolic substance was observed in mushroom samples due to the soaking and boiling process during canning. Choi et al. (2006) determined that the amount of bound and free phenolic substances in mushrooms that were heat-treated at 121 °C for 15 and 30 min decreased significantly compared to fresh mushrooms. It is stated in some studies that the amount of phenolic substances increases due to the deterioration of the cell wall and cell structure with heat treatment. In contrast, some others state that there is no decrease or change in phenolic components and antioxidant activity with heat treatment (Kim et al., 2006).

Table 9. CCRD experimental data for the thermal and chemical properties of cooked mushrooms

Cooking Method	Exp. No	To (°C)	ΔH (J/g)	GI	AA (µmol trolox/g)	TPC (mg GA/g dm)
Deep Frying	1	39.01±0.12	36.76±0.52	42.82±0.11	925.51±0.47	101.52±3.55
	2	36.33±0.50	39.90±0.65	43.23±0.31	1155.62±0.84	81.39±0.58
	3	32.81±0.34	32.57±0.84	44.91±0.25	1198.36±0.44	93.39±0.29
	4	29.36±0.37	41.17±1.25	45.83±0.52	1737.48±0.34	77.52±0.86
	5	35.71±0.61	34.06±1.64	43.58±0.85	1076.73±0.84	95.42±0.67
	6	36.36±0.34	27.36±1.45	44.18±0.82	1366.01±0.47	79.76±0.58
	7	37.11±0.33	37.02±1.45	42.99±0.37	1244.38±0.58	92.44±0.29
	8	29.61±0.15	39.51±1.25	45.07±0.55	1471.21±0.96	87.49±0.38
	9	28.02±0.14	35.47±1.84	43.70±0.35	1303.55±0.25	91.22±0.29
	10	33.27±0.11	34.74±1.64	43.64±0.61	1273.97±0.33	92.03±0.29
	11	29.08±0.55	35.58±1.35	43.75±0.22	1313.41±0.15	91.02±0.00
	12	28.15±0.34	35.33±0.20	43.65±0.10	1300.26±0.14	91.49±0.29
	13	32.88±0.64	34.45±0.40	43.48±0.11	1333.14±0.22	90.61±0.58
Hot air Baking	1	29.74±0.44·	34.46±0.23	48.86±0.12	1793.36±0.31	162.47±0.32
	2	31.51±0.15	30.52±0.24·	52.14±0.31	2039.91±0.25	157.31±0.42
	3	29.16±1.64	42.34±0.33	51.16±0.25	1911.70±0.13	160.03±0.77
	4	33.11±1.94	26.97±0.25	55.45±0.22	2184.55±0.41	154.40±0.55
	5	32.71±1.20	37.65±0.16·	49.75±0.31	1882.12±0.75	160.64±0.21
	6	29.33±1.35·	42.86±0.85	53.97±0.55	2118.81±0.34	155.75±0.14
	7	37.97±0.33·	42.51±0.16	52.25±0.34	1951.15±0.24	159.21±045
	8	36.04±0.40	47.29±0.38·	55.39±0.74	2115.52±0.35	155.82±0.34
	9	32.28±0.34	33.30±0.77	53.19±0.91	2033.34±0.24·	157.52±0.57
	10	33.09±1.65·	32.86±0.58·	53.15±0.93	2007.04±0.52	158.06±0.75
	11	32.79±0.51	33.52±1.26	53.21±0.82	2016.90±0.95	157.86±0.34
	12	32.69±0.38	33.33±1.30	53.26±0.87	2049.77±0.88	157.18±0.24
	13	32.97±0.77	33.51±1.54·	53.43±1.15	2062.92±0.63	156.91±0.24

GI: Glycemic index, AA: Antioxidant activity, TPC: Total phenolic content

The food composition, the interaction of components with each other, the technological processes, the time and temperature of the heat treatment, the solvents used to extract the phenolic substances during the analysis can influence antioxidant activity and phenolic components. Some phenolic and antioxidant substances can be destroyed or reduced by heat treatment. It is expected that the antioxidant activity and the total amount of phenolic substance would decrease with the heat treatment. Perla et al. (2012) stated that the heat treatment reduced the antioxidant activity and the total amount of phenolic substances of different potato varieties. Amin and Lee (2005) found that the antioxidant activity and phenolic components were significantly reduced in cabbage species with 5-10 minutes of boiling at 98 °C. In our study, when the analysis results of potatoes, carrots and mushrooms cooked by different cooking techniques were examined, antioxidant capacity and total phenolic content decreased due to the increase in cooking temperature and time.

Effects of different cooking methods on the estimated glycemic index (eGI)

According to the results, the eGI values of the cooked potatoes, carrots, and cultivated mushrooms were ranged in 48.83-59.11 (Table 3), 49.77-68.50 (Table 6), and 42.82-55.45 (Table 9), respectively. ANOVA analyses results showed that both deep-frying and hot-air baking affected significantly ($p<0.05$) the eGI values of all samples (Tables 4, 7, and 10). The eGI values of the deep-fried potato and carrot samples were higher than their baked counterparts were higher than the hot-air baked equivalents. In contrast to cooked potatoes and carrots, the eGI values of the deep-fried cultivated mushrooms were determined lower than the hot-air baked samples. Based on the glycemic index considering glucose as a reference, foods are classified as low glycemic index food and high glycemic index food when the glycemic index lower than 55 and higher than 70, respectively (Ferng et al. 2016). According to this classification, all samples tested in our study appear to have low glycemic indexes, with the exception of deep-fried carrot samples.

Table 10. ANOVA evaluation of linear, quadratic and interaction terms for each response variables for mushrooms

Variation Coefficients	*L*	*a*	*b*	ΔE	To (°C)	ΔH (J/g)	GI	AA (µmol trolox/g)	TPC (mg GA/g dm)
	p-Values for deep frying								
Model	<0.0001	0.1161	0.0016	<0.0001	0.0594	0.2619	< 0.0001	0.0001	< 0.0001
β_1	<0.0001	0.1448	0.0003	<0.0001	0.4172	0.5319	< 0.0001	< 0.0001	< 0.0001
β_2	<0.0001	0.6248	0.2874	<0.0001	0.0140	0.9556	< 0.0001	< 0.0001	0.0002
$\beta_1\beta_2$	0.0015	0.0208	0.3121	0.0009	0.8862	0.4116	0.0348	0.0199	0.0635
β_1^2	<0.0001	0.2617	0.0033	0.0002	0.0618	0.2104	0.0109	0.0197	0.0028
β_2^2	0.9224	0.4764	0.8868	0.1300	0.6272	0.0338	0.0005	0.2055	0.6699
Lack of Fit	0.0006	<0.0001	0.0001	0.0011	0.4744	0.0004	0.5226	0.0180	0.0524
R^2	0.9900	0.6564	0.9084	0.9874	0.7246	0.5422	0.9927	0.9586	0.9875
Adj-R^2	0.9829	0.4109	0.8430	0.9784	0.5278	0.2152	0.9875	0.9290	0.9786
	p-Values for hot air baking								
Model	< 0.0001	< 0.0001	<0.0001	< 0.0001	0.1675	0.7885	<0.0001	< 0.0001	< 0.0001
β_1	< 0.0001	0.0001	<0.0001	< 0.0001	0.6508	0.4135	<0.0001	< 0.0001	< 0.0001
β_2	0.0003	< 0.0001	<0.0001	0.0004	0.8594	0.5916	<0.0001	< 0.0001	< 0.0001
$\beta_1\beta_2$	0.0214	0.0062	0.0461	0.0319	0.6064	0.4165	0.0251	0.5355	0.5892
β_1^2	< 0.0001	< 0.0001	0.1812	< 0.0001	0.0156	0.6529	<0.0001	0.0106	0.0115
β_2^2	0.1892	0.8247	0.0994	0.2121	0.1239	0.5091	0.0083	0.4795	0.5014
Lack of Fit	< 0.0001	0.1973	0.0039	0.0001	0.0003	<0.0001	0.0523	0.7179	0.7021
R^2	0.9550	0.9923	0.9768	0.9731	0.6102	0.2513	0.9945	0.9790	0.9787
Adj-R^2	0.9550	0.9868	0.9603	0.9539	0.3318	-0.2834	0.9906	0.9640	0.9635

β_1: Cooking temperature, β_2: Cooking time, GI: Glycemic index, AA: Antioxidant activity, TPC: Total phenolic content

Miao et al. (2015) stated that the eGI is affected by the specific properties of the food, the properties of the applied process, lipid and protein matrix, and starch content. Tian et al. (2016) reported that crushed and boiled potatoes had a higher eGI than fried, microwaved or conventionally baked potatoes due to the effects of the degree of gelatinization. They stated that during frying, the water in the cells inside the food causes the starch to be fully gelatinized; on the other hand, the high temperature on the surface causes the amylose-lipid complex (resistant starch) to form. Our study found similar results for specifically cultivated mushrooms and partly for potato samples. On the other hand, Tuta et al. (2017) have reported that the eGI of potatoes was low in the baking process recommended as a healthy cooking method.

Sensory properties of the samples

ANOVA of the sensory analysis results of potato, carrot and mushroom samples cooked with different cooking techniques is given in Table 11. When the color, odor and taste characteristics of different cooking techniques were averaged, the most accepted samples were deep-fried samples.

Table 11. Sensory properties of the cooked potatoes, carrots and cultivated mushrooms

Sensory properties	Potatoes		Carrots		Cultivated mushroom	
	Deep frying	Hot-air baking	Deep frying	Hot-air baking	Deep-frying	Hot-air baking
Appearance	$3.69^a \pm 0.52$	$3.61^a \pm 0.78$	$3.53^a \pm 0.08$	$3.03^b \pm 0.01$	$3.26^b \pm 0.08$	$3.53^a \pm 0.14$
Color	$4.00^a \pm 0.05$	$3.88^b \pm 0.36$	$3.42^a \pm 0.03$	$3.38^a \pm 0.16$	$3.61^b \pm 0.03$	$3.76^a \pm 0.02$
Odor	$3.84^a \pm 0.02$	$3.76^a \pm 0.04$	$3.76^a \pm 0.11$	$3.80^a \pm 0.09$	$2.92^b \pm 0.03$	$3.69^a \pm 0.07$
Taste	$4.00^a \pm 0.12$	$3.30^b \pm 0.06$	$3.38^a \pm 0.06$	$3.30^b \pm 0.02$	$3.30^a \pm 0.01$	$3.30^a \pm 0.04$
Texture	$3.07^a \pm 0.07$	$3.03^a \pm 0.05$	$2.84^a \pm 0.07$	$2.34^a \pm 0.03$	$3.30^a \pm 0.10$	$3.03^b \pm 0.08$
General acceptance	$4.07^a \pm 0.13$	$3.53^b \pm 0.07$	$3.23^a \pm 0.02$	$2.76^b \pm 0.11$	$2.84^b \pm 0.06$	$3.26^a \pm 0.04$

Results are means ± standard error, different letter is same row for each factor shows significant (p<0.05) difference.

Xu and Kerr (2012) produced corn chips by vacuum drying and deep-frying methods. They reported that the deep-fried samples got higher scores in all sensory criteria than the vacuum dried samples. In another study, carrot samples were deep-fried and the sensory analyzes were carried out during storage. It was determined that deep-fried carrot samples were appreciated by the panellists and received high scores on the hedonic scale (Sulaeman et al., 2003). Guiné et al. (2019) obtained different products using mushrooms in their study. They served the mushroom-based products to the panelists by frying them in deep oil. It has been reported that deep-fried mushrooms and mushroom-based products receive high scores from panelists. In a previous study, mushroom samples were fried and baked, similar to our current study; the most liked samples by the panelists were the samples in which the frying process was applied (Doğan et al., 2020). In many fruits and vegetables that have been tried with different cooking methods, the panelists most liked the method of frying. It has been reported that frying increases the sensory properties of vegetables and is preferred by panellists (Troncoso et al., 2009; Devi et al., 2020).

CONCLUSION

In conclusion, potato, carrot and cultivated mushroom samples, rich in different main compounds such as starch, pectin and protein, respectively, gave different reactions to deep-frying and hot-air baking methods. The deep-frying process caused significant differences in the samples' Hunter *L, a*, and *b* values. It was observed that deep-fried samples had redder and darker color due to advanced non-enzymatic browning reactions. According to the thermal analysis results, To and ΔH values changed significantly depending on cooking. Different cooking techniques change the granular structure of the starch of the samples and make it easier to gelatinize. All samples tested in our study appear to have low (<55) glycemic indexes, except for deep-fried carrot samples, which have still moderate eGI values (<70). The samples' antioxidant activity and total phenolic contents were also significantly influenced by different cooking techniques and baking provided higher functional properties. As a result, deep-frying is not recommended due to the loss of functional compounds, although consumers appreciate the process due to its desired sensory properties. It has been determined that the baking technique stated in many studies in the literature as a healthy method can also be used for potatoes, carrots and mushrooms.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution

Ayşe Nur Edis: conceptualization, data curation, formal analysis.

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Hilal Şahin-Nadeem: Conceptualization, data curation, formal analysis, funding procurement, original draft writing, review, and editing.

Zehra Günel: Conceptualization, data curation, formal analysis, funding acquisition, writing (original draft), writing (review & editing)

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Studies on *in vitro* germination on endemic *Salvia* L. species

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DergiPark AKADEMİK

Abstract
This study used seeds of two endemic sage plants (*Salvia siirtica* and *Salvia kronenburgii*) as a starting material. Mucilage causes dormancy in the seeds of these plants. Therefore, it is important to improve the germination performance of these plants' seeds, and in this study, some treatments were applied to the seeds before or during sowing. To this end, sodium hypochlorite, ethyl alcohol, gibberellic acid, seed cracking, removal of the seed coat, pre-cold treatment, and sulfuric acid treatments were applied to the seeds of the two species separately or in combination, and their germination performances were investigated in comparison with a control group. Considering the results higher germination rates were obtained for both plants compared to the control group in all treatments except sulfuric acid treatments. The best germination rate for both plants was obtained from the treatments where the seed coat was mechanically removed. In this treatment, the germination rate in *S. siirtica* increased 3.3 times, while it increased 2.4 times in *S. kronenburgii* compared to the control group. Additionally, GA treatments for *S. siirtica* and cold pre-treatments for *S. kronenburgii* significantly increased germination rates. In light of these results, the removal of the seed coat, gibberellic acid, and cold pre-treatment effectively broke dormancy in sage seeds and increased germination rates.

Keywords: Dormancy, Germination, *In vitro*, Endemic, *S.siirtica*, *S.kronenburgii*

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INTRODUCTION

Seed germination is one of the most critical stages of plant life and forms the basis of agricultural production (El-Keblawy and Al-Rawai, 2005). Detailed knowledge of germination patterns is important for successful cultivation (Pająk et al., 2019), and understanding the mechanism underlying species germination barriers (dormancy, abiotic factors, etc.). Seed germination, which significantly impacts the plant growth cycle, is temporarily delayed in some plants due to dormancy (Bu et al., 2008). The presence of germination-inhibitory substances in the seed coat, embryo, and seed is among the factors affecting seed dormancy (Latifi, 2001; Elamin et al., 2013). Seed germination studies, which are important for biodiversity protection, should know the germination conditions of seeds for the species studied.

The Lamiaceae, which has an important place among medicinal and aromatic plants, is the third largest family in Türkiye by the number of taxa and the fourth largest by the number of species. Among the members of the Lamiaceae family, which includes many economically and medicinally important species, *Salvia*, the largest genus of the family, contains approximately 950-1000 taxa. The genus *Salvia* is represented by 107 taxa in Türkiye, of which 58 are endemic and have an endemism rate of 54% (Celep and Dirmenci, 2017). *Salvia* is derived from the word "salvare," meaning healing (Amiri, 2007), and was used in ancient times as tea, for pain relief, sore throat, and influenza (Zhou et al., 2005; Mayekiso et al., 2008). Many studies have reported that the contents of *Salvia* species mainly include phenolic acids, flavonoids, terpenes, and terpenoids, and these secondary metabolites provide pharmaceutical and biological benefits due to their antimicrobial, antifungal, antiseptic, analgesic, anticancer, and insecticide properties (Lu and Yeap Foo, 2002; Fidan et al., 2021; Uysal et al., 2023). In Türkiye, which has rich biodiversity, the germination requirements of natural species are mostly unknown, and therefore,

studies on seed germination are insufficient. Various studies have recently investigated seed germination in the Lamiaceae, Asteraceae, and Brassicaceae families (Surgun-Acar et al., 2017).

With recent climatic changes, factors such as sudden rainfall, floods, droughts, desertification, and salinity, as well as excessive and unconscious collection of plants, overgrazing and human pressure have brought many plants to the point of extinction. Especially the future of endemic populations with few individuals is at a higher risk (Rakotonandrasana et al., 2023). Various *in vitro* tissue culture methods (micropropagation, germplasm preservation, etc.) are applied to conserve and produce endangered and difficult-to-propagate species. In these methods, seeds are an alternative explant source and enable the production of as many seedlings as desired indefinitely under aseptic conditions for various purposes, saving time and space (Babaoğlu et al., 2002; Gökdoğan et al., 2022). *Salvia* is propagated by seeds. However, since their seeds have a mucilaginous seed coat and dormancy, i.e., factors that inhibit germination (Tursun, 2019), tissue culture techniques can be considered an effective alternative method in the germination of these species.

S. siirtica Kahraman, Celep & Doğan, which is 'Critically Endangered' (CR) with very few individuals and confined to a very narrow range (Kahraman et al., 2011), and *S. kronenburgii* Rech.fil., which is categorized as 'Endangered' (EN), are endemic taxa growing in Türkiye (Kuşaksız, 2019). Seed germination studies on rare and endemic species are important in determining species conservation strategies for these plants. These species are in danger of extinction in many cases. Therefore, an accurate and precise understanding of the germination ability of these taxa is important for the continuity, conservation, and development of the species (Arslan et al., 2017). For all of the above-mentioned reasons, this study was conducted to determine the effect of different treatments on breaking the germination barrier and improving the germination potential of seeds of endangered endemic *S. siirtica* and *S. kronenburgii* plants using tissue culture methods.

MATERIALS AND METHODS

Plant material

The mature seeds of endemic *S. siirtica* (Siirt Tillo Çatılı Village - 37º 58' 19" N / 42º 01' 51" E, 1491 m) and *S. kronenburgii* (Van Tuşba Ayanis Village - 38º 41' 56" N / 43º 11' 44" E, 1741 m) used as starting material in this study were collected and identified by Hüseyin EROĞLU (PhD).

Seed Surface Sterilization

After the seeds were rinsed in tap water, they were kept in 70% ethanol (EtOH) for 30 s and then in 5% sodium hypochlorite (NaOCI) for 10 min for surface sterilization. After surface sterilization, the seeds were rinsed thoroughly with sterile distilled water to remove the chemicals.

Treatments

Different chemical substances and their combinations were used to break the seed coat's dormancy, increase germination rates, and reveal differences between the treatments in improving the germination potential. To this end, the mature seeds of each plant, whose surfaces were sterilized, were subjected to the following treatments for germination: 48% H_2SO_4 for 60 and 120 s; 96% H_2SO_4 for 60 and 120 s; -20ºC for 10 h with and without cracking; sanding; 250 and 500 ppm GA for 4 h; 250 and 500 ppm GA-supplemented ¼ Murashige&Skooge (Murashige and Skooge, 1962) medium cultivation and mechanical removal of seed coat (Table 1). The treatment group that was only pre-sterilized in 70% EtOH for 30 s and then in 5% NaOCI for 10 min was considered the “control” group.

Table 1. Different treatments for breaking the germination barrier

Treatments			
1	Control	8	Sanding
2	48% H_2SO_4	9	250 ppm GA
3	48% H_2SO_4	10	500 ppm GA
4	96% H_2SO_4	11	250 ppm GA + MS
5	96% H_2SO_4	12	500 ppm GA + MS
6	-20ºC (cracking)	13	Removal of the seed coat
7	-20ºC (non-cracking)		

To determine the effect of different treatments on dormancy breaking in seeds, a randomized experimental design was established with three replicates. A total of 100 seeds were sown for both plants, four seeds in each Magenta GA-7 culture vessel for each treatment.

Culture Conditions

A ¼ MS medium supplemented with 30 g L^{-1} sucrose and pH 5.8 was used for seed germination. Then, 5.465 g agar was added to the medium and sterilized in an autoclave at 1 atm and 121°C for 25 min. The prepared medium was equally divided into Magenta GA-7 culture vessels. The sterilized and pretreated seeds were cultured in these containers and left to germinate in the plant growth chamber where aseptic conditions were provided with 16 hours light/8 hours dark photoperiod and 24±1°C (during 24 hours) temperature. The emergence of a radicle on the seeds was considered a germination criterion, and germinated seeds were counted daily for about two weeks. Germination rates were calculated as % for each treatment.

Statistical analysis

All experiments were performed in triplicate. The results of the activity assays are shown as means ± standard error (SE). The data analysis was performed using SPSS 20.0 for Windows (SPSS Inc., Chicago, USA). The significance of differences was tested using a one-way analysis of variances (ANOVA) and Tukey's test at a 0.05 level of significance.

RESULTS

Table 2 and Figure 1 show the effects of different treatments and their combinations on the germination of *S. siirtica* and *S. kronenburgii* seeds.

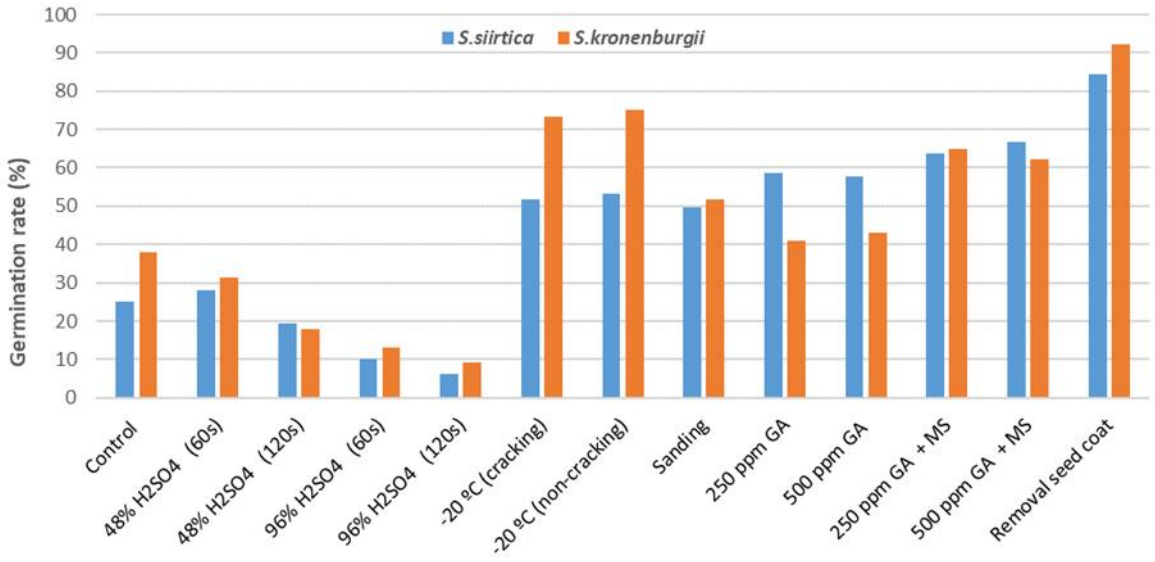

Figure 1. Effects of different treatments on the germination percentage of *S. siirtica* and *S. kronenburgii* seeds under *in vitro* conditions (%)

Considering the results for *S. siirtica*, the highest germination rate was obtained from the treatment where the seed coat was mechanically removed at 84.4%, followed by the treatment where seeds were cultured in a GA-supplemented MS medium with 66.7% and 63.6% germination rates and these treatments were found to be statistically different. Approximately half of the seeds germinated in cold pretreatment and sanding treatments. However, cracking/non-cracking treatment did not contribute significantly to the germination rate. Sulfuric acid treatments reduced the germination rate of seeds compared to the control group (except 48% 60 s). Furthermore, the germination rate decreased significantly in these treatments with the increased concentration and duration. These decreases were found to be statistically significant. For *S. kronenburgii*, all treatments, except sulfuric acid treatments, resulted in higher germination rates in comparison with the control group. The highest germination rate (92.2%) was obtained from the treatment where the seed coat was mechanically removed, followed by -20ºC cold pretreatment with 75.0% and 73.3% germination rates, while cracking had no significant effect. The effects were not statistically different. Seeds cultured on the GA-supplemented MS medium had higher germination rates than those pretreated with GA (Figures 2 and 3).

Table 2. Effects of different treatments on the germination percentage of *S. siirtica* and *S. kronenburgii* seeds under *in vitro* conditions (%)*

No.	Treatments	Time	*S.siirtica*	*S.kronenburgii*
1	Control	10 minutes	25.1±0.37^{l}	38.0±0.54^{f}
2	48% H_2SO_4	60 seconds	28.0±0.23^{k}	31.5±0.69^{g}
3	48% H_2SO_4	120 seconds	19.3±0.15^{m}	17.8±0.81^{h}
4	96% H_2SO_4	60 seconds	10.0±0.73^{n}	13.1±0.32^{k}
5	96% H_2SO_4	120 seconds	6.1±0.18^{o}	9.2±0.15^{l}
6	-20ºC (cracking)	10 hours	51.9±0.42^{g}	73.3±1.29^{b}
7	-20ºC (non-cracking)	10 hours	53.4±0.95^{f}	75.0±1.09^{b}
8	Sanding	-	49.6±1.03^{h}	51.7±0.87^{d}
9	250 ppm GA	4 hours	58.8±0.45^{d}	41.0±0.73^{e}
10	500 ppm GA	4 hours	57.9±1.27^{e}	43.0±0.35^{e}
11	250 ppm GA + MS	-	63.6±0.75^{c}	64.8±0.29^{c}
12	500 ppm GA + MS	-	66.7±1.02^{b}	62.1±0.99^{c}
13	Removal of the seed coat	-	84.4±0.87^{a}	92.2±1.09^{a}

*Vertical bars indicate ± SE. Letters (a-k) indicate significant differences ($p \leq 0.05$) compared to the control group.

Figure 2. 28-day *in vitro* development of *S. siirtica* with the seed coat removal treatment

Figure 3. 28-day *in vitro* development of *S. kronenburgii* with the seed coat removal treatment

DISCUSSION

Seeds of most medicinal plants adapt to environmental conditions differently. Therefore, it is necessary to create optimal conditions for the germination of seeds of medicinal plants and determine the ecological/physiological factors affecting dormancy in their production (Khakpoor et al., 2015). Studies on the seed pretreatment of *Salvia* plants have shown that they exhibit dormancy caused by mucilage in the hard seed coat (Khakpoor et al., 2015; Yaman, 2020). Therefore, to break this dormancy and improve the germination potential, some pretreatments (cold pretreatment, soaking in gibberellic acid, immersion in sulfuric acid, mechanical removal of the seed coat, etc.) were applied to the seeds in the present study. In our study, germination by the mechanical removal of the seed coat gave the highest germination rate (92.2% and 84.4%, respectively) compared to the other treatments for both species (*S. kronenburgii* and *S. siirtica*). Likewise, in a study conducted to improve the germination of *Vitex doniana*, whose population was declining due to low yield, long germination time and overharvesting, removing the seed coat followed by germination on filter paper resulted in the highest germination percentage (87%) (Haruna et al., 2024). In another study, to increase the *in vitro* seed germination success of *Jatropha curcas*, some pretreatments (imbibition, stratification, scarification, and removal of the seed coat) were performed before sowing. The dormancy of the seeds was broken only by removing the seed coat and the seeds were kept at 25 ± 2˚C in the dark and cultured in a 1/1 MS medium and 100% germination was achieved.

Furthermore, many methods, such as chemical etching, physical etching, and hormone treatments, are used to break dormancy in various plant seeds (Uludağ and Özer, 1999; Serim and Sözeri, 2011; Bozdoğan et al., 2019). Among the dormancy-breaking procedures performed on *Adonis aestivalis* seeds, the most effective method was GA treatment at 150 ppm, and the germination rate was 20.0% (Taşkesen, 2007). Another study reported that 250 and 300 ppm GA solutions applied to *Hippomarathrum microcarpum* seeds increased the germination rate above 50.0% (Ertuş et al., 2011). In support of the researchers, Tursun (2019) obtained the highest seed germination rate (74%) for breaking dormancy in *Salvia verticillata* seeds with 2000 ppm GA and complete darkness treatment. Subaşı and Güvensen (2010) detected very low germination in the pre-chilling and non-pre-chilling groups for *Salvia smyrnaea* seeds and revealed that pre-chilling was insufficient for breaking dormancy and gibberellin was also necessary. Our results regarding the significant increase in the germination percentage of the seeds cultivated in the GA-supplemented ¼ MS medium agree with the data in the literature. In their study on breaking the high dormancy in *Sinapis arvensis* seeds, Ateş and Üremiş (2021) provided the best germination rates with 100% in 2000 ppm GA_3 and 91.9% in H_2SO_4 (60 s), respectively. Tursun (2019) reported that when the exposure time to sulfuric acid was increased from 60 seconds to 15 minutes in *Salvia verticillata* seeds, the germination rate also increased but then decreased significantly after 15 minutes. Likewise, Tuncer and Ummuhan (2017) found high germination rates for Molehiya (*Corchorus olitorius* L.) seeds subjected to sulfuric acid treatment for 5-10 minutes and reported statistically significant decreases with the increased duration. Bhardwaj et al. (2016) examined the effects of stratification, scarification, acids (H_2SO_4, HNO_3, and HCl), gibberellic acid, and alcohol on germination to break dormancy in various medicinal plants. The best germination rate for all plants was obtained in seeds treated with H_2SO_4 alone. In the present study, H_2SO_4 treatments displayed the lowest germination percentage for both species, generally lower than the control group.

Ismaili et al. (2023) examined the effects of different temperature treatments to determine the optimum germination conditions for *Stachys mouretii* and obtained the highest germination percentage (66.5%) at 25/10°C. Young and Young (1992) reported that most *Salvia* taxa require cold pretreatment for germination. Yücel and Yılmaz (2009) determined high germination rates (78%) in *Salvia cyanescens* after pre-chilling at constant temperature of -5ºC for 5 minutes. In our study, cold pretreatment significantly increased the germination rate for both plants, while this rate was higher (75%) for *S. kronenburgii*.

Özcan et al. (2014) determined that gibberellin (95.1% and 50.2%, respectively) for *Salvia officinalis* and *Salvia fruticosa* and pre-drying (42.0%) and ethylene (47.6%) for *Salvia pomifera* species were the best media for germination. The researchers reported significant differences between the species' germination rate and germination strength values. Another study by Arslan et al. (2017) measured the highest germination and rooting rates (21.25% and 17.97%, respectively) for *S. siirtica* seeds in a 28-day stratification treatment. In the study, GA, citric acid, and +4ºC retention treatments applied to *S. siirtica* seeds did not achieve success in germination. In another study in which seeds of *Salvia verticillata* species were treated with sanding and hot water, the effect of sanding on germination was higher, at 53% (Khapor et al., 2011). In a study on the effects of different dormancy-breaking treatments on the germination of *Zaleya pentandra* seeds, the treatment with sandpaper was highly effective in breaking seed dormancy (Munawar et al., 2015). The effects of different concentrations of gibberellic acid, potassium nitrate, and mechanical scarification were investigated to break dormancy and improve germination in yarrow seeds. It was stated that sanding by mechanical scarification increased germination (Nejad et al., 2022). In our study, sanding before sowing germinated about half of the seeds in both plant species.

CONCLUSION

S. siirtica and *S. kronenburgii*, which are the subject of our study, are endangered plant species whose seed coat contains mucilage inhibiting germination. In our research conducted to improve germination in these plant species, different pretreatments were applied to the seeds before germination under *in vitro* conditions and mechanical removal of the seed coat promoted germination at the highest rate. However, the other treatments (except the H_2SO_4 treatment) generally increased the germination by 50% or more compared to the control group. In conclusion, the removal of the seed coat used to break mucilage-induced dormancy may improve the germination potential of other species and enable mass multiplication. Furthermore, these results provide basic information on possible treatments to restore endemic and endangered species in their natural habitat.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contribution

P.O., İ.S.K.- Analysis, interpretation, literature review and writing. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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The volatile compounds of some edible wild plants consumed in the Mediterranean region

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Abstract

In this study, 13 different edible weed species [*Centaurea depressa* Bieb., *Cichorium intybus* L., *Lactuca serriola* L., *Malva neglecta* Wallr., *Papaver dubium* L., *Polygonum cognatum* Meissn., *Rumex patientia* L., *Scorzonera cana* (C.A.Mey.Hoffm.), *Silene alba* (Mill.) Krause, *Stellaria media* L., *Sonchus oleraceus* L., *Taraxacum officinale*, *Tragopogon longirostris* Bisch] were collected from the same location in the Mediterranean region. Then, the leaves of all species were analyzed by the SPME-GC/MS method for the detection of volatile compounds. The compounds were grouped according to their structures as alcohols, aldehydes, alkanes, ester, furans, hydrocarbons, ketones, sulfur compounds, and terpenes. The percentages of the terpenes, aldehydes and alcoholic compounds were found to have the highest ratios of volatile compounds, respectively. The species found with the highest total terpene percentage was *Sonchus oleraceus* L. (78.84%), while the lowest one was *Stellaria media* L. (51.03%). Similarly, the highest total aldehydes percentage was found in *Stellaria media* L. (38.41%), and the lowest was in *Centaurea depressa* Bieb. (4.62%). Lastly, the highest total alcohol percentage was observed in *Centaurea depressa* Bieb. (9.92%) and the lowest was in *Malva neglecta* Wallr. (1.11%). The limonene, which is an important monoterpene, among 63 components, was found to be the major component in all species with a range of approximately 51-79%. Among them, *Sonchus oleraceus* L. had the highest limonene content (78.84%).

Keywords: Volatile compounds, Terpene, Limonene, SPME GC/MS, Wild species

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INTRODUCTION

Vegetables play a crucial role in the human diet due to their rich nutritional content and health benefits. They are essential components of a balanced diet as they provide a wide range of essential nutrients, including dietary fiber, phytochemicals, vitamins, and minerals (Dias & Imai, 2017; Amao, 2018), while their regular consumption contributes to improve the overall health (Leyo et al., 2022). In addition, consumption of vegetables is also associated with various health benefits, including improved gastrointestinal health, enhanced vision, reduced risk of heart disease, stroke, chronic diseases like diabetes, and certain forms of cancer (Dias, 2012). Vegetables are also known to be rich in phytonutrients such as phenolics, flavonoids, and carotenoids, which have antioxidant properties and contribute to overall well-being (Liu, 2013). Additionally, vegetables provide a diverse range of tastes, aromas, textures, and colors, as well as enhanced the variety in food choices and satisfying personal preferences (Hong & Gruda, 2020). Furthermore, vegetables are a significant source of vitamins and minerals like vitamin C, folate, potassium, and beta-carotene, which are essential for various bodily functions and overall health (Power et al., 2011). World Health Organization recommends the consumption of at least five portions of fruits and vegetables per day to promote health and prevent chronic conditions (Fulton et al., 2011). Increasing vegetable intake and reducing the consumption of energy-dense foods are important targets for nutrition interventions to maintain good health and prevent diseases (Pearson et al., 2010).

Thirteen species were chosen among the mostly used plants by residents of Bahtiyar village and served as the base of this study. One of them is *Taraxacum officinale*, commonly known as dandelion, a perennial herbaceous

plant belonging to the Asteraceae family. The primary bioactive components found in *T. officinale* include phenolic compounds, flavonoids, triterpenes, polysaccharides, and inulin. Among them, phenolic compounds are one of the most significant groups found in *T. officinale*. Some of compounds, like caffeic acid and chlorogenic acid, are known to be strong antioxidants that help the body to fight free radicals and reduced the oxidative stress (García-Carrasco et al., 2015; Khan et al., 2018). Flavonoids are another important class of bioactive compounds found in *T. officinale*, include quercetin and kaempferol. These flavonoids are known for their anti-inflammatory, antimicrobial and anticancer activities (Dongare et al., 2021). The presence of these compounds contributes to the overall health benefits of dandelion, making it a valuable addition to dietary and medicinal applications (Epure et al., 2023). Triterpenes, such as taraxasterol and taraxerol, are also prominent in extract of *T. officinale*, particularly in its roots. These compounds have been associated with various pharmacological activities, including anti-inflammatory and anticancer effects. Researchers have found that taraxasterol can control immune responses and fight against tumors, which makes *T. officinale* even more useful as a medicine (Ren et al., 2022). The presence of volatile compounds in *T. officinale* have been linked to its antimicrobial properties generally. Research has shown that extracts from dandelion roots exhibit significant antimicrobial activity against various bacterial strains, including methicillin-resistant *Staphylococcus* aureus and *Bacillus cereus* (Kenny et al., 2015). This antimicrobial action is attributed to the presence of specific volatile compounds that disrupt bacterial cell membranes and inhibit growth (Jerković et al., 2015). The identification of nitriles as dominant volatile molecule in dandelion honey further underscores the plant's potential as a source of natural antimicrobial agents (Jerković et al., 2015; Siegmund et al., 2017).

Centaurea depressa Bieb. (Asteraceae), is consumed for its nutritional value and potential health benefits. Piperitone, elemol, β-eudesmol, and spathulenol are some of the important chemicals that have been studied and found to have medicinal and nutritional value (Carev et al., 2022). The presence of fatty acids like hexadecenoic acid and dodecanoic acid further enhances its profile, suggesting potential applications in dietary practices (Erdoğan et al., 2014). Moreover, *C. depressa* is in a broader trend towards utilizing wild leafy vegetables, which are often underappreciated in modern diets. People are increasingly recognizing these plants on their potential to improve food security and enhance nutritional diversity. Consuming such wild plants not only supports dietary variety, but also contributes to sustainable agricultural practices by promoting the use of native flora (Baydoun et al., 2023). The role of volatile components in the consumption of *Centaurea depressa* Bieb. is multifaceted, encompassing aspects of flavor, aroma, and ecological interactions. In the case of *C. depressa*, the presence of specific volatile organic compounds (VOCs) can improve the sensory experience of consuming the plant, making it more attractive to consumers (Xu et al., 2023).

People consume *Cichorium intybus* L., (Asteraceae), is commonly known as chicory, for its nutritional value, medicinal properties, and culinary versatility, among other reasons. This plant is rich in bioactive compounds, including inulin, flavonoids, and phenolic acids, which contribute in health benefits and make it a valuable addition to the diet. Various cultures have traditionally use of *Cichorium intybus* for its medicinal properties, in addition to its nutritional benefits. Studies have reported that chicory exhibits hepatoprotective effects, reducing enzyme levels in lever and improve its function in toxicity models (Maletha et al., 2022; Andalib et al., 2021). Moreover, the antimicrobial properties of *C. intybus* are noteworthy, with research indicating that its extracts possess activity against various pathogenic microorganisms. The volatile compounds in chicory have been shown to exhibit antibacterial effects against strains such as *Escherichia coli* and *Staphylococcus aureus*, suggesting their potential use as natural preservatives in food products (Ghaderi et al., 2012; Bezerra et al., 2022). This antimicrobial activity is particularly relevant in the context of increasing resistance to conventional antibiotics, positioning *C. intybus* as a valuable resource in the search for alternative antimicrobial agents.

Lactuca serriola L. (Asteraceae), commonly known as prickly lettuce, is consumed for its nutritional benefits, medicinal properties, and culinary versatility. This wild relative of cultivated lettuce (*Lactuca sativa*) is rich in various phytochemicals, including flavonoids, phenolic acids, and lactones, which contribute to its health-promoting effects. For instance, the presence of compounds such as lactucin and lactucopicrin has been linked to gastrointestinal relief and respiratory benefits, making it a valuable addition to traditional medicine. Furthermore, studies have shown that *L. serriola* can inhibit α-glucosidase activity, which may aid in regulation of blood sugar, thus providing potential benefits for individuals with diabetes. In addition to its health benefits, the volatile components of *L. serriola* play a significant role in its consumption. Traditional uses of *L. serriola* include its application as a sedative, diuretic, and antispasmodic agent, which have been corroborated by scientific studies. For instance, extracts from *L. serriola* have demonstrated significant sedative effects in experimental models, suggesting that its volatile constituents may play a crucial role in modulating the central nervous system (Aziz et al., 2016; İlgün et al., 2020). The presence of compounds such as lactucin and lactucopicrin in the volatile profile has been linked to these sedative effects, indicating their potential utility in treating anxiety and sleep disorders (Kim et al., 2022). These volatile compounds also contribute in development of plant's aroma and flavor, enhancing its appeal as a food source. The unique profile of volatile organic compounds (VOCs) in *L. serriola* not only influences its sensory characteristics but also affects consumer preferences (Abdul-Jalil, 2020; Kim et al., 2022).

Malva neglecta Wallr. (Malvaceae), commonly known as low mallow or common mallow. Its leaves, flowers, and roots are edible, and it can be consumed raw in salads or as cooked in various dishes. They are known to have a mild flavor, which makes them suitable for incorporation into a variety of culinary preparations (Yeşil et al., 2019; Akbulut & Zengin, 2023). The plant is rich in mucilage, flavonoids, and phenolic compounds, which contribute to its health benefits, including anti-inflammatory and antioxidant properties (Jędrzejczyk & Rewers, 2020; Saleem et al., 2020). These attributes make *M. neglecta* a popular choice among foragers and those interested in wild edible plants, as it is often regarded as a nutraceutical or functional food that promotes health (Yeşil et al., 2019). In addition to its antioxidant properties, *M. neglecta* exhibits notable antimicrobial activity. Research has shown that extracts from the leaves and flowers of *M. neglecta* possess significant antibacterial effects against various pathogenic bacteria, including *Escherichia coli* and *Staphylococcus aureus* (Bushra et al., 2012; Al-Qurashy, 2023). This antimicrobial action is attributed to the presence of specific volatile compounds that can disrupt bacterial cell membranes and inhibit growth, making *M. neglecta* a potential candidate for natural preservatives in food products (Keyrouz et al., 2017).

People consume *Papaver dubium* L. (Papaveraceae), also known as blind poppy or horned poppy, for its nutritional value, medicinal properties, and its role in traditional practices. The leaves of the plant are rich in vitamins and minerals, making them a valuable addition to salads and cooked dishes Honěk & Martinková (2010). Traditional medicine has utilized *P. dubium* for its therapeutic properties. Like other species in the *Papaver* genus, it contains alkaloids that may have health benefits. While *P. dubium* is less studied than its more famous relative, *Papaver somniferum*, and it is believed that *P. somniferum* possess same type of beneficial properties, such as anti-inflammatory and analgesic effects (Butnariu et al., 2022). Folk medicine has used plant to treat various ailments, including respiratory issues and digestive problems (Casado-Hidalgo et al., 2021). The presence of specific volatile compounds, phenolic compounds and alkaloids are believed to play a crucial role in antimicrobial action, making *P. dubium* a candidate for natural preservatives in food products. Moreover, the antimicrobial properties of *P. dubium* align with its traditional uses in folk medicine for treating infections and wounds (Mohammed, 2023).

Polygonum cognatum Meissn. (Polygonaceae), commonly known as "madimak" in Türkiye. One of the primary reasons for consuming *P. cognatum* is its nutritional profile. The plant is rich in vitamins A and C, minerals, and dietary fiber, which contribute to its health benefits (Demirgül et al., 2022; Akpınar, 2023). The presence of phenolic compounds and other bioactive substances contributes to its therapeutic potential, making it a valuable resource in herbal medicine (Kaplan & Gökşen Tosun, 2023; Yıldırım et al., 2002). Research indicates that essential oils and extracts from various parts of the plant possess significant antibacterial properties against a wide range of pathogenic bacteria. This antimicrobial action is attributed to specific volatile compounds that can disrupt bacterial cell membranes and inhibit their growth, making *P. cognatum* a best candidate for natural preservatives in food products. Its traditional use in folk medicine for treating infections further supports its relevance in modern therapeutic applications (Kaplan & Gökşen Tosun, 2023; Eruygur et al., 2020). Moreover, the volatile composition of *P. cognatum* has been linked to its antidiabetic properties. The plant has been reported to exhibit hypoglycemic effects, which may be associated with its ability to enhance insulin sensitivity and reduce blood glucose levels (Gözcü, 2023).

Rumex patientia L., also referred to as patience dock or garden patience, is a perennial herb. This perennial herb's leaves, belonging to the Polygonaceae family, are high in vitamins A and C, as well as minerals such as calcium and iron, making them a valuable addition to diets (Kaya et al., 2020). Research has indicated that extracts from this plant exhibit anti-inflammatory and antioxidant activities, which can be beneficial for managing various health conditions (Jovin et al., 2011). Furthermore, it is used in folk medicine to treat ailments such as respiratory issues and digestive disorders (Küpeli et al., 2007). *R. patientia* has been recognized for its anti-inflammatory effects. The plant has shown potential in reducing inflammation in various models, which may be beneficial for conditions such as arthritis and other inflammatory diseases (Gürbüz et al., 2005). The presence of bioactive compounds such as flavonoids and phenolic acids in the volatile profile is believed to contribute to these anti-inflammatory effects, highlighting the potential of *R. patientia* in managing inflammatory conditions.

Scorzonera cana (C.A. Mey. Hoffman), is a member of Asteraceae, commonly known as gray scorzonera. The bioactive compounds found in *S. cana* include flavonoids, phenolic acids, triterpenes, and other phytochemicals that are responsible for its health benefits. *S. cana*'s rich content of flavonoids and phenolic acids contributes to its antioxidant properties. These antioxidants help neutralize free radicals in the body, reducing oxidative damage and lowering the risk of chronic diseases such as heart disease and cancer (Deveci, 2022; Monteiro et al., 2018). In addition to its antioxidant properties, *S. cana* has demonstrated notable antimicrobial activity. This antimicrobial action is attributed to specific volatile compounds that can disrupt bacterial cell membranes. The traditional use of *S. cana* in folk medicine for treating infections further supports its relevance in modern therapeutic applications (Sakul et al., 2021).

Silene alba (Mill.) Krause (Caryophyllaceae), has several bioactive components that makes it nutritious and therapeutic. In addition to flavonoids, it contains saponins and glycosides, which have been reported to exhibit antimicrobial properties (Akgöz, 2014). These chemicals have antioxidant, anti-inflammatory, and antibacterial activities. In addition, they can inhibit the growth of various pathogens, thus contributing to the plant's traditional

use in folk medicine for treating infections (Ullah et al., 2019). They reduce oxidative stress by scavenging free radicals. Preventing cardiovascular disease and cancer requires antioxidant capacity. *S. alba* also contains quinic and malic acids, which boost its nutrition value. These acids improve food flavor, preservation activity and aid metabolism. Hesperidin, a flavonoid glycoside, improves vascular health and lowers blood pressure, making the plant healthier (Zengin et al., 2018). Research has shown that the floral scent of *S. alba* is specifically adapted to attract nocturnal pollinators, such as moths, which are critical for its reproductive success. The composition of these volatile compound can vary based on environmental conditions and the plant's phenological state, even influenced by effectiveness of pollination (Barthelmess et al., 2005). Moreover, its volatile profile is also indicative of its defense strategies against herbivores. The presence of certain terpenoids and other secondary metabolites in the essential oils has been correlated with antifungal and antibacterial properties, which may protect the plant from pathogens and pests (Hussein et al., 2019). Additionally, the ecological significance of *S. alba* extends to its role in community dynamics. The interactions facilitated by its volatile compounds can influence the composition and structure of plant communities. This suggests that the volatile composition of *S. alba* not only serves its immediate ecological functions but also contributes to broader ecological processes (Ramseier et al., 2005).

Stellaria media L. (commonly known as chickweed) is a member of the Caryophyllaceae family. One of the most prominent groups of bioactive compounds is polyphenols, which are known for their strong antioxidant properties. Flavonoids are another important class of compounds, have been extensively studied for their health benefits. The flavonoid content in *S. media* has been quantified, revealing levels of at least 1.2% in raw plant material, while different extraction process showed extraction of higher concentrations (Demján et al., 2022). Research has shown that extracts from *S. media* can inhibit enzymes such as xanthine oxidase, which is involved in the production of uric acid, thus suggesting potential benefits for conditions like gout (Taskin & Bitis, 2013). The volatile profile of *S. media* has implications for its use in traditional medicine. The plant has been utilized for its anti-inflammatory and antiviral properties, with extracts demonstrating efficacy against various pathogens (Ma et al., 2012). The presence of specific volatile compounds may enhance these therapeutic effects, making *S. media* a valuable candidate for further pharmacological research and development. Additionally, the plant's essential oils have been studied for their potential applications in food preservation and as natural antimicrobial agents, highlighting the versatility of its volatile composition (Chak et al., 2021).

The phytochemical profile of *Sonchus oleraceus* L. (Asteraceae), includes flavonoids, phenolic acids, sesquiterpene lactones, and various vitamins and minerals, which collectively offer a range of health benefits. These compounds are well-known for their antioxidant properties, which help in neutralizing free radicals and reducing oxidative stress in the body. The presence of flavonoids such as quercetin and kaempferol has been documented, and these compounds are associated with anti-inflammatory, antimicrobial, and anticancer activities (Hussein & Gobba, 2014; Nouidha, 2023; Li & Yang, 2018). Additionally, phenolic compounds, including hydroxycinnamic acids like caffeic acid, contribute significantly to the plant's antioxidant capacity (Nouidha, 2023; Chen et al., 2019). Understanding the volatile composition of *S. oleraceus* can aid in developing integrated weed management strategies. For instance, the allelopathic effects of its extracts on the germination and growth of other plants can be harnessed to suppress weed populations in agricultural settings (Gomaa et al., 2014). Additionally, the identification of specific VOCs that inhibit the growth of competing species can inform practices aimed at reducing the impact of this weed on crop yields (Ibrahim et al., 2022).

Tragopogon longirostris Bisch, a member of the Asteraceae family, exhibits a range of botanical and biochemical properties that are significant for both ecological and medicinal contexts. Biochemically, it is notable for its phytochemical composition, which includes various phenolic compounds and flavonoids known for their antioxidant properties. Similar species, like *Tragopogon porrifolius*, have been studied and found to have high levels of antioxidants like caffeic acid and flavonoids. These antioxidants help the plant's to possess the medicinal potential (Al-Rimawi et al., 2016; Abdalla & Zidorn, 2020). These compounds are associated with various health benefits, including anti-inflammatory and anticancer activities, as evidenced by studies on other *Tragopogon* species (Unver, 2023; Suleimen et al., 2019). In addition to their ecological significance, the volatile compounds of *T. longirostris* may possess medicinal properties. Research on related species within the *Tragopogon* genus has indicated that they contain bioactive compounds with antioxidant and anti-inflammatory activities. For example, a study on *T. graminifolius* found that its essential oil exhibited antioxidant and antimicrobial properties (Farzaei et al., 2014). These properties are often attributed to the presence of phenolic compounds and other secondary metabolites found in the essential oils of these plants.

The relationship between taste and volatile compounds in vegetables is a complex interplay that significantly contributes to the overall sensory experience of consuming vegetables. Volatile compounds are key contributors to the aroma and flavor of vegetables, working in conjunction with non-volatile compounds to create a holistic taste profile (Alasalvar et al., 2012). Non-volatile compounds such as organic acids, sugars, and free amino acids primarily influence taste perception, while, volatile compounds like aldehydes, alcohols, ketones, and aromatic compounds play a crucial role in providing the characteristic aroma of vegetables. The characteristic taste and aroma of vegetables are often determined by specific volatile compounds present in them. For example, the cabbage-like flavor of certain vegetables is attributed to key volatile compounds, many of which contain sulfur

(Meinert et al., 2011; Alasalvar et al., 2012). These volatile compounds not only contribute to the aroma but also influence the overall taste perception of vegetables. In the case of vegetable soybeans, a variety of volatile compounds have been identified, with specific compounds like 1-octen-3-ol, hexanal, and nonanal playing significant roles in defining the flavor profile (Guo et al., 2022). Moreover, the sensory flavor of vegetables is a combination of taste and smell, with both non-volatile and volatile compounds, which play essential roles in shaping the overall taste experience (Fan et al., 2022). While, non-volatile compounds stimulate taste receptors, in the same way, volatile compounds stimulate olfactory receptors, hence, both collectively contribute in the perception of taste and aroma in vegetables (Caporaso & Sacchi, 2021).

Terpenes, the major components of essential oil, are produced by many plant species and have many roles, including herbivore, pathogen protection and plant development. The terpene basic chemical structure is isoprene (Mewalal et al., 2017). Many types of specialized plant cells generate terpenes via metabolic processes (Zulak & Bohlmann, 2010; Cho et al., 2017). In prevention from illness and its treatment, terpenes have shown antibacterial, anti-allergenic, antioxidant, anti-inflammatory, and immunomodulatory activities (Theis & Lerdau, 2003). Their lipophilicity and low molecular weight lend the terpenes widespread usage in medicine (Miguel, 2010; Rufino et al., 2015). Limonene is one of the most common terpenes in nature as a colorless liquid which exists in two optical isomeric forms of d- or l-limonene, and as a racemic mixture (Zulaikha, 2015). This volatile compound found in various fruits and vegetables, contributes significantly to the flavor profile of vegetables. Limonene imparts a fruity aroma and is recognized as a key flavor compound that influences the odor of vegetables Xu et al. (2023). In the food industry, limonene is utilized as a flavoring agent in a wide range of food products, including vegetables, herbs, citrus juices, candies, beverages, and ice creams (Tang et al., 2022). The presence of limonene in vegetables enhances their sensory appeal by providing a distinct citrus-like flavor and aroma. Furthermore, limonene plays a crucial role in defining the taste and aroma of vegetables due to its characteristic properties. In plants, limonene is involved in biosynthetic pathways that lead to the production of various volatile compounds responsible for flavor and aroma. For instance, the 6-hydroxylation of limonene by specific enzymes is a crucial step in the biosynthesis of volatile compounds like carvone, which contributes to the flavor of spices such as caraway fruit (Dudareva et al., 2004). Limonene's citrus-like flavor makes it a popular choice as a flavoring agent in perfumes, creams, soaps, household cleaning products, and various food products, including fruit beverages and ice creams (Espina et al., 2013).

The aim of this study was to determine the volatile compounds content of some uncultivated weed species consumed as vegetables. The increasing interest in using various plants as human food for a healthy life, shaped this objective.

MATERIALS AND METHODS

Plant Materials

Thirteen different species (*Taraxacum officinale*, *Centaurea depressa* Bieb., *Cichorium intybus* L., *Lactuca serriola* L., *Malva neglecta* Wallr., *Papaver dubium* L., *Polygonum cognatum* Meissn., *Rumex patientia* L., *Scorzonera cana* (C.A. Mey. Hoffm.), *Silene alba* (Mill.) Krause, *Stellaria media* L., *Sonchus oleraceus* L., *Tragopogon longirostris* Bisch.), used in various ways as vegetables are naturally cultivated in the Akdeniz region of Türkiye.

All plants were collected from Bahtiyar village, Yalvaç distinct in Isparta (Latitude: 38.190574, Longitude: 31.163447), during the vegetation period in May-June, 2021. At least 10 individuals per species were collected in the early hours of the day randomly. Then they brought to the Suleyman Demirel University, Innovative Technologies Application and Research Center under controlled conditions. Fresh plant samples transported to the laboratory with a cold chain were stored at -20 degrees until SPME analysis. In SPME analysis, homogenized leaves were studied in triplicate.

Determination of Volatile Compounds by Solid-Phase Micro Extraction Gas Chromatography/ Mass Spectrometry

A 2-g sample was weighed in a 15-mL vial closed by a silicone septum. The sample was placed on a heating block at 60°C and held for 15 minutes to achieve temperature equilibrium. A Carboxen/polydimethylsiloxane manual solid-phase microextraction (SPME) fiber (75-µm Fused Silica, Supelco Ltd., Bellefonte, PA, USA) was inserted into the vial and kept for 30 minutes at 60°C to absorb volatile compounds from leaves. The fiber was then inserted into the injection port of gas chromatograph for 5 minutes at 250°C for the desorption of aroma compounds. Gas extraction/mass spectrometry (GC/MS) analyses were performed using a Shimadzu GC-2010 gas chromatograph equipped with an MS-QP2010 plus a mass spectrometer (Shimadzu Corporation, Kyoto, Japan). The analyses conditions are as follows: column, Rxi-5Sil MS (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Restek, Bellefonte, PA, USA); temperature program, from 40°C (2 minutes) to 250°C (5 minutes) at 4°C/min; injection temperature, 250°C; inlet pressure, 83.5 kPa; carrier gas, He [linear velocity (ū): 44.2 cm/s]; injection mode, split (10:1); MS interface temperature, 250°C; MS mode, electron ionization; detector voltage, 1.5 kV; mass range, 35–450 m/z; scan speed, 1428 u/s; interval, 0.30 seconds (2 Hz). Data handling was made through GCMS solution 2.5 (Shimadzu). GC/MS analysis was accomplished in the scan mode in the 40–300 amu mass range (Yılmazer et al., 2016).

Statistical Analysis

All the experiments were performed in triplicate and their statistical significance of differences between the averages in the obtained data was analyzed with the ANOVA and Duncan ($P<0.05$) multiple comparison test in SPSS software (IBM-SPSS Inc., Armonk, NY, USA) version 25.0, and the values are given as mean ± SD (standard deviation) (Zhang et al., 2022).

RESULTS AND DISCUSSION

A total of 63 volatile compounds belonging to 13 species were identified with SPME-GC and presented (Sample chromatogram is shown in Fig. 2). These compounds were grouped according to their structures as alcohols, aldehydes, alkanes, ester, furans, hydrocarbons, ketones, sulfur compounds, and terpenes (Table 1-2). These groups were calculated for each plant species. When the total percentages were analyzed, terpene was in highest concentration compare to other volatile compound contents in all plants following terpenes, aldehydes and alcohols in the groups (Figure 1).

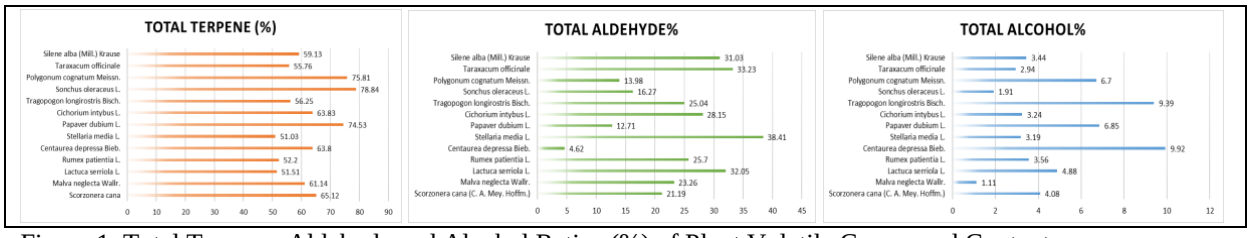

Figure 1. Total Terpene, Aldehyde and Alcohol Ratios (%) of Plant Volatile Compound Contents

In this study volatile component content of *Silene alba* consists of 59.13% terpene, 31.03% aldehyde and 3.44% alcohol (Figure 1). In headspace studies with different *Silene* species, high levels of monoterpenes (myrcene 23% in *Silene chlorantha*, trans-β-ocimene 27.2% in *S. nutans* and 34.9% in *S. sericea*, fenchyl acetate 12.7% in *S. chlorantha*, β-linalool 40.5% in *S. chlorantha* and 14.5% in *S. italica*) were found in all *Silene* species (Jürgens et al., 2002). The genus *Silene* comprises over 700 species, each exhibiting distinct volatile profiles that play critical roles in pollinator attraction and ecological interactions. The volatile components of different *Silene* species vary significantly, influenced by factors such as species-specific adaptations and environmental conditions. In their comparative study, Page et al. noted that the floral scent of *Silene species*, including *S. latifolia*, contains common volatile compounds that influence the preferences of pollinators like *Hadena bicruris* (Page et al., 2014). Moreover, Vivaldo et al. emphasized that the chemical composition of volatiles is often species-specific, with terpenes and nitrogen-containing compounds being prevalent in some *Silene* species, while others may emit sulfur-containing volatiles. This chemical diversity is further supported by the findings of Mamadalieva et al., who documented over 450 different secondary metabolites in *Silene*, indicating a rich array of volatile compounds that may serve various ecological functions (Mamadalieva et al., 2014; Vivaldo et al., 2017; Unlu et al., 2017).

The presence of volatile component content of *Taraxacum officinale* consists of 55.76% terpene, 33.23% aldehyde and 2.94% alcohol. A study on volatile compound was carried out on leaves and roots of *Taraxacum officinale* by HS-SPME/GC-MS. As terpene compounds, it was mentioned that caryophyllene, farnesene, -elemene, neofitadiene detected in the leaves of some varieties of dandelion have anti-inflammatory, antioxidant and anti-tumour like biological activities (Kiyama, 2017; Zhang et al., 2022). It was found that the most intense volatile compound group was dominated by esters and ketones. It was thought that those results, which are not very compatible with our findings, may be due to the difference in habitat condition of the plants.

The volatile component content of *Polygonum cognatum* consists of 75.81% terpene, 13.98% aldehyde and 6.7% alcohol. In another study, essential oils of different *Polygonum* species were extracted by hydro-distillation and analyzed by GC. Their results were found quite similar to our findings and presence of terpene groups were found to be dominant among the volatile compounds. Moreover, it was also observed that essential oil contains 33 different components, among them more than half identified groups were belonging to terpenes (Demirpolat, 2022).

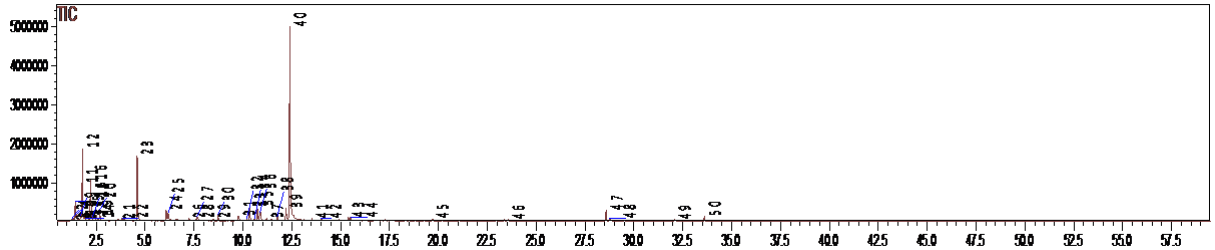

Figure 2. SPME/GC-MS chromatogram of *R. patientia*

The presence of volatile component content in *Sonchus oleraceus* consists of 78.84% terpene, 16.27% aldehyde and 1.91% alcohol. In already published study, gas chromatography mass spectrometry (GC-MS) data showed the identification of ten volatile compounds in hexane fraction of the Sonchus oleraceus, among them two were found to be the major components (40.92% 9 Octadecenamide (CAS) and 21.01% 1 Hexacosanol). Moreover, other thirty-six compounds isolated from different parts of S. oleraceus have also been reported. Among them flavonoids and terpene lactones have been the most reported secondary metabolites in these species (Sánchez-Aguirre et al., 2024). Hence, this information also supports our findings.

The volatile component content of *Tragopogon longirostris* consists of 56.25% terpene, 25.04% aldehyde and 9.39% alcohol. Riu-Aumatell et al. (2011) analyzed *Tragopogon porrifolius'* volatile composition using HS-SPME and SDE, connected to GLC/MS. There were around 80 volatile components from different chemical classes. They found that the SDE approach can identify sesquiterpenes such as β-elemene, α-humulene, α-cadinol, and farnesol.

The volatile component content of *Cichorium intybus* consists of 56.25% terpene, 25.04% aldehyde and 9.39% alcohol. It has been reported in the literature that Cichorium intybus is a rich source of terpenoid and phenolic compounds (Nasimi Doost Azgomi et al., 2021).

The presence of volatile component content ration in *Papaver dubium* consists of 74.53% terpene, 12.71% aldehyde and 6.85% alcohol. Direct information on the terpene content in *Papaver dubium* is limited in the literature. However, related studies on Papaver species like *Papaver somniferum* shed light on the presence of terpenes in poppies (Muthukumaran et al., 2019). Research on *Papaver somniferum* has highlighted the role of tyrosine aminotransferase in benzylisoquinoline alkaloid biosynthesis, indicating the involvement of various compounds, including terpenes, in the metabolic pathways of poppies. Additionally, a study on the phytochemical analysis of *Papaver somniferum* identified the presence of terpenoids among other secondary metabolites (Karioti et al., 2014).

The volatile component content of *Stellaria media* consists of 51.03% terpene, 38.41% aldehyde and 3.19 % alcohol. The results of phytochemical analysis of *Stellaria media,* revealed the presence of various secondary metabolites including terpenoids, flavonoids, phenols, alkaloids, tannins, glycosides and saponin (Chak et al., 2021).

In this study volatile component content of *Centaura depressa* consists of 63.8% total terpene (Figure 1), 4.62% aldehyde and 9.92% alcohol (Figure 1). In another study, the oil of *C. depressa* consisted of four oxygenated monoterpenes (36.5%), six sesquiterpene hydrocarbons (5.9%), 10 oxygenated sesquiterpens (39.7%), five aliphatichydrocarbons (4.4%) and one aliphatic acid (4.0%) (Esmaeili et al., 2005). The chemical composition of *Centaurea scabiosa* hydro-distilled volatile oil was determined using the GC/MS chromatographic technique and 32 volatile oil or chemical compounds were detected, which represent 90.21% of volatile oils. The chemical compounds are grouped in terpenes (43.73%) (Carev et al., 2022). Similar to our findings, the highest volatile compound group was reported in literature appears to be terpenes. However, differences in the percentage of volatile compounds may be due to differences in the species, the method of analysis or the place of cultivation.

The volatile component content of *Rumex patientia* consists of 52.2% terpene, 25.7% aldehyde and 3.56 % alcohol. Data on the presence of volatile component contents of the same species were not found in the literature. However, the contents of a different species belonging to the same genus were found and compared with our findings. According to this study, presence of volatile constituents prepared from *Rumex visicarius* by hydro-distillation was viscous, with yellow in color and unpleasant nauseating odor; results of GC-FID and GC/MS analysis of the essential oil revealed the identification of 26 compounds representing 90.66% of the total sample. Thirteen terpenoid compounds were found (47.23%), of which 3 monoterpenes (7.71%) and sesquiterpenes (39.52%); 5 oxygenated terpenes (28.96%); and 5 esters were identified representing 14.99% (El-Hawary et al., 2011). These results are consistent with our findings.

The volatile component content of *Lactuca serriola* consists of 51.51% terpene, 32.05% aldehyde and 4.88% alcohol. In already published data, *L. serriola* essential oil was obtained by hydro-distillation of plant leaves and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). As a result of the GS-MS analysis, hexadecanoic acid, oleic acid, myristic acid, hexahydrofarnesyl acetone, β-ionone, and n-tetradecyl butanoate were found as dominant components in the plant essential oil with the rate of 6.61%, 4.84%, 2.05, 1.55%, 1.3%, and 1.09% (v/v), respectively. The difference between the ratios may be due to a different methodology. One was analyzed by SPME directly from the leaf, while the other was analyzed by GC after extracting the essential oil. In addition, seven terpene compounds were isolated from the above-ground parts of *Lactuca indica* L. by column chromatographic separation of MeOH extract (Kim et al., 2008).

Table 1. The Volatile Compounds of *S. cana, M. neglecta, L. serriola, R. patientia, C. depressa, S. media, P. dubium*

COMPOUNDS	RT	*Scorzonera cana* (C. A. Mey. Hoffm.)	*Malva neglecta* Wallr.	*Lactuca serriola* L.	*Rumex patientia* L.	*Centaurea depressa* Bieb.	*Stellaria media* L.	*Papaver dubium* L.
ALCOHOLS								
3-Methyl-1-butanol	3.296	-	-	-	-	-	0.04±0.00 a	-
Pentanol	3.889	0.14±0.01 ab	0.14±0.00 ab	0.15±0.01 ab	0.25±0.08 a	-	0.17±0.02 ab	0.16±0.04 ab
E-2-Penten-1-ol	3.931	0.35±0.01 cd	0.33±0.06 cd	0.73±0.14 b	0.33±0.05 cd	-	0.15±0.00 de	0.51±0.12 bc
Z-3-Hexen-1-ol	6.166	2.59±0.03 bc	-	2.90±0.05 b	2.06±0.08 bc	5.77±0.14 a	-	3.04±0.78 b
E-2-Hexen-1-ol	6.539	-	-	-	-	0.33±0.09 c	-	0.19±0.04 c
Hexanol	6.631	0.73±0.01 cd	0.43±0.01 de	0.90±0.02 bc	0.29±0.02 e	2.20±0.00 a	2.38±0.15 a	0.72±0.19 cd
1-Octen-3-ol	10.583	-	-	-	0.30±0.03 b	-	0.22±0.01 b	1.83±0.40 a
Octan-3-ol	11.263	-	-	-	-	0.33±0.03 a	-	-
Benzyl alcohol	12.650	-	-	-	-	0.55±0.06 a	-	-
Phenethyl alcohol	15.655	-	-	-	-	0.18±0.00 a	-	-
2-Ethylhexanol	32.100	0.27±0.00 de	0.21±0.01 de	0.20±0.01 e	0.33±0.01 cde	0.56±0.02 b	0.23±0.03 de	0.40±0.07 bcd
ALDEHYDE								
Isobutanal	1.625	0.06±0.01 cd	-	0.30±0.02 a	-	-	0.06±0.01 cd	0.23±0.08 ab
3-Methylbutanal	2.224	0.49±0.00 cde	0.26±0.01 de	1.76±0.26 b	4.55±0.10 a	-	0.21±0.01 de	1.02±0.36 c
2-Methylbutanal	2.313	0.35±0.01 cdef	0.18±0.01 def	1.21±0.11 a	0.59±0.05 bc	-	0.15±0.02 ef	0.65±0.21 bc
Pentanal	2.677	0.71±0.03 cd	-	1.15±0.07 b	2.01±0.08 a	-	0.46±0.01 de	0.42±0.12 e
2-Methyl-2- butenal	3.388	-	-	-	-	-	0.14±0.00 a	-
E-2-Pentenal	3.601	1.07±0.01 bc	1.54±0.00 a	0.83±0.05 cd	0.37±0.06 ef	0.14±0.04 fg	0.96±0.00 bcd	0.42±0.11 e
Hexanal	4.588	3.15±0.23 ef	4.56±0.00 e	6.83±0.07 cd	12.81±0.89 b	0.16±0.01 g	15.29±1.08 a	0.46±0.10 g
Furfural	4.992	-	-	-	-	-	0.12±0.02 a	-
E-2-Hexenal	6.070	8.69±0.13 d	11.57± bcd	14.45±0.78 ab	2.41±0.25 e	2.74±0.57 e	15.20±0.01 ab	7.99±2.05 d
Heptanal	7.657	1.44±0.05 a	1.14±0.00 b	1.32±0.01 a	0.72±0.02 d	0.23±0.01 f	1.39±0.06 a	0.41±0.00 e
Z-2-Heptenal	9.657	0.11±0.01 cd	-	-	-	-	0.43±0.00 a	-
Benzaldehyde	9.770	1.09±0.14 b	1.75±0.00 a	0.87±0.09 b	1.13±0.06 b	-	0.97±0.06 b	-
E,E-2,4-Heptadienal	11.164	1.80±0.16 b	0.65±0.00 d	1.25±0.22 c	-	-	0.64±0.01 d	-
Octanal	11.433	0.49±0.01 bc	0.54±0.00 b	0.41±0.02 cd	0.25±0.01 e	-	0.38±0.01 d	-
E,Z-2,4-Heptadienal	11.766	-	-	-	-	-	0.66±0.06 b	-
Benzeneacetaldehyde	12.925	0.32±0.01 b	0.18±0.00 b	0.41±0.08 ab	-	1.35±0.67 a	-	0.34±0.06 b
Nonanal	15.385	1.26±0.02 cd	0.81±0.00 def	1.16±0.21 cde	0.86±0.03 def	-	1.24±0.04 cd	0.67±0.08 ef
Decanal	19.275	0.16±0.01 bc	0.08±0.00 d	0.10±0.01 cd	-	-	0.11±0.00 cd	0.10±0.02 cd
ALKANE								
2,2-Dimethyltetradecane	23.370	0.22±0.03 d	0.35±0.00 c	0.11±0.00 ef	-	-	0.09±0.00 f	0.19±0.02 de
2,2,11,11-Tetramethyldodecane	23.562	0.25±0.02 de	0.38±0.00 c	0.15±0.00 fg	0.25±0.00 d	-	0.11±0.02 gh	0.25±0.00 de
ESTER								
2-Propenoic acid, 2-methyl-, methyl ester	2.859	-	-	--	-	0.30±0.02 a	-	-

Table 1. Continued

COMPOUNDS	RT	*Scorzonera cana* (C. A. Mey. Hoffm.)	*Malva neglecta* Wallr.	*Lactuca serriola* L.	*Rumex patientia* L.	*Centaurea depressa* Bieb.	*Stellaria media* L.	*Papaver dubium* L.
FURAN								
2-ethyl- Furan	2.691	-	0.51±0.00 a	-	-	-	-	-
HYDROCARBONS								
o-Xylene	6.540	0.55±0.00 a	0.32±0.05 b	0.21±0.01 c	-	-	-	-
Styrene	7.249	0.61±0.00 a	0.66±0.05 a	0.42±0.02 b	0.48±0.03 b	-	0.17±0.02 d	-
1,2,3-Trimethylbenzene	10.995	0.33±0.00 a	-	-	-	-	-	-
p-Dichlorobenzene	11.755	1.47±0.00 a	1.28±0.10 a	1.45±0.15 a	0.72±0.04 b	-	-	0.30±0.07 c
γ-Cadinene	29.908	-	-	-	-	1.30±0.07 a	-	-
δ -Cadinene	30.079	-	-	-	-	1.97±0.09 a	-	-
KETONES								
1-Penten-3-one	2.518	0.85±0.06 bcdefg	1.56±0.08 ab	1.66±0.12 a	1.30±0.16 abcd	0.19±0.00 g	0.29±0.01 fg	1.35±0.46 abc
2,3-Pentanedione	2.655	-	0.39±0.05 a	-	-	-		0.52±0.16 a
3-Pentanone	2.666	-	-	-	-	0.26±0.00 a	-	-
2-Heptanone	7.278	-	-	-	-	0.14±0.00 a	-	-
6-Methyl-5-hepten-2-one	10.730	1.93±0.02 bc	1.78±0.09 bc	2.20±0.04 b	3.41±0.29 a	-	0.58±0.04 def	1.81±0.49 bc
3-Octanone	10.772	-	-	-	-	1.32±0.12 a	-	-
3,5-Octadien-2-one	14.005	0.32±0.01 de	0.51±0.02 abc	0.48±0.03 bcd	0.57±0.04 ab	-	0.65±0.06 a	0.27±0.06 ef
E- β -Ionone	28.763	0.06±0.03 cd	0.12±0.01 cd	0.16±0.01 c	0.60±0.07 a	-	0.41±0.06 b	0.14±0.03 c
SULFUR COMPOUNDS								
Dimethyl sulfide	1.496	1.13±0.02 c	4.20±0.51 a	2.55±0.24 b	-	-	-	-
Carbon disulfide	1.559	0.52±0.01 b	0.61±0.01 a	-	-	-	-	-
TERPENE								
α-Thujene	8.486	-	0.08±0.01 cde	0.13±0.00 cd	0.32±0.03 b	-	-	0.10±0.01 cd
α-Pinene	8.727	-	0.08±0.02 de	0.49±0.06 b	0.94±0.05 a	0.92±0.14 a	0.15±0.01 cde	0.36±0.09 bc
Sabinene	10.223	-	-	0.13±0.02 b	0.31±0.04 a	-	-	0.10±0.01 bcd
β-Pinene	10.362	-	0.17±0.03 ef	0.98±0.15 bc	2.29±0.12 a	-	0.46±0.02 de	1.16± 0.18 b
β-Myrcene	10.903	3.49±0.06 a	3.53±0.13 a	1.98±0.01 bcd	1.03±0.02 f	2.20±0.19 bc	1.06±0.05 f	1.68±0.30 cde
δ-3-Carene	11.212	-	-	-	0.30±0.01 a	-	-	-
β -Ocimene	11.594	-	-	-	-	-	-	-
p- Cymene	12.213	4.88±0.00 a	4.43±0.08 ab	3.35±0.12 cde	2.52±0.10 f	2.59±0.04 f	2.84±0.03 ef	3.15±0.32 def
Limonene	12.447	56.16±0.16 b	52.35±0.54 bc	44.08±2.42 c	44.13±0.81 c	53.82±1.57 bc	45.68±0.77 c	67.39±5.80 a
γ- Terpinene	13.535	0.59±0.00 abc	0.50±0.01 bc	0.37±0.02 c	0.36±0.00 c	0.80±0.04 a	0.84±0.03 a	0.59±0.14 abc
Linalool	15.237	-	-	-	-	0.22±0.03 a	-	-
α -Cedrol	29.259	-	-	-	-	1.22±0.11 a	-	-
Bicyclogermacrene	29.355	-	-	-	-	1.55±0.08 a	-	-
α -Muurolene	29.451	-	-	-	-	0.48±0.04 a	-	-
OTHERS		1.40±0.03 ef	1.84±0.03 def	2.18±0.06 def	11.20±0.76 b	16.19±0.83 a	5.07±0.16 c	1.08±0.19 ef

* Shows values with insignificant difference ($p < 0.05$) for each column shown with same letters (± standard deviation)

Table 2. The Volatile Compounds of *C. intybus*, *T. longirostris*, *S. oleraceus*, *P. cognatum*, *T. officinale*. *S. alba*

COMPOUNDS	RT	*Cichorium intybus* L.	*Tragopogon longirostris* Bisch.	*Sonchus oleraceus* L.	*Polygonum cognatum* Meissn.	*Taraxacum officinale*	*Silene alba* (Mill.) Krause
ALCOHOLS							
3-Methyl-1-butanol	3.296	-	-	-	-	-	-
Pentanol	3.889	-	0.16±0.00 ab	0.05±0.00 bc	0.23±0.02 a	-	0.09±0.01 bc
E-2-Penten-1-ol	3.931	0.11±0.01 de	0.46±0.03 bc	0.09±0.00 de	1.49±0.09 a	0.07±0.01 de	0.28±0.01 cde
Z-3-Hexen-1-ol	6.166	2.43±0.21 bc	3.05±0.06 b	1.50±0.03 c	1.99±0.04 bc	1.90±0.10 bc	2.25±0.07 bc
E-2-Hexen-1-ol	6.539	-	2.40±0.15 a	-	1.58±0.27 b	-	-
Hexanol	6.631	0.28±0.02 e	2.39±0.10 a	0.27±0.00 e	1.15±0.06 b	0.37±0.05 de	0.45±0.03 de
1-Octen-3-ol	10.583	0.11±0.00 b	0.07±0.01 b	-	0.11±0.00 b	0.13±0.01 b	0.10±0.00 b
Octan-3-ol	11.263	-	-	-	-	-	-
Benzyl alcohol	12.650	-	-	-	-	-	-
Phenethyl alcohol	15.655	-	-	-	-	-	-
2-Ethylhexanol	32.100	0.31±0.02 cde	0.86±0.05 a	-	0.15±0.02 ef	0.47±0.09 bc	0.27±0.02 de
ALDEHYDE							
Isobutanal	1.625	-	0.15±0.00 bc	-	-	0.11±0.01 bcd	0.10±0.01 cd
3-Methylbutanal	2.224	0.33±0.01 de	0.84±0.03 cd	0.37±0.04 cde	-	0.38±0.00 cde	0.63±0.00 cde
2-Methylbutanal	2.313	0.44±0.00 bcde	0.52±0.04 bcd	0.16±0.00 def	-	0.56±0.04 bc	0.70±0.03 b
Pentanal	2.677	0.49±0.01 de	0.86±0.00 c	-	-	0.73±0.08 cd	0.79±0.06 c
2-Methyl-2- butenal	3.388	-	-	-	-	-	-
E-2-Pentenal	3.601	-	0.77±0.00 d	0.46±0.01	0.34±0.05 ef	1.19±0.02 b	0.89±0.11 cd
Hexanal	4.588	4.05±0.18 e	3.82±0.16 e	1.14±0.02 fg	1.15±0.19 fg	5.08±0.47 de	7.63±0.27 c
Furfural	4.992	-	-	-	-	-	-
E-2-Hexenal	6.070	16.44±0.06 a	14.26±1.34 abc	11.66±0.12 bcd	11.55±0.41 bcd	14.72±0.56 ab	10.12±1.05 cd
Heptanal	7.657	1.18±0.03 b	0.94±0.00 c	0.61±0.02 d	0.27±0.00 f	-	1.45±0.04 a
Z-2-Heptenal	9.657	0.09±0.00 d	-	0.05±0.00 e	-	0.24±0.00 b	0.12±0.02 c
Benzaldehyde	9.770	0.46±0.06 c	0.20±0.01 cd	0.36±0.01 cd	0.08±0.02 d	1.95±0.03 a	1.11±0.15 b
E,E-2,4-Heptadienal	11.164	1.36±0.08 c	1.14±0.07 c	0.36±0.00 de	0.22±0.03 de	1.98±0.02 ab	2.41±0.02 a
Octanal	11.433	0.55±0.02 b	-	0.13±0.00 f	-	0.75±0.07 a	0.70±0.01 a
E,Z-2,4-Heptadienal	11.766	0.71±0.06 b	-	-	-	1.88±0.23 a	1.76±0.02 a
Benzeneacetaldehyde	12.925	0.22±0.01 b	0.25±0.03 b	0.17±0.00 b	-	0.37±0.03 ab	0.25±0.00 b
Nonanal	15.385	1.64±0.06 c	1.19±0.18 cde	0.80±0.01 def	0.37±0.01 fg	2.94±0.19 a	2.21±0.08 b
Decanal	19.275	0.19±0.00 b	0.10±0.01 cd	-	-	0.35±0.04 a	0.16±0.01 bc
ALKANE							
2,2-Dimethyltetradecane	23.370	-	0.92±0.01 a	0.09±0.00 f	0.15±0.02 def	0.78±0.03 b	0.31±0.00 c
2,2,11,11-Tetramethyldodecane	23.562	-	1.04±0.02 a	0.08±0.01 h	0.19±0.02 ef	0.83±0.02 b	0.36±0.00 c

Table 2. Continued

COMPOUNDS	RT	*Cichorium intybus* L.	*Tragopogon longirostris* Bisch.	*Sonchus oleraceus* L.	*Polygonum cognatum* Meissn.	*Taraxacum officinale*	*Silene alba* (Mill.) Krause
ESTER							
2-Propenoic acid, 2-methyl-, methyl ester	2.859	-	-	-	-	-	-
FURAN							
2-ethyl- Furan	2.691	-	-	-	0.52±0.05 a	-	-
HYDROCARBONS							
o-Xylene	6.540	0.12±0.01 d	-	-	-	0.20±0.01 cd	-
Styrene	7.249	0.17±0.02 d	0.30±0.03 c	-	0.12±0.00 d	0.12±0.02 d	0.10±0.01 de
1,2,3-Trimethylbenzene	10.995	-	-	-	0.14±0.00 c	0.30±0.00 a	0.22±0.03 b
p-Dichlorobenzene	11.755	-	0.71±0.03 b	0.34±0.02 c	0.21±0.03 cd	-	-
γ-Cadinene	29.908	-	-	-	-	-	-
δ -Cadinene	30.079	-	-	-	-	-	-
KETONES							
1-Penten-3-one	2.518	0.42±0.03 efg	1.13±0.02 abcde	0.42±0.00 efg	0.94±0.06 abcdef	0.58±0.07 defg	0.73±0.06 cdefg
2,3-Pentanedione	2.655	0.49±0.01 a	-	0.56±0.02 a	-	-	-
3-Pentanone	2.666	-	-	-	-	-	-
2-Heptanone	7.278	-	-	-	-	-	-
6-Methyl-5-hepten-2-one	10.730	1.38±0.06 bcd	1.59±0.04 bc	0.63±0.02 def	0.53±0.04 ef	1.15±0.06 cde	1.09±0.04 cde
3-Octanone	10.772	-	-	-	-	-	-
3,5-Octadien-2-one	14.005	-	0.36±0.03 cde	0.13±0.01 fgh	0.09±0.02 gh	0.32±0.02 e	0.23±0.01 efg
E- β -Ionone	28.763	-	-	0.09±0.00 cd	-	0.12±0.01 cd	0.10±0.00 cd
SULFUR COMPOUNDS							
Dimethyl sulfide	1.496	-	-	0.76±0.03 cd		0.51±0.03 cd	0.84±0.14 c
Carbon disulfide	1.559	-	-	-	-	-	-
TERPENE							
α-Thujene	8.486	0.15±0.02 c	0.07±0.01 cde	-	0.05±0.01 de	1.33±0.05 a	-
α-Pinene	8.727	0.35±0.03 bcd	0.15±0.00 cde	0.09±0.00 cde	0.13±0.03 cde	0.11±0.00 cde	0.26±0.01 bcde
Sabinene	10.223	0.12±0.01 bc	0.12±0.00 bc	0.05±0.00 cde	0.03±0.01 de	-	0.08±0.00 bcd
β-Pinene	10.362	0.92±0.12 bc	0.32±0.00 def	0.31±0.00 def	0.37±0.04 def	0.28±0.04 def	0.62±0.01 cd
β-Myrcene	10.903	1.80±0.10 cde	-	1.25±0.01 ef	1.43± 0.05 def	2.48±0.04 b	1.89±0.01 cd
δ-3-Carene	11.212	-	0.30±0.03 a	-	-	-	-
β -Ocimene	11.594	-	0.09±0.01 a	-	-	-	0.10±0.01 a
p- Cymene	12.213	2.78±0.06 ef	2.96±0.05 def	3.55±0.13 cd	2.65±0.05 f	4.01±0.09 bc	3.90±0.23 bc
Limonene	12.447	57.27±0.76 b	51.79±1.04 bc	72.92±0.61 a	70.17±0.87 a	47.42±0.58 bc	51.82±1.11 bc
γ- Terpinene	13.535	0.44±0.03 c	0.34±0.02 c	0.60±0.01 abc	0.74±0.10 ab	-	0.46±0.02 c
Linalool	15.237	-	0.11±0.01 bc	0.07±0.00 c	0.24±0.02 a	0.13±0.01 b	-
α -Cedrol	29.259	-	-	-	-	-	-
Bicyclogermacrene	29.355	-	-	-	-	-	-
α -Muurolene	29.451	-	-	-	-	-	-
OTHERS		2.21±0.16 def	3.26±0.16 d	0.97±0.01 ef	0.58±0.06 f	3.15±0.06 d	2.42±0.15 de

* Shows values with insignificant difference ($p < 0.05$) for each column shown with same letters (± standard deviation)

The presence of volatile component content in *Malva neglecta* consists of 61.14% terpene, 23.26% aldehyde and 1.11 % alcohol. SPME analysis of *M. neglecta* was not found in the literature. However, *M. neglecta* crude methanolic extract (Mn.Cme) was chemically characterized using GCMS analysis. In GC-MS analysis, oleic acid (19.67%), taurine (17.60%), ethylene dimercaptan (14.67%), isoeugenol (14.61%), patchoulane (10.36%), methyl 12-methyltetradecanoate (8.47%) and isopropyl myristate (7.02%) were highly abundant compounds. In another study, the efficacy of solvent-free microwave extraction (SFME) was investigated for the extraction of essential oils from the above-ground parts of *Malva neglecta* Wallr. The essential oils were then injected onto the HP-5MS column of a commercially available GC/MS (Hewlett-Packard 5973), resulting in a chromatogram of 24 compounds, accounting for 99.9% of the oil composition. In terms of general categories, non-terpene hydrocarbons and sesquiterpene hydrocarbons were found to be the main fractions of the chemical profiles (Mohammadhosseini, 2021.

The volatile component content of *Scorzonera cana* consists of 65.12% terpene, 21.19% aldehyde and 4.08 % alcohol. In another study published by Lendzion et al. (2021), presence of many bioactive compounds like triterpenoids, sesquiterpenoids, flavonoids, or caffeic acid and quinic acid derivatives were found in extracts obtained from aerial and subaerial parts of *Scorzonera* species. On the other hand, *Scorzonera* species are found to be highly rich in terpenes compounds: such as monoterpenes, sesquiterpene lactones, and triterpenes (Acikara et al., 2013; Yang et al., 2016). These studies also support our results.

As mentioned above, terpenes were the dominant group of volatile compounds in all plants and limonene was the major component among them. The interaction of abiotic factors such as soil characteristics and climate can influence the volatile profiles of all species. For instance, environmental conditions can affect the emission rates and types of volatiles produced, leading to variations in the chemical cues available to pollinators and herbivores. Some of the different results we obtained in our study compared to the literature are probably due to this interaction of abiotic factors. The volatile components may play a role in enhancing the sensory attributes of the plant, making it more appealing for culinary uses. The aroma and flavor profile of volatile compounds can significantly affect consumer preferences. For example, terpenes, which are prevalent in many plants, contribute to the characteristic scents and flavors that can enhance the palatability of foods (Fukuda et al., 2013). This sensory enhancement can encourage the consumption of vegetables that contain terpene aromas, thereby increasing their nutritional intake. In conclusion, the high terpene content species serves crucial ecological functions such as pollinator attraction, defense against herbivores, and potential allelopathy. Nutritionally, terpenes contribute to the health benefits, flavor, and potential medicinal properties of these plants, enhancing their value as food sources. The interplay between ecological roles and nutritional benefits highlights the importance of terpenes in these species' survival and utility.

Limonene is frequently identified as one of the most highly volatile compounds found in plants, and this prominence can be attributed to several factors related to its chemical properties, ecological roles, and biosynthetic pathways. Limonene is a monoterpene, characterized by its low molecular weight and relatively simple structure, which contributes to its high volatility. The volatility of a compound is influenced by its molecular weight, boiling point, and vapor pressure; limonene has a low boiling point (approximately 177°C) and high vapor pressure, making it readily evaporate at room temperature. This property allows limonene to be emitted in significant quantities into the atmosphere, where it can serve various ecological functions (Erasto & Viljoen, 2008). Limonene plays a crucial role in attracting pollinators. Its pleasant citrus aroma is appealing to many insects, including bees and butterflies, which are essential for the pollination of many flowering plants. The ability to attract these pollinators enhances the reproductive success of plants that produce limonene, thereby promoting genetic diversity and plant population stability (Zhao & Kang, 2002). Limonene also serves as a defense compound against herbivores and pathogens. Its insecticidal properties have been documented, with studies showing that limonene can deter certain insect pests, thereby reducing herbivory (Lackus et al., 2018). Additionally, limonene has antifungal properties, which can help protect plants from fungal infections. This dual role as both an attractant and a deterrent underscore the ecological significance of limonene in plant survival (Quintana-Rodríguez et al., 2014). The emission of limonene and other volatile compounds can trigger induced resistance in neighboring plants. When a plant is damaged by herbivores, it may release limonene, which can signal nearby plants to bolster their own defenses against potential threats (Erasto & Viljoen, 2008). This phenomenon enhances community resilience against herbivory and disease. Limonene is biosynthesized from geranyl pyrophosphate (GPP) through the action of limonene synthase, an enzyme that catalyzes the conversion of GPP into limonene in a single step. This pathway is common among many plant species, contributing to the widespread occurrence of limonene in various plant genera, particularly in citrus fruits and aromatic herbs. The ability of plants to produce limonene from a common precursor facilitates its prevalence in the plant kingdom (Maruyama et al., 2001; Chen, 2024). The highest limonene content in all species was found in *Sonchus oleraceus* L. with approximately 73%, while the lowest content was found in *Lactuca serriola* L., and *Rumex patientia* L. with 44%. In the food sector, limonene can be utilized as a flavoring agent to mask bitter tastes, and it also possesses antioxidant, antimicrobial, anti-carcinogenic, chemo-preventive, and antidiabetic properties that are beneficial for pharmaceutical purposes (Hidajat et al., 2020). The limonene aroma likely contributes to the local people's preference for collecting and

consuming these 13 species. For this reason, limonene can have a positive effect on consumers' vegetable consumption rates.

CONCLUSION

This study provides important data for determining plants' volatile compound profiles. The volatile components play a significant role in the sensory attributes of food, influencing both aroma and flavor. They are produced by plants in response to various stimuli, including biotic and abiotic stresses, and are crucial for attracting pollinators, deterring herbivores, and facilitating seed dispersal. The complexity of these compounds contributes to the overall sensory experience of food, as they can evoke specific flavors and aromas that enhance consumer appeal. They are integral to the sensory characteristics of food, influencing aroma, flavor, and even food safety. The findings from this study will contribute to obtaining more information about plants potential biological activities and applied areas. Future studies should compare the volatile compound profiles of the same species growing in different habitats or different species growing in the same habitat to better understand the effects of environmental factors. Furthermore, conducting similar analyses on various plant species may increase the general results' validity. These studies will be plants complex interactions and biosynthetic pathways present opportunities for enhancing food quality through agricultural and processing innovations.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

ACT: design, writing and laboratory studies. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Shoot tip culture of Bilecik İrikarası, Sarı Üzüm, Kartal Çavuş and Razakı grape varieties grown in Bilecik province

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Abstract
The aim of this study was to determine the propagation potential of some local varieties using the in vitro shoot-tip culture method. Bilecik İrikarası, Sarı Üzüm, Kartal Çavuş and Razakı were used as materials. As a result of the study, the values of rooting rate, number of roots, length of roots, number of shoots, length of shoots, number of leaves and number of nodes of the grape varieties were determined. When the cultivars were compared in terms of rooting characteristics, the highest rooting rate was 54.7%, the highest root number was 3.71 per plant, and the highest average root length value was 17.93 cm from Bilecik İrikarası. When the cultivars were evaluated in terms of shoot length, the highest shoot length value was determined in Razaki variety with 5.72 cm. Similarly, considering the number of leaves and nodes in the shoots were determined, with the highest leaf number value was of 8.71, the highest node number value was of 6.71 in Razakı variety. As a result of the study, local varieties that used as material in this study showed positive results in in vitro propagation.

Keywords: Bilecik, Grape, Tissue Culture, Local Variety, Shoot Tip Culture

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INTRODUCTION

Local grape varieties are the most important grapevine genetic resource and has an important role in breeding studies (Sağlam and Çalkan Sağlam, 2018; Ekbiç and Yılmaz, 2018). In addition to using these varieties for breeding purposes, their local production is also in demand (Sağlam and Çalkan Sağlam 2017; Ergönül and Çelik, 2018).

When it comes to the production of local varieties for different purposes, insufficient production material is often the most important problem. For this reason, production methods that can produce as much as possible from this scarce material are needed. Tissue culture is one of these methods. Tissue culture is also used for the critical materials protected under controlled conditions. On the other hands, with tissue culture, it is possible to carry out breeding and production activities that would take years in a much shorter time. It also enables large quantities of production to be carried out in a small area.

With the tissue culture method, it is possible to obtain a new plant under in vitro conditions by taking parts from various parts of the plant (meristem, shoot tip, embryo, anther, etc.) (Ergül et al., 2017). There are many studies on tissue culture practices and meristem culture in viticulture (Stamp et al., 1990; Adıyaman, 1998; Péros et al., 1998; Baydar, 2000; Park et al., 2001; Karaca, 2006; Jaskani et al., 2008; Ekbiç et al., 2015; Sağlam et al., 2016; Ergönül and Çelik, 2018; Balı et al., 2020).

According to studies conducted on grapevine; It has been determined that the regeneration rate in grape varieties varies depending on the variety (Miljani´c et al., 2022). On the other hand, in studies conducted on different grape varieties, it has been determined that the contents of the growing medium used are important in terms of development criteria (Skadia et al., 2010; Ayman et al., 2011; Batukaev et al., 2021).

In addition, the concentration of growth regulators added to the growth medium significantly affects the success of tissue cultures (Gomes et al., 2004; Skadia et al., 2010; Ayman et al., 2011; Khan et al., 2015; Kumsa, 2016; Kavitha and Surakhshitha, 2023). As a matter of fact, while there was no callus development in the Cabernet Franc grape variety in the medium containing no Benzyl adenine purine (BA), callus development occurred in the medium containing 10mg BA (Garcia et al., 2023).

In this study, the suitability of Bilecik İrikarası, Sarı Üzüm, Kartal Çavuş and Razakı grape varieties, which are genetic resource of Bilecik, Turkey, for clonal propagation by shoot tip culture, which is one of the tissue culture techniques, was determined.

MATERIALS AND METHODS

This study was carried out in the Tissue Culture and Plant Breeding laboratory within Bilecik Şeyh Edebali University Biotechnology Application and Research Center in 2019-2021.

Materials

Bilecik İrikarası, Kartal Çavuş, Sarı Üzüm and Razakı varieties were used as material in the research.

The long term of climatically data for Bilecik were found in table 1.

Table 1. Bilecik province long-term climate data averages (1991-2020) (Anonymus, 2024)

BILECIK	January	February	March	April	May	June	July	August	September	October	November	December	Yearly average
Average temperature (°C)	2.7	4.1	7.2	11.6	16.5	20.4	22.9	23.0	19.1	14.4	8.9	4.5	12.9
Average maximum temperature (°C)	6.2	8.5	12.4	17.5	22.7	26.8	29.6	30.0	25.9	20.2	13.7	7.9	18.4
Average minimum temperature (°C)	0.0	0.8	3.2	6.9	11.3	14.8	17.0	17.4	13.9	10.2	5.5	1.9	8.6
Average sunshine duration (h)	3.1	3.8	4.8	6.4	8.0	9.6	10.6	10.0	8.1	5.5	4.2	2.8	6.4
Average number of rainy days	13.63	13.20	12.70	10.77	10.27	8.67	3.77	3.77	5.60	8.67	9.57	13.43	114.0
Monthly total rainfall average (mm)	48.0	44.4	48.7	48.6	43.9	50.6	16.4	11.2	27.0	50.1	37.1	56.1	482.1
Maximum temperature (°C)	22.0	24.6	30.2	33.3	35.8	38.2	41.0	40.6	38.4	34.3	27.4	25.0	41.0
Minimum temperature (°C)	-16.0	-14.3	-11.6	-6.0	1.0	6.0	7.7	8.2	3.2	-0.8	-9.2	-14.5	-16.0

Methods

During the resting period, cuttings were taken from the grape varieties used as material and planted with 3-4 buds in pots containing a mixture of 10-liter peat-perlite. The pots were left to develop in a climate chamber at 25±1 °C, 16 hours of light, 8 hours of darkness, and 4000 lux illumination. After shoots were formed from these shoots, the shoot tips taken from these shoots were transferred to the nutrient medium.

Figure 1. Cuttings planted in pots

Figure 2. Shoot formation in cuttings

MS (Murashige and Skoog) medium was used in the study. The medium was prepared manually. While preparing the medium, the pH of the medium was adjusted to 5.7-5.8. Then, 1 mgL^{-1} BAP, (Benzyl Amino Purine) was added to this solution (Fotini et al., 2010; Ekbiç ve Yılmaz, 2018; Beza, 2010; Baydar, 2000) to encourage shoot formation and 2 mgL^{-1} IBA (Indole-3-Butyric Acid) (Ekbiç vd., 2015; Jaskani vd., 2008) was added to encourage root formation. The prepared medium was sterilized in an autoclave at 121 ºC at 1 atm pressure for 20 minutes. Then, this medium was transferred to sterilized 150 ml jars, 20 ml per jar, before it solidified.

Shoot tips, which served as study material, were collected from the shoots developed in the climate chamber and subjected to sterilization. For sterilization, 10% sodium hypochlorite solution was used. For this purpose, the material was kept in a shaker in sodium hypochlorite solution for 10 minutes, then washed with pure water in a shaker three times for five minutes each. After sterilization, these shoot tips were planted in MS medium and left to develop in a climate chamber with a temperature of 25±1 ºC, relative humidity of 50-60% and a photoperiod of 16 hours light and 8 hours dark with 4000 lux illumination.

Figure 3. Development in in vitro climate chamber

Data were taken by measurements 4-6 weeks after planting, depending on the development status. In the study, data on the number of rooted plants, number of roots, root length (cm), number of shoots, shoot length (cm), number of nodes and number of leaves were collected and evaluated. The study was designed in a Randomized Parcels Trial design with 3 replications, and 25 shoot tips were planted in each replication. All data obtained were subjected to statistical evaluation with the help of JMP 16.0 package program.

RESULTS AND DISCUSSION

When the rooting status of the varieties was evaluated statistically, it was found to be significant within the 95% confidence interval. Data regarding rooting rate are given in Table 2. When Table 2 is examined; the highest rooting value was in the Bilecik İrikarası variety with 54.7%.

Table 2. Number of roots, root length, number of shoots, shoot length and number of nodes values obtained from the study

Variety	Root ratio (%)	Root number/plant (n)	Average Root Length (cm)	Average Shoot Length (cm)	Average Number of Leaves(n)	Average Number of Nodes(n)
Bilecik İrikarası	54,7 a	3,71 a	17,93 a	4,71 ab	6,29 ab	5,55 ab
Razakı	18,7 b	3,50 a	17,60 ab	5,72 a	8,71 a	6,71 a
Kartal Çavuş	26,7 ab	1,55 b	12,32 bc	3,01 b	2,48 c	2,48 c
Sarı Üzüm	20,0 ab	3,53 a	9,14 c	4,93 ab	4,40 bc	4,67 b

In the study, when the lengths of the shoots formed from the planted shoot tips were evaluated statistically, the differences were found to be significant within the 95% confidence interval. If the varieties were compared in terms of shoot lengths, the highest shoot length value was obtained from the Razakı variety with 5.72 cm. Razakı variety was followed by Sarı Üzüm with 4.93 cm and Bilecik İrikarası with 4.71 cm, and the length value of the Kartal Çavuş variety was 3.01cm (Table 2).

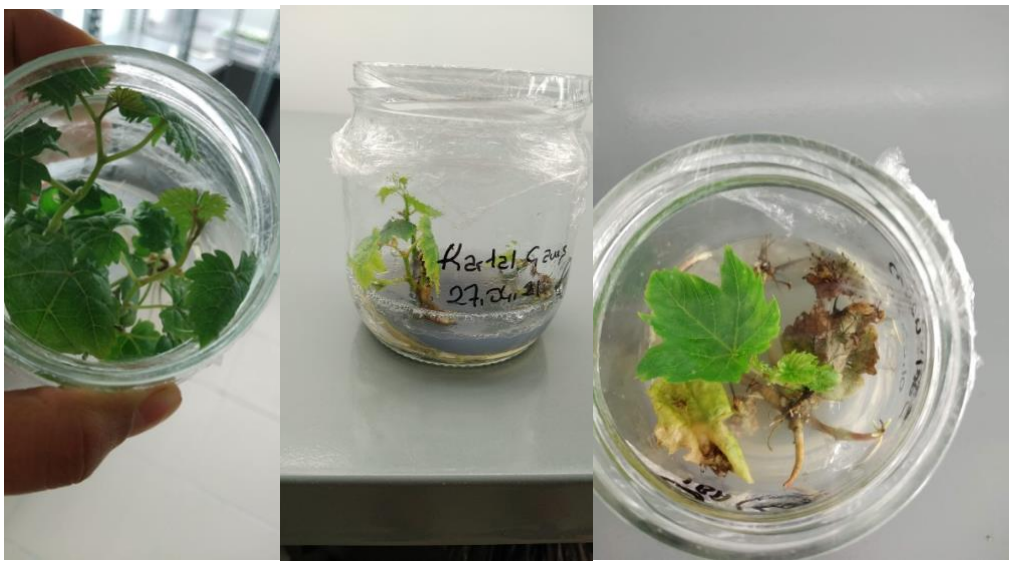

Figure 4. In vitro shoot development

When the root numbers are examined; It is seen that the highest number of roots was obtained from Bilecik İrikarası variety with 3.71 per plant. Sarı Üzüm ranked second with 3.53, and Razakı ranked third with 3.50 (Table 2). Differences in the number of roots between varieties were found to be statistically significant at a 95% confidence interval.

Figure 5. Root formation

Considering the length values of the roots formed from the planted shoot tips; The highest average root length value was obtained from Bilecik İrikarası variety with 17.93 cm (Table 2). As a result of the statistical analysis,

the differences between varieties in terms of root length were found to be statistically significant at a 95% confidence interval.

In terms of the number of leaves on the shoots, the highest leaf number value was obtained from the Razakı variety with 8.71, while the lowest leaf number value was obtained from the Kartal Çavuş variety with 2.48 (Table 2). If the leaf number data were subjected to statistical evaluation, the differences between the varieties were found to be significant within the 95% confidence interval.

Considering the varieties were compared in terms of number of nodes, the highest number of nodes was obtained from the Razakı variety with 6.71, and the lowest value was obtained from the Kartal Çavuş variety with 2.48 (Table 2). When the number of nodes values were evaluated statistically, the differences between the varieties were found to be significant within the 95% confidence interval.

As a result of the study, it was determined that there were differences between the varieties in terms of root and shoot formation. The Bilecik İrikarası variety had higher values compared to other varieties in terms of rooting rate, number of roots and root length. The Razaki variety came to the fore in terms of shoot length, number of leaves and nodes. The differences between the varieties considering rooting, number of roots, root length, shoot length, number of leaves and number of nodes are similar to the results obtained from previous studies. Previous studies have found that there are differences between varieties within a species as well as between species (Karoğlan et al., 1990; Péros et al., 1998; Adıyaman, 1998; Baydar, 2000; Ekbiç and Yılmaz, 2018). It has been determined that different BAP concentrations produce different results in shoot development and callus formation (Gomes et al, 2004; Skadia et al., 2010; Ayman et al., 2011; Kumsa, 2016; Kavitha and Surakhshitha, 2023).

According to the results of the study, the rooting rate of the Bilecik İrikarası variety is higher than the others. Although different IBA doses were not tested in the study, it is thought that more positive results can be obtained if different doses are used (especially at doses higher than $2mgL^{-1}$). Supporting this, different IBA doses were used in some previous studies. In the Balıkçı Siyahı grape genotype, IBA was used at concentrations of 0-4 mg/L for rooting and the best results were obtained in the medium containing 4 mgL^{-1} IBA (Ekbiç and Yılmaz, 2018), while the most suitable IBA concentration for rooting in Sultani Çekirdeksiz and Cheema Sahabi grape varieties is 1 mgL^{-1}(Aazami, 2010). In another study, the most appropriate IBA dose for different grape varieties was found to be 0.1 mgL^{-1} (Mozafari et al., 2016).

As a result of the study, it was determined that Bilecik İrikarası, Razakı, Sarı Üzüm and Kartal Çavuş varieties could be propagated by the shoot tip culture method. According to Ekbiç et al. (2015), although the highest rooting rate for the Isabella grape variety was obtained from MS medium supplemented with 2 mgL^{-1} IBA, 2 mgL^{-1} IBA was not found sufficient for the varieties used in this study. Another studies conducted previously, the best result was given at a BAP concentration of 1 mgl^{-1}, similar to this study (Beza, 2010; Jaskani vd., 2008).

CONCLUSION

With this study, the in vitro propagation possibilities of four local grape varieties, which are of economic importance for the Bilecik region in Türkiye was determined and the basis was laid for future studies such as determining the resistance of these varieties to biotic and abiotic stress conditions and obtaining virus-free plants.

Considering the data obtained from the study, it is thought that different IBA concentrations should be used. Similarly, it is recommended to use different BAP concentrations in these varieties in future studies.

Because of the different BAP and IBA concentrations produce different results in shoot development and callus formation, it is thought that studies should be carried out to determine the appropriate IBA and BAP doses for each variety, and different plant growth regulators should also be investigated to promote rooting.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

Thre are not any conflict of interest

Author contribution

This article was prepared from a part of Seda Özdemir Memiş's master's thesis. Hayri Sağlam is the thesis advisor.

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