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From the Editorial Board

As the number of scientific publications rises, readers are increasingly seeking concise information, precise results, and definitive answers within these materials. Many authors gather a substantial amount of data for their experimental works in an attempt to understand the mechanisms and etiology of stress. However, this needs laborious writing, careful arrangement, and lengthy papers. Of course, this would discourage readers from perusing the entire article and hinder their ability to quickly grasp the concept. Almost in all areas of scientific studies, we use sophisticated software, statistical tools, and graphical illustrations with excellent quality. As we noted from the recent publications, internationally reputed scientific authors have started using new tools to add value to their manuscripts. Unlike the manuscripts in the previous decade, the authors have started to use new tools to analyze their data to reach a definite verdict. Researchers are now evaluating the same old manuscripts using a new concept.

Correlation-based network analysis (CNA) and pathway-based metabolic analysis have reached out to a broad audience, and they have been employed in many recent scientific articles and books. With a single illustration, we could say many things about what we are going to say. We could easily evaluate, understand, interpret, and develop mechanisms and establish a network between metabolites. So, rather than comparing two parameters in terms of significance, we could see the whole picture and understand the mechanism in normal and stress conditions. By means of this approach, we could even notice the relations that could have a high impact under stress conditions before the occurrence of stress. When we want to breed or develop a new variety, most of the time we choose or select a gene or gene groups either on the basis of references from the published works or on the basis of the “try and error” principle. This approach, which has been used, is costly, time-consuming, and needs meticulous work. A modification, upregulation, or downregulation of a gene would be assessed at a later stage after the modification has been completed. By using biochemicals on living things and watching what happens afterward, we could make them more tolerant or resistant without changing their genomic structures. All scientific disciplines, such as botany, zoology, medicine, and agriculture, could debate this approach. However, if we know what we are going to assess and if we are able to predict the potential
changes in advance of the application, we would be very pleased, and we are going to follow the right pathway in a living cell. As long as you have data, you can follow up on any minor changes in a cell using metabolic pathway and correlation network analyses. Data do not have to belong to one family of compounds; they may vary and be quite distant from each other in terms of their nature. For example, you can compare plant height, weight, biochemical compounds, and even gene regulations in a single pathway employing network analysis. With the use of artificial intelligence and machine learning, we could even analyze the cells with millions of possibilities without an invasive approach.

Correlation-based network analysis (CNA) uses complex dendrograms to show multiple correlations between components, with similarities and differences between them being mathematically defined. "Heads" or "nodes" represent metabolites or parameters, while lines called "edges" represent relationships among them. Edges could be directed or undirected, which means connections between two nodes may be bilateral, directed, or not. The thickness of the edges determines the level of significance of the interactions. The color of the edges represents positive or negative correlations. The analysis of parameters determines the node size, which in turn represents the value of the parameters. With the use of CNA and pathway-based metabolic analyses, we do not have to go into data reduction. Every single data point, regardless of its magnitude, holds a significant value in the network map. We could describe this with a simple example. For example, cities in a country connected to each other via roads represent a map. However, ignoring side roads or village roads prevents us from understanding the city transition. Some important products may be transferred via shortcut roads to save energy between cities. The cell mechanisms follow the same example. This approach allows for a detailed evaluation of proteomics and metabolomics studies. We believe publications employing these methods might have a higher chance and value in highly regarded scientific journals.

Some free package programs that could be used with these programs are Pajek, Gephi, Cytoscape, social network analysis (SNA), and network visualization software (NVS). As long as the codes that define metabolites are written correctly in an algorithmic way, these programs could be used with no hassle.

As old in age but new in concept, we are happy to accept good quality articles written within the above frame. Since we are a strong candidate for the Scopus and WOS databases, we could only achieve this with authors submitting their high-quality works and readers appreciating and citing the high-quality papers. We recently renewed our Editorial Board with dynamic and highly regarded scientists worldwide to achieve our goals. Our members of the team are from different universities around the globe. We are now welcoming new members to join us to expand our team capacity. Meanwhile, we would like to express our sincere gratitude to the former Editorial Board members who worked hard to bring the journal to this stage.

With our kind regards,

Prof. Dr. Murat Dikilitaş,
On Behalf of the Editorial Board

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Research Article/Araştırma Makalesi

The determination of antifeedant effect of Neem Azal T/S on almond leaf bee *Cimbex quadrimaculata* (Müller, 1766) (Hymenoptera: Cimbicidae) larvae

Neem Azal T/S'nin badem yaprak arısı, Cimbex quadrimaculata (Müller, 1766) (Hymenoptera: Cimbicidae) larvaları üzerinde beslenme engelleyici etkisinin belirlenmesi

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ABSTRACT

In this study, the antifeeding index (AFI) value of Neem Azal T/S plant extract against the larvae of the almond leaf bee Cimbex quadrimaculata Müller, 1766 (Hymenoptera: Cimbicidae) was determined under laboratory conditions. The leaf dipping method was employed, and the measurement concentrations were set at 0% (control), 0.8%, 1.0%, 1.2%, 2%, 4%, 6%, 8%, and 10%. The extract was applied to the 3rd and 4th larval period of the pest. The antifeeding indexes of different doses of the extract were calculated using both choice and non-choice methods, and the differences between the data were assessed using the Kruskal-Wallis test. The differences between the choice and non-choice methods were also statistically determined using the Mann-Whitney U test. As a result of the study, the highest AFI value in both larval stages was recorded for the 10% concentration in both choice and no-choice methods. The lowest AFI value was observed at the lowest concentration of 0.8% in both methods during the 4th larval stage of the pest. It was found that as the concentration of extract increased, the C. quadrimaculata larvae consumed fewer leaves. In conclusion, it was determined that the highest AFI values occurred at the of 8% and 10%, indicating that the effectiveness of Neem Azal T/S increased with increasing dosage.

Key Words: Neem Azal T/S, *Cimbex quadrimaculata*, larvae, Maximum antifeeding index (AFI), Efficacy

ÖZ

Bu çalışmada, badem yaprak arısı *Cimbex quadrimaculata* (Hymenoptera: Cimbicidae) larvalarına karşı Neem Azal T/S bitki ekstraktının laboratuvar koşullarında maksimum beslenme engelleyici indeks (AFI) değeri belirlenmiştir. Çalışmada, yaprak daldırma yöntemi uygulanmış ve ölçüm konsantrasyonları %0 (Kontrol), %0.8, %1.0, %1.2, %2, %4, %6, %8 ve %10 olarak belirlenmiştir. Ekstrakt, zararlının 3. ve 4. dönem larvalarına karşı uygulanmıştır. Ekstraktın farklı dozlarının anti beslenme indeksleri seçenekli ve seçeneksiz metoda göre hesaplanmış, veriler arasında farkın olup olmadığı Kruskal Wallis testi ile belirlenmiştir. Seçenekli ve seçeneksiz metotlar arasındaki farklar da istatistiki olarak Mann Whitney U testi ile belirlenmiştir. Çalışma sonucunda, her iki larva döneminde de seçenekli ve seçeneksiz metotlar içerisinde en yüksek AFI değeri % 10 konsantrasyon için kaydedilmiştir. En düşük AFI değeri ise iki metotta da her iki larva döneminde en düşük konsantrasyon olan %0.8'lik dozda ve ve zararlının 4. larva döneminde belirlenmiştir.

Ekstraktın konsantrasyonu arttıkça, *C. quadrimaculata* larvalarının daha az yaprak tükettiği saptanmıştır. Sonuç olarak, en yüksek AFI değerlerinin %8 ile %10'luk dozlarda gerçekleştiği, Neem Azal T/S'ın doz artışıyla birlikte etkililiğinin arttığı belirlenmiştir.

Anahtar Kelimeler: Neem Azal T/S, *Cimbex quadrimaculata*, Larva, Maksimum beslenme engelleyici indeks (AFI), Etkinlik

Introduction

Almond leaf bee *Cimbex quadrimaculata* Müller, 1766 (Hymenoptera: Cimbicidae) is a significant pest both in Türkiye and worldwide. This pest causes considerable damage in almond orchards. In a study conducted in the provinces of Diyarbakır, Elâzığ, and Mardin, the almond leaf bee *C. quadrimaculata* was identified as the dominant species at a rate of 51% (Bolu et al., 2005). This pest damages not only almonds but also cherries, apricots, peaches, and pears in the region. There is no effective application for controlling this pest. Therefore, the absence of a licensed pesticide against this pest has led to random and unconscious chemical control practices among farmers. In addition to determining the biology and population dynamics of this pest, studies have been conducted on the important factors affecting its populations, the identification of significant natural enemies, and alternative chemical control methods using some pyrolysis wood vinegar products (Özgen et al., 2021 a, b, c; Özgen et al., 2022 a, b; Koç et al., 2024).

In this study, the antifeeding effect of Neem Azal T/S, which has insecticidal, repellent, and antifeeding effects against pests, was investigated against the larvae of *C. quadrimaculata.* Neem extracts are known to have insecticidal effects on over 550 pests worldwide, and the number of pests affected is increasing day by day (Saxena and Basit, 1982; Isman, 1999; Durmuşoğlu et al., 2003; Whalon et al., 2008; Özgen and Karsavuran, 2011; Cura and Gencer, 2019).

Neem Azal T/S contains 1% active ingredient azadirachtin. Azadirachtin is a triterpenoid found in the neem tree, scientifically known as *Azadirachta indica* A. (Juss) (Meliaceae). The importance of this active ingredient is increasing due to its low toxicity to mammals and minimal environmental harm. The established efficiency of this active ingredient against various pests will contribute to organic and sustainable agricultural approaches in combating this pest, which is increasingly damaging almonds, a significant hard-shelled fruit in the Eastern and Southeastern Anatolia regions. As there is no licensed pesticide recommended in technical instructions for chemical control of this pest, the determination of the effectiveness of this plant-based product, used in organic farming, against this pest for the first time will contribute to integrated pest management of *C. quadrimaculata* in almonds.

Material and Method

The study was conducted in the laboratories of the Department of Bioengineering at Fırat University. The larvae, all from the same habitat, were collected in their first larval stage from Sütlüce village, Central district of Elazığ province. They were reared in the laboratory until they reached the third and fourth larval stages, which were the targeted biological stages for the study, and the experiments were initiated at the appropriate biological stage. While separating the third and fourth larval stages of the pest, the publication by Bolu (2016) was taken into consideration. Neem Azal T/S (1%) preparation containing active ingredient azadirachtin was used as insecticide for the pest larvae. The leaf dipping method was used in the experiments (Park et al., 2002). The measurement concentrations were set at 0% (Control), 0.8%, 1.0%, 1.2%, 2%, 4%, 6%, 8%, and 10%. "The concentrations have been mixed with pure water. Concentrations should be given as ppm or mg l^{-1}.

In the study, leaf disks with a diameter of 3 cm were immersed in extract dilutions for 30 seconds and dried in a fume hood for 1-2 hours. They were then placed on a damp filter paper inside a plastic container (7 cm in diameter and 3 cm high)

to prevent the leaves from drying out. Ten disks were used in each trial, and three replicates of leaf disks were used for each treatment. For each dose application, one individual was placed in each container, and leaf consumption was recorded with 10 replications. A total of 340 larvae (chıice: 160/ non choice: 180) of the same biological stage (3rd or 4th instar larvae of the same age) were used during each trial. Experiments were conducted on a total of 340 larvae. The larvae were starved for 4 hours before being placed on the extract-treated leaf disks using both choice and no-choice options. The feeding areas of every ten larvae were summed according to the dose, and the average consumption area for each dose was calculated using the arithmetic mean.

For the choice method of each dose application, 10 3rd or 4th stage larvae were placed on the extract-treated or untreated (control) leaf disks in a plastic container. For the non-choice method, extract-treated leaf disks and controls were placed in separate plastic containers (7 cm in diameter and 3 cm high). After 24 hours, the larvae were removed, and their feces were brushed off the leaf disks. The remaining surfaces of the leaf disks were photographed, and the areas consumed and not consumed were measured and recorded using the IMAGEJ computer program (version 1.410, available at http://rsb.info.nih.gov/ij).For the choice method, the Antifeeding Index (AFI) was calculated using the formula (AFI), (C-T)/(C+T)*%100 , while for the non-choice method, the formula (C-T)/C*%100 was used (Arivoli and Tennyson, 2013) In these formulas, C and T (cm) represent the consumed leaf area of the control and extract-treated disks, respectively. Additionally, a scale was created to number the damage rates on the leaves. The Kruskal-Wallis test was used to determine if there were differences between the data. The differences between the choice and non-choice methods were statistically analyzed using the Mann-Whitney U test (Nachar, 2008).

Figure 1. Effects of the prepared solutions and the Neem Azal T/S on the larvae in the application

Results and Discussion

The results of the antifeeding effects of Neem Azal T/S formulations in both choice and no-choice applications against *Cimbex quadrimaculata* are presented in Table 1 and Table 2.

Table 1. AFI determined after 24 hours in *Cimbex quadrimaculata* larvae using the choice method with Neem Azal T/S formulation.

Concentration	Consumed area by 3rd larval stage (T) (cm)	Consumed area (C) (cm)	AFI for 3rd instar larvae	Consumed area by 4th larval stage (T) (cm)	Consumed area (C) (cm)	AFI for 4th instar larvae
0.8%	0.22	1.49	74.26	0.29	1.59	69.14
1%	0.20	1.5	76.47	0.25	1.61	77.27
1.2%	0.16	1.46	80,24	0,24	1.6	76.7
2%	0.16	1.44	80	0.17	1.58	80.57
4%	0.16	1.48	80.48	0.16	1.55	81.28
6%	0.10	1.49	87.42	0.10	1.57	88.02
8%	0.08	1.51	89.93	0.09	1.62	89.47
10%	0.04	1.5	97.07	0.08	1.54	90.12

Table 2. AFI determined after 24 hours in *Cimbex quadrimaculata* larvae using the non-choice method with Neem Azal T/S formulation.

Concentration	Consumed area by 3rd larval stage (T) (cm)	AFI for 3rd instar larvae	Consumed area by 4th larval stage (T) (cm)	AFI for 4rd instar larvae
0.8%	0.28	83.62	0.26	85.22
1%	0.27	84.21	0.24	86.36
1.2%	0.20	88.3	0,21	88.06
2%	0.21	87.71	0.18	89.77
4%	0.19	88.88	0.17	90.34
6%	0.15	91.22	0.15	91.47
8%	0.10	94.15	0.11	93.75
10%	0.07	95.9	0.09	94.88
Control	1.71		1.76	

A normality test was conducted to determine whether there were differences between the doses in terms of larval stages within each method, as well as to identify the differences among larvae between the choice and no-choice methods. Since the obtained values did not conform to a normal distribution, non-parametric tests, specifically the Kruskal-Wallis and Mann-Whitney U tests, were performed.

Table 3. Results of the Kruskal-Wallis Test for different doses applied with choice and no-choice methods in terms of larval stages.

	Dose	Median	H	*p*	Pairwise comparisons
3rd instar choice	0.8	73.90	71.773	<0.001	%0.8 < %6, %8, %10 %1 < %6, %8, %10 %1.2 < %10 %2 < %10 %4 < %10
	1	75.88			
	1.2	80.02			
	2	80.31			
	4	80.14			
	6	88.67			
	8	89.89			
	10	98.09			
4rd instar choice	0.8	69.10	71.335	<0.001	%0.8 < %2, %4, %6, %0.8 < %8, %10, %1 < %6, %8, %10, %1.2 < %6 %8, %10
	1	77.27			
	1.2	76.20			
	2	80.57			
	4	81.31			
	6	88.02			
	8	89.60			
	10	89.85			
3rd instar non-choice	0.8	83.65	63.360	<0.001	%0.8 <%6, %8, %10 %1 < %6, %8, %10 %1.2 < %10 %2 < %10 %4 < %10
	1	84.21			
	1.2	87.87			
	2	87.69			
	4	88.88			
	6	91.56			
	8	94.15			
	10	95.80			
4rd instar non-choice	0.8	85.05	67.362	<0.001	%0.8 < %6, %8, %10 %1 < %6, %8, %10 %1.2 < %8, %10 %2 < %10
	1	86.36			
	1.2	88.03			
	2	89.68			
	4	90.26			
	6	91.47			
	8	93.99			
	10	94.94			

Upon examining Table 3, a statistically significant difference was found between the application dose groups for the AFI values of the 3rd and 4th instar larvae in both choice and non-choice methods ($p < 0.05$). The highest AFI values were observed at the 8% and 10% doses, indicating that the neem extract was quite effective. The results of the Kruskal-Wallis Test multiple comparisons for different doses applied with choice and no-choice methods in terms of larval stages are also presented in Table 3 and Figures 2-5.

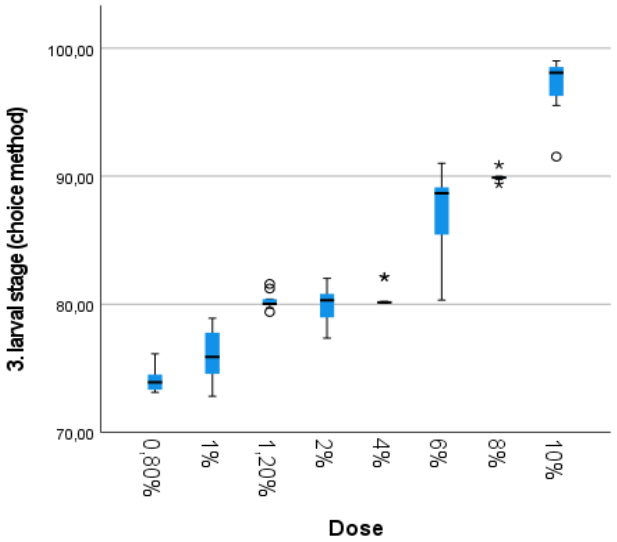

Figure 2. 3rd instar choice larvae AFI values

Upon examining Table 3 and Figure 2, it can be seen that the 6%, 8%, and 10% concentrations are significantly more effective than the 0.8% and 1% concentrations for the AFI values of the 3rd instar choice larvae ($p < 0.05$). Additionally, the 10% concentration is significantly more effective than the 1.2%, 2%, and 4% concentrations ($p < 0.05$).

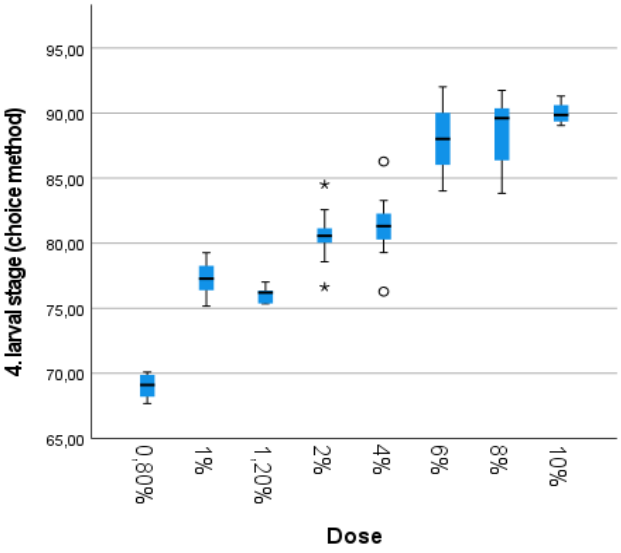

Figure 3. 4th instar choice larvae AFI values

Upon examining Table 3 and Figure 3, it can be seen that the 2%, 4%, 6%, 8%, and 10% concentrations are significantly more effective than the 0.8% concentration for the AFI values of the 4th instar choice larvae ($p < 0.05$). Furthermore, the 6%, 8%, and 10% concentrations are significantly more effective than the 1% and 1.2% concentrations ($p < 0.05$).

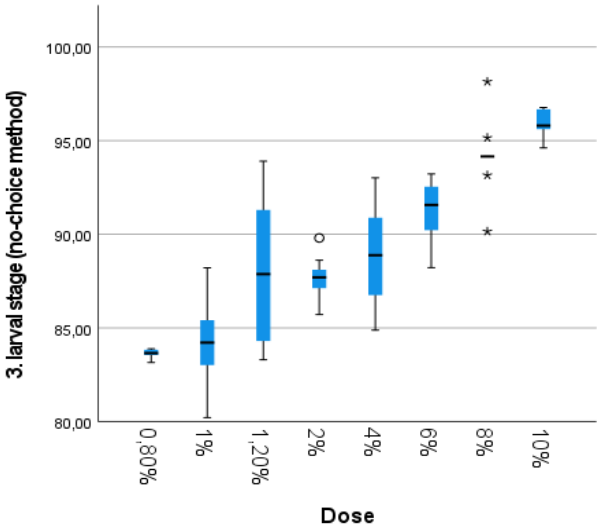

Figure 4. 3rd instar no-choice larvae AFI values

Upon examining Table 3 and Figure 4, it can be seen that the 6%, 8%, and 10% concentrations are significantly more effective than the 0.8% and 1% concentrations for the AFI values of the 3rd instar no-choice larvae ($p < 0.05$). Additionally, the 10% concentration is significantly more effective than the 1.2%, 2%, and 4% concentrations ($p < 0.05$).

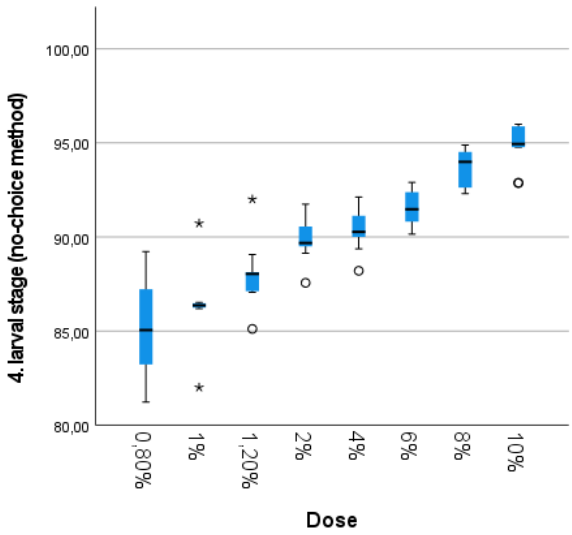

Figure 5. 4th instar no-choice larvae AFI values

Upon examining Table 3 and Figure 5, it can be seen that the 6%, 8%, and 10% concentrations are significantly more effective than the 0.8% and 1% concentrations for the AFI values of the 4th instar no-choice larvae ($p < 0.05$). The 8% and 10% concentrations are significantly more effective than the 1.2% concentration ($p < 0.05$). Additionally, the 10% concentration is significantly more effective than the 2% concentration ($p < 0.05$).

Upon examining Table 3 and Figure 5, it can be

Table 4. Results of the Mann-Whitney U Test determining the differences in larval stages between choice and no-choice methods

	Dose	Median	U	*p*
Choice	3rd term	80.27	2961.00	0.415
	4th term	81.11		
Non-choice	3rd term	88.74	3554.50	0.226
	4th term	90.15		

Upon examining Table 4, there is no significant difference between the choice 3rd instar and choice 4th instar ($p > 0.05$). Similarly, there is no significant difference between the no-choice 3rd instar and no-choice 4th instar ($p > 0.05$).

Table 5. Results of the Mann-Whitney U Test determining the differences in larval stages (3rd and 4th Instars) between choice and no-choice methods

	Dose	Median	U	*p*
3rd term	Choice	80.27	4855.50	<0.001
	Non-choice	88.74		
3rd term	Choice	81.11	5440.50	<0.001
	Non-choice	90.15		

Upon examining Table 5, there is a significant difference between the choice 3rd instar and no-choice 3rd instar ($p < 0.05$). It appears that the no-choice 3rd instar is better than the choice 3rd instar. Additionally, there is a significant difference between the choice 4th instar and no-choice 4th instar ($p < 0.05$), with the no-choice 4th instar being better than the choice 4th instar.

In this study, within the optional method, the maximum antifeedant index (AFI) value was recorded at a concentration of 10%. Additionally, in the experiments conducted with the optional method, the antifeedant index for 3rd larvae was determined to be at its lowest at a concentration of 0.8%. In clear terms, as the concentration of Neem Azal T/S extract increased, *C. quadrimaculata* larvae consumed less leaf material. This situation was also observed in 4th larvae (Table 1). In the study, although there was an increase in the area of almond leaves consumed by 3rd and 4th larvae, this increase is believed to be directly proportional to larval size.

In the no-choice method study, the area of leaves consumed on leaves that were not treated by 3rd instar larvae was measured at concentrations of 0.8, 1, 1.2, 2, 4, 6, 8, and 10% as follows: 0.28, 0.27, 0.20, 0.21, 0.19, 0.15, 0.10, and 0.07 cm (Table 2). The highest AFI values occurred at concentrations of 6%, 8%, and 10%. The lowest AFI value was observed at concentrations of 0.8% and 1%, where the concentration of the insecticide was low.

Despite a tenfold increase in the dose of Neem Azal T/S, an effectiveness difference of 9.36% was found between the lowest dose of 0.8% and the 10% dose for the AFI value (94.88-85.22=9.36%). It was determined that the antifeedant and repellent effects against the pest were quite high at all doses. Similarly, in no-choice method studies, it was determined that the area of leaves consumed increased with the dose in the 4th larval stage (Table 2).

Overall, it was determined that the highest AFI values occurred at the highest doses of 8% and 10%, and that the effectiveness of the neem extract generally increased with the dose against the pest. In all applications, maximum leaf consumption was observed on untreated leaves. Although the amounts of leaf consumption between doses were similar, the lowest leaf consumption was observed with a dose of 10% in the optional method and again with a dose of 10% in the non-choice method during the 4th larval stage. These values were higher in both

methods due to the greater control leaf consumption by the 4th instar larvae, while the control consumption in the 3rd instar larvae was somewhat lower.

The antifeedant effects of neem extracts have been tested on many pests other than the one studied here and positive results have been found. Particularly, repellent and antifeedant effects have been identified on Lepidoptera pests, certain species belonging to the Heteroptera order (*Nezara viridula*), and species from the Cicadellidae family (Isman, 1999; Saxena and Basit, 1982; Durmuşoğlu et al., 2003). The results of this study in the literature indicate that it provides antifeedant and repellent effects in parallel with increased doses applied to the plant (Sharma and Gupta, 2009). Some other studies on larvae should be given with doses. In the study conducted to determine the effectiveness of Neem Azal T/S, increases in the amount of AFI effect of the pest was observed with increasing doses.

As a result, these data indicate that the extract of *A. indica* prevents the consumption of almond leaves and has a significant antifeedant effect against *C. quadrimaculata.* This stimulatory effect is referred to in entomology as "Stimulodeterrent diversionary.", and it was determined in this study that it acts against *C. quadrimaculata* with "push-pull" activity (Charleston et al., 2005).

As suggestions;

Therefore, in orchard studies, concentrations of 6%, 8%, and 10% will be applied to trees against both 3rd and 5th instar *C. quadrimaculata* larvae to determine the antifeedant and repellent effects of neem extract against the larvae.

These results are also supported by the statistical results that will be specified below, showing differences due to the increase in doses among the applications. In these applications, the effects of neem extract on the natural enemy fauna (predators and parasitoids) will also be observed, and differences will be compared with the control plots. Additionally, individuals that transition to the pupal stage from trees treated with different doses will be collected from the field and brought to the laboratory to determine the effects of the applied doses on parasitism rates.

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Conflict of interest

Author declares that no financial or competing interest.

Author's Contributions

The authors declare that they have contributed equally to the article.

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Research Article/Araştırma Makalesi

Effects of the foliar cedar tar treatment on the control of the Ascochyta blight caused by Ascochyta rabiei

Sedir Katranının Ascochyta rabie'nin neden olduğu nohut yanıklıklığının kontrolü üzerine etkisi

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ABSTRACT

Ascochyta blight (*Ascochyta rabiei*) is a significant fungal disease that affects chickpea crops all around the world. Synthetic fungicides, which have harmful environmental effects, are commonly used to control the disease. Alternatively, various plant extracts have been explored for disease management. In this study, the antifungal activity of cedar tar, its potential to control the disease, and its disease prevention mechanism in chickpeas were evaluated. The antifungal activity results indicated that whereas 10%, 25%, and 50% cedar tar application doses had an inhibitory effect greater than 50% but were harmful to the plant, 1% and 5% cedar tar application doses suppressed mycelial growth by less than 50%. Therefore, doses of 0.5 $\%^{-1}$, 1 %, and 2 %were selected to determine the disease prevention potential of cedar tar at different application times. Cedar tar treatment applied 72 hours before *Ascochyta rabiei* inoculation effectively prevented disease development in chickpea plants. This treatment also decreased MDA content, indicating the membrane was protected from pathogen attack. These results suggest that cedar tar can be considered an effective bio-fungicide formulation for future integrated pest management programs.

Keywords: Ascochyta blight, Ascochyta rabiei, Cedrus libani, Cicer arietinum

ÖZ

Ascochyta yanıklığı (*Ascochyta rabiei*), dünya çapında nohutu etkileyen önemli bir fungal hastalıktır. Zararlı çevresel etkileri olan sentetik fungisitler hastalığın kontrolünde yaygın olarak kullanılmaktadır. Alternatif olarak hastalık yönetimi için çeşitli bitki ekstraktları araştırılmaktadır. Bu çalışmada sedir katranının antifungal aktivitesi, hastalığı kontrol etme potansiyeli ve nohutta hastalık önleme mekanizması değerlendirilmiştir. Antifungal aktivite sonuçları, sedir katranının %1 ve %5'lik uygulama dozlarının misel gelişimini %50'den daha az engellediğini, %10, %25 ve %50'lik dozların ise %50'den daha büyük bir inhibisyon etkisine sahip olduğunu ancak bitki için toksik olduğu görülmüştür. Bu nedenle farklı uygulama zamanlarında sedir katranının hastalıkları önleme potansiyelini belirlemek için %0,5, %1 ve %2'lik dozlar uygulanmıştır. Ascochyta rabiei aşılamasından 72 saat önce uygulanan sedir katranı uygulaması nohut bitkilerinde hastalık gelişimini etkili bir şekilde önlemiştir. Bu uygulma aynı zamanda MDA içeriğini de azalttığı; bu da zarın patojen saldırısından korunduğunu göstermiştir. Sonuçlar, sedir katranının gelecekteki entegre mücadele yönetimi programları için etkili bir biyo-fungusit olarak değerlendirilebileceğini göstermektedir.

Anahtar Kelimeler: Nohut yanıklığı, *Ascochyta rabiei*, *Cedrus libani*, *Cicer arietinum*

Introduction

The self-pollinating legume chickpea (*Cicer arietinum* L.) has been farmed for generations in the Mediterranean, Central America, South America, the Far East, and the Near East.

Approximately 10% of cropland worldwide is used for legumes. Chickpeas (*Cicer arietinum* L.) are among the most commonly farmed legumes, behind common beans (*Phaseolus vulgaris* L.) and peas (*Pisum sativum* L.) (FAO 2023; Iqbal et al. 2006). It can be produced in a variety of climates, including subtropical, temperate, desert, and semiarid areas, in at least fifty countries. (Jukanti et al. 2012; Zhang et al. 2020). ChiEmail:ckpea occupies 17.8 million hectares of farmland, representing over 15% of global legume production, with an annual output of 17.2 million tonnes. India contributes approximately 72% of global chickpea production, with other major producers including Turkey, the USA, Canada, Australia, and Mexico. In 2022, global chickpea imports reached approximately 1.89 million tonnes, while exports amounted to approximately 2.05 million tonnes (FAO 2023).

Chickpea production is affected by numerous phytopathogenic factors, with more than 50 pathogens known to affect yield and quality at various levels. Ascochyta blight, which is brought on by *Ascochyta rabiei*, is one of the most serious biotic hazards to chickpeas. (Pass.) Labr. (Teleomorph *Didymella rabiei* (Kov.) v. Arx). Crop losses were recorded as early as the 1930s, and the illness was first mentioned in 1867. Over the following two decades, Ascochyta blight and its associated losses have been studied by researchers from various countries, including Morocco, Bulgaria, Greece, Pakistan, and Spain (Deokar et al. 2019; Nene 1982; Salotti et al. 2021).

To manage Ascochyta blight, numerous research groups are currently striving to create disease-resistant genotypes or select resistant ones using a variety of breeding techniques (Gayacharan et al. 2020). However, the genetic basis of resistance to Ascochyta rabiei is extremely complex (Sharma et al. 2016), and the chickpea gene pool is known to be quite limited (Toker et al. 2021; Tekin et al. 2018). Furthermore, breeding efforts have been hampered by the pathogen's great genetic variety (Kumar et al. 2020; Gayacharan et al. 2020). It has been noted that despite the development of cultivars with a certain degree of resistance to this pathogen, fungicides are still required to control Ascochyta blight (Kurt et al. 2008; Rani et al. 2020). However, fungicides may occasionally have more negative effects on the environment than positive effects.

The antimicrobial and antifungal properties of various plant extracts, such as monoterpenoids, sesquiterpenoids, and hydrocarbons, obtained from cedar *(Cedrus libani*), have been tested against various plant pathogens (Kızıl et al. 2002; Kurt et al. 2008 Ghanem and Olama 2014; Takci et al. 2021; Venditti et al. 2020). Among these extracts, cedar tar (CT) is known as a byproduct of the distillation of the dry, resinous wood of the Taurus cedar species at high temperatures (Kurt and Işık 2012; Kurt el al. 2008). It has a wide range of applications ranging from controlling harmful insects, pathogenic fungi, and bacteria to combating various parasites (Kurt et al. 2008; Takci et al. 2021). It has been reported that CT can exhibit protective effects against a wide range of pathogenic organisms (Kurt and Işık 2012; Kurt et al. 2008). Additionally, some components of CT's chemical composition have been found to have antifungal properties.

The objective of this study was to determine the potential of cedar tar (CT) treatment to control Ascochyta blight (AB) development in chickpea plants. For this purpose, the antifungal activity of CT was investigated using the agar plate method and a pot experimental design. Antioxidant enzyme analyses, proline content and, lipid peroxidation, were also analyzed to understand the disease prevention mechanism of CT. To the best of our knowledge, this is the first study to investigate the efficacy of CT against AB.

Materials and Methods

Preparation of Ascochyta rabiei inoculum and plant material

An isolate was provided as a single spore culture from the collection of *Ascochyta rabiei* pathotype IV, maintained by Assoc. Prof. Kadir AKAN (Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Plant Protection). In previous decades, pathotype IV has been categorized according to variations in the pathogen's virulence (Udupa et al. 1998; Imtiaz et al. 2011). Pathotype I is less aggressive, pathotype II is more aggressive, pathotype III is more aggressive and pathotype IV is extremely aggressive. Therefore, this study employed the pathotype IV group, which is recognized as the most aggressive pathotype of the disease. Chickpea Seed Meal Agar (CSMDA; 40 g of chickpea seed meal, 20 g of dextrose, and 18 g of agar in 1 L of sterilized distilled water) was used to cultivate this isolate for 7–10 days at 20–22°C. Spores were resuspended using a flamed wire loop after the plates were filled with sterile distilled water. According to Trapero-Casas and Kaiser (1992), the suspension's spore concentration was determined using a hemocytometer, adjusted to 5×10^5 pycnidiospores mL^{-1} using sterile distilled water, and treated with 0.15% Tween 20 (polyoxyethylene–sorbitan monolaurate) (MERCK®, Nottingham, UK).

Evaluation of antifungal activity of cedar tar against Ascochyta rabiei in agar plate

The antifungal activity of cedar tar was assessed using the poisoned food approach. To achieve the desired percentage concentrations of cedar tar, a specific amount of tar was dissolved in the PDA medium mix. The concentration of cedar tar diluted with DMSO at a ratio of 50% was considered as 100%, and subsequent dilutions were made accordingly. The preparation of Potato Dextrose Agar (PDA) prepared by autoclaving at 121°C and cooling to 40°C. CT was added to PDA media after being dissolved in ½ of dimethyl sulfoxide (DMSO). CT doses were changed to 1, 5, 10, 25, and 50% at the end. The mycelial discs were made from the tips of a 7-day-old AB culture using a sterile cork borer with a 5 mm diameter. Once the PDA medium had solidified, they were placed in the middle of each Petri plate. The plates were incubated for 14 days at 25±2°C. Control treatments were prepared without the use of CT extract. Each treatment was set up with two replicates and three replicates. AB's mycelial growth diameters were measured daily. The growth of control plates and mycelial growth in plates with varying doses were compared, and the inhibition rates of various CT doses were computed using the following formula (Deans and Soboda 1990):

$$(C-T)/C\,x100$$

Where C=Length of control hyphae (mm) and T=Length of treated hyphae (mm).

Evaluation of potential of cedar tar treatment against Ascochyta rabiei in-vitro

Different doses of CT (% 0.5, 1 and 2) with varying disease implementation times were applied to determine the potential of CT treatment against *Ascochyta rabiei in-vitro*. In the first treatment, CT and *Ascochyta rabiei* inoculation were applied to the test material simultaneously (T0-D0), while in the second treatment, CT was applied 72 h before the inoculation of *Ascochyta rabiei* (T1-D2). For the third treatment, CT was performed 72 h after the inoculation (T2-D1).

The chickpea cultivar Uzunlu, which is susceptible to *Ascochyta rabiei*, was used in the study. Three seeds were planted in separate pots that contained peat: perlite. (2:1 v/v). The pots were grown under greenhouse conditions with 14 h of light and 10 h of darkness at 25±5°C until the two-leaf stage. Plants were inoculated with a 5×10^5 pycnidiospores mL^{-1} suspension of spores using a pressure sprayer. A group of plants was treated with only sterile distilled water (negative control) or inoculated only with *Ascochyta rabiei* spores (positive control). A humidifier with a

100% constant humidity setting was then attached to the polythene tent, keeping the plants moist for 48 hours at 18–22°C (12 hours of continuous darkness and 12 hours of light). All plants were then covered with clear polythene. As shown in Table.1, a 0–9 rating scale was used to evaluate the illness response to Ascochyta rabiei 14 days after inoculation (Reddy and Singh 1987). A fully randomized design was used to conduct the study. The following formula was used to determine the percentage of disease incidence (PDI) based on the 0–9 rating scale (Sankara and Acharyya 2012).

Percentage of Disease Incidence (PDI) = Number of diseased plants / Total Number of plants observed X 100

Table 1. 1-9 scale to be used in reaction evaluation of *Ascochyta rabiei.*

Scale	Disease Intensity	Reactions
0	No minor lesions or symptoms,	Immune (I)
1	Lesions on the apical stems are few and tiny,	Highly Resistant (HR)
2	On certain branches, there are tiny girdling stem lesions deep down,	Resistant (R)
3	One or two branches are broken and lesions up to 5–6 mm in size	Resistant (R)
4	The majority of the plant clearly has lesions (2–5 mm in size), and several of its branches are damaged,	Moderately Resistant (MR)
5	Defoliation has begun, half of the branches are damaged, and there are numerous big lesions,	Moderately Susceptible (MS)
6	More defoliation, damaged stems, and dry branches are among the lesions similar to those in 5.	Susceptible (S)
7	As in 5 and 6, there are several huge lesions with clear defoliation, up to 70% of branches are damaged, and up to 25% of plants are destroyed,	Susceptible (S)
8	As in 7, symptoms include up to 50% of plants being killed.	Highly Susceptible (HS)
9	Symptoms as in number eight, with every plant dead,	Highly Susceptible (HS)

Determination of the malondialdehyde (MDA) content

Malondialdehyde (MDA) content was measured to assess lipid peroxidation (Ohkawa et al. 1979). Five milliliters of a 5% trichloroacetic acid (TCA) solution was used to homogenize 0.5 grams of leaf tissue. Following centrifugation of the homogenate, equal quantities of TCA and thiobarbituric acid solutions were added to the supernatant in the tubes. The tubes were then incubated for 25 minutes at 96°C. The tubes were placed in a cold bath to stop the reaction, and then they were centrifuged for five minutes at 6,000 rpm. A spectrophotometer (Shimadzu Corporation UV-VIS Spektrofotometre UV-1280) was used to measure the absorbance of the final mixture at 532 nm and 600 nm. The extinction coefficient was used to determine the MDA content.

Measuring the amount of free proline

A homogenate of 0.5 g of leaf tissue was mixed with 3% sulfosalicylic acid and centrifuged for 3 minutes at 3,000 rpm. Subsequently, the glacial acetic acid, acid ninhydrin, and supernatant were combined in equal amounts and incubated for one hour at 100°C. Four milliliters of cold toluene were added to the tubes to stop the reaction. After being evaporated, the toluene phase was measured at 520 nm wavelength using a spectrophotometry (Shimadzu Corporation UV-VIS Spektrofotometre UV-1280). A standard curve was used to calculate the proline levels (Bates et al. 1973).

Evaluation of antioxidant enzymes

Catalase (CAT) and superoxide dismutase (SOD) enzyme activity tests were performed using the same extraction technique. Five milliliters of extraction solution comprising 0.1 M potassium phosphate buffer (pH 6.8), 100 milligrams of polyvinylpyrrolidone (PVP), and 0.1 mM ethylenediaminetetraacetic acid (EDTA) were used to homogenize 0.5 grams of fresh leaves. After centrifuging the homogenate for five minutes at 16,000 ×g, the supernatant was

utilized to analyze SOD and CAT. SOD (superoxide dismutase; EC 1.15.1.1) activity was measured as described by Beyer and Fridovich (1987). A solution containing methionine, nitroblue tetrazolium, EDTA, riboflavin, and phosphate buffer (pH: 7.8) was mixed with 200 µL microliters of the extract. The reaction was conducted at 25°C in a chamber illuminated by a fluorescent lamp. The fluorescent lamp was turned on to begin the reaction, then it was turned off after five minutes. At 560 nm, blue formazan—which is created when NBT is photoreduced in the presence of light—was observed. The quantity of enzyme required to block 50% of the NBT was determined to be one SOD unit. Aebi (1983) defined CAT activity (catalase; EC 1.11.1.6) as the consumption of H_2O_2 at 240 nm for 30 s. Three milliliters of a solution containing 50 milliliters of potassium phosphate buffer (pH 7.0) and 20 milliliters of $H2O_2$ were mixed with fifty microliters of the enzymatic extract. At 240 nm, the absorbance drop was observed. The extinction coefficient was used to compute the enzyme activity, which was then represented as µmol H_2O_2 oxidized g^{-1} mg protein.

Statistical analysis

Using SAS software, the findings of the agar plate and pot studies were statistically evaluated using one-way ANOVA and post-hoc Tukey's testing with %95 confidence (JMP version 8, SAS Institute Inc.). Two-way factorial analysis of variance (ANOVA) was used to examine the results from the proline content, lipid peroxidation, and antioxidant enzyme tests, with or without treatment and dose interaction. Descriptive statistics, such as mean, standard deviations, and errors, were calculated using base packages of R version 4.1.2 with RStudio (RStudio Team 2021). The ANOVA of the interaction between all groups and controls was found to be statistically significant ($P < 0.05$). Therefore, Tukey-Kramer test and Only when treatment and dosage interactions were not present, Duncan's multiple range test was performed with %95 confidence to compare the groups. Using the Rcmdr package of the R environment, two-way ANOVA and post-hoc tests were conducted (Fox 2017; R Core Team 2021, RStudio Team 2021).

Results and Discussion

Assessment of cedar tar's antifungal efficacy against Ascochyta rabiei

Using the agar plate method, the effect of five distinct CT dosages on the mycelial development of the fungus was examined. It was determined that the application doses of 25% and 50% completely inhibited mycelial growth, whereas a dose of 10% was found to inhibit mycelial growth by 50%. The doses of 1% and 5% inhibited mycelial growth by less than 50% (Fig.1). The effects of CT applications on mycelial growth inhibition were statistically significant, except for the difference between the doses of 25 % and 50 %.

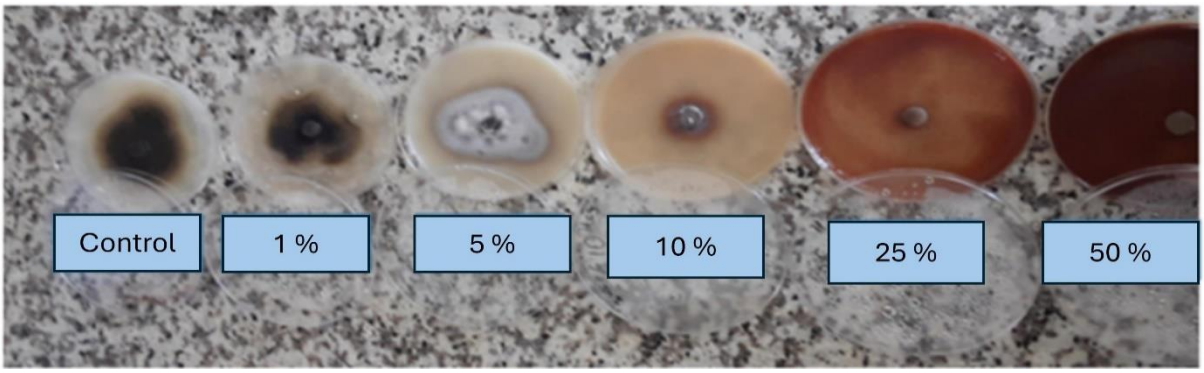

Figure 1. Images of the petri dishes showing the antifungal activity of CT against *Ascochyta rabiei.*

Evaluation of potential of cedar tar treatment against Ascochyta rabiei in-vitro

The Chickpea plants were toxically affected by application levels of 5, 10, 25, and 50 %, even though these doses inhibited mycelial growth by below and above 50%, respectively. As a result, studies were carried out *in-vitro* using application dosages between 0.5 % and 2 %. The assessment of AB severity on chickpea plants was assessed within 14 days of inoculation. Statistical analysis was performed using percentage disease incidence (PDI) results. In the 1st and 2nd experiments, it was observed that AB was notably prevented by CT, with no significant differences between the different doses of CT treatment. In the 3rd experiment, it was determined that none of the CT treatments prevented AB development (Fig. 2).

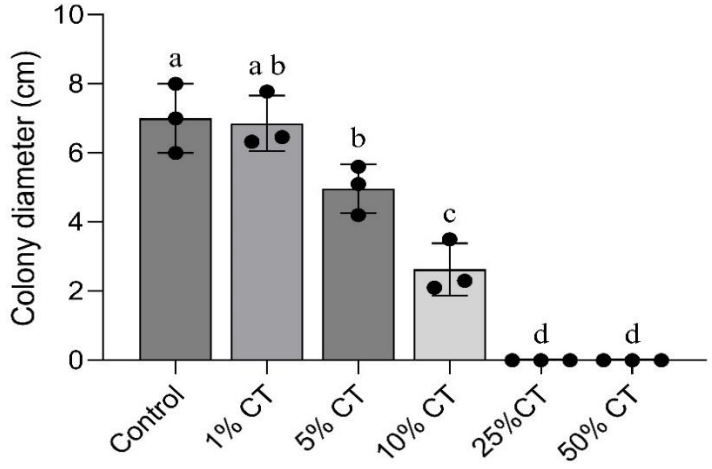

Figure 2. Cedar tar's antifungal effects against *Ascochyta rabiei in vitro*.

The lowercase letters refer to statistical significance among different CT treatments at *p≤0.05*.

Proline and MDA content

Ascochyta rabiei inoculation and CT treatment did not significantly change the proline content, except in the T2-D1 2 % treatment (Fig. 3) in contrast to the control.

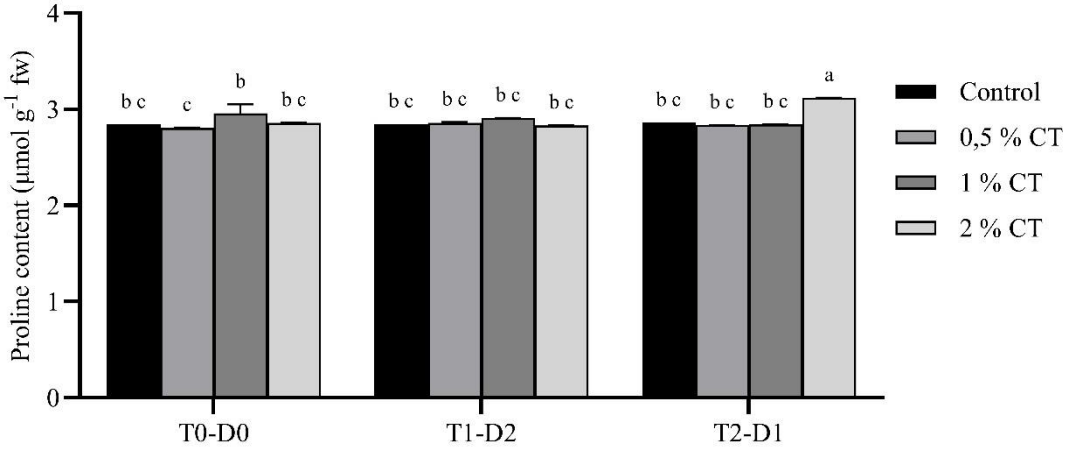

Figure 3. Chickpea plant proline content.

The means of the three plants make up the data. Two-way factorial ANOVA was conducted between the groups. Significant differences (*p < 0.05*) are indicated by asterisks (*).

Inoculation of chickpea plants with *Ascochyta rabiei* increased malondialdehyde (MDA) content

in all CT treatments except in the T1-D2 treatment ($p<0.05$). It was found that T1-D2's MDA content was comparable to that of the positive control's. The T2-D1 therapy had the highest MDA content (Fig. 4).

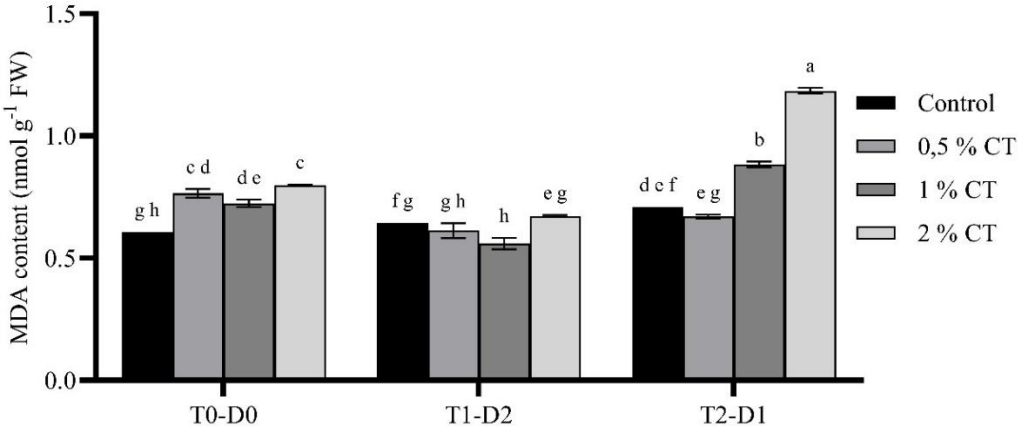

Figure 4. MDA content of chickpea plants

The data represent the mean of three plants. Between groups, a two-way factorial ANOVA was conducted. Significant differences are indicated by asterisks at $p \leq 0.05$ (*) and $p \leq 0.01$ (**).

Antioxidant enzyme analysis

The highest catalase activity was observed in T0-D0 and T1-D2, except in the T2-D1 1 % treatment ($p<0.05$) (Fig. 5).

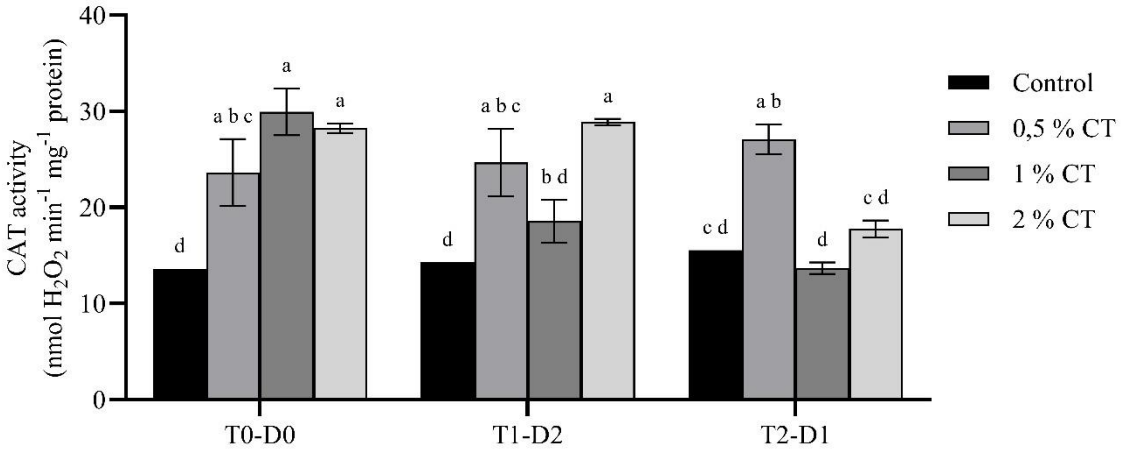

Figure 5. Catalase activity of chickpea plants

The data represent the mean of three plants. Two-way factorial ANOVA was performed between groups. Asterisks denote significant differences at $p \leq 0.05$ (*)

Following the inoculation and CT treatment, all groups' superoxide dismutase (SOD) activity in all groups increased ($p<0.05$) in compared to that in the control group. The T1-D2 group exhibited the highest SOD activity (Fig. 6).

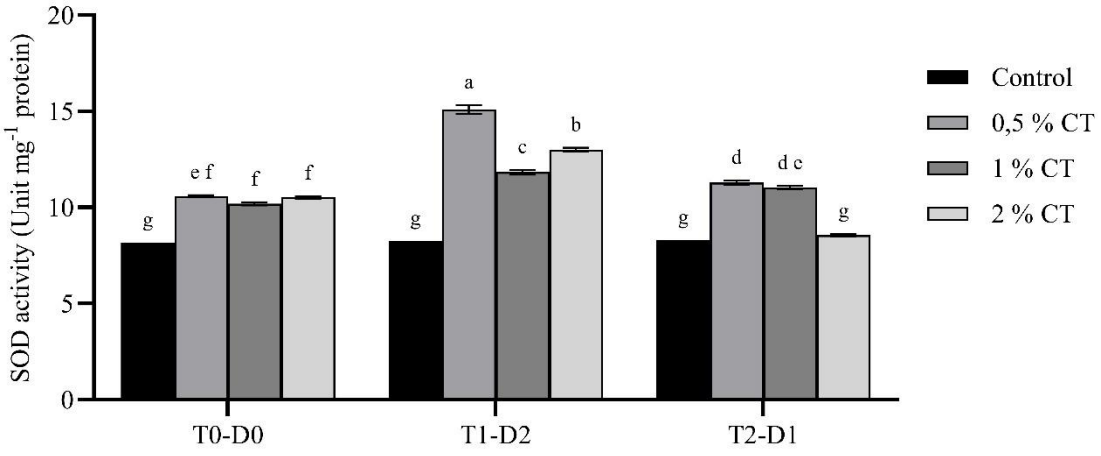

Figure 6. SOD activity of chickpea plants

The data represent the mean of three plants. Two-way factorial ANOVA was performed between groups. Asterisks denote significant differences at $p \leq 0.05$ (*), $p \leq 0.01$ (**)

In this study, the potential use of CT against AB, which causes foliar infection in chickpea, was analyzed. The efficacy of different doses of CT as an antifungal agent against the pathogen *Ascochyta rabiei* was examined. Antifungal activity test findings unequivocally demonstrated that CT treatment prevented *Ascochyta rabiei* from growing mycelially. Exogenous CT treatment also inhibited the development of the disease. Our results showed that CT can have a potential effect in delaying disease symptoms and reducing susceptibility to *Ascochyta rabiei* in chickpea. This beneficial impact of CT is prominent in both consecutive and simultaneous applications. This is the first report to reveal the antifungal activity of CT against *Ascochyta rabiei,* and it is promising that the plant extract used in this study could be an effective approach against a new and highly virulent *Ascochyta rabiei* isolate (pathotype IV). Limited information is available on the antifungal activity of different plant extracts in reducing AB in chickpea. *Ascochyta rabiei* was evaluated to determine the antifungal efficacy of various concentrations of *Chenopodium album* leaf methanolic extract (1%, 2.5%, 4%, 5.5%, and 7%). The highest fungal biomass reduction (68%) was noted with a 7% dose of *C. album* methanolic extract (Sherazi et al.2016).

Withania somnifera, a weed species, was used to control the AB. According to the findings, pathogenic fungal growth was effectively suppressed by a 0.2% dose of *W. somnifera* leaf extract in methanol (Javaid et al. 2020). Tests against five distinct isolates of *Ascochyta rabiei* were carried out *in-vitro* to determinate the antifungal activity of *Mentha spicata* L. essential oils. Consequently, it was discovered that a 10 µL dose of *Mentha spicata* essential oil inhibited the fungus's mycelial growth (Bayar 2018). According to the study's findings, CT might include a number of antifungal substances that can successfully stop *Ascochyta rabiei* from growing.

No significant change was observed in proline content after *Ascochyta rabiei* inoculation and CT treatment in any of the groups. However, increases in proline levels in chickpea plants following *Ascochyta rabiei* inoculation have been demonstrated by various researchers (Hasanian et al. 2020; Üstün and Dolar 2001). Additionally, there has been an increase in the expression of genes linked to proline synthesis that help plants tolerate *Ascochyta rabiei* (Coram and Pang 2006). Although proline's function as an osmoprotectant is its most well-known characteristic (Chun et al. 2018), it also has direct or indirect antioxidant functions (Szabados and Savouré 2009). In addition, chickpea cultivars that are more tolerant to *Ascochyta rabiei* have a lower proline content (Hasanian et al. 2020). The finding of proline content in this study, which is close to that of the positive control in all treatments, suggests that CT treatment may lessen the adverse effects of the

disease (Hasanian et al. 2020).

MDA, one of the end products of lipid peroxidation, is a good indicator of the level of membrane damage caused by oxidative stress (Çevik 2021). An increase in MDA content after *Ascochyta rabiei* inoculation was shown by Bahmani et al. (2020). In the same study, tolerant cultivars had lower MDA contents than sensitive cultivars under *Ascochyta rabiei* inoculation. CT treatment before *Ascochyta rabiei* inoculation decreased MDA content in the T1-D2 group. Low MDA content under pathogen attack indicates that CT treatment helps preserve the membrane integrity of the plant. Providing this protection with pre-disease treatment is also a positive outcome for the potential field application of CT. The PDI of the T2-D1 group was much higher than that of the T1-D2 and T0-D0 groups (Fig. 2); this result was also consistent with the MDA results. Considering the MDA results, it can be concluded that pre-disease CT treatment protects plant membranes from the effects of the disease.

Following stress and CT treatments, CAT and SOD activity increased in every group. Although the T0-D0 group showed the highest catalase activity, the T1-D2 group had the highest overall catalase activity. The two crucial components of the plant antioxidant systems are CAT and SOD. The antioxidant system's first line of defense is SOD, which catalyzes the conversion of superoxide radicals into $H2O_2$ and O_2. The transformation of $H2O_2$ into H2O and O_2 is catalyzed by CAT (Kaur et al. 2021). In addition to a significant increase in CAT under disease pressure (Hasanian et al. 2020), upregulated defense enzyme activities have also been observed against *Ascochyta rabiei* inoculation (Kaur et al. 2021). These investigations unequivocally demonstrated that, in an infected environment, resistant genotypes exhibit lower MDA concentrations and higher antioxidant enzyme activities than susceptible genotypes. Higher antioxidant enzyme activities following tar treatment in response to AB may indicate the suppression of oxidative stress caused by the pathogen. However, isozyme analysis is are required to better understand the effects of tar treatment on the antioxidant system during infection.

The cost performance of CT was also evaluated comparatively with that of a fungicide used to control AB. The "partial budgeting" method was preferred, based on the principle that all expenditures are the same except for the cost of the fungicide and CT. The approximate cost of 500 mL of a fungicide containing “25% Boscalid, 12% Pyraclostrobin” is 37 USD, while the approximate cost of 500 mL of CT is 4 USD. It was calculated that the cost of supplying CT is 90% lower than the fungicide. Fungicide application is recommended at a dose of 500 mL ha^{-1}, and the cost per hectare is calculated to be 37 USD. It was calculated that 100 L of water would be used for a one-hectare area, and 2 mL of CT is sufficient for the 2% application dose, with the approximate cost for this area being 8 USD. The unit area cost of the 2% application dose of CT is 78% lower than the fungicide. Considering that large areas are cultivated; greater profit will be achieved owing to the reduction of input costs by 90% for the initial supply cost and by 78% for the unit area. In fact, if the profit margin is reduced slightly reduced, the probability of finding customers in the market is quite high. In this case, it is predicted that this will improve the production cost performance (Jukanti et.al. 2012; Shuping. et al. 2017; Wani et.al.2022)

Fungicides are the main methods for controlling AB in chickpeas. The potential for new fungicide-resistant races to emerge or possible shifts in the pathogen's population structure present the greatest obstacles to both chemical control techniques and resistance breeding research. Using CT can assist in reducing on the use of fungicides or serve as an alternative to them. The findings of the study demonstrated that CT has a great potential for use against infections.

Author information

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Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

All data available within the manuscript.

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Research Article/Araştırma Makalesi

The effects of different cutting times on morphological, agronomic and quality characteristics of wooly-pod vetch (*Vicia villosa* Roth ssp. *dasycarpa* (Ten) Cavi.)

Tüylü meyveli fiğde (Vicia villosa Roth ssp. dasycarpa (Ten) Cavi.) farklı biçim zamanlarının morfolojik, agronomik ve kalite özelliklerine etkileri

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ABSTRACT

In arid and semi-arid regions, vetch species are typically cultivated in row crop rotation or in the fallow year for the purpose of forage production. In order for vetch to be incorporated more effectively into this agricultural system, it is essential that it does not negatively impact the yield of the subsequent main crop. Therefore, vetches need to leave the field as soon as possible. For this reason, the morphological traits (main stem length, natural plant height), agronomic traits (forage yields) and quality traits (ADF and NDF ratio, digestible dry matter ratio, relative feed value) at four different growth periods were investigated in woolly pod vetch, Seğmen-2002 variety. This study was conducted at the Gölbaşı/İkizce research farm of the Central Research Institute of Field Crops between 2010 and 2013 in a randomised block design with four replications. The results indicated that the main stem length varied between 56.2 and 118.9 cm, while the natural plant height varied between 41.5 and 63.3 cm. The highest yields of fresh and dry forage were obtained at full flowering, with 2157.7 kg ha^{-1} and 354.1 kg ha^{-1}, respectively. While crude protein content was 19.7% at the beginning of flowering, it decreased gradually throughout the growth periods and decreased to 16.4% at the pod setting period. The highest crude protein yield was obtained at full flowering, with 67.0 kg ha^{-1}. The relative feed value was found to be the highest at the beginning of flowering (111.3) and to decrease to the lowest level (98.5) at full flowering. This research indicates that it is appropriate to cut wooly-pod vetch at full flowering in terms of yield, quality, and early completion of the harvesting process.

Keywords: Wooly-pod vetch, forage yield, crude protein yield, relative feed value

ÖZ

Kurak ve yarı–kurak bölgelerde fiğ türleri nadas yılında ya da ekim nöbeti içerisinde genellikle ot amaçlı olarak yetiştirilmektedir. Fiğlerin bu tarım sistemi içerisinde daha fazla yer bulması için, kendinden sonra gelen ana bitkinin verimini olumsuz etkilememesi önemlidir. Bu nedenle fiğlerin tarlayı mümkün olduğunca erken terk etmesi gereklidir. Bu çalışmada, tüylü meyveli fiğ 4 farklı dönemde biçilerek, morfolojik (ana sap uzunluğu, doğal bitki boyu), agronomik (yeşil ve kuru ot verimi) ve kalite

özellikleri (ADF ve NDF oranı, sindirilebilir kuru madde oranı, nisbi yem değeri) incelenmiştir. Bu araştırmada, tüylü meyveli fiğin (*Vicia villosa Roth* ssp. *dasycarpa* (Ten) Cavi.) Seğmen çeşidi kullanılmış, 2010-2013 yılları arasında Tarla Bitkileri Merkez Araştırma Enstitüsünün Gölbaşı/İkizce araştırma çiftliğinde tesadüf blokları deneme deseninde dört tekerrürlü olarak yürütülmüştür. Araştırma sonucunda, ana sap uzunluğu 56.2-118.9 cm, doğal bitki boyu 41.5-63.3 cm arasında değişmiştir. En yüksek yeşil ot ve kuru ot verimi 2157.7 kg da^{-1}, 354.1 kg da^{-1} ile tam çiçeklenme döneminde elde edilmiştir. Ham protein oranı çiçeklenme başlangıcında %19.7 iken, biçim dönemlerinde azalarak, bakla bağlama döneminde % 16.4'e düşmüş, en yüksek HPV 67.0 kg da^{-1} ile tam çiçeklenme döneminde alınmıştır. Nisbi yem değeri çiçeklenme başlangıcında en yüksek (111.3) olmuş ve tam çiçeklenme döneminde de en düşük (98.5) seviyeye inmiştir. Bu araştırma sonucunda, verim, kalite ve tarlanın erken boşaltılması açısından tüylü meyveli fiğin tam çiçeklenme döneminde biçilmesinin uygun olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Tüylü meyveli fiğ, ot verimi, ham protein verimi, nisbi yem değeri.

Introduction

Vetch species play a significant role in forage crops, providing valuable high-quality roughage for animal nutrition. Despite a decrease in cultivated area in recent years, farmers continue to cultivate the plant due to its high adaptability to various climatic and soil conditions, which range from arid to cool and humid. Although the area under vetch cultivation is currently estimated at 342 thousand hectares, there is potential for further expansion. The Central Anatolia Region, which is characterised by arid and semi-arid climatic conditions, is the region where fallow is the most prevalent practice, with an area of 2.96 million hectares (Anonymous, 2024). In the fallow year, when the field remains uncultivated and in a state of rotation, it is possible to sow vetch species as a winter intermediate crop, prior to the cultivation of summer crops for the production of forage. It is possible to cultivate hay without reducing the yield of cereals sown after it (Eser et al., 1997; Avcı et al., 2007). Vetch can be cultivated as a pre-crop prior to the cultivation of summer crops, including vegetables, sugar beet, cotton, sunflower, and maize. This practice allows for the production of forage and green manure. In irrigated agricultural areas where industrial products are grown, planting annual legume forage crops as winter intercrops can be carried out without reducing the yield of the main product (Kaplan and Gökkuş, 2018). They positively impact the soil structure, which is beneficial for summer crops (Eser et al., 1997; Kaya, 2009). The incorporation of annual legumes into the soil prior to planting maize has been demonstrated to positively influence the yield of the latter crop by facilitating the fixation of nitrogen in the soil (Kalkan, 2019). Among the different plants sown as a preplant, the highest nitrogen content was obtained from the application of hairy vetch (Liebmana et al., 2018).

Woolly pod vetch (*Vicia villosa* Roth ssp. *dasycarpa* (Ten) Cavi.), a subspecies of hairy vetch (*Vicia villosa* Roth), is also known as false hairy vetch (Açıkgöz 2021). Although it is a subspecies, it exhibits a number of distinctive characteristics that set it apart from hairy vetch. In contrast to the pubescence observed on the stems and leaves of other species, this variety exhibits a light and bright green colouration. Although it is less winter-hardy than hairy vetch (*V. villosa*), it emerges earlier than it. As it is approximately 10 days earlier than Hungarian vetch and grows earlier and more rapidly in the cool period of spring, it can be cultivated in fallow areas to a greater extent than Hungarian vetch (Mutlu, 2012). In a study on mixed sowing of vetches and cereals, it was observed that grazing sheep demonstrated a preference for common vetch, hairy vetch, and Hungarian vetch, followed by oats, barley, and rye (Munzur 1982). Since it is more preferred by animals and it has an early characteristic, it shows that wooly pod vetch has the potential to be grown more for forage purposes.

Vetches can be harvested for forage at various stages, beginning with flowering and ending with the full formation of pods (Açıkgöz, 2021). In the cereal-fallow system, earlier removal of vetch from the field before the main crop is planted

allows vetch to be grown as a forage crop in more areas. The aim of this study was to determine the optimal cutting time for wooly pod vetch, focusing on forage yield and quality at different growth stages.

Material and Methods

The research was conducted between 2010 and 2013 at the Research and Application Farm of the Central Research Institute of Field Crops, located at the Gölbaşı/İkizce site. The soil structure of the test area was clayey-loamy textured, with an organic matter content of 0.92%. The phosphorus content was moderate, exhibiting a very calcareous character and a high potassium content. The pH was slightly alkaline (Table 1).

Table 1. Soil analysis results of the test area

Test area	Total Salt (%)	Organic matter (%)	Lime $CaCo_3$ (%)	Phosphorus P_2O_5 (kg da^{-1})	Potassium K_2O (kg da^{-1})	pH	Soil structure
Gölbaşı/İkizce	0.023	0.92	25.36	6.28	178.98	7.91	Clay-loamy

When the precipitation distribution in the years studied is examined, it is clear that there are significant differences in the distribution and amount of precipitation, particularly during the first two production seasons (Table 2). In the first production season, 343.6 mm of rain fell, which was close to the long-term average.

The precipitation was concentrated, particularly during the vetch development period. In the second production season, the rainfall total of 166 mm was considerably below the long-term average. The precipitation of 1.8 mm in March and April is considerably below the long-term average of 94.1 mm. The third-year rainfall is found to be very close to the long-term average.

Table 2. Precipitation (mm) and temperature data (°C) for the experimental area over the 2010-2013 period and for longer time periods

Months	Total Precipitation (mm)				Average temperature (°C)			
	2010-2011	2011-2012	2012-2013	1989-2010	2010-2011	2011-2012	2012-2013	1989-2010
September	0.0	1.6	3.6	19.4	17.0	17.1	19.0	16.9
October	81.6	34.0	46.3	23.7	11.3	12.3	14.5	12.4
November	10.0	2.2	34.7	34.4	11.8	6.4	6.9	5.4
December	13.2	19.8	60.4	50.0	4.3	1.0	1.3	0.4
January	28.0	56.4	27.0	29.1	0.2	-0.5	0.1	-1.5
February	5.0	3.6	26.8	32.9	-0.6	-0.3	3.8	-0.2
March	42.0	0.0	37.2	43.0	2.6	4.8	6.5	3.9
April	40.4	1.8	49.4	51.1	7.6	10.1	10.0	9.2
May	86.6	46.8	59.8	44.4	12.4	16.1	16.5	13.9
June	36.8	0.0	13.0	26.1	16.8	20.1	20.0	18.4
Total	343.6	166.2	358.2	354.1				
Average					8.3	8.7	7.9	7.9

Woolly pod vetch, Seğmen-2002 (*Vicia villosa* Roth ssp. *dasycarpa* (Ten) Cavi.) cultivar, was used as the material in this trial. This study examined four various growth stages of vetch: the initial flowering stage (IFP), the mid-flowering stage (MFP), the full flowering stage (FFP), and the period when grains in the lower pods reach full maturity (PP). This latter period is typically recommended as the optimal cutting time for vetch, as outlined by Balabanlı (2009) and

Anlarsal (2009). The experiment was conducted in accordance with a randomised block design, with four replicates. Each plot comprised six rows of five metres in length, with a spacing of 26 centimetres between rows. The thousand grain weight of vetch was calculated, and 250 seeds per square metre (Munzur et al. 1995) were sown by hand at a depth of 3-4 cm. The remaining 4.16 m² area within the plots was harvested after the side rows had been mown, with a clearance of 50 cm from both sides of the row. The plot yield was determined by weighing the mown hay and fresh forage yield (FFY) was calculated for each cutting time according to the methodology proposed by Altınok and Hakyemez (2000). Prior to harvesting, the natural plant height (NPH) (Balabanlı, 1992) and main stem length (MSL) (Fırıncıoğlu et al., 2009; Bedir, 2010) were determined in five randomly selected plants. The growth habits were observed at five different growth stages (1:erect, 3:semi-erect, 5:non-erect) (Fırıncıoğlu et al., 2009).

A total of 500 g of fresh forage was randomly selected from each plot and subsequently dried. The dry forage yield (DFY) was determined according to the methodology described by Ünal (2011), while the crude protein ratio (CPR) was calculated using the approach outlined by Tekkanat and Soylu (2005). The crude protein yield (CPY) was obtained based on the method developed by Bedir (2010). Additionally, the acid detergent fibre (ADF) and neutral detergent fibre (NDF) analyses were conducted in accordance with the procedures outlined by Kutlu (2008). The digestible dry matter ratio (DDMR) and relative feed value (RFV) were determined using the techniques described by Starkey et al. (1993) and Demirbağ et al. (2015), respectively. The statistical analysis of the research results was performed using the statistical software package SAS. All averages were grouped according to the least significant difference (LSD) test.

Results and Discussion

Morphological traits

Growth habit

The growth habits observed at five different growth stages are presented in Table 3. Upon analysis of Table 3, it can be observed that woolly pod vetch exhibited a value of 2.7, indicating a medium level of development at the beginning of flowering. Subsequently, it demonstrated a semi-erect and non-erect development. The lowest value of main stem length was observed in the second and third years, coinciding with the onset of flowering. Accordingly, an evaluation of the main stem length and natural plant height (Table 4) reveals a tendency for lodging in wooly pod vetch from the time of the beginning of flowering.

Number of cutting days

Table 3 shows the number of days that should be cut in order to harvest fresh forage from woolly pod vetch at each growth stage. The mean numbers of day required cutting the four growth stages were 204.3, 210.7, 216.7 and 237.0 days, respectively. The prolongation of the PP in the first rainy year demonstrates that the vegetative growth period of woolly pod vetch is prolonged in accordance with rainfall and temperature. The results obtained in the present study were lower than those reported by Hakyemez et al. (1997), who found a duration of 194-240 days, and higher than those reported by Özpınar and Sabancı (2014), who found a duration of 97-118 days in the MFP period of hairy vetch. The difference may be attributed to the varying climatic conditions across the years.

Table 3. Growth habit and cutting day numbers of plants at different growth stages

	Growth habit				Cutting day numbers			
Developmental stages	2010-2011	2011-2012	2012-2013	Average	2010-2011	2011-2012	2012-2013	Average
IFP	4.0	2.0	2.0	2.7	204.0	208.0	201.0	204.3
MFP	5.0	3.0	3.0	3.7	212.0	216.0	204.0	210.7
FFP	5.0	3.0	4.0	4.0	220.0	223.0	207.0	216.7
PP	5.0	4.0	5.0	4.7	246.0	238.0	227.0	237.0
Average	4.8	3.0	3.5	3.8	220.5	221.3	209.8	217.2

IFP: initial flowering period, MFP: mid-flowering period, FFP: full flowering period, PP: the period when grains in the lower pods reach full maturity

Table 4. Main stem length (cm) and natural plant height (cm) of plants at different growth stages

	Main stem length				Natural plant height			
Developmental stages	2010-2011	2011-2012	2012-2013	Average	2010-2011	2011-2012	2012-2013	Average
IFP	88.0 d	37.6 c	42.9 b	56.2 d	58.3	26.2 d	40.1 b	41.5 c
MFP	121.6 c	47.0 b	41.9 b	70.1 c	56.9	33.2 c	39.1 b	43.0 bc
FFP	145.4 b	54.0 b	48.5 b	82.6 b	59.1	38.3 b	44.3 b	47.2 b
PP	202.9 a	77.6 a	76.3 a	118.9 a	61.2	67.9 a	61.0 a	63.3 a
Average	139.5 a	54.0 b	52.4 b	82.0	58.9 a	41.4 c	46.1 b	48.8
$F_{(cutting)}$	77.5**	51.2**	31.5**	143.0**	1.0	144.9**	8.4**	47.9**
$F_{(years)}$				652.8**				52.3**
$F_{(cutting\ x\ years)}$				23.8**				11.5**
$LSD_{(cutting)\ (0.05)}$	17.6	7.6	9.2	6.5	5.6	4.9	11.3	4.2
CV (%)	7.9	8.8	11.0	9.5	6.0	7.4	15.3	10.3

*IFP: initial flowering period, MFP: mid-flowering period, FFP: full flowering period, PP: the period when grains in the lower pods reach full maturity, **: Significant difference at 1% level, CV: Coefficient of variation (%)*

Main stem length

The results of the variance analysis of main stem length (cm), as presented in Table 4, indicate that the factors of years ($P < 0.01$), cutting periods ($P < 0.01$) and cutting x years interaction ($P < 0.01$) were found to be statistically significant. The mean main stem length (MSL) was found to be greatest during the PP, at 118.9 cm, followed by the FFP, MFP and IFP, which exhibited mean lengths of 82.6, 70.1 and 56.2 cm, respectively. The highest MSL value was recorded during the PP in all three years. While the mean MSL values measured in the second and third years were similar, the MSL value measured in the first year was notably high at 139.5 cm, due to the occurrence of heavy rainfall. The MSL values obtained in the first year were higher than those reported by Doğan (2014), who found 62.3 cm in the PP, and were also close to those reported by Özdemir et al. (2021), who found 102.8-194.3 cm. They were, however, higher than those reported by Hakyemez et al. (1997) found 31-80 cm in the MFP, and which is consistent with the findings of Altınok and Hakyemez (2000), who observed 82 cm in the FFP period. Additionally, the results align with those of Mihailovic et al. (2007), who recorded 98 cm, and Şahar (2006), who noted 89.3 cm. The high MSL values observed in the first year can be attributed to the climate conditions prevailing during that period.

Natural plant height (NPH)

The analysis of variance revealed statistical significance for NPH, years ($P<0.01$), cutting periods ($P<0.01$), and the interaction between cutting and years (Table 4). The mean NPH values were highest during the PP (63.3 cm), followed by the FFP, MFP, and IFP (47.2, 43.0, and 41.5 cm, respectively). The highest NPH was observed in the first year, with a value of 58.9 cm, while the second and third years exhibited similar values, at 41.4 and 46.1 cm, respectively. The mean NPH values obtained in this study were similar to those reported by Tenikecier et al. (2020), who found 43.3, 53.6, and 39.3 cm for Hungarian vetch in the IFP, MFP, and FFP, respectively.

Agronomic traits

Fresh forage yield

The analysis of variance found statistically significant for fresh forage yield (FFY) at cutting stages, year, and cutting x year interaction (P<0.01). The mean results indicated that the highest FFY was observed during the FFP, with a yield of 2157.7 kg da^{-1}. This was followed by the MFP, IFP, and PP, with yields of 1758.6, 1686.8, and 1161.9 kg da^{-1}, respectively. The highest FFY was obtained in the first rainy year, with an annual average of 3793.1 kg da^{-1}, followed by the third and second years, with annual averages of 872.9 and 407.7 kg da^{-1}, respectively. The highest FFY was obtained in the FFP in the first year, in accordance with the established cutting periods. In contrast, the highest FFY was obtained in the PP in the subsequent years. The difference observed in the initial year can be attributed to the rotting of the lower branches, which was caused by the lodging of the overgrown plants due to the excessive rainfall. The differentiation between the harvesting periods according to the years resulted in the form-by-year interaction being statistically significant. The high FFY values observed in the PP during the second and third years led to the conclusion that wooly pod vetch effectively utilised the rainfall in May, resulting in an increased FFY in both years. The highest FFY was obtained in the FFP in our study, as evidenced by the average values. Our findings were higher than those reported by Desalegn and Hassen (2015), who observed a mean of 1534.1 kg da^{-1} during the MFP, and Coşkun and Çaçan (2019), who noted a range of 449-1901 kg da^{-1} during the PP. The differing climatic conditions may be the reason for this discrepancy. The results obtained in this study were lower than those reported by Özdemir et al. (2021), who found 1841-2591 kg da^{-1} in the PP, and Hakyemez et al. (2005), who found 2157-2310, 2160-2002, 1448-1467 kg da^{-1} in the IFP, FFP, and PP, respectively. Additionally, the findings were lower than those reported by Mihailovic et al. (2007), who found 3120 kg da^{-1} in the PP. The discrepancy in FFY values may be attributed to the different vetch species involved.

Dry forage yield

Dry forage yields (DFY) were statistically significant years (P < 0.01), cutting stages (P < 0.01), and the cutting x year interaction (P < 0.01) (Table 5). The highest FFY was obtained in the FFP, with an average of 354.1 kg da^{-1}. This was followed by the PP, MFP, IFP and IFP, which had average DFYs of 319.9, 285.6, 271.1 and 271.1 kg da^{-1}, respectively. The highest average DFY was observed in the first rainy year, with a value of 482.0 kg da^{-1}. In the second and third years, the highest DFY value was obtained from cutting conducted during the PP period, whereas in the first year, it was obtained from cutting conducted during the FFP period. This discrepancy resulted in a statistically significant cutting x years interaction. The results demonstrate that the rotting of the lower branches and leaves during the PP, caused by the lodging of the overgrown plants in the first year, was an effective phenomenon. The results obtained in this study were higher than those reported by Coşkun and Çaçan (2019), who found a mean yield of 225 kg ha^{-1} during the PP. This discrepancy can be attributed to variations in climatic conditions. Among researchers working on hairy vetch, Ova and Uslu (2021) harvested in the same periods (593.4, 887.4, 803.9, 852.8 kg da^{-1}), Özdemir et al. (2021) harvested 280-559 kg da^{-1}in the PP, Hakyemez et al. (2005) harvested in the IFP, MFP, and PP (461.9-526.5, 510-554.7, 583-549.4 kg da^{-1}), and Mihailovic et al. (2007) harvested 570 kg da^{-1} in the PP period. The discrepancy in values may be attributed to the different species of vetch involved.

Table 5. Fresh forage (kg da^{-1}) and dry forage yield (kg da^{-1}) values at different growth stages

	Fresh forage yield				Dry forage yield			
Developmental stages	2010-2011	2011-2012	2012-2013	Average	2010-2011	2011-2012	2012-2013	Average
IFP	4225.5 b	208.5 d	626.2 c	1686.8 b	665.3 b	38.9 bc	109.1 bc	271.1 c
MFP	4281.1 b	308.3 c	686.3 bc	1758.6 b	683.5 b	51.4 c	121.8 c	285.6 bc
FFP	5232.9 a	415.6 b	824.6 b	2157.7 a	843.3 a	67.7 b	151.3 b	354.1 a
PP	1432.9 c	698.1 a	1354.7 a	1161.9 c	482.0 c	171.1 a	306.4 a	319.9 ab
Average	3793.1 a	407.7 c	872.9 b	1691.2	668.5 a	82.3 c	172.2 b	307.6
$F_{(cutting)}$	98.4**	73.7**	31.2**	54.5**	15.2**	73.0**	62.6**	5.6**
$F_{(years)}$				1466.5**				547.9**
$F_{(cutting\ x\ years)}$				127.6**				20.5**
$LSD_{(cutting)\ (0.05)}$	529.0	78.7	189.9	159.2	121.5	22.6	36.9	**44.8**
CV (%)	8.7	12.1	13.6	11.3	11.4	17.2	13.4	**17.5**

*IFP: initial flowering period, MFP: mid-flowering period, FFP: full flowering period, PP: the period when grains in the lower pods reach full maturity, **: Significant difference at 1% level, CV: Coefficient of variation (%)*

Quality traits

Crude protein ratio (CPR)

The analysis of variance (Table 6) of crude protein ratios (%) determined at harvesting periods revealed statistically significant findings for the factors of harvesting periods ($P < 0.01$), harvesting x year interactions ($P < 0.01$), and years ($P < 0.01$). The highest crude protein ratio was observed during the MFP period, with an average of 19.9%. This was followed by the IFP, FFP and PP periods, which exhibited crude protein ratios of 19.7%, 18.9% and 16.4% HP, respectively. The highest crude protein ratio was recorded during the MFP period, which was influenced by the second year's rainfall, which led to vegetative growth in the plants and consequently increased the crude protein ratio (CPR) during the MFP and PP periods. This resulted in the highest mean crude protein ratio of the year, with a value of 19.5%. This is due to the significant interaction between cutting and year. The three-year average of our study revealed that the crude protein ratio (CPR) was higher than that reported by Desalegn and Hassen (2015), who found 18.9% in the MFP period, and higher than that reported by Coşkun and Çaçan (2019), who found 21.8% in the PP period. However, it was lower than that reported by Doğan (2014), who found 20%. The percentage was 26% in the PP period, which is lower than the findings of Özdemir et al. (2021), who reported a range of 17.1-18.7% for the same period. It is also close to the results of Ova and Uslu (2021), who observed values of 20.0%, 18.0%, 18.0%, 18.0%, and 18.0% for the same periods of hairy vetch. The difference in the results can be explained by the differences in the climatic conditions of the year and the type of vetch.

Table 6. Crude protein ratio (%) and crude protein yield (kg da^{-1}) at different growth stages

	Crude protein ratio				Crude protein yield			
Developmental stages	2010-2011	2011-2012	2012-2013	Average	2010-2011	2011-2012	2012-2013	Average
IFP	20.3 a	19.3 b	19.4 a	19.7 a	135.1 a	7.5 c	21.2 b	54.6 b
MFP	19.7 ab	21.2 a	18.7 a	19.9 a	134.6 a	10.9 bc	22.8 b	56.1 b
FFP	19.2 b	21.3 a	16.3 b	18.9 a	161.9 a	14.4 b	24.47b	67.0 a
PP	16.5 c	16.2 c	16.5 b	16.4 b	79.5 b	27.7 a	50.6 a	52.6 b
Average	18.9 a	19.5 a	17.7 b	18.7	127.8 a	15.1 c	29.8 b	57.6
$F_{(cutting)}$	5.8*	47.5**	5.5*	20.1**	12.9**	39.3**	55.1**	3.8*
$F_{(years)}$				8.5**				498.4**
$F_{(cutting\ x\ years)}$				4.4**				22.3**
$LSD_{(cutting)\ (0.05)}$	2.3	1.1	2.2	1.0	30.0	4.6	6.0	9.0
CV (%)	7.5	3.5	7.6	6.6	14.9	18.7	12.7	19.0

*IFP: initial flowering period, MFP: mid-flowering period, FFP: full flowering period, PP: the period when grains in the lower pods reach full maturity, **: Significant difference at 1% level, *: Significant difference at 5% level, CV: Coefficient of variation (%)*

Crude protein yield (CPY)

The analysis of variance (Table 7) of crude protein yield (CPY) values (kg da-1) determined for years (P<0.01), cutting periods (P<0.05) and cutting x year interactions (P<0.01) revealed statistically significant results. The mean CPY was highest at the FFP stage, at 67.0 kg da^{-1}, while the CPYs obtained at the MFP, IFP and PP growth stages were 56.1, 54.6 and 52.6 kg da^{-1}, respectively. The highest CPY was obtained in the first rainy year, as compared to subsequent years. The highest CPY was obtained during the FFP period in the initial year and during the PP period in the second and third years. The difference in outcomes observed between the two years led to the conclusion that the interaction between the cutting factor and the year factor was statistically significant. The highest CPY was observed in the first year, with an average of 127.8 kg da^{-1}, followed by the third and second years, with average CPY values of 29.8 and 15.1 kg da^{-1}, respectively. The CPY findings obtained from our research were found to be lower than those reported by Coşkun and Çaçan (2019) and Doğan (2014). The former reported 63.4 kg da^{-1}, while the latter reported 61.89 kg da^{-1} in the PP period. Similarly, among researchers engaged in studies on hairy vetch, the findings were observed to be lower than those reported by Ova and Uslu (2021), who conducted harvesting during the same periods (120.8, 162.3, 147.3, 157.0 kg da^{-1}), and Özdemir et al. (2021), who undertook harvesting during the PP period (47. 8-103.8 kg da^{-1}), Altınok and Hakyemez (2000) in FFP period (87.0 kg da^{-1}), Hakyemez et al. (2005) in IFP, FFP periods (75.8-88.7, 67.8-79.4 kg da^{-1}). The low CPY results can be attributed to the reduced rainfall levels experienced during the second and third years of the growth period for the plants in question.

Acid detergent fibre (ADF)

The analysis of variance (Table 7) revealed that the ADF ratios of woolly pod vetch exhibited statistically significant differences (P<0.01) across years and cutting periods. However, the cutting x year interaction was found to be insignificant. The highest ADF rate was observed in the FFP period, with an average of 41.5%, followed by the MFP, PP and IFP periods, which exhibited average rates of 39.9, 38.3 and 38.1%, respectively. In general, the ADF rate increased with the progression of the development period. In the first year and the third year, the ADF rate increased until the FFP period and then decreased in the PP period. In contrast, in the second year, the highest ADF rate was determined in the IFP period and then showed a slight decrease with the progression of the development period. The highest ADF rate was identified in the initial year, with a value of 43.0%. This was followed by rates of 40.4% and 34.6% in the third and second years, respectively. It was determined that the lowest ADF rate in the second year and the decline in ADF rate with the progression of the development period, in contrast to the other years, was attributable to the vegetative development of the plants, which was influenced by rainfall in May. The difference between the ADF ratios determined for the development periods was found to be statistically significant in the third year, while the other years were found to be insignificant. The ADF ratio values obtained in this study were higher than those reported by Desalegn and Hassen (2015) for the MFP period (37.3%) and by Coşkun and Çaçan (2019) for the PP period (37.6%). The results were higher than those reported by Ova and Uslu (2021) for the same periods (34.0%, 39.0%, 35.0%, 37.0%), with the exception of the MFP period. Furthermore, they were higher than those reported by Özdemir et al. (2021) for the PP period (35.4-39.5%). The discrepancy may be attributed to variations in climatic conditions and species-specific differences.

Neutral detergent fibre (NDF)

The analysis of variance (Table 7) revealed that the NDF ratios of woolly pod vetch were statistically significant (P < 0.01) for both the years and cutting periods. However, the cutting x year interaction was found to be insignificant. The mean NDF ratio was highest during the FFP period

(55.5%), followed by PP (54.7%), MFP (51.7%), and IFP (50.5%) periods. The difference between the NDF ratios determined for cutting periods was statistically significant (P < 0.01) in the third year, but not in the first or second years. The lowest NDF ratio was observed in the second year, with a value of 46.6%. In the initial and third years, the NDF ratio demonstrated an increase throughout the growth period, reaching its peak during the FFP period, and subsequently declined during the PP period. In contrast, in the second year, the NDF ratio exhibited a different trend, displaying a decline from the IFP to the FFP period and attaining its highest level during the PP period. It was thus concluded that this result was due to the vegetative development of the plants, which was once again stimulated by the rainfall in May. The NDF ratios obtained in the present study exhibited higher values than those reported by Desalegn and Hassen (2015) for the MFP period (47.1%) and Coşkun and Çaçan (2019) for the PP period (47.2%). In hairy vetch, the values were higher than those reported by Ova and Uslu (2021) for the same periods (42.0%, 54.0%, 46.0%, 50.0%) and Özdemir et al. (2021) for the PP period (47.5-49.8%). The elevated NDF ratios observed may be attributed to climatic conditions and species-specific factors.

Table 7. Acid detergent fibre (%) and neutral detergent fibre ratios (%) of plants at different growth stages

	Acid detergent fibre				Neutral detergent fibre			
Developmental stages	2010-2011	2011-2012	2012-2013	Average	2010-2011	2011-2012	2012-2013	Average
IFP	41.2	35.2	37.9 c	38.1 b	58.0	46.2	47.2 c	50.5 c
MFP	43.1	34.9	39.9 b	39.3 b	59.3	45.5	50.4 bc	51.7 bc
FFP	46.0	34.0	44.6 a	41.5 a	64.3	44.9	57.4 a	55.5 a
PP	41.5	34.1	39.3 bc	38.3 b	62.9	49.9	51.4 b	54.7 ab
Average	43.0 a	34.6	40.4 b	39.3	61.1 a	46.6 c	51.6 b	53.1
$F_{(cutting)}$	2.0	0.5	25.5**	4.6**	1.2	2.9	13.0**	3.6*
$F_{(years)}$				46.4**				44.5**
$F_{(cutting\ x\ years)}$				1.9				1.5
$LSD_{(cutting)\ (0.05)}$	5.0	2.7	1.8	2.1	8.6	4.2	3.8	3.7
CV (%)	7.2	4.9	2.8	6.4	8.8	5.6	4.6	8.3

*IFP: initial flowering period, MFP: mid-flowering period, FFP: full flowering period, PP: the period when grains in the lower pods reach full maturity, **: Significant difference at 1% level, *: Significant difference at 5% level, CV: Coefficient of variation (%)*

Digestible dry matter ratio (DDMR)

The analysis of variance (Table 8) revealed that the digestible dry matter ratios (DDMR) of woolly pod vetch exhibited statistically significant differences (P < 0.01) between years and cutting periods. However, the cutting x year interaction was found to be insignificant. The highest DDMR was identified during the IFP period, with a value of 59.2%. This was followed by the PP, MFP and FFP periods, which exhibited DDMRs of 59.1%, 58.3% and 56.5%, respectively. The difference between the DDMRs determined for the cutting periods was statistically significant in the third year (P < 0.01), but not in the first and second years. The highest DDMR was obtained in the second year, with a value of 62.0%. In the first and third years, DDMR decreased from IFP to FFP, reflecting the progression of the development period. In contrast, it increased in the PP period. In the second year, however, it exhibited a slight increase from IFP to FFP and a decrease in the PP period. This discrepancy in DDMR between years can be attributed to the rainfall in May and the vegetative growth of the plants. The DDMRs obtained according to the three-year averages of our study were similar to those reported by Coşkun and Çaçan (2019) for the PP period (59.6%), comparable to the MFP period of Ova and Uslu (2021) who mowed hairy vetch in the same periods (62.3, 58.6, 61.6, 60.0%), and lower than the other periods. These findings are

consistent with those reported by Özdemir et al. (2021) in the PP period of hairy vetch (58.1-61.3%). The discrepancy in results between cropping periods may be attributed to species-specific differences.

Relative feed value (RFV)

The analysis of variance (Table 8) revealed that the relative feed value (RFV) of woolly pod vetch was statistically significant for both years ($P < 0.01$) and cutting periods ($P < 0.05$). However, the cutting x year interaction was found to be insignificant. The mean results indicated that the highest RFV was observed in the IFP period, with a value of 111.3, followed by the MFP, PP, and FFP periods, which exhibited mean values of 107.1, 102.4, and 98.5, respectively. The discrepancy between the RFVs determined for the cutting periods was statistically significant in the third year, but not in the other years. The highest RFV was observed in the second year, which was characterised by a lack of precipitation, with a value of 124.2. This result can be attributed to the impact of precipitation in May on vegetative development in plants. The RFV values obtained in this study were found to be lower than reported by Coşkun and Çaçan (2019) during PP period (118.6). It was found to be higher than the YY period and lower than other periods of Ova and Uslu (2021), who harvested hairy vetch in the same periods (138.3, 100.78, 124.60, 111.82). It was found to be lower than (112-120) Özdemir et al.(2021) In the BB period for hairy vetch. The RFV that we obtained may be due to the change in climatic conditions. The difference in the RFV can be explained by the differences in the climatic conditions of the year and the type of vetch.

Table 8. Digestible dry matter ratio (%) and relative feed value of plants at different growth stages

	Digestible dry matter ratio				Relative feed value			
Developmental stages	2010-2011	2011-2012	2012-2013	Average	2010-2011	2011-2012	2012-2013	Average
IFP	56.8	61.5	59.3 a	59.2 a	91.8	124.3	117.7 a	111.3 a
MFP	55.3	61.7	57.9 b	58.3 a	87.4	126.4	107.3 ab	107.1 ab
FFP	53.0	62.4	54.2 c	56.5 b	78.1	129.5	87.9 c	98.5 b
PP	56.6	62.3	58.3 ab	59.1 a	84.6	116.7	106.1 b	102.4 ab
Average	55.4 c	62.0 a	57.4 b	58.3	85.5 c	124.2 a	104.7 b	104.8
$F_{(cutting)}$	2.0	0.5	25.5**	4.6**	1.2	2.9	13.0**	3.6*
$F_{(years)}$				46.4**				52.6**
$F_{(cutting\ x\ years)}$				1.9				1.5
$LSD_{(cutting)\ (0.05)}$	5.0	2.7	1.8	2.1	8.6	4.2	3.8	3.7
CV (%)	7.2	4.9	2.8	6.4	8.8	5.6	4.6	8.3

*IFP: initial flowering period, MFP: mid-flowering period, FFP: full flowering period, PP: the period when grains in the lower pods reach full maturity, **: Significant difference at 1% level, *: Significant difference at 5% level, CV: Coefficient of variation (%)*

Conclusion

The main purpose of vetch cultivation is to obtain more economic income and provide feed production without losing yield in the main product. Different climatic conditions emerged each year in this study, which covered three years. Woolly pod vetch produced a significant yield in the study's first year under ideal rainfall and temperature conditions. This clearly shows that he has high potential. Considering the variable climate conditions, the most suitable time for cutting may vary according to the producer's preferences. In later years, when plant development slows down due to decreased rainfall during the growing season, the most suitable harvesting time is when the grains in the lower pods reach full maturity. The three-year average results show that harvesting woolly pod vetch at full flowering, when grown for hay, is a good practice. However, it is possible to delay cutting by 15 days in the second year and 20 days in the third year, i.e. to leave the field for a longer period. In this instance, the producer must decide between the potential gain from forage and the possibility of yield loss in the main crop resulting from delayed vetch cutting. Additionally, it is crucial to note that woolly pod vetch, which

exhibits horizontal growth characteristics, should be sown in conjunction with cereals in regions experiencing a rainy spring season.

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Authors' Contribution: : ZM designed the study and set up the experiments, ZM, SÜ and BE conducted the study, ZM and SÜ analyzed the data, and ZM, SÜ and BE wrote the article.

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Araştırma Makalesi/Research Article

Şeker pancarında (*Beta vulgaris* L.) bor dozu ve sulama zamanlamasının verim ve bazı kalite parametreleri üzerine etkisi

Effect of boron dosage and irrigation timing on yield and some quality parameters in sugar beet (Beta vulgaris L.)

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ÖZ

Bu araştırma, bor dozu ve sulama uygulamasının şeker pancarının (*Beta vulgaris* L.) kök verimi ve mineral madde konsantrasyonları üzerindeki etkilerini belirlemek amacıyla yapılmıştır. Araştırma 2022 yılında Kayseri ili Pınarbaşı ilçesi Pazarönü köyünde Tesadüf Bloklarında Bölünmüş Parseller Deneme Desenine göre üç tekerrürlü tarla denemesi olarak yürütülmüştür. Araştırmada üç şeker pancarı çeşidi (Salama, Portofina, Indira), 3 sulama uygulama zamanı (çıkış öncesi, 1. sulama ve 3. sulama) ve 3 Bor dozu (0=kontrol, 200 ve 400 g da^{-1}) uygulanmıştır. Araştırmada; pancar kök verimi, şeker oranı, azot (N), potasyum (K), sodyum (Na) ve toplam bor içeriği incelenmiştir. Araştırma sonucu; çeşitler arasında incelenen bütün parametreler bakımından önemli farklılığın olduğu, sulama zamanları arasında verim, K ve Bor içeriği bakımından, Bor uygulama dozları arasında ise verim, Na, K ve Bor içeriği bakımından önemli farklılığın olduğu belirlenmiştir. İlave olarak interaksiyonlardan çeşit x sulama interaksiyonunun Na ve K miktar; çeşit x Bor dozu interaksiyonunun verim, N, K ve Bor içeriği; çeşit x sulama zamanı x Bor dozu üçlü interaksiyonunun verim ve şeker oranı üzerinde önemli farklılıklar oluşturduğu belirlenmiştir. En yüksek pancar kök verimi 8682.10 kg da^{-1} ile Indira çeşidinden çıkış öncesi sulama ve 200 g da $^{-1}$ bor dozu uygulamasından, en yüksek şeker oranı %16.54 ile Portofina pancar çeşidinin 1. Sulama zamanında bor uygulamasının kontrol grubundan elde edilmiştir. Elde edilen bulgular; dikkate alındığında Salama ve Portofina şeker pancarı çeşitlerinde sulamanın 3. zamanda ve 200 da-1 bor dozu kullanılarak yapılmasının, Indira şeker pancarı çeşidinde ise sulamanın çıkış öncesi ve 200 da-1 bor dozu kullanılarak yapılması önerilebilir.

Anahtar Kelimeler: Şeker pancarı, Bor gübrelemesi, sulama yönetimi, verim, mineral madde

ABSTRACT

This research was conducted to determine the effects of Boron dose and irrigation application on the root yield and mineral substance concentrations of sugar beet (*Beta vulgaris* L.). For this purpose, a three-replication field experiment was conducted in Pazarönü village of Pınarbaşı district of Kayseri province in 2022 according to the Split Plots Experiment Design in Randomized Blocks. In the research, three sugar beet varieties (Salama, Portofina, Indira), 3 irrigation application times (pre-emergence,

1st Irrigation and 3rd Irrigation) and 3 Boron application doses (0=control, 200 and 400 g da-1) were applied. In the research, beet root yield, sugar content, nitrogen (N), potassium (K), sodium (Na) and total boron content were examined. As a result of the research, it was determined that there were significant differences in all parameters examined among the varieties, significant differences in yield, K and Boron content among irrigation times, and significant differences in yield, Na, K and Boron content among Boron application doses. In addition, it was determined that variety x irrigation interaction caused significant differences on Na and K content, variety x Boron dose interaction caused yield, N, K and Boron content, variety x irrigation time x Boron dose triple interaction caused yield and sugar content. The highest sugar beet yield of 8682.10 kg da-1 was obtained from Indira variety with pre-emergence irrigation and 200 g da -1 boron dose application, and the highest sugar content of 16.54% was obtained from Portofina beet variety with boron application at the 1st irrigation time from the control group. Considering the findings, it can be recommended that Salama and Portofina sugar beet varieties should be irrigated at the 3rd time using a boron dose of 200 da-1, while Indira sugar beet variety should be irrigated before emergence using a boron dose of 200 da-1.

Key Words: Sugar beet, Boron fertilization, irrigation management, yield, mineral substance

Giriş

Şeker pancarı (*Beta vulgaris* L.), dünya genelinde şeker üretiminde yaygın olarak kullanılan ve ekonomik değeri yüksek olan bir bitkidir. Şeker pancarı tarımı, sağlamış olduğu istihdam olanakları ve yarattığı katma değer ile ülkemizin tarım sektörü ve şeker endüstrisi için hayati önem taşımaktadır. Türkiye, yıllık 20 milyon ton civarında şeker pancarı ve 2.5-3 milyon ton civarında şeker üretimi yapmaktadır (TÜİK, 2024). Türkiye'deki şeker pancarı verimi dünya ortalamasına göre yüksek olup, genellikle hektar başına 60-70 ton civarındadır. Bu değer, modern tarım tekniklerinin kullanımına, sulama olanaklarına ve iklim koşullarına bağlı olarak bölgesel farklılıklar göstermektedir (Kanat, 2023).

Sürdürülebilir tarım sistemlerinde, verim ve kaliteyi optimize edebilmek için sulama, gübreleme, ilaçlama gibi yetiştirme tekniği paketi uygulamalarının yetiştirme sürecinde bitkiye zamanında ve dengeli bir şekilde uygulanması gerekir. Bor, bitki büyümesi ve gelişimi için hayati öneme sahip bir mikro besin elementidir. Özellikle hücre duvarı sentezi, membran bütünlüğü, karbonhidrat ve azot metabolizması gibi süreçlerde rol oynamaktadır (Marschner, 2012). Şeker pancarı, bor ihtiyacı nispeten yüksek olan bitkilerden birisi olup, özellikle kök gelişimi, şeker birikimi ve şeker oranı üzerinde önemli bir etkiye sahiptir. Bor eksikliğinde veya fazlalığında şeker pancarında büyüme, verim ve kalite olumsuz olarak etkilenmektedir (Saenz, 2001). Bor elementinin çok boyutlu işlevleri dikkate alındığında; topraktaki bor noksanlığı ya da fazlalığı durumu bitki yetiştiriciliği açısından dikkate alınması gereken bir konudur. Toprakta bor noksanlık düzeyi 0,5 mg/kg olarak kabul edilmiş olmakla birlikte topraklardaki B yeterli sınıfında yer alsa bile B isteği yüksek olan şeker pancarında noksanlık belirtilerinin görülebileceği (Pendias, 2010), Kahramanmaraş ilinde tarım alanlarının yarayışlı B miktarının 0.01-0.99 mg kg^{-1} B ile çok az ve az sınıfında yer aldığı (Yılmaz, 2020), Orta Anadolu'daki tarım alanlarının %62'sinde B noksanlığının olabileceği (Eyüpoğlu ve ark., 2002) rapor edilmiştir

Şeker pancarı bitkisi ile mikro besin elementi (Bor) ilişkisi birçok araştırmaya konu olmuştur. Nitekim yapılan araştırmalarda; en yüksek şeker verimi ve en iyi şeker kalitesi 100 ppm bor konsantrasyonunda elde edildiği (Abdel-Motagally, 2015), dekara 0,18 kg B uygulamasının yumru verimini artırdığı (Durak ve Ulutaş, 2017), şeker pancarına yaprak gübresi olarak Trisert-CB'nin uygulaması ile yaprak veriminin %19.4, kök–gövde veriminin %7.4 arttığı (Okurcan ve Er, 1999), yapraktan 1.0 kg ha^{-1} Bor uygulaması ile kontrole göre kök veriminin %19.4 ve şeker veriminin %39.5 arttığı (Kristek ve ark., 2006), kontrole göre verimin; topraktan B uygulaması ile %6, yapraktan B uygulaması ile %8.7 arttığı, toprakta noksanlığı durumunda B uygulamasının verimi ve mineral madde miktarını artırdığı (Şener, 2015), Orta Anadolu koşullarında şeker pancarında 0.3 kg da^{-1} bor'un toprak+yaprak, toprak ve yaprak şeklinde uygulamasının kök verimini sırası ile %12.5, %12.1 ve %11.1 ve

arıtılmış şeker verimini sırasıyla %8.7, %18.3 ve %3.5 oranında artırdığı (Gezgin ve ark., 2001), yaprak B konsantrasyonunun; yapraktan B uygulamasında 64 mg kg^{-1}, topraktan bor uygulamasında 42 mg kg^{-1} ve toprak+yaprak B uygulamasında 58.5 mg kg^{-1} olduğu, kök veriminin kontrole göre toprağa 0.30 kg da^{-1} borun uygulamasında %5.7 ve toprak+yaprağa 0.45 kg da^{-1} bor uygulamasında %7.4 arttığını, şeker veriminin kontrole göre; toprağa ve toprak+yaprağa 0.45 kg da^{-1} bor uygulaması ile sırasıyla %3.8 ve %7.3 oranlarında arttığı (Gezgin ve ark., 2007) rapor edilmiştir.

Kahramanmaraş Elbistan'da toprak ve bitki örneklerinin büyük bir kısmının bor açısından kritik sınırların altında olduğunu, dekara 450 g B uygulamasının arıtılmış şeker veriminde %18.7'lik bir artış sağladığı ve bor gübrelemesinin şeker pancarı tarımında verim ve kaliteyi artırmada önemli bir rol oynadığı (Kara, 2022), aynı bölgede dekara 150 g B ve üzeri uygulamaların kök verimi ve arıtılmış şeker veriminde anlamlı artışlar sağladığı ve bor gübrelemesinin şeker pancarı tarımında verim ve kaliteyi artırmada etkili olduğu (Ceylan, 2021) ve aynı bölgede şeker pancarı üretiminde ekonomik ve çevresel açıdan 2-3 yılda bir dekara 0.6 kg B uygulaması gerektiği (Bilir ve Saltalı, 2023) rapor edilmiştir. Ayrıca yapılan araştırmalar bor uygulanma zamanının önemli olduğu, en yüksek kök ağırlığının çıkışı itibaren 60.günde 1.22 kg ha^{-1} bor uygulamasından elde edildiği (Armin ve Asgharipour, 2012), 100 ve/veya 150 ppm B'un ekimden 90 gün sonra yapraktan uygulaması durumunda en yüksek kök ve şeker veriminin elde edildiği (Nemeata Alla,2017) rapor edilmiştir

Şeker pancarında verim ve kaliteyi etkileyen diğer önemli bir tarımsal faaliyet de sulamadır. Şeker pancarı su ihtiyacı fazla olan bir bitki olup, büyüme ve gelişim süreçlerinde su kritik bir rol oynar. Yetiştirme sezonu boyunca su stresine maruz kalması durumunda şeker pancarı verimi azalır, şeker oranı düşer. Dolayısıyla bitkinin su ihtiyacının çimlenme, vejetatif büyüme, kök ve şeker birikimi dönemi gibi kritik dönemlerde karşılanması, yüksek verim ve kaliteli şeker elde edilmesi açısından hayati derecede önem taşımaktadır (Hoffmann ve ark., 2009). Nitekim Ankara koşullarında damla sulama yöntemi ile beş farklı N dozu (dekara 10, 13, 16, 19 ve 21 kg N) uygulanan şeker pancarında en yüksek şeker oranı dekara 10 kg N dozunda elde edildiği (Pişkin ve Ünal, 2014), Orta Anadolu koşullarında uygulanan farklı sulama programlarının şeker pancarının şeker oranı, potasyum (K), sodyum (Na) ve alfa-azot (αN) içerikleri üzerinde önemli etkileri olduğu, özellikle, sulama aralığı ve miktarının optimize edilmesinin, şeker pancarının kalite özelliklerini iyileştirdiği (Tarı ve ark., 2016), Kırşehir koşullarında sulama suyu miktarının artırılmasıyla kök veriminin ve şeker oranının arttığı, ancak, su israfını önleme açısından optimum sulama programlarının belirlenmesinin önemli olduğu (Kara ve Öztürk, 2022) bildirilmiştir.

Şeker pancarında bor dozu ve sulama zamanı arasındaki etkileşim, bitki verimi ve kalitesini doğrudan etkileyen diğer kritik bir faktördür. Bor elementinin bitki tarafından alınması ve verimli bir şekilde kullanılabilmesi için suyun zamanında ve yeterli miktarda sağlanması gerekir. Bor elementinin taşınması ve kullanılabilir hale gelmesinde toprak nemi kritik rol oynadığı için Bor uygulaması ile sulama yönetiminin birlikte ele alınması, borun bitki tarafından alımını artırmak için sulama zamanının doğru bir şekilde tespit edilmesi gerekir (Nable ve ark., 1997). Yetersiz ya da aşırı sulama, Bor elementinin toprakta çözünürlüğünü ve bitki tarafından alımını olumsuz etkiler (Goldbach ve ark., 2001). Bu nedenle, şeker pancarı yetiştiriciliğinde Bor uygulaması ve sulama zamanlamasının birlikte ele alınması, bitkinin su ve besin elementlerine dengeli bir şekilde ulaşmasını sağlayacak koşulların sağlanması gerekir.

Bor mikro elementinin şeker pancarının verim ve kalitesine etkileri yukarıda da örnekleri verildiği gibi çeşitli araştırıcılar tarafından araştırılmasına karşın, bu araştırmanın yapıldığı bölgede, bu konu bugüne kadar araştırılmamıştır. Ayrıca, araştırma alanı topraklarının analizi sonucunda bor miktarının 0.47 mg kg^{-1} olarak noksan seviyesinde

olduğu tespit edilmiştir. Dolayısıyla bu araştırma; bölgede şeker pancarının verimini ve kalitesini olumsuz yönde etkileyen bor eksikliği sorununu, Bor gübrelemesini ve su yönetimini birlikte değerlendirerek, araştırmak amacıyla yürütülmüştür.

Materyal ve Metot

Araştırma alanının toprağı; hafif alkali, fazla kireçli ve toprak elektriksel iletkenliği (EC) hafif tuzsuz olup, araştırmanın yürütülmesinde önem arz eden alınabilir B içeriği ise kritik (0.47 mg/kg) seviyededir (Çizelge 1).

Çizelge 1. Deneme toprağının bazı fiziksel ve kimyasal özellikleri
Table 1. Some physical and chemical properties of the experimental soil

Özellik ***Feature***	**Değer** ***Value***	**Sonuç** ***Result***
pH	7.70	Hafif Alkali
EC	1.21 dS m^{-1}	Hafif
Tuz	% 0.0605	Tuzsuz
Kireç	% 19.30	Fazla kireçli
Fosfor	9.09 kg da^{-1}	Yeterli
Potasyum	142.30 kg da^{-1}	Yeterli
Bakır	0.32 mg kg^{-1}	Yeterli
Demir	2.24 mg kg^{-1}	Az
Çinko	1.02 mg kg^{-1}	Yeterli
Mangan	2.26 mg kg^{-1}	Yeterli
Kalsiyum	6744.0 mg kg^{-1}	Fazla
Magnezyum	543.1 mg kg^{-1}	Fazla
Sodyum	85.2 mg kg^{-1}	Fazla
Bor	0.47 mg kg^{-1}	Az

Araştırmada bitki materyali olarak Salama (kök tipi), Portofina (şeker tipi) ve Indira (kök-şeker tipi) olmak üzere bölgede sözleşmeli şeker pancarı tarımı yaptıran KWS şirketine ait 3 pancar çeşidi kullanılmıştır. Bor uygulaması çıkış öncesi, 1. sulama (6 yapraklı dönen) ve 3. sulama (10 yapraklı dönem) olmak üzere 3 farklı sulama zamanında sulama suyu ile damla sulama sistemi içinde homojen bir biçimde ve aynı yoğunluk ve su basıncında olacak şekilde uygulanmıştır. Bor gübresi kontrol, 200 g da^{-1} ve 400 g da^{-1} olmak üzere 3 farklı uygulama dozu olacak şekilde uygulanmıştır.

Deneme yeri toprağı ekime hazır duruma getirildikten sonra bütün parsellere dekara 10 kg N ve 10 kg P_2O_5 olacak şekilde üre gübresi formunda serpilip karıştırılarak uygulanmıştır. Daha sonra dekara 5 kg N ikinci çapa ve 5 kg N'da birinci su öncesi (üre gübresi formunda) olmak üzere ekim öncesiyle birlikte dekara toplam 20 kg saf N uygulanmıştır.

Deneme, Tesadüf Bloklarında Bölünen Bölünmüş Parseller Deneme Desenine göre 3 tekerrür olarak kurulmuştur. Ekim, 2022 yılı Nisan ayının son haftasında, pnömatik mibzer kullanılarak yapılmıştır. Denemede; çeşitler ana parsellerde, sulama uygulaması alt parsellerde ve Bor gübre dozları alt-alt parsellerde yer almıştır. Her bir parselde 4,5 m uzunluğunda 6 sıra yer almış olup, sıra arası 45 cm ve sıra üzeri 18 cm olarak ayarlanmıştır. Denemede her bir parsel alanında (4.5mx0.45x6=12.15 m^2) toplam 150 (25x6) bitki yer almıştır. Denemenin yürütüldüğü periyot boyunca; şeker pancarı yetiştiriciliğinde rutin olarak önerilen bakım işlemleri yapılmıştır. Denemenin hasadı, şeker pancarı şeker pancarı bitkileri fizyolojik olgunluğa eriştikleri 15.10.2023 tarihinde yapılmıştır. Hasat öncesi, her bir parselin her iki başından 0.54 m (3'er bitki) ve her iki kenardan birer sıra (0.45x2=0.9 m) kenar tesiri olarak atılmıştır. Geriye kalan hasat alanındaki (1.80x3.42 m= 6.156m^2) bitkiler (19x4=76 bitki) hasat beli kullanılarak, kökleri zarar görmeyecek hassasiyette sökülmüştür.

Araştırmada pancar verimi (kg da^{-1}) için her parselin hasat alanından elde edilen toplam yumru ağırlıkları tartılıp, dekara çevrilmiş ve kg olarak kaydedilmiştir. Araştırmada incelenen

şeker oranı, azot, potasyum ve Na miktarı için gerekli analizler Kayseri Şeker Fabrikası Kalite Analiz Laboratuvarında yapılmıştır. Şeker oranı (%) için her parselden rastgele seçilen 10 yumru örneği ortadan ikiye ayrılmış ve orta kısımdan bir damlaya çıkacak şekilde pancar sıyrılmıştır. Dijital refraktometre ile kuru madde içeriğinden elde edilen ve cihazda okunan değer 0.8 ile çarpılarak şeker oranı belirlenmiştir. Daha sonra ölçülen 10 yumru örneğinin ortalaması alınarak yumrudaki ortalama şeker oranı (%) hesaplanmıştır. Toplam azot (N) içeriği Kjeldahl Yöntemi kullanılarak (Chapman ve Pratt, 1961) belirlenmiştir. Toplam potasyum (K) miktarını hesaplamak için bitki örnekleri kül fırınında yakıldıktan sonra 3N HCl ile ekstrakt edilmiş ve çözeltideki K alev fotometresinde okunarak bulunmuştur. Toplam sodyum (Na) miktarını hesaplamak için Nitrik asit ve perklorik asit ($HNO_3/HClO_4$) ile yakılan yaş bitki numunelerinde Na içeriği ICP-OES cihazı ile belirlenmiştir. Toplam Bor miktarının analizi ICPOes Yöntemine (Kacar ve İnal, 2008) göre OMÜ, Ziraat Fakültesi, Toprak Bilimi ve Bitki Besleme Bölümü laboratuvarında yapılmıştır.

Elde edilen verilerin analizinde SPSS Statistics Programı Ver 17.0 kullanılmış olup, ortalamalar arasındaki farklılıkların belirlenmesinde Duncan Çoklu Karşılaştırma Testi (Gomez ve Gomez, 1984) kullanılmıştır.

Araştırma Bulguları ve Tartışma

Pancar kök verimi

Yapılan analiz sonucu; çeşit, sulama zamanı, bor uygulaması, çeşit x bor uygulama interaksiyonu ve çeşit x sulama zamanı x bor uygulama üçlü interaksiyonlarının kök verimi bakımından istatistiki anlamda önemli farklılıklar oluşturduğu saptanmıştır (Çizelge 2 ve Çizelge 3). En yüksek kök verimi Indira çeşidinden (8047.15 kg da^{-1}) elde edilmiştir. Ayrıca en yüksek kök verimi 7789.44 kg da^{-1} ile çıkış öncesi sulamadan, 8311.04 kg da^{-1} ile 200 200 g da^{-1} bor uygulama dozunda elde edilmiştir. Çeşit x bor uygulaması interaksiyonu incelendiğinde en yüksek kök verimi Salama çeşidinin 200 g da^{-1} ve 400 g da^{-1} bor dozu (sırasıyla 8545.27 ve 8477.88 kg da^{-1}) uygulamasında belirlenmiştir. Çeşit x sulama x bor dozu üçlü interaksiyon bakımından en yüksek kök verimi Indira çeşidinin çimlenme öncesi sulama zamanının 200 g da^{-1} bor dozu uygulamasından (8682.10 kg da^{-1}) ve Salama çeşidinin 3. sulama zamanı ile çıkış öncesi sulama zamanında 200 g da^{-1} bor dozu uygulamalarından (sırasıyla 8660.49 ve 8635.80 kg da^{-1}) elde edilmiştir (Çizelge 2 ve Çizelge 3).

Pancar kök verimi, birim alandan elde edilen pancar miktarını ifade eder ve şeker pancarı yetiştiriciliğinde üretim miktarının değerlendirilmesinde en önemli göstergedir. Kök verimi, pancar üretiminde karlılığı doğrudan etkileyen bir faktördür. Kök verimi, pancarın büyüklüğü, kök gelişimi ve genel sağlığıyla da ilişkilidir. Yüksek kök verimi, bitkinin sulama, gübreleme ve besin elementlerinden iyi bir şekilde yararlandığının da bir göstergesidir. Şeker pancarı, suya oldukça hassas bir bitkidir ve yeterli su temini bitkinin verimini doğrudan etkiler. Yetersiz sulama bitkinin gelişimini sınırlandırabileceği gibi kök büyümesini de engelleyebilir. Aşırı sulama ise köklerde çürüme ve hastalıkların etkinliğinin artmasına zemin hazırlayarak verimi düşürebilir. Optimum sulama zamanı, bitkinin maksimum verim kapasitesine ulaşmasına olanak sağlar. Bu nedenle, sulama zamanlaması ve miktarının dengeli bir şekilde uygulanması zorunludur. Şeker pancarında sulama yönetimi özellikle su yetersizliğine duyarlı olan bitki gelişiminin erken aşamalarında kritik bir rol oynar (Kenter ve ark., 2006*)*. Nitekim bu araştırmada çıkış öncesi yapılan sulamadaki pancar veriminin (7789.44 kg da^{-1}) 1. Sulama zamanındaki pancar verimine göre %0.83 (7725.31 kg da^{-1}) ve 3. sulama zamanındaki pancar verimine göre ise %1,57 (7668.90 kg da^{-1}) daha fazla olduğu tespit edilmiştir. Eksik sulama stratejilerinin şeker pancarı verimi ve kalitesi üzerindeki etkilerinin incelendiği bir araştırmada da suyun uygun zamanda ve optimal düzeyde kullanılması ile şeker pancarı kök veriminde bir düşüş olmadan su tasarrufu sağlanabileceği ancak, büyüme döneminde şeker pancarına yeterli miktarda su verilmemesi durumunda kök

veriminin önemli ölçüde azalmakta olduğu (Kıymaz ve Ertek, 2015), sulama aralığı ve miktarının optimize edilmesinin şeker pancarında kalite özelliklerini iyileştirdiği (Tarı ve ark., 2016) belirlenmiştir.

Bor, hücre bölünmesi ve büyümesinde rol oynayan önemli bir elementtir. Özellikle kök gelişimi ve şeker birikimi için kritik öneme sahiptir. Bor yetersizliğinde köklerin gelişimi ve kök verimi olumsuz etkilenir. Bor uygulaması köklerin daha güçlü gelişmesini sağlayarak, kök verimini artırır (Enan ve ark., 2016; Nemeata Alla, 2017; Abdel-Nasser ve Ben Abdalla, 2019). Yapılan bir araştırmada bor uygulaması ile kök veriminin kontrole (7223 kg da^{-1}) göre %12.65 daha yüksek (8137 kg da^{-1}) olduğu belirlenmiştir (Pişkin, 2022). Bu araştırmada da 200 g da^{-1} bor uygulaması ile kontrole (7235.94 kg da^{-1}) göre %14.86 daha fazla kök verimi (8311.04 kg da^{-1}) elde edildiği orta Anadolu'da yapılan bir başka araştırma da dekara 0.3 kg bor uygulamasının kök verimini %12,1 oranında artırdığı (Gezgin ve ark., 2001) belirlenmiştir.

Bu araştırmada her ne kadar sulama zamanı ve bor interaksiyonu istatistiki anlamda önemli bulunmamış olmakla birlikte çıkış öncesi, 1. sulama (6 yapraklı) ve 3. sulama (10 yapraklı) olmak üzere her üç dönemde de su ve bor uygulamasının kontrole göre pancar veriminde artış ortaya koyduğu tespit edilmiştir. Diğer taraftan çeşit x sulama x bor üçlü interaksiyonunun istatistiki anlamda önemli olduğu belirlenmiştir. Bu bulgu; sulama ve bor uygulaması yapılacaksa çeşit faktörünün de daima göz önünde bulundurulması gerektiğini göstermektedir. Nitekim üçlü interaksiyonlar dikkate alındığında bütün çeşitlerde ve bütün sulama zamanlarında bor uygulamasının kontrole göre pancar verimini artırdığı belirlenmiştir. Bor eksikliği özellikle kuru koşullarda daha yaygındır ve yetersiz sulama borun bitki tarafından kullanılabilirliğini sınırlar. Yetersiz veya düzensiz sulama koşullarında bitkinin bor alımı azalacağı için özellikle şeker pancarı gibi bor ihtiyacı yüksek bitkilerde verim kaybına neden olur (Brown ve ark., 2002).

Şeker oranı (%)

Araştırma sonucu; çeşit ve çeşit x sulama zamanı x bor uygulaması üçlü interaksiyonunun şeker oranı bakımından önemli farklılıklar oluşturduğu belirlenmiştir. En yüksek şeker oranı %16.24 ile Portofina pancar çeşidinden elde edilmiştir. Üçlü interaksiyon bakımından en yüksek şeker oranı ise Portofina çeşidinin 1. ve 3. sulama zamanlarının kontrol grubundan (sırasıyla %16.54 ve %16.45) ve Indira çeşidinin 1. sulama zamanı ile 200 g da^{-1} bor dozu uygulamasından (%16.46) elde edilmiştir (Çizelge 2 ve Çizelge 3).

Şeker oranı, şeker pancarı üretiminin ana hedeflerinden biridir. Şeker pancarının kökünde biriken şeker miktarı, yetiştiricilikte ekonomik açıdan büyük önem taşımaktadır. Şeker oranı, doğrudan pancarın kalitesini ve elde edilecek şeker miktarını belirler. Şeker pancarının şeker oranı üzerine çeşit, iklim, bitki besin elementleri, sulama, hastalık vb. birçok faktör etkilidir. Şeker pancarında oluşan ürün miktarı arttıkça şeker miktarı düşmektedir. Bu araştırmada elde edilen sonuçlar da bu durumu teyit etmektedir. Sulama zamanı, bitkinin şeker üretimi üzerinde doğrudan etkilidir. Aşırı sulama, pancar içinde su birikmesine ve şekerin köklerde yeterince yoğunlaşmamasına neden olabilir, bu da şeker oranını düşürür. Yetersiz sulama ise bitkide su stresine yol açarak fotosentez kapasitesini ve dolayısıyla şeker üretimini azaltır. Nitekim şeker pancarına uygulanan sulama rejimlerinin bitki büyümesi ve şeker kalitesi üzerinde belirgin bir etkisi olduğu, özellikle büyümenin kritik dönemlerinde doğru sulama yapılmamasının hem şeker oranını hem de verimi olumsuz etkilediği, yetersiz sulama durumunda ise kök büyümesinin sınırlanmış olduğu ve kökteki şeker oranının azaldığı tespit edilmiştir (Milford ve ark., 1985). Diğer taraftan şeker oranı ve veriminin artırılması için bor uygulamasının sulama rejimiyle birlikte ele alınması gerektiği (Abdel-Nasser ve Ben Abdalla, 2019), bor eksikliğinin şeker pancarında hücre bölünmesini ve şeker birikimini engelleyerek şeker oranının azalabileceği (Kacar ve ark., 2009), borun bitki metabolizmasında

karbonhidrat sentezi ve taşınmasında kritik rol oynadığı bilinmektedir. Bununla birlikte kullanılan çeşitlerin sulama ve bor uygulamasına karşı göstermiş oldukları tepkiler şeker oranının değişmesinde de önemli bir faktördür. Nitekim bu araştırmada ele alınan çeşitler ve çeşit x sulama x bor üçlü interaksiyonu dışında diğer uygulamaların istatistiki anlamda önem arz etmemesi, bor ve sulama uygulamasının yalın olarak uygulanmasının her zaman beklendiği gibi bir sonuç ortaya koyamayacağını göstermektedir. Diğer taraftan bor uygulamasının Salama pancar çeşidinde olduğu gibi erken su uygulamaları ve yüksek bor dozlarında şeker oranını artırabileceği tespit edilmiştir. Çeşitler arasında bu tür farklılıkların ortaya çıkması hiç şüphesiz ki genotip faktörünün metabolik faaliyet üzerindeki etkisinin yoğunluğunu göstermesi bakımından önem arz etmektedir. Nitekim borun uygun düzeyde uygulanmasının şeker oranını artırdığına ilişkin birçok araştırmalar mevcuttur. Kristek ve ark. (2006) bor uygulamasıyla en yüksek %14.92 şeker oranı elde ettiklerini; Abbas ve ark. (2014) yapraktan 0.25 g L^{-1} bor uygulamasıyla şeker oranının %16.1'e yükseldiğini bildirmişlerdir.

Sodyum (Na) miktarı

Araştırmada bitkide Na miktarı çeşit, bor uygulaması ve çeşit x sulama zamanı interaksiyonu bakımından önemli farklılıklar ortaya koymuştur ($p<0.05$). Çeşit bazında en düşük Na miktarı Salama çeşidinden (1.31 mmol 100 g^{-1}) elde edilmiştir. Bor uygulaması bakımından en düşük Na miktarı kontrol dozundan (1.48 mmol 100 g^{-1}) elde edilmiştir. Çeşit ve sulama zamanı interaksiyonu değerlendirildiğinde; bitkide en düşük Na miktarı Salama çeşidinin çimlenme öncesi sulama zamanından (1.25 mmol 100 g^{-1}) elde edilmiştir (Çizelge 2 ve Çizelge 3).

Şeker pancarı gibi bazı bitkiler, düşük seviyelerde sodyumdan fayda görebilir; sodyum, su ve iyon dengesinin korunmasına yardımcı olur. Ancak, sodyumun fazla olması toprakta tuzluluk sorunlarına neden olabilir ve bu da bitkilerde osmotik stres yaratarak su ve besin alımını zorlaştırır. Şeker pancar kökünde bulunan ve şeker dışı maddeler arasında melas oluşturucular olarak bilinen sodyum varlığı büyük önem taşımaktadır. Sodyum, sakarozun suda çözünürlüğünü artırır, dolayısıyla kristalizasyonu zorlaştırarak melasın şeker miktarını yükseltir. Bu nedenle şekerin arıtılmasını güçleştiren sodyum varlığının şeker pancarı kökünde düşük olması istenir. Pancarda Na miktarının fazlalığı safiyet bozucu olarak değerlendirilir ve şeker pancarında Na içeriğinin 1.74 mmol 100 g^{-1} 'dan yüksek olması istenmez (Akyar ve ark., 1980). Yapılan bir araştırmada şeker pancarı kökünün Na içeriğinin 1.69-1.90 mmol 100 g^{-1} pancar arasında değiştiği rapor edilmiştir (Pişkin, 2021). Bu araştırmada da Na içeriğinin çeşitler arasında 1.31-1.79 mmol 100 g^{-1} arasında değiştiği tespit edilmiştir. Elde edilen bu değerlerin üst sınırı (1.79 mmol 100 g^{-1}) şeker pancarında Na'nın bulunmaması gereken üst sınır değerlerinin (1.70 mmol 100 g^{-1}) sınırında yer almaktadır. Na miktarının değişkenlik göstermesinde başta kullanılan şeker pancarı çeşitleri olmak üzere yetiştiricilikte uygulanan sulama ve gübreleme gibi yetiştirme tekniği paketi elemanları rol oynaması beklenir. Nitekim bu araştırmada Bor uygulamasının Na miktarını kontrole (1.46 mmol 100 g^{-1}) göre az da olsa bir miktar artırmış olması, bu durumu yansıtma açısından önemli bir bulgudur.

Sulama zamanı, toprakta sodyum birikimini doğrudan etkiler. Bu araştırmada da her ne kadar istatistiki anlamla önemli düzeyde olmasa da özellikle geciken sulamanın, Na miktarını az da olsa artırdığı tespit edilmiştir. Bu sonuç, şeker pancarında Na birikimi açısından sulamanın erken dönemde yapılmasının daha uygun olduğunu göstermesi bakımından önemli bir bulgu olarak değerlendirilebilir.

Çizelge 2. İncelenen parametrelere ait ana faktörlerin ortalamaları ve analiz sonucu F değerleri
Table 2. Averages of main factors of the examined parameters and F values as a result of the analysis

Faktörler Factors		Sodyum *Sodium (mmol 100 g^{-1})*	Azot (N) *Azote (mmol 100 g^{-1})*	Potasyum (K) *Potassium (mmol 100 g^{-1})*	Şeker Oranı *Sugar Rate* (%)	Pancar verimi *Sugar beet yield* (g da^{-1})	Toplam bor miktarı *Total boron amount* (ppm kg^{-1})
Çeşit	Salama (S)	1.31 a	1.66ab	4.27 a	15.79 b	7672.15 b	82 b
	Portofina (P)	1.64 b	1.51 a	4.59 b	16.24 a	7464.33 c	86 ab
	Indira (I)	1.79 c	1.94 b	4.88 c	15.89 b	8047.15 a	92 a
	F Value	10.81**	16.62*	84.73**	11.93**	79.58**	5.26**
Sulama Zamanı	Çimlenme Öncesi (ÇÖ)	1.59	1.75	4.68 b	15.92	7789.44 a	91 a
	1.sulama	1.56	1.69	4.60 b	16.03	7725.31ab	83 b
	3.sulama	1.60	1.66	4.48 a	15.98	7668.90 b	86 ab
	F Value	0.51ns	2.16ns	5.04*	0.99 ns	3.11**	3.61**
Bor Uygulaması	Kontrol	1.48 a	1.76	4.72 b	16.07	7235.94 c	78 b
	200	1.65 b	1.67	4.49 a	15.91	8311.04 a	79 b
	400	1.63 b	1.67	4.53 a	15.95	7636.66 b	104 a
	F Value	7.84 **	1.12ns	8.90**	2.06 ns	269.87**	44.65**

Bor, hücre duvarının güçlendirilmesi ve iyon dengesi üzerinde etkili olduğundan, bor eksikliği sodyum toksisitesine karşı bitkiyi daha hassas hale getirerek verim ve kalitenin bozulmasına sebep olabilir. Bu araştırmada bor uygulamasının Na miktarını kontrole göre (1.48 mmol 100 g^{-1}) az da olsa artmasına sebep olduğu tespit edilmiştir. Ancak bu artış, şeker pancarında Na miktarının üst sınır olarak kabul edilen (1.70 mmol 100 g^{-1}) değerlerin altındadır (200 g da^{-1} bor dozu için 1.65 mmol 100 g^{-1} ve 400 g da^{-1} bor dozu için 1.63 mmol 100 g^{-1}). Şeker pancarı gibi tuza duyarlı bitkilerde, bor dozunun ve sulama zamanlamasının birlikte yönetilmesi gerektiği, bu faktörlerin sodyum birikimini doğrudan etkilediği bildirilmiştir (Grattan ve Grieve, 1999). Nitekim bu araştırmadan elde edilen bulgular, sulama x bor interaksiyonunun Na bakımından istatistiki anlamda önemli ölçüde değişmediğini ortaya koymuştur. Bununla birlikte sulama x bor interaksiyonunda sulamanın gecikme durumuna bağlı olarak Na miktarının artmış olduğu ancak bu artışa rağmen şeker pancarı için kabul edilen Na miktarının üst sınırının altında değerlere sahip oldukları belirlenmiştir. Diğer taraftan Sulama x Bor interaksiyonuna benzer bir biçimde çeşit x sulama x bor interaksiyonunda da Na miktarındaki değişmeler, Indira çeşidinin 1. sulama 400 g da^{-1} bor dozu ve 3. Sulama 200 g da^{-1} bor dozu hariç, şeker pancarı için üst sınır kabul edilen değerin altındadır. Bu sonuç; bor uygulamasının şeker pancarında sulama ve çeşit faktörü dikkate alınarak yapılması gerektiğini göstermektedir.

Azot (N) miktarı

Araştırmada bitkide N miktarının çeşit ve çeşit x bor uygulaması interaksiyonu açısından istatistiki anlamda farklılık oluşturduğu tespit edilmiştir. Çeşit bazında değerlendirildiğinde; bitkide en düşük N değeri 1.51 mg 100 g^{-1} ile Portofina çeşidinden elde edilmiştir. Çeşit x Bor uygulaması interaksiyonu bakımından ise en düşük N değer 1.46 mg 100 g^{-1} ile Portofina çeşidinin 200 g da^{-1} bor uygulamasından elde edilmiştir (Çizelge 2 ve Çizelge 3).

Azot, bitkilerde büyüme ve gelişme için en önemli makro besin elementlerinden birisidir. Özellikle şeker pancarı gibi yüksek verimli bitkilerde, azotun yeterli düzeyde alınması, bitkinin genel sağlığını ve buna bağlı olarak fotosentez kapasitesini doğrudan etkiler. Azot aynı zamanda bitkide karbonhidrat metabolizmasını düzenleyerek, şeker pancarı gibi köklerinde şeker birikimi yapan bitkilerde önem arz eder. Azotlu bileşikler şeker işlemesinde çöktürülemediğinden azot içeriğinin düşük olması istenir (Mahn ve ark., 2002). Ayrıca abiyotik stresi bulunmayan ve sağlıklı şeker pancarında azot içeriği 1.30-1.70 mmol 100 g^{-1} pancar arasında değişir (Armstrong ve Milford, 1985). Ancak bitki büyüme ve gelişmesi açısından uygulanması gereken azotlu gübrelemeler, bitki azot içeriğini

artırır ve 2.86 mmol 100 g^{-1} pancar üzerindeki değerler pancarın fabrikasyonunu olumsuz etkiler (Akyar ve ark., 1980).

Bu araştırmada çeşitler, uygulamalar ve interaksiyonlara ilişkin elde edilen N miktarı değerleri, fabrikasyon açısından sorun oluşturduğu kabul edilen değerin altındadır. Yapılan bir araştırmada da kompoze gübre kullanıldığında şeker pancarındaki azot içeriğinin 1.32-1.80 mmol 100 g^{-1} pancar arasında değiştiği belirlenmiştir (Pişkin, 2021). Azot metabolizması bor ve sulama gibi yetiştirme tekniği paketi uygulamaları ile yakından ilişkilidir (Kaur ve Gupta, 2005). Azot, bitkide protein sentezi ve büyüme için kritik bir elementtir ve bor eksikliği veya düzensiz sulama, azotun etkin kullanımını sınırlandırarak bitki büyümesini yavaşlatabilir. Ayrıca suyun bitkinin ihtiyacı olduğu zamanda alınmaması, azotun kök bölgesinden bitki bünyesine taşınmasını engeller. Bu da verim ve kalitenin azalmasına etki eder. Nitekim bu araştırmada geciken sulama uygulaması, bor dozu uygulaması ve çeşit bor interaksiyonu kontrole göre N miktarının istatistiki anlamda önemli olmasa da azalmasına sebep olduğu tespit edilmiştir.

Bitkide potasyum (K) miktarı

Araştırmadan elde edilen verilerin istatistiki değerlendirmesi sonucu; bitkide K miktarı açısından çeşit, sulama zamanı, bor uygulaması, çeşit x sulama zamanı ve çeşit x bor uygulaması interaksiyonu bakımından önemli istatistiki farklılıklar belirlenmiştir. Bitkide en düşük K miktarı Salama çeşidi (4.27 mmol 100 g^{-1}); 3.sulama zamanı (4.48 mmol 100/g); 200 g da^{-1} bor uygulamalarından (4.49 mmol 100 g^{-1}) elde edilmiştir. Ayrıca çeşit ve sulama zamanı interaksiyonu açısından bitkide en düşük potasyum miktarı Salama çeşidinin 3.sulama zamanından (4.17 mmol 100 g^{-1}) elde edilirken, çeşit ve bor uygulaması interaksiyonu bakımından en düşük potasyum miktarı Salama çeşidinin 400 g da^{-1} bor uygulamasında (4.17 mmol 100 g^{-1}) tespit edilmiştir (Çizelge 2 ve Çizelge 3).

Potasyum, bitkilerin su dengesi, hücre içi iyon dengesi ve karbonhidrat taşınımı gibi süreçlerde önemli rol oynayan bir makro besin elementidir. Potasyum, stomaların açılıp kapanmasını kontrol ederek bitkide su kullanımını düzenler. Şeker pancar kökünde bulunan ve şeker dışı maddeler arasında melas oluşturucu olarak bilinen potasyum oldukça önemlidir. Potasyum sakarozun suda çözünürlüğünü artırır, dolayısıyla şekerin kristalleşmesini zorlaştırarak melasın şeker miktarını yükseltir. Bu nedenle şekerin arıtılmasını güçleştiren ve şeker pancarı kökünün kalite değerlerinden olan potasyum varlığının düşük olması istenmektedir. Şeker pancarında potasyumun optimum düzeyde bulunması, köklerde şeker birikimini artırır ve bitkinin stres koşullarına karşı direncini yükseltir. Ancak şekerin arıtılmasını zorlaştırdığı için potasyum içeriğinin düşük olması arzu edilir. Ayrıca şeker pancarı kökünün potasyum içeriğinin 5.38 mmol 100 g^{-1} pancar değerini aşması durumunda, şekerin kristalleşmesi de engellenir (Akyar ve ark., 1980).

Yapılan araştırmalarda potasyumca yeterli olan alanda yetiştirilen şeker pancarı kökünün potasyum içeriğinin 3.1-4.9 mmol 100 g^{-1} pancar arasında değiştiği bildirilmiştir (Bee ve ark., 1997; Okut ve Yıldırım, 2004; Amrstrong ve ark., 2010). Bir başka araştırmada da kompoze gübre uygulandığında şeker pancarı kökü potasyum değerlerinin 4.13-4.32 mmol 100 g^{-1} pancar arasında değiştiği rapor edilmiştir (Pişkin, 2021). Bu araştırmada da potasyum içeriğinin 3.92 - 5.34 mmol 100 g^{-1} arasında değiştiği belirlenmiştir. Bor uygulanması veya sulamanın geciktirilmesi durumunda potasyum içeriğinin azalmış olduğu tespit edilmiştir. Yapılan bir araştırmada da bor uygulamasının potasyum içeriğini düşürdüğü rapor edilmiştir (Nemeata Alla, 2017). Ancak başka bir araştırma sonucu bor uygulamasının şeker pancarı bitkisinin potasyum içeriğini etkilemediği şeklinde rapor edilmiştir (Enan ve ark., 2016). Elde edilen bu sonuçlar, bitkideki potasyum içeriği bakımından yapılacak sulama veya bor gübrelemesi gibi kültürel uygulamaların birbirleri üzerindeki etkilerinin daima göz önünde bulundurulması gerektiğini göstermektedir. Nitekim bor ve potasyumun bitki bünyesinde

birbirleriyle etkileşimde oldukları ve her iki elementin de hücre genişlemesi, enzim aktivasyonu ve iyon dengesinde rol oynadıkları, farklı bor dozlarının ve sulama rejimlerinin, bitkide potasyum taşınımını etkilediği rapor edilmiştir (Cakmak, 2005).

Bor miktarı

Araştırma sonucu elde edilen veriler değerlendirildiğinde; bitkideki toplam bor miktarı bakımından çeşit, sulama zamanı, bor uygulaması ve çeşit x bor uygulaması interaksiyonu bakımından istatistiki farklılıklar olduğu belirlenmiştir (P<0.05). En yüksek toplam bor miktarı 92 ppm kg^{-1} ile Indira çeşidinden, sulama açısından da 91 ppm kg^{-1} ile çimlenme öncesi sulama zamanından ve bor uygulama dozu bakımından 104 ppm kg^{-1} ile 400 g da^{-1} bor dozundan elde edilmiştir. Çeşit x bor uygulaması interaksiyonu bakımından ise en yüksek bor miktarı 119 ppm kg^{-1} ile Indira 400 g da^{-1} bor uygulamasından elde edilmiştir (Çizelge 2 ve Çizelge 3).

Çizelge 3. İncelenen parametrelere ait ana faktörlerin interaksiyonlarına ilişkin verilerin ortalamaları ve analiz sonucu F değerleri

Table 3. Averages of data related to interactions of main factors of the examined parameters and F values as a result of analysis

Faktörlerin İnteraksiyonları *Interactions of factors*	Sodyum *Sodium (mmol 100 g^{-1})*	Azot (N) *Azote (mmol 100 g^{-1})*	Potasyum (K) *Potassium (mmol 100 g^{-1})*	Şeker Oranı *Sugar Rate* (%)	Pancar verimi *Sugar beet yield* (kg da^{-1})	Toplam bor miktarı *Total boron amount* (ppm kg^{-1})
Çeşit x Sulama Zamanı Interaksiyonu						
S x ÇÖ	1.25 a	1.71	4.32 b	15.91	8405.86	85
S x 1.su	1.40 c	1.68	4.31 b	15.64	8191.35	71
S x 3.su	1.31 b	1.59	4.17 a	15.83	8335.90	81
P x ÇÖ	1.74 e	1.56	4.72 de	16.12	7281.89	84
P x 1.su	1.54 d	1.45	4.61 d	16.36	7281.89	77
P x 3.su	1.65 de	1.50	4.47 c	16.26	7144.03	74
I x ÇÖ	1.79 fg	1.98	4.99 f	15.73	7680.55	105
I x 1.su	1.74 f	1.92	4.88 ef	16.10	7702.67	101
I x 3.su	1.84 g	1.88	4.79 e	15.85	7526.75	106
F Value	3.39**	0.36ns	0.19**	0.66ns	1.12ns	1.24ns
Çeşit x Bor Dozu İnteraksiyonu						
S x Kontrol	1.23	1.62 c	4.20 b	16.12	7909.98 b	89 b
S x 200	1.47	1.81 de	4.52 c	15.33	8545.27 a	70 e
S x 400	1.26	1.56 b	4.08 a	15.92	8477.88 a	78 d
P x Kontrol	1.49	1.53 b	4.75 e	16.43	7019.54 d	71 e
P x 200	1.71	1.46 a	4.46 c	16.16	7114.20 d	83 c
P x 400	1.74	1.53 e	4.59 d	16.14	7574.07 c	81 cd
I x Kontrol	1.72	2.12 f	5.26 b	15.64	7563.48 c	101 ab
I x 200	1.79	1.74 d	4.49 cd	16.24	7357.00 cd	94 b
I x 400	1.88	1.94 e	4.90 f	15.79	8089.51 b	119 a
F Value	2.37ns	4.03**	13.98**	1.07ns	15.91**	7.41**
Sulama x Bor Dozu İnteraksiyonu						
ÇÖ x Kontrol	1.55	1.88	4.88	15.93	7460.91	89
ÇÖ x 200	1.59	1.69	4.52	15.92	7742.80	84
ÇÖ x 400	1.64	1.68	4.63	15.89	8164.61	102
1.su x Kontrol	1.41	1.69	4.78	16.17	7474.34	87
1.su x 200	1.70	1.72	4.54	15.89	7699.59	78
1.su x 400	1.57	1.64	4.48	16.02	8000.00	86
3.su x Kontrol	1.48	1.69	4.56	16.10	7474.34	86
3.su x 200	1.67	1.59	4.40	15.90	7574.07	85
3.su x 400	1.66	1.69	4.47	15.93	7976.85	90
F Value	1.25ns	0.83ns	1.87ns	1.57ns	2.52ns	1.15ns

Çeşit x Sulama Zamanı x Bor Dozu İnteraksiyonu						
S x ÇÖ x Kontrol	1.22	1.84	4.40	16.13 b	7314.81e	86
S x ÇÖ x 200	1.35	1.80	4.57	15.39 e	8635.80 a	71
S x ÇÖ x 400	1.19	1.50	4.00	16.20 b	7277.78 e	92
S x 1.Su x Kontrol	1.32	1.57	4.30	15.92 c	7265.43 e	85
S x 1.Su x 200	1.68	2.00	4.71	15.02 g	8339.51 b	59
S x 1. Su x 400	1.20	1.47	3.94	15.98 c	7493.83 de	92
S x 3.Su x Kontrol	1.15	1.44	3.92	16.32 ab	6762.35 g	77
S x 3.su x 200	1.40	1.65	4.29	15.57 d	8660.49 a	78
S x 3.Su x 400	1.38	1.7	4.31	15.59 d	7299.38 e	98
P x ÇÖ x Kontrol	1.71	1.55	4.91	16.30 ab	7009.26 f	75
P x ÇÖ x 200	1.65	1.53	4.44	16.08 bc	7899.69 c	92
P x ÇÖ x 400	1.85	1.60	4.81	15.96 c	7473.76 de	99
P x 1.Su x Kontrol	1.30	1.54	4.83	16.54 a	6987.65 f	69
P x 1.Su x 200	1.77	1.42	4.43	16.20 b	7910.49 c	86
P x 1.Su x 400	1.60	1.40	4.56	16.31 ab	7530.87 d	101
P x 3.Su x Kontrol	1.47	1.48	4.51	16.45 a	7061.73 f	67
P x 3. Su x 200	1.70	1.43	4.50	16.19 b	7919.75 c	88
P x 3.Su x 400	1.78	1.59	4.41	16.14 b	7385.80 e	100
I x ÇÖ x Kontrol	1.71	2.25	5.34	15.37 e	7521.60 d	90
I x ÇÖ x 200	1.78	1.76	4.55	16.30 ab	8682.10 a	91
I x ÇÖ x 400	1.90	1.95	5.08	15.51 d	8290.13 bc	125
I x 1.su x Kontrol	1.63	1.97	5.21	16.04 bc	7592.59 d	77
I x 1.Su x 200	1.67	1.75	4.49	16.46 a	8324.07 b	67
I x 1.Su x 400	1.93	2.06	4.94	15.77 d	8083.83 c	110
I x 3.Su x Kontrol	1.81	2.16	5.24	15.52 d	7608.03 d	76
I x 3. Su x 200	1.91	1.71	4.43	15.94 c	8427.47 b	74
I x 3.Su x 400	1.82	1.80	4.69	16.08 bc	7895.06 cd	119
F Value	1.63ns	1.16ns	2.13ns	10.47**	2.96**	0.29ns

Toplam bor miktarı, bitki tarafından alınan ve dokularda biriken bor miktarını ifade eder. Bitkideki bor miktarı, borun hücresel fonksiyonlarda nasıl kullanıldığını ve bitkinin bor ihtiyaçlarının karşılanıp karşılanmadığını gösterir. Bor, özellikle hücre duvarının güçlenmesi, hücre bölünmesi, çiçeklenme ve meyve gelişimi gibi süreçlerde rol oynar. Bor eksikliği, şeker pancarı gibi bor ihtiyacı yüksek bitkilerde ciddi büyüme bozukluklarına, verim ve kalite kaybına yol açar. Öte yandan, aşırı bor uygulaması da bor toksisitesine neden olabilir ve bu da bitkide yaprak yanıklığı ve büyüme duraklaması gibi sorunlara yol açar.

Sulama zamanlaması, borun topraktaki çözünürlüğüne ve kök bölgesindeki bor konsantrasyonuna etki etmesi yanında bitkinin topraktaki boru ne kadar etkin bir şekilde kullanabileceğini belirler. Yetersiz sulama, borun bitki tarafından alınmasını zorlaştırır ve bor eksikliğine yol açar. Aşırı sulama ise borun topraktan yıkanmasına ve bitki kök bölgesinde yeterli bor bulunamaması sebebiyle bitkinin bor eksikliği yaşamasına neden olur. Doğru sulama stratejileri, bitkinin optimum bor alımını sağlar. Toplam bor miktarının ölçülmesi, uygulanan bor dozlarının etkinliğini değerlendirmeye olanak tanır.

Bor düzeyi 1 ppm'den az olan topraklar bitki büyümesini destekleyecek yeterlilikte Bor sağlayamazlar bu nedenle pancarda taç ve çürük öze oluşumu sonucu verim ve kalite azalır (Aydemir ve İnce, 1988). Şeker pancarında bor yeterlilik miktarı 31.00-200.00 ppm olduğu bildirilmiştir (Ungai ve Győri, 2008). Bu araştırmada bor içeriğinin 59 ppm kg^{-1} (Salama çeşidinin 6 yapraklı dönemde yapılan 1. sulama zamanı 200 g da^{-1} bor dozunda) ile 125 ppm kg^{-1} (Indira çeşidinin çıkış öncesi sulama zamanı 400 g da^{-1} bor dozunda) arasında değişmiş olduğu tespit edilmiştir. Elde edilen bu değerler, şeker pancarı için ön görülen yeterlilik miktarları dahilindedir. Şeker pancarında Bor uygulamasının bitkinin toplam bor içeriğini arttırdığı bildirilmesine (Gezgin ve ark., 2007; Durak ve Ulubaş, 2017) karşın bu araştırmada elde edilen sonuçlar bor uygulamasının sulama ve çeşit faktörleri ile değerlendirilmesi gerektiğini ortaya koymuştur.

Nitekim bitkideki bor miktarının; çeşitlere göre değişkenlik gösterdiği, sulamanın gecikmesine bağlı olarak azaldığı, bor uygulama dozlarının artmasına bağlı olarak arttığı, ikili veya üçlü interaksiyon durumunda ise standart bir değişime sahip olmayıp, değişkenlik göstermiş olduğu belirlenmiştir.

Sonuçlar

İncelenen parametreler birlikte ele alındığında, araştırmanın sulama ve bor yönetimi stratejilerinin şeker pancarı üretimi üzerindeki etkilerini tam anlamıyla ortaya koyarak, optimum üretim için en uygun bor dozunu ve sulama zamanını belirlemeye yardımcı olur. Şeker pancarı yetiştiriciliğinde ana hedefin verim olduğu dikkate alındığında; çeşitler açısından değerlendirildiğinde, araştırmada incelenen şeker pancarı çeşitleri arasında en yüksek pancar veriminin Indira şeker pancarı çeşidinden elde edildiği, dolayısıyla yetiştiricilikte bu diğer çeşitlere göre bu çeşidin kullanılması önerilebilir. Diğer taraftan sulama zamanı açısından değerlendirildiğinde; çok erken olmayan (1. Sulama zamanı) sulamanın verim ve şeker oranı bakımından daha avantajlı olmasına karşın, çeşitleri de dikkate aldığımızda Salama ve Portofina pancar çeşitlerinde çıkış öncesi sulama uygulaması, Indira çeşidinde ise 1. sulama zamanı, sulama zamanı olarak önerilebilir. İlave olarak bor uygulama dozu bakımından değerlendirildiğinde; her ne kadar yalın olarak 200 g da^{-1} Bor dozunun kontrol ve 400 g da^{-1} bor dozuna göre pancar verimi üzerinde daha etkili olduğu belirlenmiş ise de özellikle sulama durumu dikkate alındığında bütün sulama zamanlarında 400 g da^{-1} bor dozunun daha avantajlı olduğu dolayısıyla sulama koşullarında bor dozu olarak 400 da^{-1} bor dozunun önerilebileceği neticesine ulaşılmıştır.

Sonuç olarak; incelenen parametreler üzerinde bütün faktörlerin etkileri dikkate alındığında Salama ve Portofina şeker pancarı çeşitlerinde sulamanın 3. zamanda ve 200 da^{-1} bor dozu kullanılarak yapılmasının, Indira şeker pancarı çeşidinde ise sulamanın çıkış öncesi ve 200 da^{-1} bor dozu kullanılarak yapılması önerilebilir. İlave olarak elde edilen sonuçlar da göz önünde bulundurularak uygulanan sulama ve bor gübrelemesi gibi yetiştirme tekniği paketi ögeleri, farklı yetiştiricilik hedeflerine göre optimize edilmesinin verim ve kalite açısından gerekli olduğu kanaatine varılmıştır.

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Evaluation of teff production in Ethiopia using water footprint analysis for food security

Etiyopya'da gıda güvencesi için teff üretiminin su ayak izi analizi ile değerlendirilmesi

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ABSTRACT

Achieving Sustainable Development goal (SDG)2; Zero Hunger by 2030 in Africa requires reconsidering the challenges of food security in relation to several factors including agricultural practices and water availability. Teff crop plays a significant role in Ethiopia but the yield is low due to rain-fed production practices. The water footprint (WF) concept provides a useful perspective on the dependency of crops on precipitation, revealing the need for irrigation. So, WF analysis of teff production can help farmers to increase the yield and maintain water efficiency. In this study, the green and blue water footprints of teff production in Ethiopia were estimated for 2019/2020 season using the CROPWAT 8.0 and CLIMWAT 2.0 models. The results show that WF_{green} is dominant with a value of 1170 $m^3\,ton^{-1}$ in Tigray region to 1481 $m^3\,ton^{-1}$ in SNNPR region. On the other hand, the WF_{blue} varied significantly from 264 $m^3\,ton^{-1}$ in Amhara to 1022 $m^3\,ton^{-1}$ in Tigray, respectively, indicating the need for irrigation since water requirement is much higher than the effective precipitation. The economic water productivity of teff was found to be 0.68 USD m^{-3}, which is higher than other crops such as maize. Given the potential impact of climate change and droughts, this study suggests increasing water allocation to teff production and implementing appropriate irrigation practices at a national level. Integrating water footprint analysis into river basin-level water allocation plans would be beneficial for sustainable water resource management and food security.

Key Words: Agriculture, Ethiopia, teff production, water footprint

ÖZ

Afrika'da 2030 yılına kadar Sürdürülebilir Kalkınma Hedefleri (SDG) arasında yer alan SDG 2: Sıfır Açlık hedefine yaklaşmak için, tarımsal uygulamalar ve su mevcudiyeti gibi çeşitli faktörlerle ilgili olarak gıda güvencesi sorunlarının yeniden gözden geçirilmesi gerekmektedir. Teff ürünü Etiyopya'da önemli bir yere sahiptir, ancak yağışa dayalı yetiştiricilik uygulamaları nedeniyle verim düşüktür. Su ayak izi (WF) kavramı, ürünlerin yağışa bağımlılığı hakkında yararlı bir bakış açısı sağlayarak sulama ihtiyacını ortaya koyar. Bu nedenle, teff üretiminin WF analizi, çiftçilerin verimi artırmasına ve su verimliliğini korumasına yardımcı olabilir. Bu çalışmada, Etiyopya'daki teff üretiminin yeşil ve mavi su ayak izleri, CROPWAT 8.0 ve CLIMWAT 2.0 modelleri kullanılarak 2019/2020 sezonu için tahmin edilmiştir. Sonuçlar, yeşil su ayak izi (WF_{green})'nin Tigray bölgesinde 1170 $m^3\,ton^{-1}$, SNNPR bölgesinde ise 1481 $m^3\,ton^{-1}$ değeriyle baskın olduğunu göstermektedir. Öte yandan, mavi su ayak izi (WF_{blue}), Amhara'da 264 $m^3\,ton^{-1}$ ile Tigray'da 1022 $m^3\,ton^{-1}$ arasında önemli ölçüde değişmektedir. Bu sonuç, bitki su ihtiyacının etkili yağıştan çok

daha yüksek olması nedeniyle sulama ihtiyacını göstermektedir. Teff'in ekonomik su verimliliği, mısır gibi diğer ürünlerden daha yüksek olarak 0,68 USD m^{-3} olarak bulunmuştur. İklim değişikliğinin ve kuraklığın potansiyel etkisi göz önüne alındığında, bu çalışma teff üretimine su tahsisini artırmayı ve ulusal düzeyde uygun sulama uygulamalarının gerçekleştirilmesini önermektedir. Su ayak izi analizini nehir havzası düzeyindeki su tahsis planlarına entegre etmek, sürdürülebilir su kaynakları yönetimi ve gıda güvencesi için faydalı olacaktır.

Anahtar Kelimeler: Tarım, Etiyopya, teff üretimi, su ayak izi

Introduction

According to FAO, almost 70% of the total number of people facing severe food insecurity are found in eight countries, five of which are in Africa including Ethiopia (FSIN, 2020). Africa has a large amount of fertile land and relies heavily on agriculture, but it only produces 10% of the world's agricultural output. This is due to issues such as low productivity, lack of investment, and policies that prioritize urban areas. These challenges have led to famines and poverty in many parts of Africa (AU, 2023). Additionally, the continent suffers from water shortages despite having abundant water resources, mainly due to uneven distribution and poor management. By 2025, even more African countries will face water stress, and a significant portion of the population will experience water scarcity. Sub-Saharan countries also don't have adequate access to safe water and sanitation (WWF, 2023).

Teff (*Eragrostis tef*) is among the crucial sources of food security for African people, and it serves as a staple food for 85% of Ethiopia's population (Tadele and Hibistu, 2022). As the economy grows rapidly and cities become more populated, there is a greater need for teff in the food system, particularly in Ethiopia. Meeting the increasing demand for teff helps to improve the national policies on food and agriculture (Andreotti, et al., 2022). Apart from its importance for Africa, teff has also gained attention worldwide due to its high protein and amino acid levels, lack of gluten, and low glycemic index, making it a suitable choice for individuals with type 2 diabetes (Rosenberg et al., 2005).

Ethiopia accounts for over 90% of the world's teff production. Teff has the highest cultivated area share of any crop (24% in 2019/2020), followed by maize (18%), so teff is ranked second in terms of production volume in the country (CSA, 2020). Teff, besides being Ethiopia's second most significant revenue generator after coffee, brings in about $500 million annually for local farmers. Teff production is approximately 33% higher than coffee production (Minten et al., 2018; Mekonnen and Hoekstra, 2010). It is also grown in South Africa as a forage and cover crop, as well as in Northern Kenya as a cereal crop (FAO, 2023).

Most of Ethiopia's economy relies on agriculture, contributing to 70% of export earnings, 80% of employment, and 40% of the country's GDP (USAID, 2015). Teff production accounts for 7.6% of the real GDP (Moges, 2020*)*. Between 1997 and 2017, agriculture had the highest water consumption compared to other sectors, making it the largest water consumer (WB, 2017). Approximately 92% of all water withdrawals in the country (surface water and groundwater) are designed for agriculture, and water use is significantly higher than 70%, which is the global average (FAO, 2021). Although Ethiopia has a substantial amount of renewable water resources, less than 5% of it is used. Despite this, the country is still considered to be under "water stress" due to its fast-growing population. Ethiopia's renewable water supply was measured as 1,446 m^3 cap^{-1} $year^{-1}$, which is classified as "water-stress" according to the Falkenmark index (Daria, 2017).

Water availability impacts teff yield, which is highly variable among different parts of the continent. In South Africa, an annual teff production amount of 6,000 to 8,000 tons can be achieved by applying both irrigation and dryland methods, with a local production of at least 12,000 to 16,000 hectares (Agriorbit, 2023). However, in Ethiopia, the average teff yield is only 910 kg ha^{-1}, but by following effective agricultural practices, it is possible to consistently achieve yields of 2,000-

2,200 kg ha^{-1}. South African farms have already demonstrated the ability to reach these high yields, with reports of yields of 2,000 kg ha^{-1} (Biovision, 2022). On the other hand, in Ethiopia, the teff yield has reached 3300 kg ha^{-1}in experimental plots and farmers have achieved a maximum yield of 2500 kg ha^{-1}, but typically they produce 1000 kg ha^{-1}(Yihun, 2015).

According to the country's National Statistics Agency report (CSA, 2020), the highest amounts of teff are produced in Oromia and Amhara regions, accounting for around 85% of teff production volume and 84% of the planted area in the cropping season of 2019/2020. Southern Nations; Nationalities; and People's Region (SNNPR) and Tigray region are the third and fourth largest teff producing regions, respectively, despite their lower contribution to national teff output, estimated at 6.5% and 5.4% (CSA, 2020) (Table 1).

In Ethiopia, irrigation is underdeveloped and not widely practiced (CSA, 2020). In the 2019/2020 season, about 1.3 million farmers engaged in irrigation, covering a total area of approximately 211,047 hectares. The majority of the irrigated land was used for growing maize, sorghum, and teff, with maize occupying 53,670 hectares, sorghum 19,619 hectares, and teff 7,708 hectares. The majority of teff production during the 2019/2020 planting season relied on rainfall, as only around 0.25% of the entire teff plant area was irrigated (Table 1).

Table 1. Production data in priority teff producing regions

Region	Planted area (ha)	Irrigated area (ha)	Production		Yield (ton ha^{-1})
			(ton $year^{-1}$)	(%)	
Oromia	1,487,971	2,226	2,809,098	49	1.88
Amhara	1,156,131	2,398	2,189,237	38	1.89
SNNPR	241,009	NA	380,420	7	1.58
Tigray	188,392	2,277	311,754	6	1.65
Total	3,073,503	7,708	5,690,509	100	-

Increasing teff yield is important for food security, hence its water requirement in a growing season should be fully met. Determination of teff's water footprint can help identify whether precipitation is adequate to meet its water requirement and whether/how much irrigation water is required. In this way, irrigation frequency and amount of irrigation water can be planned. Also, adequacy of the available water resources can be assessed.

The water footprint concept was introduced to measure how much water is used and polluted in production systems (Hoekstra and Hung, 2002). As a pressure indicator, it helps manage water resources, deal with water scarcity, adjust to changing consumption patterns, and improve water efficiency. There are two approaches to analyze water footprint; the first is the Water Footprint Network (WFN) approach and the second one is life cycle analysis (LCA). A significant difference between these methods is that LCA approach focuses on the product WFN approach focuses on water management (Lovarelli et al., 2016). The LCA approach is an international standard (ISO 14046:2014) (ISO, 2014), which adds impact assessment to the WF accounting.

Models can be used to estimate WF. For example, CROPWAT 8.0 is a program developed by the FAO, by which water requirements and irrigation schedule for crops based on climate, soil, and crop data can be calculated (Swennenhuis, 2009; Allen et al.,1998). It is widely used for determining the crop water footprints as recommended in the Water Footprint Assessment Manual. The program uses data from CLIMWAT 2.0 software to determine precipitation, crop growth inputs, and soil data to calculate water requirements. Once all variables are considered, the blue and green water footprints can be determined. CLIMWAT 2.0 is a climate database that works with the CROPWAT 8.0 software. It helps calculate water needs, irrigation supply, and scheduling for different crops and weather stations globally. The FAO's Water Development

and Management Unit and the Climate Change and Bioenergy Unit worked on CLIMWAT 2.0, which offers data from over a thousand locations worldwide.

To accurately measure water usage in agriculture, it is required to take into account the amount of water evaporating from the soil, absorbed by plant roots, and evaporating from the plants themselves. The entire depth of precipitation required by the crop during times of growth is known as effective precipitation (P_{eff}) (Aldaya and Llamas, 2008). Effective precipitation is the WF_{green} that makes up a significant portion of water used in the different stages of agricultural production. The WF_{blue} of teff crop production was calculated as the blue component of crop water use, i.e. water from the groundwater or surface water such as rivers and lakes. Blue water is required when green water is not sufficient for crop growth (Hoekstra et al., 2011).

Previous studies estimated that Ethiopia's total annual water footprints were 77.8 billion $m^3 year^{-1}$ from 1996 to 2005 (Hirpa et al., 2022). The water footprints for crop production are primarily green (97%), with smaller proportions being blue (2%) and gray (1%). For industrial production, the water footprints are 5% blue and 95% gray, while for domestic water supply, they are 10% blue and 90% gray (Mekonnen and Hoekstra, 2011). A more recent study by WFN (2016) focused on the water footprints of major crops in Ethiopia, finding that 25% of the green water footprint is used for grazing and 75% for crop production. The study concluded that the annual green water footprint for agricultural production is 56.5 billion m^3, with a blue water footprint of 1.17 billion m^3. It also highlighted that the country is experiencing blue water scarcity in February and March, despite blue water only accounting for 2% of the overall water footprint.

The economic impact of the water footprint is related to inefficient water consumption. Water use efficiency can be considered at three scales: local, river basin, and global (Chapagain et al., 2006). The important question in agriculture is whether we can increase the amount of product we get for each unit of water. This can be measured as the amount of product per unit of water ($tons^{-3}$) or the economic value of the product (USD m^{-3}). The economic water productivity (EWP) is physically equal to the value obtained as a result of multiplying the water efficiency (unit of product per unit of water) by the price of the product (monetary value per unit of product).

Teff has not been extensively studied in terms of its water footprint at a national level. However, understanding and managing water consumption in agriculture is crucial for improving efficiency and sustainability. Tuyishimire et al. (2022) have found that the water footprint of food consumption has increased in Africa from 2000 to 2018. To this end, this study aims to assess the water footprint of teff production in Ethiopia, the leading producer in Africa, by measuring the green and blue water footprints using the CROPWAT 8.0 model (in $m^3 ha^{-1}$, $m^3 year^{-1}$ and $m^3 ton^{-1}$). The EWP was also calculated and compared to other crops. The findings can be used by decision makers to prioritize water use in the agricultural sector and potentially increase agricultural output to contribute to food security.

Materials and Methods

Study area

Oromia and Amhara regions, which account for 85% of total production and 84% of the cultivated area were considered. Additionally, SNNPR and Tigray regions, despite their smaller contributions to national teff production were included, as they are ranked third and fourth in production (CSA, 2020). These regions and rivers of Ethiopia are shown in Figure 1. Most of Ethiopia's river basins are interconnected and share regional territories.

Models used in the study

Data such as climate and teff crop characteristics were applied using the CROPWAT 8.0 model. The climate data of selected regions were taken from the CLIMWAT 2.0 software. The green and blue water footprints of teff crop were calculated following the framework presented by

Hoekstra and Chapagain (2002). WF_{blue} and WF_{green} of teff crops in each region was measured, considering the local climate and soil conditions. The amount of water used by the teff crops was calculated using the method and assumptions from Allen et al. (1998).

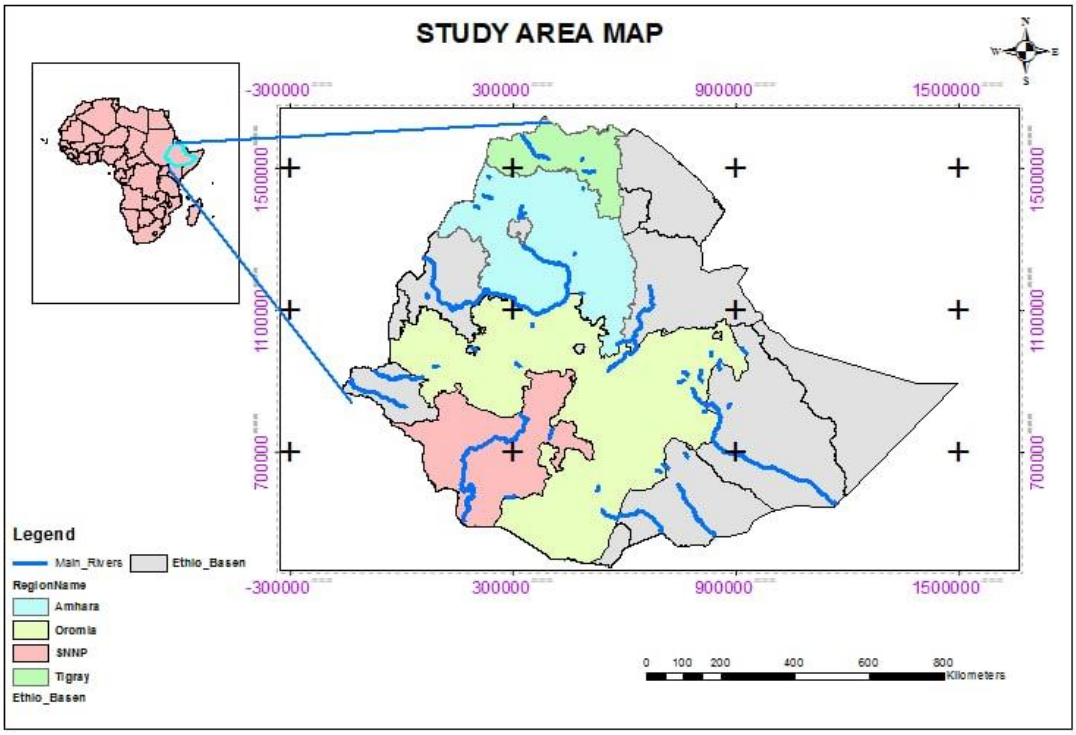


Figure 1. Ethiopian river basins and the largest teff producing regions

Evapotranspiration

Calculating evapotranspiration (ET_c) is essential to find the water footprints of crops. The ET_c of teff crop is available by the CROPWAT 8.0 software, which utilizes the FAO Penman-Monteith method for deciding reference crop evapotranspiration (ET_o). With this technique the ET_o of an area can be calculated based on the temperature, humidity, wind speed and sun data as given in Equation 1 (Allen et al., 1998).

$$ET_o = \frac{0.408\Delta(Rn-G)+\gamma\left(\frac{900}{Tmean+273}\right)u2(VPD)}{\Delta+\gamma(1+0.34u2)} \quad (1)$$

where;

ET_o : daily reference evapotranspiration [mm day^{-1}]
(For longer periods 900 becomes 37)
T_{mean} : mean air temperature at 2 m height [°C]
VPD : vapor pressure deficit [kPa]
u2 : wind speed at 2 m height [m s^{-1}]
Rn : net radiation on the surface of the crop surface [MJ m^{-2} d^{-1}]
Δ : slope vapor pressure curve [kPa $°C^{-1}$]
Γ : psychometric constant [kPa $°C^{-1}$]
G : soil heat flux density [MJ m^{-2} d^{-1}]

In this study, the "crop water requirement (CWR)" option was utilized, which means evapotranspiration was estimated under optimal conditions, i.e., crop evapotranspiration (ET_c) equals CWR (Hoekstra et al., 2011). The crop evapotranspiration (ET_c, $mmday^{-1}$) was calculated using Equation 2 (Mekonnen and Hoekstra, 2010; Allen et al., 1998).

$$ET_c = ET_o \times kc \quad (2)$$

where;

ET_c : crop evapotranspiration ($mmday^{-1}$)
ET_o : reference evapotranspiration ($mmday^{-1}$)
k_c : crop coefficient

The kc values used for teff crop were obtained from literature (Yihun, 2015) and presented in

detail in Table 2. These values represent the water consumption characteristics of teff crop during its different growth stages. Specifically, the crop coefficients for the initial, development, mid, and late stages were determined based on previous studies on teff growth under similar climatic and soil conditions, as outlined by Yihun (2015).

Green water footprint

Green water footprint (WF_{green}) equals the green effective precipitation (P_{eff}) if evapotranspiration (ET) is higher than the effective precipitation that occurs during plant growth. On the other hand, if evapotranspiration is lower than the effective precipitation, WF_{green} equals the green evapotranspiration as given in Equations 3 and 4 (Hoekstra and Chapagain 2002).

$ET \geq P_{eff}$ $WF_{green} = P_{eff}$ (3)

$ET < P_{eff}$ $WF_{green} = ET$ (4)

ET refers to actual evapotranspiration, which represents the water used by the crop (including both transpiration and soil evaporation) during the growing period. ETc (crop evapotranspiration) or ETo (reference evapotranspiration) were not used in these equations, as the focus is on the actual water used (ET) rather than potential water demand under ideal conditions (ETc or ETo).

Crop water use can come from either precipitation or irrigation. Green crop water use is calculated using Equation 5 (Hoekstra et al., 2011). The actual evapotranspiration was calculated from the day the teff crops were planted until harvest. In rain-fed vegetable crop production, blue crop water use (CWU_{blue}) is zero.

$$\text{CWU green}(\frac{\text{m3}}{\text{ha}}) = 10 * \sum_{\text{d=1}}^{\text{lgp10}} \text{ETgreen (mm)} \quad (5)$$

where;

ET_{green} : daily evapotranspiration green (mm day^{-1})

lgp : length of growing period (days)

D : factor of 10 to convert water depths in mm into water volume per hectare (m^3 ha^{-1})

The WF_{green} of teff crop production was calculated using Equation 6 as the total of rainwater evaporated from the area during the growth period. It is calculated as the green component of crop water use (CWU_{green}) divided by the yield (ton ha^{-1}) (Hoekstra et al., 2011).

$$\text{WFgreen} = \frac{\text{CWUgreen}}{\text{Y}} (\text{m3ton} - 1) \quad (6)$$

Blue water footprint

Decisively, if the evapotranspiration is higher than the effective precipitation, the blue water footprint (WF_{blue}) is potentially equal to the difference between the evapotranspiration and the effective precipitation. Otherwise, the blue water footprint is zero (Equations 7 and 8) (Hoekstra et al., 2011).

$ET \geq Peff$ $WF_{\text{blue theoretical}} = ET - Peff$ (7)

$ET < Peff$ $WF_{\text{blue theoretical}} = 0$ (8)

CWU_{blue}, which is calculated using Equation 9, is equal to the difference of simulated total ET_c during the growing period and the use of green crop water. The summation is done from the first day the crops were grown until the end of harvest (Hoekstra et al., 2011).

$$\text{CWU blue}(\frac{\text{m3}}{\text{ha}}) = 10 * \sum_{d=1}^{lgp10} ETblue\ (mm) \quad (9)$$

WF_{blue} (m^3 ton^{-1}) was calculated by dividing the CWU_{blue} (m^3 ha^{-1}) by the actual crop yield (Y) in ton/ha (Equation 10) (Hoekstra et al., 2011).

$$\text{WF blue} = \frac{\text{CWUblue}}{\text{Y}} \left(\frac{\text{m3}}{\text{ton}}\right) \quad (10)$$

As previously mentioned, the aforementioned equations were used to calculate the green and blue water footprint of teff production for

Ethiopia's biggest teff producing regions, namely, Oromia, Amhara, Tigray, and SNNPR, in terms of m^3 ha^{-1} and m^3 ton^{-1}. The green and blue water footprint components were also computed in units of m^3 $year^{-1}$. The water footprint indicator, which reflects the annual volume of water consumed (m^3 $year^{-1}$), is determined by multiplying the water footprint value in m^3 ha^{-1} by the area (ha) where the teff crop was planted in 2019/2020 (Equations 11 and 12) (Hoekstra et al., 2011).

$$\text{WFgreen}\left(\frac{\text{m3}}{\text{year}}\right) = \text{WFgreen}\left(\frac{\text{m3}}{\text{ha}}\right) * \text{planted area}\left(\frac{\text{ha}}{\text{year}}\right) \quad (11)$$

$$\text{WFblue}\left(\frac{\text{m3}}{\text{year}}\right) = \text{WFblue}\left(\frac{\text{m3}}{\text{ha}}\right) * \text{planted area}\left(\frac{\text{ha}}{\text{year}}\right) \quad (12)$$

CROPWAT 8.0 input data

Water footprints were calculated using four types of data; meteorological, soil, crop parameter and yield for the period 2019-2020.

Meteorological data

The CLIMWAT 2.0 software was used to collect weather data from 93 weather stations in Ethiopia's Oromia, Amhara, SNNPR, and Tigray regions. This data includes monthly averages of climate data such as temperature, humidity, wind speed, sunshine, solar radiation, etc.

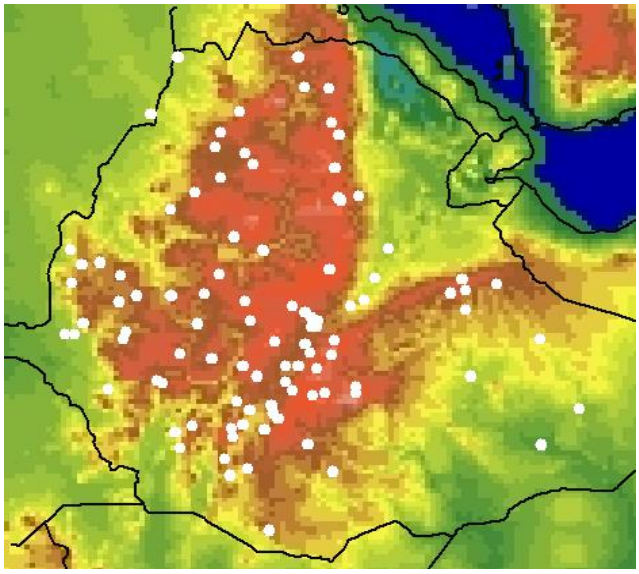


Figure 2. Weather station locations

The data from weather stations can be obtained in a format suitable for CROPWAT 8.0. Each station generates two files, one containing long-term monthly rainfall data and effective rainfall, and another containing average monthly values for climate factors, coordinates and altitude (FAO, 2022). The CROPWAT 8.0 software utilizes the Penman-Monteith formula to calculate evapotranspiration at each location. The USDA Soil Conservation Service formula is used to determine effective precipitation levels based on the total precipitation and monthly usage (Hoekstra et al., 2011).

Teff crop characteristics and soil data

The teff crop's crop coefficient, characteristics, planting and harvest dates were obtained from literature sources as they were not provided in the FAO 56 guideline. The data on teff crop parameters and the sources of literature used are listed in Table 2 (Desta and Almayehu, 2018). The plant depletion factor for teff is 0.50. The sowing and harvesting dates chosen for this study were July 15 and October 18. The overall yield response for teff production is 1.07, and the yield response factor (Ky) values vary throughout the growing season based on growth phases. The growth period of teff varies depending on the variety, and for this study, the DZ-01-976 variety with a growth period of 96 days was randomly selected.

Regarding soil information, if soil data is not available, the manual recommends using medium

soil, which is a combination of heavy and light soil. In this study, medium soil was used. The production and yield of teff crops in 2019/2020 were obtained from the Ethiopian Central Statistics Agency annual report (CSA, 2020) The report indicated that the regions of Oromia, Amhara, Tigray, and SNNPR had the highest teff production in that year. Yield data for the 2019/2020 season were obtained from the Ethiopian Central Statistical Agency.

Table 2. Teff crop parameter data

Teff crop parameters	Growing periods				Reference
	Initial	Development	Mid	Late	
Crop coefficient (k_c)	0.6	0.8	1.2	0.8	(Yihun, 2015)
lgp (days)	36	12	24	24	(Desta, et al., 2018)
Rooting depth (m)	0.6	1.0			(Steduto, et al., 2012)
Depletion factor (%)	0.5	0.5	0.5		(Allen et al., 1998)
Yield response factor (k_y)	1.07				(Hilemicael, K. 2017)
Crop height (m)	1.0				(Steduto, et al., 2012)

In Ethiopia, teff crop mainly rely on rainfall, with only a small amount being irrigated. However, a computer program was used to calculate the optimal amount of water needed for teff crop, considering both rainfall and irrigation, if necessary. The program considers the ideal water requirements for the plants' growth and yield, even though the overall water usage for teff production is low. According to weather station data, the software CROPWAT 8.0 is used to determine that certain regions do not receive enough precipitation to meet the water needs of teff crop. Therefore, the software calculates both the precipitation and irrigation requirements for optimal crop growth. In other words, the water used by the teff crop in Ethiopia is mainly from rainfall, but the software calculates the additional irrigation water needed.

Water footprint of teff crop and the economic water productivity

It is calculated as the average producer's price of teff for the period 2019/2020 (USD ton^{-1}) divided by the total (green + blue) water footprint ($m^3\,ton^{-1}$) (Equation 13) (Tewelde, 2019).

$$EWP = UP/WF_{total} \quad (13)$$

where;

WF_{total} : Total water footprint ($m^3\,ton^{-1}$)

UP : the product unit price (USD ton^{-1})

Results and Discussion

Effective rainfall and teff water requirement

CROPWAT outputs are given in Figure 3. The results show that Oromia, Amhara and SNNPR regions had teff water requirement (ET_c) of 287 – 298 mm in 2019/2020 period. On the contrary, teff water requirement was 359 mm in Tigray region, which is above the 260 - 317 mm value of Ethiopia (Araya et al., 2011). Despite having the highest water requirement, Tigray region received effective precipitation as low as 277 mm. On the other hand, the highest effective precipitation of 395 mm belonged to Amhara region. The country received 330 mm of effective precipitation on the average.

The average national teff green water requirement (ET_{green}) was recorded as 226 mm. The Amhara region had the highest ET_{green} of 245 mm, followed by Tigray region with the lowest, at 194 mm. On the other hand, the highest ET_{blue} belonged to Tigray, with a value of 169 mm. Other regions had similarly lower ET_{blue} values of 50 mm – 61 mm. The national average for ET_{blue} was found as 84 mm (Figure 3).

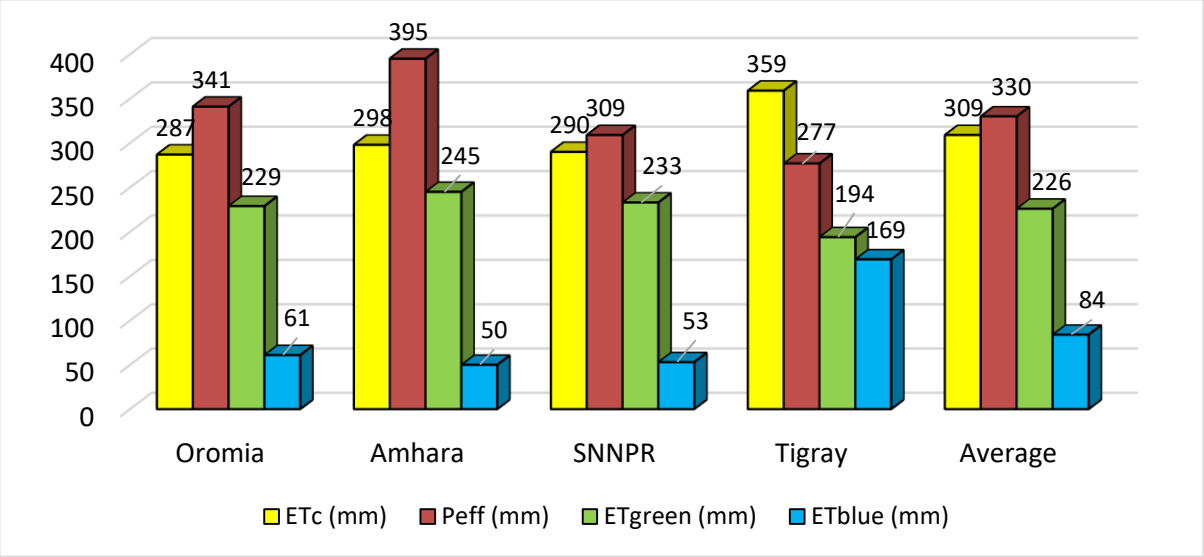


Figure 3. Teff water requirement, effective precipitation, green and blue water requirements

Water footprint of teff crop per area planted

When the WF values were calculated (Equation 9), it was found that teff in Amhara region had the highest WF_{green} with a value of 2452 m^3 ha^{-1}, followed by the SNNPR and Oromia regions (Figure 4). Teff in Tigray region had the lowest WF_{blue} with a value of 1692 m^3 ha^{-1}, which was about three times higher than those of other regions. A comparison of green versus blue WF showed that WF_{green} was four to five times higher than WF_{blue}, except for Tigray, where they were very close to each other. The average WF for growing teff in Ethiopia is 2254 m^3 ha^{-1} for green water and 835 m^3 ha^{-1} for blue water. The WF_{total} for teff production was 3089 m^3 ha^{-1}, which is representative of the national water footprint since the selected regions accounted for 99% of teff production.

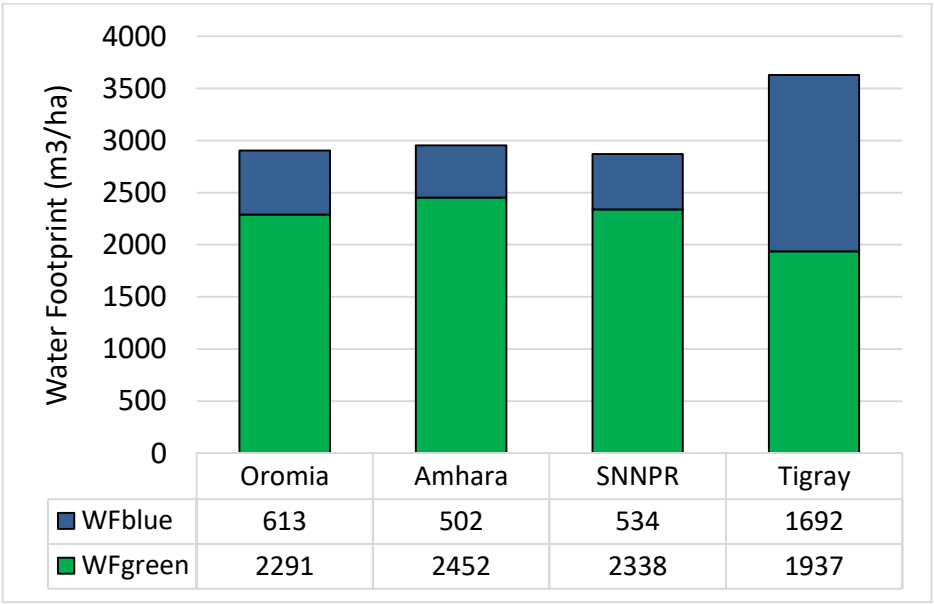


Figure 4. Green and blue water footprints (m^3 ha^{-1}) of teff crop

Water footprint of teff crop per ton of product

The green, blue, and total WF of teff production were also calculated in m^3 ton^{-1} as a measure of water efficiency using Equation 10 (Figure 5). The nation's largest WF_{total} per ton of harvested teff was observed in SNNPR region with a value of 1481 m^3 ton^{-1}, close to the average value of 1280 m^3 ton^{-1}. This was followed by Amhara, Oromia and Tigray regions with similar values of 1295 - 1170 m^3 ton^{-1}. Regarding WF_{blue}, Amhara, SNNRP and Oromia regions had low values of 264 - 343 m^3 ton^{-1}, while Tigray region had significantly higher WF_{blue} of

1023 m^3 ton^{-1}. The average blue water footprint was 492 m^3ton^{-1} and the national average total water footprint was found to be 1772 m^3 ton^{-1}.

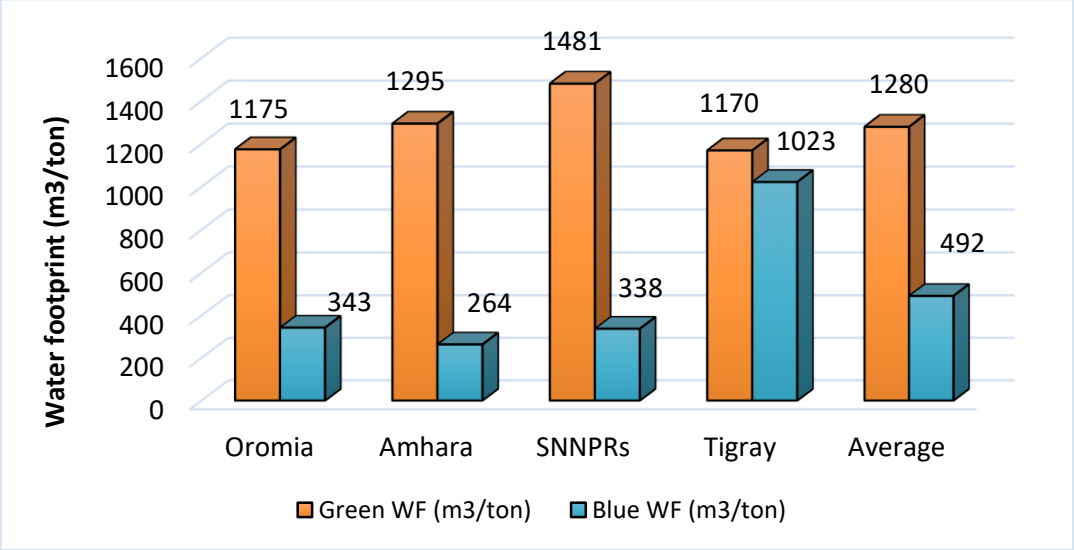


Figure 5. Green, blue and total water footprints per production quantity

A study by the WFN in 2016 found that maize mostly uses the green water, accounting for 22% of all green water footprints. Maize also has a combined water footprint of 4234 m^3 ton^{-1}, making it the third largest consumer of blue water (Figure 6). However, Mekonnen and Hoekstra (2010) had reported the global average WF of maize as 1222 m^3 ton^{-1}. Teff is the second most abundant crop in Ethiopia, and its green water footprint was 1280 m^3 ton^{-1}, reaching a total of 1772 m^3 ton^{-1} when including the blue water footprint. There may be differences in the calculation of water footprints between teff and maize due to climate, crop type, and calculation methods. Theoretical assessments of crop water use may overestimate the water footprint of crops (Fandika, 2019).

According to a recent study, the green and grey WF of teff was found as 4205 m^3 ton^{-1} and that of maize was found as 1940 m^3 ton^{-1} by Hirpa et al. (2022). The study also states that the green water footprint of the teff production was 3648 m^3 ton^{-1}, which is significantly higher than the 1280 m^3 ton^{-1} found in this study. The significant variations in crop water requirements and water footprints among the chosen crop varieties may be due to variations in growth stages and dates to maturity (Fandika, 2019). Hirpa et al. (2022) also state that spatial variation in climate, soil type, length of crop growing period (lgp) and fertilizer consumption affects and brings significant variation on the total amount of water footprint across locations. Another reason for this significant difference might be due to the fact that the study took the average teff lgp as 140 days but in this study the lgp of teff was taken as 96 days. A comparison of the results of this study with recent literature (Table 3) reveals that the national average blue water demand for the optimum gain in teff yield was highly variable. For example, WF_{blue} was found as 835 m^3 ha^{-1} in this study, however, teff production in the Debrezait area required more blue water; 1175 m^3 ha^{-1}, to meet the water that cannot be provided by available precipitation. Conversely, WF_{blue} was higher for Tigray region in this study, calculated as 1692 m^3 ha^{-1} (Figure 4).

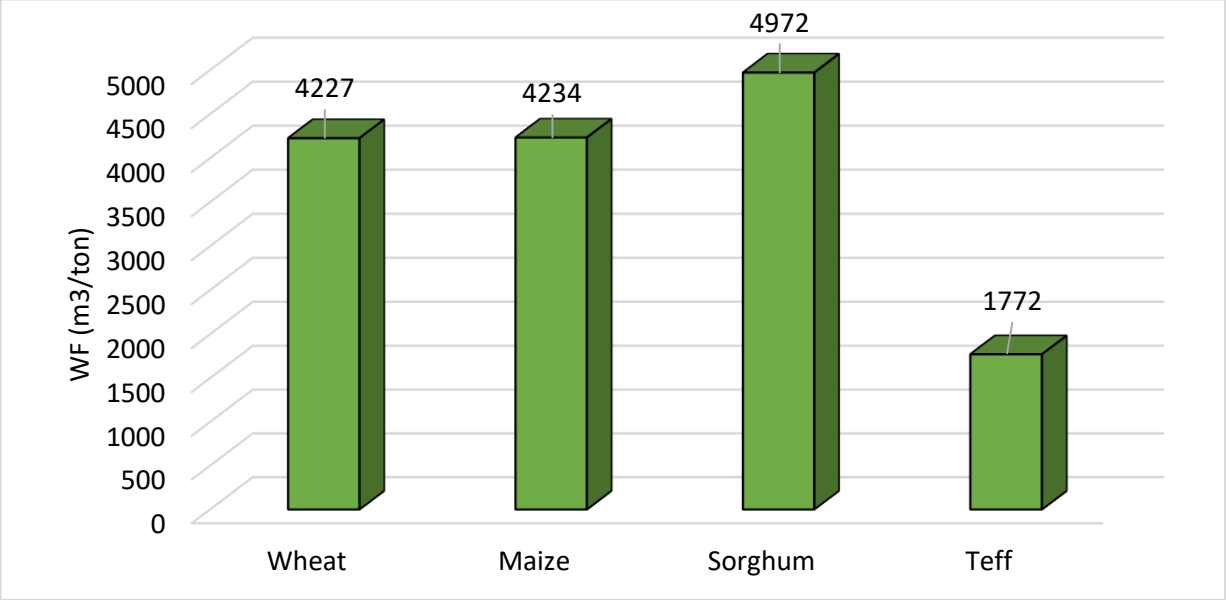


Figure 6. The water footprint of teff and other major crops in Ethiopia

Table 3. Comparison of this study with literature

Product	This study				Literature (Hirpa et al., 2022)	
	WF_{green} (m^3 ha^{-1})	WF_{blue} (m^3 ha^{-1})	WF_{green} (m^3 ton^{-1})	WF_{blue} (m^3 ton^{-1})	WF_{green} (m^3 ton^{-1})	WF_{blue} (m^3 ha^{-1})
Teff	2254	835	1280	492	3648	1175

Water footprint of teff crop per year (m^3 $year^{-1}$)

The water footprints were converted to total annual values (Equation 11 and 12). Teff in Oromia and Amhara regions used the highest green water, with 3.3 billion m^3 $year^{-1}$ and 2.8 billion m^3 $year^{-1}$, respectively. Conversely, teff in Tigray and SNNPR regions used the least green water, with 0.4 billion m^3 $year^{-1}$ and 0.6 billion m^3 $year^{-1}$, respectively. Overall, a total of 7.06 billion m^3 $year^{-1}$ of national green water was used to grow teff during the growing season (Figure 7).

Regarding blue water footprints, with 0.9 billion m^3 $year^{-1}$, teff in Oromia had the highest value. The nation's overall blue water impact from teff production was 1.9 m^3 $year^{-1}$ (Figure 7). A total of 9 billion m^3 of water was used in teff production in 2019/2020. It was estimated that WF_{green} and WF_{blue} make up 78% and 22% of WF_{total}, respectively (Figure 8).

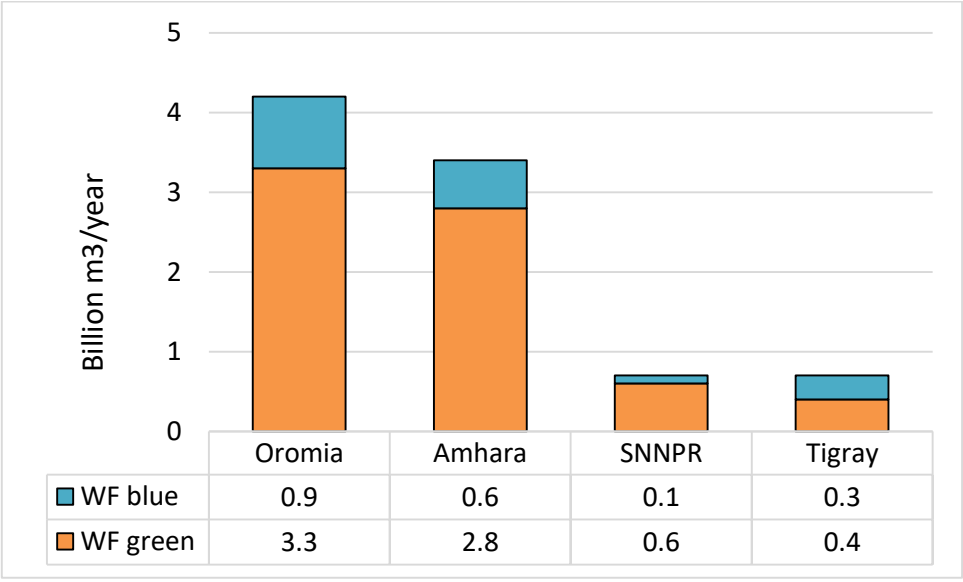


Figure 7. Green and blue water footprints (Billion m^3 $year^{-1}$) of teff crop

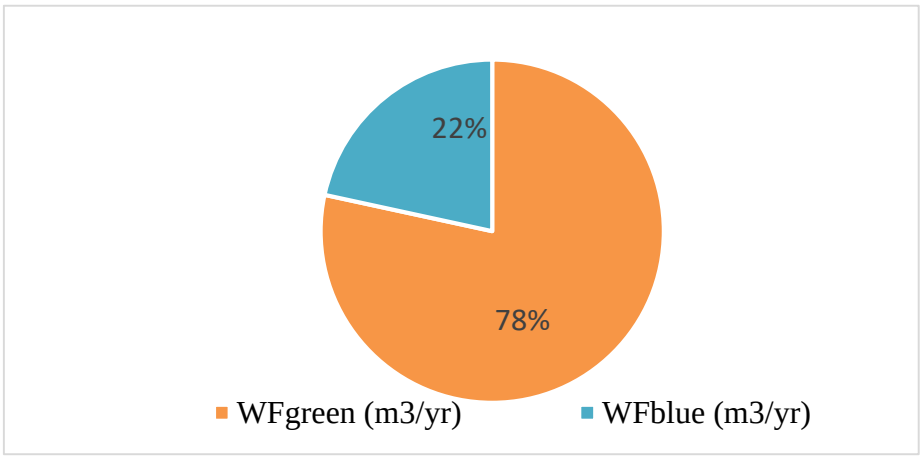
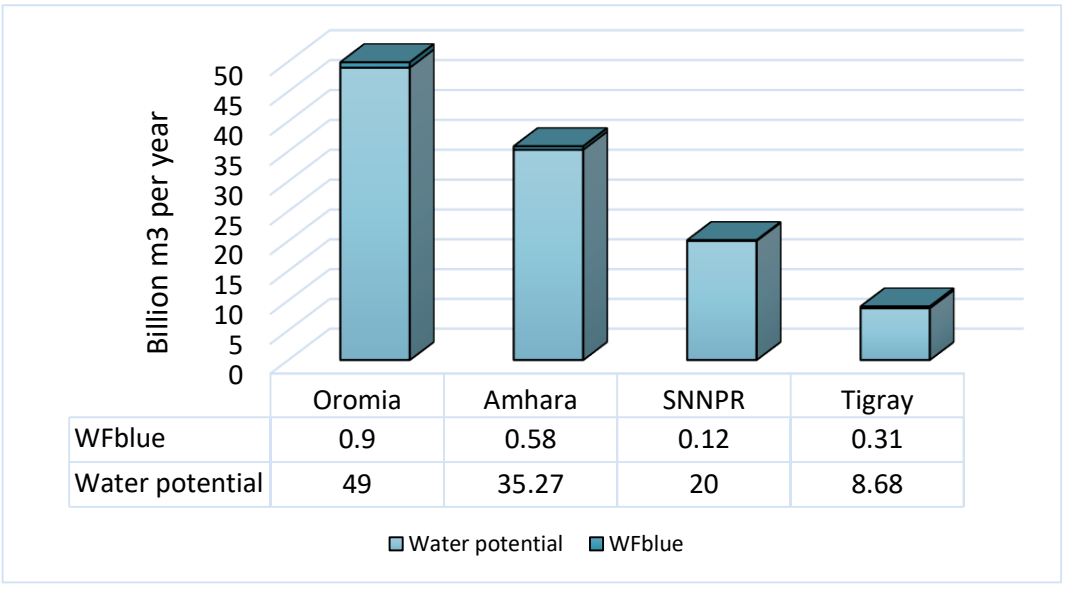


Figure 8. Share of green and blue water footprints

Figure 9 shows a comparison between the total WF of teff and the total water potential in four regions. In Oromia, the rivers have a water potential of 49 billion m^3 $year^{-1}$. If it is considered that teff cultivation requires both green and blue water, then 0.90 billion m^3 $year^{-1}$ of blue water is needed. This means that only 1.85% of the total water potential can be used for teff farming. However, this figure suggests that with additional irrigation techniques, teff can still be grown in the area, and the abundant water potential in the basin can help maximize teff production. The water footprint of teff was compared to the water potential of different regions. The Oromia region has a potential water capacity of 49 billion m^3 $year^{-1}$ and only 1.85% of it is needed for teff farming. On the other hand, teff in Amhara region requires water as low as 0.58 billion m^3 $year^{-1}$, which is about 1.63% of its water potential. Teff in Tigray region has the highest demand, needing 3.6% of its water potential.



	Oromia	Amhara	SNNPR	Tigray
WFblue	0.9	0.58	0.12	0.31
Water potential	49	35.27	20	8.68

Figure 9. Comparison of teff water footprint with water potential of regions

Economic water productivity of teff production

Teff is priced at 4200 Ethiopian Birr (77 USD) per 100 kg at the consumer level (Table 4), which displays the teff market chain for 2019/2020. The price of teff is substantially higher when it is processed into the finished product known as injera.

Table 4. Cost and price of the teff value chain (in Ethiopian units per quintal) (Jaleta, 2021)

Type of cost/price	Value (Birr per 100 kg)
Average cost for farmer	1800
Farmer's price	2600
Collector price	3200
Wholesaler price	3700
Retailer price	4200
Consumer's expected price	2411
Value-added teff to Injera	5600

The economic water productivity of teff in the October 2019-2020 season was found to be 0.68 USD m^{-3}. The EWP of maize, rice, and barley is lower than that of teff, with values of 0.003 USD m^{-3}, 0.26 USD m^{-3}, and 0.24 USD m^{-3}, respectively, according to a study conducted in Pakistan (Khan et al., 2021). The EWP can be high even if the price is low, but the water footprint is large, and vice versa. Using green water resources can be more financially beneficial and produce more income compared to using blue water resources, depending on the region. In Ethiopia, farmers should concentrate on improving water conservation techniques and effectively using green water in agricultural systems to enhance teff production. On the other hand, blue water might still be needed to help increase the yield. Unfortunately, due to various factors, about 24% of suitable teff land is expected to be lost between 2019 and 2050 (Yumba, MD, Kiambi, & Kebebew, 2014). To counteract this, it is important to improve agricultural practices and increase land productivity. This would not only lead to a decrease in water consumption but also make teff production more sustainable. To achieve this, measures such as seed selection, mulching, tillage, fertilizer application, and soil improvement should be implemented. In Ethiopia, teff is often planted late because farmers replace it after losing their first crop. However, in some areas, there is not enough rainfall for teff, so irrigation is needed. According to Yihun (2015), the current planting date for teff does not provide enough water, especially in the Tigray region with low rainfall. Planting during the rainy season does not give enough water due to unpredictable rainfall and droughts. Therefore, it is important to carefully plan the planting date or use irrigation to ensure optimal crop production. It was observed that irrigation has greatly increased teff grain yield.

Conclusion

Teff is an essential crop for Ethiopia's food security. Despite having enough water resources for irrigation, teff production in Ethiopia currently depends on rainfall, which is unpredictable due to droughts. Water footprint concept was used to figure out the adequacy of precipitation, irrigation water demand and the availability of water in corresponding regions. It was found that the amount of green water used in teff production in Ethiopia was much higher than the amount of blue water, with a range of 1170 $m^3\ ton^{-1}$ to 1481 $m^3\ ton^{-1}$ across different regions. The blue water usage varied greatly, ranging from 264 $m^3\ ton^{-1}$ to 1022 $m^3\ ton^{-1}$, with the highest usage in the Tigray region, indicating the need for irrigation. Overall, the average total water footprint for teff production was 1777 $m^3\ ton^{-1}$. The economic water productivity of teff, with a value of 0.68 USD m^{-3}, was found to be higher than those of other crops such as maize, rice, and barley.

In order for Ethiopia to improve its agriculture and maximize production, it must effectively plan and sustainably use its water resources. The study emphasizes the importance of green water, which is more influential in global food production than blue water. Despite having significant surface and groundwater resources, only a small portion is currently being utilized for irrigation. It is essential to utilize these water resources to enhance the

efficiency of teff as well as other crops. Ultimately, the study suggests that increasing teff production, a staple crop for many Ethiopians, can help improve food security and move towards SGD 2 in the region.

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Declarations

Author Contributions

Meka Taher Yimam has conducted the study, collected data, used models, evaluated the results and wrote the manuscript. Gökşen Çapar has planned and supervised the study, evaluated the results and wrote the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest between them.

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Research Article/Araştırma Makalesi

Investigation on allele and genotype frequencies of ADH1C and FASN genes in three cattle breeds in Türkiye

Türkiye'de yetiştirilen üç sığır ırkında ADH1C ve FASN genlerinde allel ve genotip frekanslarının araştırılması

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ABSTRACT

Improvement of selection programs based on candidate genes for meat yield and quality is an efficient approach for overcoming the current dilemma between the increasing human population and the decreasing population size of farm animals. Being known to be associated with meat yield and quality in cattle, *ADH1C* and *FASN* genes were investigated across three cattle breeds reared in Türkiye namely East Anatolian Red (EAR), South Anatolian Yellow (SAY), and Holstein Friesian (HF) in this study. For this purpose, 37 animals per breed were genotyped via the allele-specific polymerase chain reaction (AS-PCR) technique. The distribution of allele frequencies significantly differed between HF and native Turkish breeds. C allele frequency ranged from 0.014 (EAR) to 0.311 (HF) while T allele frequency varied between 0.689 (HF) and 0.986 (EAR) in *ADH1C* polymorphism. C and T allele frequencies were calculated as 0.068 and 0.932, respectively, in SAY breed. G was the most frequent allele across all cattle breeds regarding *FASN* gene variation. The lowest (0.014) and highest (0.365) A allele frequency were detected in EAR and HF breeds, respectively, while G allele frequency ranged from 0.027 (EAR) to 0.635 (HF). Compared to native breeds, HF had a higher heterozygosity. A large part of the total genetic variation (67%) was attributed to differences within individuals. Variations of *ADH1C* and *FASN* genes turned out to be informative enough to distinguish native Anatolian cattle breeds from HF via genetic distance-based phylogenetic analysis. No animals with superior genotypes for the *ADH1C* and *FASN* genes were observed in EAR, while two animals with AA genotype were detected for the *FASN* gene in the SAY breed. These findings imply that for the time being, these genes do not seem efficient for marker-assisted selection (MAS) studies while desired genotypes may be developed via suitable mating programs for long-term production. Further studies may focus on screening native Turkish cattle breeds regarding other meat yield and quality-related traits to develop selection strategies.

Key Words: ADH1C, AS-PCR, FASN, genetic variant, polymorphism

ÖZ

Et verimi ve kalitesinin iyileştirilmesi için aday genler temelli seleksiyon programlarının geliştirilmesi, artan insan nüfusu ile azalan çiftlik hayvanı populasyonu arasındaki mevcut ikilemi aşmak için etkili bir yaklaşımdır. Sığırlarda et verimi ve kalitesiyle ilişkili

olduğu bilinen *ADH1C* ve *FASN* genleri Doğu Anadolu Kırmızısı (DAK), Yerli Güney Sarısı (YGS) ve Siyah Alaca (SA) olarak bilinen ve Türkiye'de yetiştirilen üç farklı sığır ırkında incelenmiştir. Bu amaçla, her ırktan 37 hayvan allel spesifik polimeraz zincir reaksiyonu (AS-PZR) tekniğiyle genotiplendirilmiştir. Allel frekans dağılımı Türkiye yerli ırkları ile SA arasında önemli şekilde farklılık göstermiştir. *ADH1C* polimorfizmi bakımından C allel frekansı 0.014 (DAK) ile 0.311 (SA), T allel frekansı ise 0.689 (HF) ile 0.986 (DAK) aralığında değişmiştir. YGS ırkında C ve T allel frekansı sırasıyla 0.068 ve 0.932 olarak hesaplanmıştır. *FASN* gene polimorfizmi bakımından bütün populasyonlarda en çok görülen allel G bulunmuştur. En düşük (0.014) ve en yüksek (0.365) A allel frekansı sırasıyla DAK ve SA ırkında tespit edilirken, G allel frekansının 0.027 (DAK) ile 0.635 (SA) aralığında değiştiği belirlenmiştir. Yerli ırklarla kıyaslandığında, SA ırkında daha fazla heterozigotluk belirlenmiştir. Toplam genetik varyasyonun büyük bir kısmı (%67) bireyler arasındaki farklılıktan kaynaklanmıştır. Genetik mesafe temelli filogenetik analiz yoluyla *ADH1C* and *FASN* genlerindeki varyasyonların yerli Anadolu sığırlarının SA ırkından olan farklılığını ortaya koymada yeterince bilgi verici olduğu ortaya çıkmıştır. DAK ırkında *ADH1C* ve *FASN* genleri için arzu edilen genotipe sahip herhangi bir hayvan tespit edilemezken YGS ırkında AA genotipine sahip iki hayvanın olduğu belirlenmiştir. Bu bulgular mevcut durumda bu genlerin marker destekli seleksiyon (MDS) çalışmaları için etkili olmamakla birlikte uzun vadede uygun çiftleştirme programları sayesinde arzu edilen genotiplerin elde edilebileceğini göstermektedir. Gelecekte yapılacak çalışmalarda, seleksiyon stratejilerinin geliştirilmesi amacıyla Türkiye yerli sığır ırklarının et verimi ve kalitesiyle ilgili diğer özellikler açısından taranması üzerinde durulabilir.

Anahtar Kelimeler: ADH1C, AS-PZR, FASN, genetik varyant, polimorfizm

Introduction

The trend of increasing human population and decreasing population size in local farm animals is one of the major concerns among scientists (Aby et al. 2014; Eusebi et al. 2019). Therefore, cattle breeding remains an indispensable part of the agricultural sector, providing valuable food resources such as milk and meat. Cattle breeding is practiced at diverse production systems across almost all continents to obtain valuable food resources. As mentioned by Demir and Argun Karsli (2024), native cattle breeds are reared by smallholder farmers at a small scale in Türkiye whereas commercial companies prefer cosmopolitan cattle breeds for large-scale production. Among native Anatolian cattle breeds, East Anatolian Red (EAR) survives in a limited geographic zone of the east part of the country covering Erzurum, Kars, and Ardahan provinces (Çobanoğlu and Ardıçlı 2022) while South Anatolian Yellow (SAY), reared for both milk and meat production, was reported to be well-adapted to mountainous areas (Demir et al. 2021). On the other hand, Holstein Friesian (HF) is the most preferred cosmopolitan cattle breed by the farmers to produce milk and meat. The fact that cosmopolitan cattle breeds are advantageous in economically important traits has led to a significant decrease in the population size of local Anatolian breeds (Argun Karslı 2024). However, local populations are known to be well-adapted to a specific environment and create opportunities to shape selection programs against diverse environmental stressors. Indeed, a recent study using 211.119 bi-allelic single nucleotide polymorphisms (SNPs) obtained by next-generation sequencing (NGS) confirmed that several genes related to survival traits such as visual modality (*LGSN*), olfaction (*MOXD2*, *OR4C1F*, and *OR4C1E*), and immune response (*TRBV3-1* and *CLDN10*) have become fixed in cattle breeds reared in Türkiye (Demir et al. 2023a).

Meat yield and quality are primarily considered in selection practices by farmers to increase the profitability and sustainability of long-term production (Hozáková et al. 2020). However, these traits show quantitative inheritance meaning that they are influenced by several environmental factors and controlled by numerous genes (Raza et al. 2020). For example, feeding practices may affect meat yield while meat processing methods and storage conditions significantly impact meat quality (Grigoletto et al. 2020). On the other hand, variations in some major genes may cause differences among individuals in a certain population regarding these traits. These variations create opportunities for farmers to select advantageous genotypes for

breeding programs called marker-assisted selection (MAS) (Brito et al. 2021). In MAS studies, it is essential to investigate genetic variations of genes which are previously been confirmed to be related to meat yield and quality via affordable and accurate molecular genotyping methods. For example, Ward et al. (2012) discovered a novel SNP described as c.-64T>C in the promoter region of alcohol dehydrogenase 1 C (*ADH1C*) via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) which turned out to have a significant impact on intramuscular fat trait in Angus-cross steers. Another PCR-RFLP-based study revealed that g.17924G>A variation in fatty acid synthase (*FASN*) gene was directly linked to the concentration of some fatty acids in meat such as oleic and palmitic acid in Hanwoo cattle breeds (Bhuiyan et al. 2009). Moreover, Rempel et al. (2012) genotyped 620 cattle via 33 mutations belonging to several genes in which a variation occurred in the position of 52183794 overlapping with the *FASN* gene was reported to significantly affect meat traits such as body weight, average daily gain, and hot carcass weight. Although known variations of the genes related to meat yield and quality could be screened via several molecular genotyping methods, RT-PCR, PCR-RFLP, and direct sequencing may not be affordable by smallholder farmers due to requiring expensive chemical reagents, professional labor, equipment, and time (Lee et al. 2022). However, allele-specific PCR (AS-PCR), relying on the amplification of mutant and wild alleles with minimal chemical reagents requirement, is one of the fastest and most accurate techniques to investigate known variations. Therefore, Lee et al. (2022) have designed AS-PCR protocols to genotype cattle breeds for several genes including *ADH1C* and *FASN* to identify superior genotypes for meat yield and quality. Although this protocol seems cost-effective and simple, it has not been utilized to genotype native and cosmopolitan cattle breeds reared in Türkiye. Hence, this study aims to screen *ADH1C* and *FASN* gene variations in EAR, SAY, and HF breeds via the AS-PCR technique. Besides these variations were evaluated in terms of whether they were informative enough to distinguish native and cosmopolitan cattle breeds via genetic distance-based molecular approaches.

Materials and Methods

Sampling and DNA isolation

A total of 111 cattle were chosen from at least three representative herds of EAR (n=37), SAY (n=37), and HF (n=37) sampled from populations reared in Erzurum, Şanlıurfa, and Antalya provinces, respectively. An oral interview was carried out with farmers to select unrelated animals. Blood samples collected from the jugular vein were subjected to a salting-out method to isolate DNA (Miller et al. 1988). 1% agarose gel electrophoresis was employed to assess the success of the DNA isolation stage.

AS-PCR genotyping

By using specific primer combinations recommended by Lee et al. (2022), c.-64T>C and g.17924G>A polymorphisms of *ADH1C* and *FASN* genes, respectively, were investigated across three cattle breeds reared in Türkiye (Table 1).

Table 1. An overview of mutation and primer sequences of the studied polymorphisms in the AS-PCR protocol

Gene	SNP	Amplicon	Expected band sizes (bp)	Primer combinations	
				Forward	Reverse
ADH1C	c.-64T>C	Control region	762	**ADH1C-OF:** ACTGGTGTCTGATTTCTCTGTTGTGAAG	**ADH1C-OR:** AGAATTCCAGTTGAGCTATTCCAGATCC
		Variant allele (T)	492	**ADH1C-OF:** ACTGGTGTCTGATTTCTCTGTTGTGAAG	**ADH1C-IR:** TTACAGACTTACAGGCTCTTCCCTGTTAAA
		Wild allele (C)	330	**ADH1C-IF:** AATCTGTGCAATCTATCTCTTGTATGTCCC	**ADH1C-OR:** AGAATTCCAGTTGAGCTATTCCAGATCC
FASN	g.17924 G>A	Control region	830	**FASN-OF:** GGGAAATCCGGCAGCTCACAATCCACAA	**FASN-OR:** GTGTAGGCCATCACGAAGGTGTGCGAGC
		Variant allele (G)	507	**FASN-OF:** GGGAAATCCGGCAGCTCACAATCCACAA	**FASN-IR:** GGCCATAGGTGGGGATGCTGAGCTTTGC
		Wild allele (A)	377	**FASN-IF:** CACCACCGTGTTCCACAGCCTGGACA	**FASN-OR:** GTGTAGGCCATCACGAAGGTGTGCGAGC

As highlighted in Table 1, different primer combinations were utilized to amplify the variant and wild type for two polymorphisms. Additionally, control regions at 762 and 830 bp length for the *ADH1C*, and *FASN* genes were amplified for all genotypes to confirm that targeted regions were monitored. An optimized PCR reaction (5 µl template DNA, 10 pmol/µl each primer, 12,50 µl EcoTech 2X Master Mix, and 5,50 µl ddH_2O) and cycler program (30 s at 95 ºC for pre-denaturation followed by 35 cycles of 30 s at 95 ºC for denaturation, 30 s at 61 ºC for annealing, and 30 s at 72 ºC for extension) were used to amplify expected bands. The final extention stage was optimized at 72 ºC for 5 min. %3 agarose gel electrophoresis was used to separate PCR fragments for genotyping animals.

Statistical analysis

Allele and genotype frequencies, observed (H_O) and expected (H_E) heterozygosity as well as chi-square ($\chi 2$) based Hardy-Weinberg equilibrium (HWE), were calculated by Popgene v.1.32 (Yeh et al. 1997). Analysis of molecular variance (AMOVA) test was carried out with the option of 999 permutations by GenAlex software (Peakall and Smouse 2012) to categorize sources of total genetic variation. Nei's standard genetic distance among breeds was estimated via GenAlex software (Peakall and Smouse 2012). The genetic distance matrix was processed in MEGA 11 software (Kumar et al. 2008) to construct a Neighbour-Joining (NJ) tree per breed.

Results and Discussions

Agarose gel electrophoresis-based genotyping revealed that all animals carried 762 bp and 830 bp length fragments for the *ADH1C* and *FASN* genes, respectively, indicating that targeted genomic regions were amplified during the AS-PCR process (Figure 1).

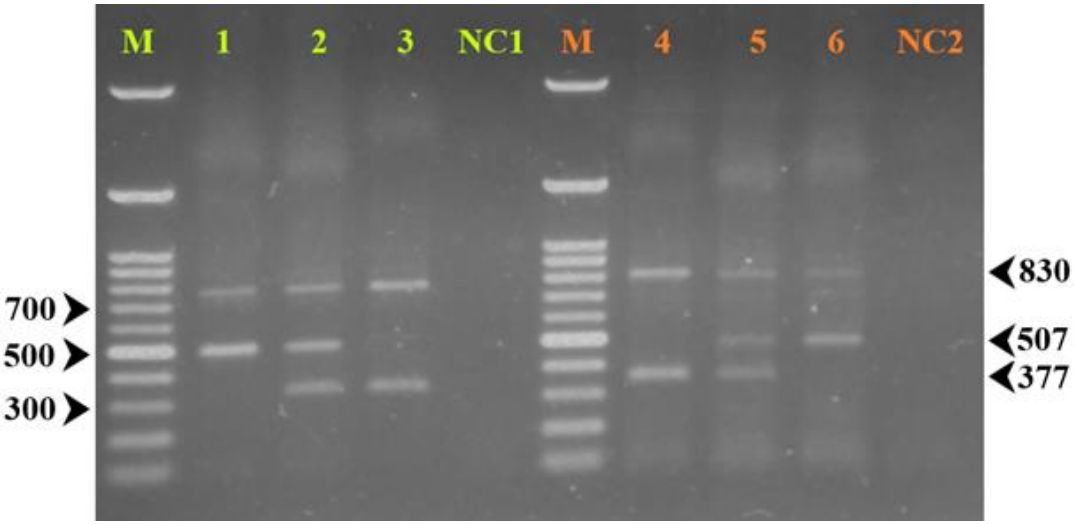

Figure 1. Agarose gel (3%) image of some representative amplified PCR fragments for *ADH1C and FASN* polymorphisms in three cattle breeds.

M: molecular weight marker (100 bp); **1-3:** TT (762 and 492 bp), CT (762, 492, and 330 bp), and CC (762 and 330 bp) genotypes for the *ADH1C* gene; **NC1:** negative control for the *ADH1C* gene; **4-6:** AA (830 and 377 bp), AG (830, 507, and 377 bp), and GG (830 and 507 bp) genotypes for the *FASN* gene; **NC2:** negative control for the *FASN* gene.

The results of allelic diversity and genetic variability parameters were summarised in Table 2 in which T and G alleles turned out to be represented with higher frequency across all breeds in terms of the *ADH1C* and *FASN* polymorphisms, respectively. However, allelic distribution significantly differed between native Anatolian cattle populations and the HF. Indeed, no animals with CC genotype were detected in EAR and SAY breeds leading to a low frequency of the C allele (0.014 and 0.068) in terms of the *ADH1C* polymorphism. 2 and 5 animals were recorded as CT genotype in EAR and SAY populations, respectively. On the other hand, 4 and 14 animals carried CC and CT genotypes for the *ADH1C* gene in the HF breed. Similar results were also detected for the *FASN* gene polymorphism in which the frequency of the A allele was higher in the HF breed (0.365) compared to Anatolian cattle breeds (0.014-0.135) (Table 2). No animals with the AA genotype were detected in the EAR breed while only 2 animals carried the AA genotype in the SAY breed. 1 and 6 animals turned out to be heterozygous for the *FASN* gene in EAR and SAY breeds, respectively. On the other hand, 7 and 13 animals were recorded as AA and AG genotypes, respectively, in the HF breed. Due to conserving a higher number of heterozygous individuals, the HF breed showed higher heterozygosity for both the *ADH1C* (H_O = 0.405) and *FASN* (H_O = 0.351) polymorphisms compared to native Anatolian cattle breeds (Table 2).

Table 2. A summary of allele frequencies and genetic variability of the *ADH1C* and *FASN* genes in EAR, SAY, and HF cattle breeds

Gene	***ADH1C***					***FASN***				
Parameter	**Allele frequency**		**Genetic variability**			**Allele frequency**		**Genetic variability**		
Breed	C	T	H_O	H_E	χ^2	A	G	H_O	H_E	χ^2
EAR	0.014	0.986	0.027	0.027	0.007^{ns}	0.014	0.986	0.027	0.027	0.007^{ns}
SAY	0.068	0.932	0.135	0.126	0.194^{ns}	0.135	0.865	0.162	0.234	3.470^{ns}
HF	0.311	0.689	0.405	0.428	0.107^{ns}	0.365	0.635	0.351	0.463	2.165^{ns}

EAR: East Anatolian Red: **SAY:** South Anatolian Yellow: **HF:** Holstein Friesian; **H_O:** Observed heterozygosity; **H_E:** Expected heterozygosity, **χ^2:** Chi-square test value; **ns:** non-significant deviation from HWE ($\chi^2_{0.05;1}$: 3.84).

AMOVA analysis categorized total genetic variation into three levels such as among populations, among individuals, and within individuals in which a large part of it (67%) was attributed to within individuals (Table 3). The differences between breeds corresponded to 19% of the total genetic variation, which was in agreement with the genetic differentiation value (F_{ST}) of 0.195.

Table 3. Summary of AMOVA analysis across EAR, SAY, and HF cattle breeds

SV	df	SS	MS	EV	%
Among populations	2	8.423	4.212	0.053	19
Among individuals	108	27.784	0.257	0.036	13
Within individuals	111	20.500	0.185	0.185	67
Total	**221**	**56.707**	-	**0.274**	**100**

SV: source of variation; **df:** degree of freedom; **SS:** sum of squares; **MS:** mean square; **EV:** estimated variance.

The lowest genetic distance (0.546) was detected between EAR and SAY, while the highest value (1.927) was observed between HF and SAY. Similarly, EAR and SAY were clustered together in NJ tree analysis while HF constituted a separate branch (Figure 2). This confirms that even two genes related to economically important traits are sufficient to genetically distinguish native Anatolian cattle from cosmopolitan breeds.

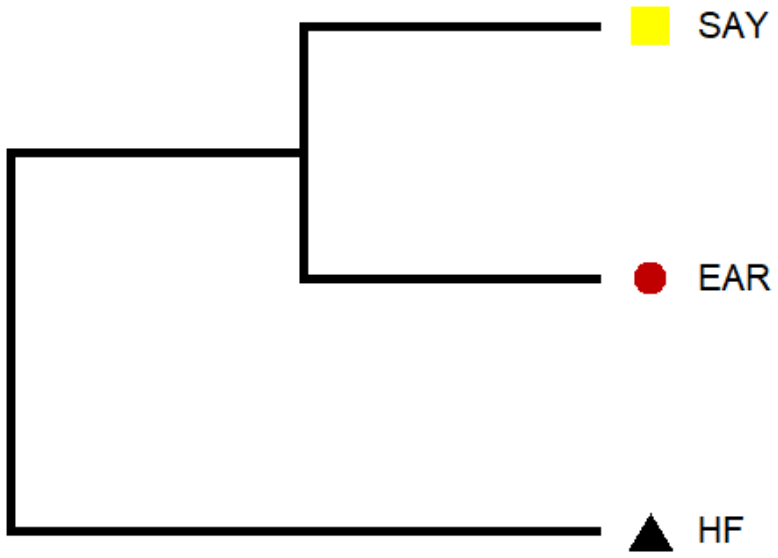

Figure 2. NJ tree-based phylogenetic analysis at breed level
EAR: East Anatolian Red: **SAY:** South Anatolian Yellow: **HF:** Holstein Friesian.

The variations of the *ADH1C* (Ward et al. 2012) and *FASN* (Bhuiyan et al. 2009; Rempel et al. 2012) genes and their effects on phenotype were clarified by several studies. Ward et al. (2012) investigated the impact of vitamin A restriction together with the *ADH1C* variation in 130 Angus steers in which animals with TT genotype had 23% higher intramuscular fat compared to animals with CC genotype in the treatment group without vitamin A supplementation. Moreover, unsupplemented vitamin A animals with the TT genotype were reported to show 24% greater intramuscular fat compared to vitamin A-supplemented animals with the TT genotype (Ward et al. 2012). The number of animals with CC, CT, and TT genotypes for Angus steers was reported to be 30 (0.230), 50 (0.385), and 50 (0.385), respectively (Ward et al. 2012). On the contrary, studying the effects of the *ADH1C* variations on meat-related traits, Peng et al. (2017) reported that animals with CT genotype are advantageous over TT genotypes in terms of eye muscle area, marbling, and carcass weight in 60 Korean native steers. The frequency of TT and CT genotypes was reported as 85% and 15%, respectively, while no animals with CC genotype

were observed (Peng et al. 2017). The results of the current study show similarities with the findings reported in the literature. As known, beef cattle are expected to carry advantageous genotypes for meat yield and quality compared to native cattle breeds. As observed in native Korean steers (Peng et al. 2017), no animals with CC genotype were detected in two Anatolian cattle breeds (EAR and SAY) in this study. Besides, a small number of animals turned out to carry CT genotypes (2 and 5 animals from EAR and SAY) which is the desired genotype for meat yield and quality. On the other hand, the CT genotype detected in Angus breed by Wang et al. (2012) was also observed in the HF breed reared in Türkiye. Similar to the *ADH1C* polymorphism, the *FASN* variations also significantly differed between HF and native Anatolian breeds in which no animals with the AA genotype were detected in the EAR breed while only 2 samples carried the AA genotype in the SAY breed. The A allele frequency ranged from 0.014 (EAR) to 0.135 (SAY) in Anatolian cattle while a higher frequency (0.365) was detected in the HF breed. Similarly, a higher AA genotype frequency (0.299) was reported in HF breed raised in China (Zhou et al. 2023). These results demonstrate that local cattle breeds adapted to specific environmental conditions cannot compete with breeds specialized for beef production in terms of meat yield and quality. However, as seen in this study, local breeds may conserve advantageous alleles and/or genotypes at low frequencies. This fact creates significant opportunities to detect and subject animals with desired genotypes for MAS studies to improve meat yield and quality, while it seems to take longer times of intensive breeding practices.

In this study, the highest proportion of the total genetic variation (67%) was detected within individuals by AMOVA analysis, which was consistent with previous studies. For example, Demir and Balcioglu (2019) assessed the genetic diversity and population structure of three native and HF cattle breeds via 20 microsatellite markers, in which the highest part of total genetic variation (80.068%) was attributed to the within individuals. Another study based on 22 microsatellite loci confirmed that 88.90% of the total genetic variation could be explained by the differences within individuals in five native Turkish cattle breeds (Öner et al. 2019).

It is known that denser genetic data have a significant potential to distinguish local cattle populations from cosmopolitan breeds at a molecular level. Indeed, several studies based on microsatellite and single nucleotide polymorphism (SNP) demonstrated that native Turkish cattle breeds were genetically different from cosmopolitan cattle breeds via several phylogenetic analyses (Demir and Balcioglu 2019; Karayel and Karsli 2022; Demir et al. 2023b). Similarly, in this study, genetic variations of two genes (*ADH1C* and *FASN*) were found enough to distinguish native Anatolian cattle from HF breed while genome-wide genetic data seems required to reveal the genetic distinctiveness of SAY and EAR breeds (Demir et al. 2022). Indeed, a recent study utilizing 211.119 SNP highlighted that EAR was genetically distinct from the other native Turkish cattle breeds (Demir et al. 2023b).

Conclusions

In this study, two previously known variations in *ADH1C* (c.64T>C) and *FASN* (g.17924G>A) genes were investigated via AS-PCR technique in EAR, SAR, and HF cattle breeds. The desired genotypes regarding meat-related traits were detected at sufficient frequencies in HF breed, while no animals with CC genotype for *ADH1C* and AA genotype for *FASN* genes were detected in EAR and SAY cattle breeds. This finding supports the idea that the genome of native Turkish cattle breeds has been mainly shaped by environmental challenges while desired genotypes are maintained in cosmopolitan cattle breeds due to ongoing selection studies. The current results imply that the *ADH1C* and *FASN* genes do not seem to be effective in improving meat yield and quality traits in Anatolian cattle breeds due to low genetic variability, while a larger sample size

covering more geographic locations has the potential to obtain more heterozygous animals. Alternatively, the current populations could be monitored in terms of other meat-related genes in further studies.

Ethical Statement

Blood samples used in this study were previously collected during routine visits of qualified veterinarians. Therefore, no ethical permission was required to carry out this study.

Conflict of interest

The author declares no conflict of interest.

Author contributions

ED conceptualized the study, developed the methodology, validated the results, and wrote the original draft.

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Araştırma Makalesi/Research Article

Müşküle (*V. vinifera* L.) üzüm çeşidinde salkımların omcalar üzerinde bekletilmesinin tane kalitesi ve biyokimyasal özelliklere etkisi

The effect of keeping clusters on vines on berry quality and biochemical properties of Müşküle (V. vinifera L.) grape variety

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ÖZ

Bu araştırma, 'ÇOMÜ Dardanos Yerleşkesi Ziraat Fakültesi Bitkisel Üretim Araştırma ve Uygulama Birimi'nde yer alan 'Sofralık Üzüm Çeşitleri Uygulama ve Araştırma Bağı'nda, 2020 ve 2021 yıllarında yürütülmüştür. Araştırmada, Müşküle (*V. vinifera* L.) üzüm çeşidinde salkımların omca üzerinde bekletilmesinin tane kalitesi ile biyokimyasal özelliklere etkilerinin belirlenmesi amaçlanmıştır. Bu kapsamda, hasat olgunluğundan (EL–38; 23.10.2020–13.10.2021) itibaren altı hafta boyunca, birer haftalık periyotlarla örnekleme yapılmıştır. İki yıllık bulgulara göre; tane eni, tane boyu ve pH değerlerinde haftalar arasında önemli bir değişiklik tespit edilememiştir. Tane ağırlığı hafif dalgalı seyir izlemiş ancak 6. haftada (4.35 g tane^{-1}) en yüksek değere ulaşmış, kabuk kalınlığı 0.250 mm tane^{-1}'den, 6. ve 5. haftalarda 0.180 ve 0.160 mm tane^{-1}'ye düşmüştür. Haftalar ilerledikçe olgunlaşmaya bağlı olarak tanelerde kabuk renginin yeşilimsi–sarıdan daha sarı tonlara doğru değiştiği, parlaklığın ve renk canlılığının azaldığı tespit edilmiştir. SÇKM değeri ilk haftalara kıyasla 6. haftada yaklaşık %0.50 artış göstermiş, asitlik değeri haftalar ilerledikçe giderek azalmış, olgunluk indisi ise giderek artış göstererek 51'den 56'ye yükselmiştir. Toplam fenolik bileşik ve tanen miktarlarında olgunluk ilerledikçe azalmaların meydana geldiği belirlenerek, en düşük değerlerin 6. haftada sırasıyla 3.09 mg GAE 100 ml^{-1} ve 0.557mg kg^{-1} olduğu tespit edilmiştir. Bu araştırma, erken sonbahar donlarının görülmediği yıllarda Çanakkale gibi ekolojilerde, üzüm salkımlarının pazarlama değerinin yüksek olduğu bir dönemde satışa sunulabilmesi için omcalar üzerinde bekletilmesinin ekonomik avantajlar sağlayacağını ortaya koymaktadır.

Anahtar Kelimeler: Fenolik bileşik, kalite, Müşküle, olgunluk, *V. vinifera* L.

ABSTRACT

This research was conducted in the 'Table Grape Varieties Application and Research Vineyard' located in the 'ÇOMÜ Dardanos Campus, Faculty of Agriculture, Plant Production Research and Application Unit'in 2020 and 2021. The aim was to determine the effects of keeping the clusters on the vine in the Müşküle (*V. vinifera* L.) grape variety on berry quality and biochemical properties. In this context, sampling was carried out in one-week periods for six weeks, starting from harvest maturity (EL–38; 23.10.2020–13.10.2021). According to the two–year findings; no significant change was detected between weeks in berry width, berry length and pH values. Berry weight followed a slightly fluctuating course but reached the highest value in the 6th week (4.35 g berry^{-1}),

and skin thickness decreased from 0.250 mm berry^{-1} to 0.180 and 0.160 mm berry^{-1} in the 6th and 5th weeks. It was determined that as the weeks progressed, the skin color of the berries changed from greenish–yellow to more yellow tones due to ripening, and the brightness and color vibrancy decreased. The TSS value increased by approximately 0.50% in the 6th week compared to the first weeks, the acidity value gradually decreased as the weeks progressed, and the maturity index gradually increased from 51 to 56. It was determined that total phenolic compound and tannin amounts decreased as maturity progressed, and the lowest values were determined to be 3.09 mg GAE 100 ml^{-1} and 0.557 mg kg^{-1} in the 6th week, respectively. This research reveals that in early autumn years when frosts are not seen in ecologies such as Çanakkale, keeping grape clusters on vines so that they can be offered for sale during a period when their marketing values are high will provide economic advantages.

Key Words: Phenolic compound, quality, Müşküle, maturity, *V. vinifera* L.

Giriş

Üzüm, hem ılıman hem de tropikal bölgelerde yetişen en önemli ticari meyve ürünlerinden biridir. Zengin besin içeriği, insan sağlığına fayda sağlayan biyoaktif bileşikler barındırması ve çok yönlü değerlendirme imkânları sunması gibi özellikleri, bu ürünü oldukça değerli ve popüler kılmaktadır. Türkiye, dünya bağcılığında öne çıkan ülkeler arasında yer almakta olup, geniş üzüm çeşitliliği, uygun iklim koşulları ve elverişli toprak özellikleri sayesinde bağcılık açısından stratejik bir öneme sahiptir (Kesici ve ark., 2010). Dünyada, 2022 yılında toplam 87.615.444 ton üzüm üretimi gerçekleştirilmiş olup, Türkiye 384.537 ha bağ alanı ile İspanya, Fransa, İtalya ve Çin'den sonra dünyada beşinci, 4.165.000 ton üzüm üretimiyle Çin, İtalya, Fransa, İspanya ve ABD'den sonra altıncı sırada yer alarak önemli bir konumda bulunmaktadır (FAO, 2024). Elde edilen üzüm üretiminin %50.42'sini sofralık üzüm (2.099.859 ton), %40.38'ini kurutmalık üzüm (1.681.808 ton) ve %9.20'sini şaraplık üzüm (383.333 ton) çeşitleri oluşturmaktadır. Çanakkale ilinde 2022 yılında 45.657 da üzüm üretim alanından, 16.959 ton sofralık, 28.504 ton şaraplık olmak üzere toplam 45.463 ton üzüm üretimi gerçekleştirilmiştir (TÜİK, 2024).

Klimakterik olmayan bir meyve olan üzüm, hasattan sonra daha fazla olgunlaşamamakta olup, yeme olumunda hasat edilmesi gerekmektedir. Üzümün olgunlaşma süreci, 'ben düşme' evresiyle başlayarak hasat dönemine kadar devam eden; ağırlık, şeker oranı, asitlik, renk, fenolik bileşikler ve aroma gibi pek çok fiziksel ve biyokimyasal özelliğin değişimini içeren karmaşık bir süreçtir(Winkler ve ark., 1974; Gomez ve ark., 1995; Piazzolla ve ark., 2015; Harput ve Dardeniz, 2020; Altın Dünya ve Dardeniz, 2023; Şahin ve ark., 2024). Olgunlaşma sürecinde gerçekleşen fiziksel ve özellikle biyokimyasal değişimler eşzamanlı olarak meydana gelmemekte, her bir bileşik farklı dinamiklerle gelişim göstermektedir. Üzümdeki asit miktarı, tanenin birinci ve ikinci gelişim evrelerinde arttmakta, 'ben düşme' başlangıcı olan üçüncü evrede en yüksek seviyeye ulaşmakta ve bu aşamadan sonra hızlı bir şekilde azalmaktadır (Ağaoğlu, 2002). Ben düşme döneminden hasat dönemine kadar SÇKM (Suda Çözünebilir Kuru Madde) ve pH değerlerinde sürekli bir artış gözlenmektedir (Ağaoğlu, 2002; Cangi ve ark., 2011; Yüksel, 2014; Şan, 2016). Optimum üzüm kalitesinin sağlanabilmesi için, tane ağırlığı, kabuk rengi, tanenin saptan kopma direnci, SÇKM, pH, asitlik ve tanen içeriği gibi biyokimyasal ve duyusal özelliklerin dikkate alınarak, üzümlerin en uygun olgunluk aşamasında hasat edilmesi son derece önemlidir (Altındişli ve ark., 1997). Bununla birlikte, üzüm kalitesine etki eden faktörlerin sayısının artması, kalite değerlendirme sürecini daha karmaşık ve zor hale getirebilmektedir.

Sofralık üzümlerde SÇKM oranı, pazarlama standartlarının belirlenmesinde önemli bir kriterdir. Türk Standartları Enstitüsü'nün Çekirdeksiz Sofralık Üzüm Standardı'na (TS 101) göre; SÇKM değerinin en az 16 °Brix olması gerekmektedir. Ancak, çekirdekli sofralık üzümler için bir standart henüz ülkemizde oluşturulmamıştır. Üzüm tanelerindeki fenolik bileşikler, renk, tat ve koku oluşumunda temel rol oynamalarının yanı sıra, insan beslenmesi ve sağlığı üzerinde de önemli etkilere sahiptir (Kunter ve

ark., 2013; Keskin ve ark., 2017). Bu bileşikler, üzüm tanesi histokimyasında şeker ve organik asitlerden sonra en fazla bulunan önemli bileşik grubunu oluşturur. Üzüm tanelerinde fenolik bileşiklerin profili ve yoğunluğu, üzüm çeşidi, ekolojik koşullar, bağda uygulanan kültürel işlemler ve olgunluk aşamaları gibi faktörlere bağlı olarak değişkenlik göstermektedir (Ribéreau–Gayon ve ark., 2000). Hem dünya genelinde hem de Türkiye'de gözlemlenen döngüsel mevsim kaymalarına bağlı olarak, üzüm çeşitlerinin fenolojik tarihlerinde olduğu gibi birçok diğer türde de değişimler gözlenmektedir. Üzüm tanesinin gelişim aşamalarında, tane büyüklüğü ve rengi gibi dışsal değişimlerin yanı sıra, olgunluk düzeyi, fenolik bileşik profili, aroma bileşenleri ve bunların yoğunluklarında da farklılıklar meydana gelmektedir.

Üzüm salkımlarının hasat olgunluğundan itibaren kalitenin korunarak yaklaşık bir ay kadar omcalar üzerinde bekletilmesiyle, pazar değerinin daha yüksek ve talebin daha güçlü olduğu bir dönemde satışa sunulabilme imkânı doğmaktadır (Kara ve Çoban, 2002).

Bu araştırmada, 'ÇOMÜ Dardanos Yerleşkesi Ziraat Fakültesi Bitkisel Üretim Araştırma ve Uygulama Birimi'nde yer alan 'Sofralık Üzüm Çeşitleri Uygulama ve Araştırma Bağı'nda yetiştirilen Müşküle (*V. vinifera* L.) üzüm çeşidinde salkımların omcalar üzerinde bekletilmesinin tane kalitesi ve biyokimyasal özelliklere etkisinin belirlenmesi amaçlanmıştır.

Materyal ve Metot

Bu araştırma, 40° 4' 26.40" K enlem ve 26° 21' 42.84" D boylam derecelerinde bulunan 'ÇOMÜ Dardanos Yerleşkesi Ziraat Fakültesi Bitkisel Üretim Araştırma ve Uygulama Birimi'ndeki 'Sofralık Üzüm Çeşitleri Uygulama ve Araştırma Bağı'nda, kurak şartlar altında yetiştirilen Müşküle üzüm çeşidi üzerinde, 2020 ve 2021 vejetasyon yıllarında yürütülmüştür. Araştırmanın yürütüldüğü bağ 2.0 da büyüklüğünde ve 3.0 x 1.5 metre aralık ve mesafede tesis edilmiştir. 5BB Amerikan asma anacı üzerine aşılı omcalar tek kollu sabit kordon terbiye şekline sahip olup, araştırmanın başlatıldığı yıl itibariyle 17 yaşındadır. Müşküle üzüm çeşidi salkımları; kanatlı konik şekilli, orta büyüklükte (200–300 g), seyrek sıklıkta olup, taneleri; yeşil–sarı, eliptik şekilli, orta irilikte, 1–3 adet çekirdekli, tane eti sert–sulu, kabuk kalınlığı kalın ve nötral aromaya sahiptir. Kış budaması kısa yapılan bir üzüm çeşidi olup, geç mevsimde olgunlaşmaktadır (Çelik, 2006).

Çanakkale ilinin 2020 yılının Ekim ve Kasım aylarına ait iklim verileri şu şekildedir: ortalama sıcaklık değerleri 18.7°C ve 14.3°C; maksimum sıcaklık 24.7°C ve 17.0°C; minimum sıcaklık 14.8°C ve 9.2°C; toplam yağış miktarı 48.7 kg m^{-2} ve 0.1 kg m^{-2}, nispi nem %77.5 ve %79.4'tür. Çanakkale ilinin 2021 yılının Ekim ve Kasım aylarına ait iklim verileri şu şekildedir: ortalama sıcaklık değerleri 18.1°C ve 15.8°C; maksimum sıcaklık 21.5°C ve 19.1°C; minimum sıcaklık 15.4°C ve 13.0°C; toplam yağış miktarı 74.5 kg m^{-2} ve 24.6 kg m^{-2}, nispi nem %64.8 ve %68.2'dir.

Müşküle üzüm çeşidinde salkımların omcalar üzerinde bekletilmesinin tane kalitesi ile biyokimyasal özelliklere etkilerinin belirlenmesinin amaçlandığı bu araştırmada; hasat olgunluğundan (EL–38) itibaren birer haftalık periyodlarda toplam altı hafta boyunca tane örnekleri alınmıştır (1. hafta: 23.10.2020 ve 13.10.2021; 2. hafta: 30.10.2020 ve 19.10.2021; 3. hafta; 6.11.2020 ve 26.10.2021; 4. hafta; 13.11.2020 ve 02.11.2021; 5. hafta: 20.11.2020 ve 09.11.2021; 6. hafta: 27.11.2020 ve 16.11.2021). Farklı olgunluk dönemlerinde alınan bu tane örnekleri ÇOMÜ Bahçe Bitkileri Pomoloji Laboratuvarı'na getirilerek, tane eni (mm $tane^{-1}$), tane boyu (mm $tane^{-1}$), tane ağırlığı (g $tane^{-1}$), tane boyut indeksi (tane boyu $tane\ eni^{-1}$), kabuk kalınlığı (mm $tane^{-1}$), tane kabuk rengi (L, Chroma ve Hue), SÇKM (%), pH, asitlik (%) (Cemeroğlu, 2007), olgunluk indisi, toplam fenolik bileşik miktarı (mg GAE 100 ml^{-1}) (Singleton ve Rossi, 1965; Slinkard ve Singleton, 1977; Göttingerová ve ark., 2021) ve toplam tanen miktarı (mg kg^{-1}) (AOAC, 1998; Tangolar ve ark., 1999) parametreleri incelenmiştir.

Müşküle üzüm çeşidinde kış budaması; araştırmanın yürütüldüğü yılların mart ayı

içerisinde 2–3 gözden kısa budama şeklinde gerçekleştirilmiştir. Yaz budaması ise; dip sürgünler ile obur sürgünlerin alınması, yazlık sürgünlerin en alt boğumlarındaki 2–3 adet dip yaprak ile koltuk sürgünlerinin dipte bir yaprak kalacak şekilde uçlarının alınması ve ikinci sürgün bağlama teli seviyesinin 5–10 cm üzerinden uç alma işleminin yapılması şeklinde uygulanmıştır. İlkbahar döneminde sıra aralarında mekanik toprak işleme, sıra üzerlerinde ise çapalama işlemi gerçekleştirilmiş, kış budaması sonrasında ölü kol (*Phomopsis viticola* Sacc.) için omcalara %5'lik bordo bulamacı ile bağ küllemesi (*Uncinula necator* "Schwein" Burr) ve bağ mildiyösü (*Plasmopara viticola*) için yazlık sürgünlerin 5–10 cm'ye (nisan sonu) ulaşmasıyla birlikte ben düşme dönemine kadar kimyasal mücadelelere devam edilmiştir.

Yürütülen bu araştırma, tesadüf parselleri deneme desenine göre 3 tekerrürlü olarak yapılmış ve her tekerrürde 3'er adet omcaya yer verilmiştir. Elde edilen araştırma bulguları 'SAS 9.1.3. Portable' istatistik paket programı kapsamında varyans analizi ile belirlenmiş, incelenen parametrelerde dönemler arasındaki farklılık LSD çoklu karşılaştırma testiyle $p<0.05$ düzeyinde değerlendirilmiştir.

Araştırma Bulguları ve Tartışma

Bu araştırmadan elde edilen tane özelliklerine ait değerler; Çizelge 1 ve Çizelge 2'de, tane rengine ait değerler; Çizelge 3'te, tane olgunluğuna ait değerler; Çizelge 4 ve Çizelge 5'te, toplam fenolik bileşik ve tanen miktarı ise; Çizelge 6, Şekil 1 ve Şekil 2'de gösterilmiştir.

Müşküle üzüm çeşidinde 2020 ve 2021 yılları ile iki yıllık ortalama tane eni ve tane boyu değerlerinde haftalar arasında önemli bir farklılık tespit edilememiştir. İki yıllık ortalama değerlerine bakıldığında; tane eni 17.41 mm tane^{-1} (3. hafta) ile 17.68 mm tane^{-1} (5. hafta) arasında; tane boyu ise 19.17 mm tane^{-1} (3. hafta) ile 19.42 mm tane^{-1} (1. hafta) arasında değişkenlik göstermiştir. 2020 yılındaki en yüksek tane ağırlığı; 4. haftadan (4.20 g tane^{-1}), en düşük değer ise 1. haftadan (3.94 g tane^{-1}) elde edilmiş olup, sırasıyla 3. hafta (3.96 g tane^{-1}), 2. hafta (4.11 g tane^{-1}), 5. hafta (4.15 g tane^{-1}) ve 6. hafta (4.17 g tane^{-1}) ara grupta yer almıştır. 2021 yılındaki en yüksek tane ağırlığı ise; son dönem olan 6. haftada (4.53 g tane^{-1}), en düşük tane ağırlığı ise 4. haftada (4.23 g tane^{-1}) saptanmıştır. Sırasıyla 3. hafta (4.28 g tane^{-1}), 1. hafta (4.39 g tane^{-1}), 5. hafta (4.45 g tane^{-1}) ve 2. hafta (4.47 g tane^{-1}) ara grubu oluşturmuştur. İki yıllık ortalama tane ağrılıklarına bakıldığında; en düşük değer 3. haftadan (4.12 g tane^{-1}), en yüksek değer 6. haftadan (4.35 g tane^{-1}) elde edilirken, sırasıyla 1. hafta (4.16 g tane^{-1}), 4. hafta (4.22 g tane^{-1}), 2. hafta (4.29 g tane^{-1}) ve 5. hafta (4.30 g tane^{-1}) farklı ara grupları meydana getirmiştir (Çizelge 1).

Tane boyut indeksi değerlerine bakıldığında; 2021 yılında en yüksek değer 4. haftadan (1.137), en düşük değerler ise sırasıyla 5. hafta (1.107) ve 2. haftadan (1.111) belirlenmiştir. Bunları sırasıyla 6. hafta (1.117), 3. hafta (1.123) ve 1. hafta (1.130) takip etmiştir. İki yıllık ortalama tane boyut indeksi değerlerine göre; en yüksek değer 4. haftada (1.110), en düşük değer ise 5. haftada (1.087) saptanmıştır. Sırasıyla 6. hafta (1.097), 1. hafta (1.100), 2. hafta (1.100) ve 3. hafta (1.100) ara grubu oluşturmuştur. 2020 yılı tane kabuk kalınlığı incelendiğinde; en yüksek değer 1. haftadan (0.280 mm tane^{-1}), en düşük değerler ise sırasıyla 5. hafta (0.183 mm tane^{-1}) ve 2. haftadan (0.193 mm tane^{-1}) alınmıştır. Sırasıyla 3. hafta (0.207 mm tane^{-1}), 4. hafta (0.227 mm tane^{-1}) ve 6. hafta (0.247 mm tane^{-1}) farklı ara gruplarda yer almıştır. 2021 yılı en yüksek tane kabuk kalınlığı; 2. haftadan (0.237 mm tane^{-1}), en düşük ise 6. haftadan (0.113 mm tane^{-1}) elde edilmiştir. Sırasıyla 5. hafta (0.133 mm tane^{-1}), 4. hafta (0.160 mm tane^{-1}), 3. hafta (0.187 mm tane^{-1}) ve 1. hafta (0.223 mm tane^{-1}) farklı ara grupları oluşturmuştur. İki yıllık ortalama değerlere göre; en yüksek tane kabuk kalınlığı 1. haftada (0.250 mm tane^{-1}), en düşük ise 5. haftada (0.160 mm tane^{-1}) belirlenmiş olup, 6. hafta (0.180 mm tane^{-1}), 4. hafta (0.190 mm tane^{-1}), 3. hafta (0.200 mm tane^{-1}) ve 2. hafta (0.217 mm tane^{-1}) farklı ara grupları meydana getirmiştir (Çizelge 2).

Müşküle üzüm çeşidinde tane renk değerlerinden parlaklığı ifade eden L değerlerine göre; 2020 yılında en yüksek değer 4. haftadan (36.74), en düşük değer ise son dönem olan 6. haftadan (35.19) elde edilmiştir. Sırasıyla 2. hafta (35.65), 1. hafta (36.30), 5. hafta (36.35) ve 3. hafta (36.51) farklı ara grupları teşkil etmiştir. 2021 yılı L değerleri incelendiğinde; en yüksek değer 1. haftada (34.59) en düşük değer ise yine son dönem olan 6. haftada (26.16) saptanmıştır. Sırasıyla 5. hafta (28.31), 2. hafta (32.20), 3. hafta (32.27) ve 4. hafta (33.57) farklı ara gruplarda yer almıştır. İki yıllık ortalama L değerlerinde; en yüksek değer 1. haftada (35.45) elde edilirken, en düşük değer 6. haftada (30.67) belirlenmiştir. Bunu sırasıyla 5. hafta (32.33), 2. hafta (33.93), 3. hafta (34.39) ve 4. hafta (35.15) izlemiştir. 2020 yılında en yüksek Chroma değeri; 2. haftada (10.67), en düşük değerler ise sırasıyla 5. hafta (9.22), 6. hafta (9.38) ve 4. haftada (9.62) tespit edilirken, 3. hafta (9.90) ve 1. hafta (10.40) farklı ara grupları oluşturmuştur. 2021 yılı en yüksek Chroma değerleri sırasıyla 3. hafta (11.20), 4. hafta (10.99), 2. hafta (10.79) ve 1. haftadan (10.52), en düşük değerler sırasıyla 5. hafta (5.78) ve 6. haftadan (6.07) alınmıştır. İki yıllık ortalama değerlere göre; en yüksek Chroma değerleri sırasıyla 2. hafta (10.73), 3. hafta (10.55), 1. hafta (10.46) ve 4. haftada (10.30), en düşük değerler sırasıyla 5. hafta (7.50) ve 6. haftada (7.72) belirlenmiştir (Çizelge 3).

Müşküle üzüm çeşidinde 2020 yılına ait Hue değerlerine göre; en yüksek değerler sırasıyla 2. hafta (98.71), 1. hafta (97.78) 3. hafta (96.55) ve 4. haftadan (95.89) elde edilirken, en düşük değerler sırasıyla 6. hafta (92.57) ve 5. haftadan (92.80) alınmıştır. 2021 yılında en yüksek Hue değeri 1. haftadan (102.73), en düşük değer ise 6. haftadan (97.87) alınmıştır. 3. hafta (100.27), 4. hafta (100.82), 5. hafta (101.38) ve 2. hafta (101.85) farklı ara grupları oluşturmuştur. İki yıllık ortalama Hue değerlerine göre; en yüksek değerler sırasıyla 2. hafta (100.28) ve 1. haftada (100.26), en düşük değer ise 6. haftada (95.22) saptanmıştır. Sırasıyla 5. hafta (97.09), 4. hafta (98.35) ve 3. hafta (98.41) ara grubu meydana getirmiştir (Çizelge 3). Elde edilen iki yıllık tane rengi değerlerine göre; hasat olgunluğundan itibaren 6. haftanın sonuna kadar tane kabuğunda pus tabakasının oldukça belirginleşmesiyle birlikte, parlaklığın ve renk canlılığının da azaldığı görülmektedir. Böylece tane renginin ise yeşilimsi–sarıdan daha sarı tonlara doğru değişim gösterdiği tespit edilmiştir.

Tane kabuk rengi ve olgunlaşma arasında yakın bir ilişki mevcuttur. Üzüm çeşidine özgü olmak üzere koyu mor ve kırmızı çeşitlerde renk parlak–koyu, renksiz çeşitlerde ise açık ve kehribar sarısı renk oluşturma durumu olgunluğu yansıtmaktadır. Üzüm çeşitlerinde kabuk ve salkım sapının rengi üzümler olgunlaştıkça değişim göstermektedir (Çoban, 2023). Khalil ve ark. (2023)'nın yürütmüş oldukları konuyla ilgili bir araştırmada, Sultanina, Flame Seedless ve NARC Black üzüm çeşitlerinde erken olgunluk dönemlerinde L değerinin yüksek olmasıyla tanelerin daha parlak, olgunlaşmanın ilerlemesiyle birlikte L değerinin azalmasıyla birlikte ise tane üzerinde pus tabakası oluşumunun ve donuklaşmanın göstergesi olduğunu bildirmişlerdir. Elde edilmiş olan araştırma bulgularıyla literatür bildirişleri uyum içerisindedir.

Müşküle üzüm çeşidinde 2020 yılı SÇKM değerlerinde dönemler arasında önemli bir farklılık tespit edilememiş olup, %20.97 (2. hafta) ile %21.63 (5. hafta) değerleri arasında değişkenlik göstermiştir. 2021 yılı SÇKM değerlerine bakıldığında; en yüksek değerler sırasıyla 6. hafta (%20.37), 4. hafta (%20.23) ve 5. haftada (%20.17), en düşük değerler ise; sırasıyla 2. hafta (%19.40), 3. hafta (%19.57) ve 1. haftada (%19.67) belirlenmiştir. İki yıllık ortalama SÇKM değerlerine göre; en yüksek değerler sırasıyla 6. hafta (%20.95), 5. hafta (%20.90) ve 4. haftadan (%20.88), en düşük değer ise; 2. haftadan (%20.18) elde edilmiştir. 1. hafta (%20.41) ve 3. hafta (%20.52) ara grupta yer almıştır. 2020 yılı ve iki yıllık ortalama pH değerlerinde dönemler arasında önemli bir farklılık belirlenememiştir.

Çizelge 1. Müşküle üzüm çeşidinin tane özelliklerine ait değerler
Table 1. The values of berry characteristics of the Müşküle grape variety

Dönemler *Periods*	Tane eni (mm tane^{-1}) *Berry width (mm berry^{-1})*			Tane boyu (mm tane^{-1}) *Berry length (mm berry^{-1})*			Tane ağırlığı (g tane^{-1}) *Berry weight (g berry^{-1})*		
	2020	2021	Ort.	2020	2021	Ort.	2020	2021	Ort.
1. Hafta	17.11	18.17	17.64	18.38	20.47	19.42	3.94 b	4.39 ab	4.16 bc
2. Hafta	17.02	18.32	17.67	18.45	20.35	19.40	4.11 ab	4.47 ab	4.29 ab
3. Hafta	16.79	18.03	17.41	18.04	20.29	19.17	3.96 ab	4.28 ab	4.12 c
4. Hafta	16.93	17.97	17.45	18.31	20.40	19.36	4.20 a	4.23 b	4.22 abc
5. Hafta	17.10	18.27	17.68	18.30	20.21	19.26	4.15 ab	4.45 ab	4.30 ab
6. Hafta	16.93	18.38	17.66	18.16	20.51	19.34	4.17 ab	4.53 a	4.35 a
LSD (0.05)	ÖD	ÖD	ÖD	ÖD	ÖD	ÖD	0.254	0.287	0.161

ÖD: Önemli Değil. LSD: Least Significant Difference.

Çizelge 2. Müşküle üzüm çeşidinin tane özelliklerine ait değerler
Table 2. The values of berry characteristics of the Müşküle grape variety

Dönemler *Periods*	Tane boyut indeksi (tane boyu tane eni^{-1}) *Berry size index (berry length berry width^{-1})*			Tane kabuk kalınlığı (mm tane^{-1}) *Thickness of berry skin (mm berry^{-1})*		
	2020	2021	Ort.	2020	2021	Ort.
1. Hafta	1.073	1.130 ab	1.100 ab	0.280 a	0.223 ab	0.250 a
2. Hafta	1.087	1.111 b	1.100 ab	0.193 c	0.237 a	0.217 ab
3. Hafta	1.073	1.123 ab	1.100 ab	0.207 bc	0.187 abc	0.200 b
4. Hafta	1.083	1.137 a	1.110 a	0.227 bc	0.160 bcd	0.190 bc
5. Hafta	1.073	1.107 b	1.087 b	0.183 c	0.133 cd	0.160 c
6. Hafta	1.077	1.117 ab	1.097 ab	0.247 ab	0.113 d	0.180 bc
LSD (0.05)	ÖD	0.024	0.019	0.051	0.067	0.039

ÖD: Önemli Değil. LSD: Least Significant Difference.

Çizelge 3. Müşküle üzüm çeşidinin tane rengine ait değerler
Table 3. The values of berry color of the Müşküle grape variety

Dönemler	L			Chroma			Hue		
Periods	2020	2021	Ort.	2020	2021	Ort.	2020	2021	Ort.
1. Hafta	36.30 ab	34.59 a	35.45 a	10.40 ab	10.52 a	10.46 a	97.78 a	102.73 a	100.26 a
2. Hafta	35.65 bc	32.20 b	33.93 c	10.67 a	10.79 a	10.73 a	98.71 a	101.85 ab	100.28 a
3. Hafta	36.51 ab	32.27 b	34.39 bc	9.90 bc	11.20 a	10.55 a	96.55 a	100.27 b	98.41 b
4. Hafta	36.74 a	33.57 ab	35.15 ab	9.62 c	10.99 a	10.30 a	95.89 a	100.82 b	98.35 b
5. Hafta	36.35 ab	28.31 c	32.33 d	9.22 c	5.78 b	7.50 b	92.80 b	101.38 ab	97.09 b
6. Hafta	35.19 c	26.16 d	30.67 e	9.38 c	6.07 b	7.72 b	92.57 b	97.87 c	95.22 c
LSD (0.05)	0.983	1.644	0.958	0.692	0.859	0.576	2.954	1.682	1.815

LSD: Least Significant Difference.

Çizelge 4. Müşküle üzüm çeşidinin tane olgunluğuna ait değerler
Table 4. The values of berry maturity of the Müşküle grape variety

Dönemler ***Periods***	**SÇKM (%)** ***TSS (%)***			**pH**		
	2020	**2021**	**Ort.**	**2020**	**2021**	**Ort.**
1. Hafta	21.15	19.67 b	20.41 ab	3.83	3.70 b	3.77
2. Hafta	20.97	19.40 b	20.18 b	3.87	3.74 b	3.80
3. Hafta	21.47	19.57 b	20.52 ab	3.73	3.71 b	3.73
4. Hafta	21.53	20.23 a	20.88 a	3.74	3.73 b	3.74
5. Hafta	21.63	20.17 a	20.90 a	3.63	3.72 b	3.68
6. Hafta	21.53	20.37 a	20.95 a	3.61	3.91 a	3.76
LSD (0.05)	ÖD	0.463	0.644	ÖD	0.061	ÖD

ÖD: Önemli Değil. LSD: Least Significant Difference.

Çizelge 5. Müşküle üzüm çeşidinin tane olgunluğuna ait değerler
Table 5. The values of berry maturity of the Müşküle grape variety

Dönemler ***Periods***	**Asitlik (%)** ***Acidity (%)***			**Olgunluk indisi (%SÇKM%asitlik^{-1})** ***Maturity indeks (TTS acidity^{-1})***		
	2020	**2021**	**Ort.**	**2020**	**2021**	**Ort.**
1. Hafta	0.350 b	0.463 a	0.410 a	60.47 a	42.55 d	51.51 b
2. Hafta	0.413 a	0.383 e	0.397 ab	51.24 c	50.73 a	50.99 b
3. Hafta	0.410 a	0.400 de	0.400 ab	53.51 bc	49.24 a	51.37 b
4. Hafta	0.350 b	0.443 ab	0.397 ab	61.53 a	45.76 c	53.65 ab
5. Hafta	0.370 ab	0.440 bc	0.403 ab	58.28 ab	46.12 bc	52.20 b
6. Hafta	0.347 b	0.420 cd	0.383 b	62.41 a	48.82 ab	55.62 a
LSD (0.05)	0.047	0.021	0.021	6.125	2.909	3.083

LSD: Least Significant Difference.

Çizelge 6. Müşküle üzüm çeşidine ait toplam fenolik bileşik ve tanen miktarı
Table 6. Total phenolic compound and tannin content of the Müşküle grape variety

Dönemler ***Periods***	**Toplam fenolik bileşik miktarı (mg GAE 100 ml^{-1})** ***Total phenolic compound content (mg GAE 100 ml^{-1})***			**Toplam tanen miktarı (mg kg^{-1})** ***Total tannin content (mg kg^{-1})***		
	2020	**2021**	**Ort.**	**2020**	**2021**	**Ort.**
1. Hafta	4.55 a	5.63 a	5.09 a	0.862 a	1.284 a	1.073 a
2. Hafta	4.00 ab	5.52 a	4.76 ab	0.830 ab	1.184 a	1.007 ab
3. Hafta	3.84 abc	4.94 b	4.39 bc	0.730 ab	1.180 a	0.955 ab
4. Hafta	3.80 abc	4.81 b	4.30 b	0.669 bc	1.119 a	0.894 bc
5. Hafta	3.69 bc	3.75 c	3.72 c	0.659 bc	0.916 b	0.787 c
6. Hafta	3.05 c	3.12 d	3.09 d	0.541 c	0.573 c	0.557 d
LSD (0.05)	0.813	0.322	0.489	0.172	0.176	0.163

LSD: Least Significant Difference.

Müşküle üzüm çeşidinin 2021 yılı pH değerleri incelendiğinde; en yüksek değerin son dönem olan 6. haftada (3.91), en düşük değerlerin ise; sırasıyla 1. hafta (3.70), 3. hafta (3.71), 5. hafta (3.72), 4. hafta (3.73) ve 2. haftadan (3.74) alındığı saptanmıştır (Çizelge 4).

Üzüm tanesinin olgunlaşma dönemine bağlı olarak SÇKM ve pH değerlerinin artış gösterdiği, özellikle 2021 yılı ve iki yıllık ortalama verilerde belirlenmiştir. Literatürde Cabernet Sauvignon (Bindon ve ark., 2013), Gewürtztraminer, Pinot Noir, Syrah, Narince (Cangi ve ark., 2011), Sultani Çekirdeksiz, Yuvarlak Çekirdeksiz, Çalkarası ve Şiraz (Otağ, 2015) üzüm çeşitlerinde olgunlaşmaya bağlı olarak SÇKM ve pH değerlerinde artışlar olduğu belirtilmektedir. Bu araştırmadan elde edilmiş olan araştırma bulgularıyla, mevcut literatürde elde edilen sonuçlar uyum içerisindedir.

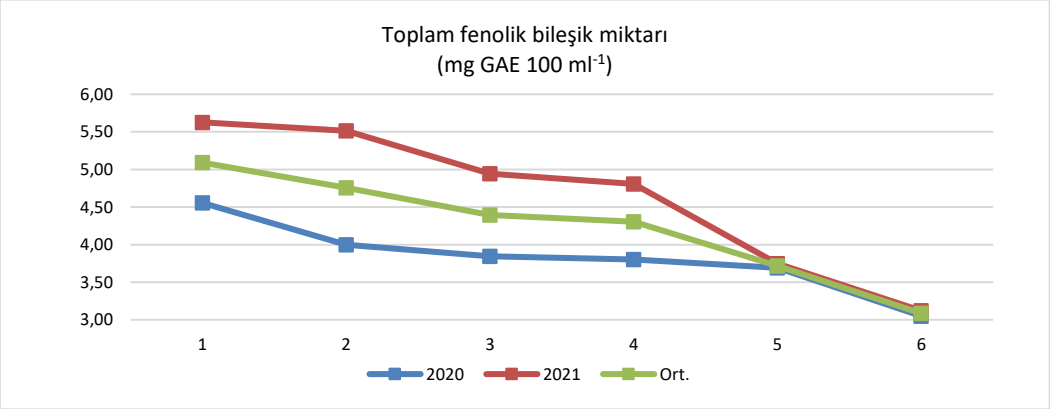
Müşküle üzüm çeşidinde 2020 yılına ait asitlik değerine göre; en yüksek değerler sırasıyla 2. hafta (%0.413) ve 3. haftadan (%0.410), en düşük değerler ise sırasıyla 6. hafta (%0.347), 1. hafta (%0.350) ve 4. haftadan (%0.350) elde edilmiş olup, 5. hafta %0.370 asitlik değeriyle ara grupta yer almıştır. 2021 yılında en yüksek asitlik değeri; 1. haftada (%0.463), en düşük değer ise 2. haftada (%0.383) belirlenmiştir. Sırasıyla 3. hafta (%0.400), 6. hafta (%0.420), 5. hafta (%0.440) ve 4. hafta (%0.443) farklı ara grupları teşkil etmiştir. İki yıllık ortalama asitlik değerlerine göre; en yüksek değer 1. haftada (%0.410), en düşük değer ise 6. haftada (%0.383) saptanmıştır. 2. hafta (%0.397), 4. hafta (%0.397), 3. hafta (%0.400) ve 5. hafta (%0.403) ara grupta yer almıştır (Çizelge 5).

Üzüm tanesinin ilk gelişim aşamasından ben düşme dönemine kadar asitlik değerinin arttığı, bu dönemden sonra ise hızlı bir şekilde azalmaya başladığı ve olgunlaşmaya yakın dönemden itibaren bu azalmanın daha yavaş bir seyir izlediği, çeşitli araştırıcılar tarafından ortaya konulmuştur (Ağaoğlu, 2002; Şen, 2007). Literatürde özellikle olgunlaşma dönemine yakın süreçte asitlik değerindeki azalmanın yavaşladığına dair bulgular, bu araştırmada elde edilen sonuçlarla uyum göstermektedir.

Müşküle üzüm çeşidinde 2020 yılına en yüksek olgunluk indisi değerleri; sırasıyla 6. haftada (62.41), 4. haftada (61.53) ve 1. haftada (60.47), en düşük değer ise 2. haftada (51.24) tespit edilmiştir. Sırasıyla 3. hafta (53.51) ve 5. hafta (58.28) farklı ara grupları meydana getirmiştir. 2021 yılında en yüksek olgunluk indisi; sırasıyla 2. hafta (50.73) ve 3. haftadan (49.24), en düşük değer ise 1. haftadan (42.55) alınmıştır. Sırasıyla 4. hafta (45.76), 5. hafta (46.12) ve 6. hafta (48.82) farklı ara grupları oluşturmuştur. İki yıllık ortalama olgunluk indisi değerlerine göre; en yüksek değer 6. haftada (55.62), en düşük değerler ise sırasıyla 2. hafta (50.99), 3. hafta (51.37), 1. hafta (51.51) ve 5. haftada (52.20) belirlenmiştir. 4. hafta ise 53.65 olgunluk indisi değeriyle ara grupta yer almıştır (Çizelge 5).

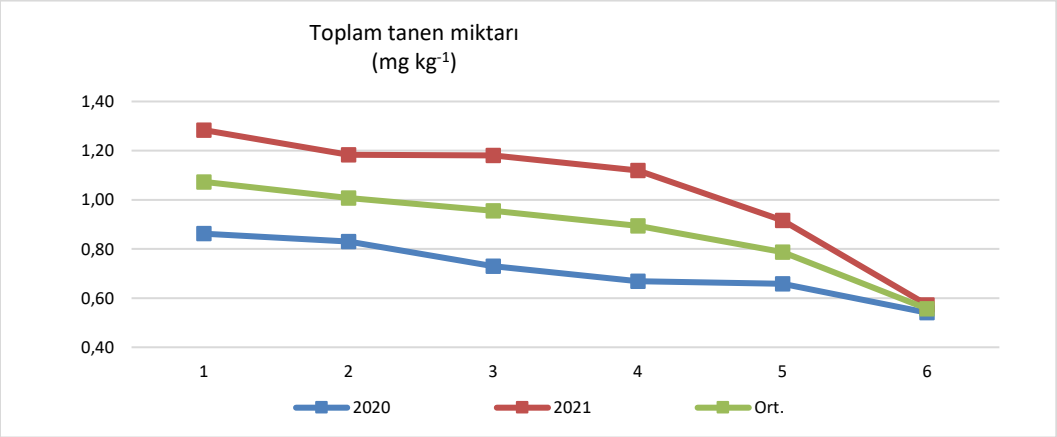
Müşküle üzüm çeşidinde, olgunlaşma süreci boyunca SÇKM değerindeki artış ve asitlik değerindeki azalmaya paralel olarak olgunluk indisinin de yükseldiği belirlenmiştir. Bu araştırmadan elde edilen bulgular, farklı olgunluk dönemlerinde üzüm olgunluk özelliklerini inceleyen birçok araştırıcılar tarafından (Aydın, 2015; Otağ, 2015; Özdemir ve Sessiz, 2018; Doğan ve ark., 2018; Demir, 2019; Şahin ve ark., 2024) literatürde rapor edilen sonuçlarla uyum içerisindedir.

Müşküle üzüm çeşidinde 2020 yılında en yüksek toplam fenolik bileşik miktarı; 1. haftada (4.55 mg GAE 100 ml^{-1}), en düşük değer ise 6. haftada (3.05 mg GAE 100 ml^{-1}) tespit edilmiştir. Sırasıyla 5. hafta (3.69 mg GAE 100 ml^{-1}), 4. hafta (3.80 mg GAE 100 ml^{-1}), 3. hafta (3.84 mg GAE 100 ml^{-1}) ve 2. hafta (4.00 mg GAE 100 ml^{-1}) farklı ara grupları oluşturmuştur. 2021 yılında en yüksek toplam fenolik bileşik miktarı; sırasıyla 1. haftada (5.63 mg GAE 100 ml^{-1}) ve 2. haftada (5.52 mg GAE 100 ml^{-1}), en düşük değer ise 6. haftada (3.12 mg GAE 100 ml^{-1}) saptanmıştır (Çizelge 6).



Şekil 1. Müşküle üzüm çeşidinde toplam fenolik bileşik miktarı grafiği

Figure 1. Total phenolic compound content graph in the Müşküle grape variety



Şekil 2. Müşküle üzüm çeşidinde tanen miktarı grafiği

Figure 2. Total tannin content graph in the Müşküle grape variety

Toplam fenolik bileşik miktarında 5. hafta (3.75 mg GAE 100 ml^{-1}), 4. hafta (4.81 mg GAE 100 ml^{-1}) ve 3. hafta (4.94 mg GAE 100 ml^{-1}) farklı ara grupları teşkil etmiştir. İki yıllık ortalama değerler incelendiğinde; en yüksek toplam fenolik bileşik miktarı 1. haftada (5.09 mg GAE 100 ml^{-1}), en düşük 6. haftada (3.09 mg GAE 100 ml^{-1}) bulunmuştur. Sırasıyla 5. hafta (3.72 mg GAE 100 ml^{-1}), 4. hafta (4.30 mg GAE 100 ml^{-1}), 3. hafta (4.39 mg GAE 100 ml^{-1}) ve 2. hafta (4.76 mg GAE 100 ml^{-1}) farklı ara grupları meydana getirmiştir (Çizelge 6).

Müşküle üzüm çeşidinde 2020 yılında en yüksek toplam tanen miktarı; 1. haftada (0.862 mg kg^{-1}), en düşük değer ise 6. haftada (0.541 mg kg^{-1}) tespit edilmiştir. Sırasıyla 5. hafta (0.659 mg kg^{-1}), 4. hafta (0.669 mg kg^{-1}), 3. hafta (0.730 mg kg^{-1}) ve 2. hafta (0.830 mg kg^{-1}) farklı ara grupları oluşturmuştur. 2021 yılında en yüksek toplam tanen miktarı; sırasıyla 1. haftadan (1.284 mg kg^{-1}), 2. haftadan (1.184 mg kg^{-1}), 3. haftadan (1.180 mg kg^{-1}) ve 4. haftadan (1.119 mg kg^{-1}) elde edilirken, en düşük değer 6. haftada (0.573 mg kg^{-1}) tespit edilmiştir. 0.916 mg kg^{-1} değeriyle 5. hafta ara grupta yer almıştır. İki yıllık ortalama değerler incelendiğinde; en yüksek değer 1. haftada (1.073 mg kg^{-1}), en düşük değer ise 6. haftada (0.557 mg kg^{-1}) belirlenmiştir. Sırasıyla 5. hafta (0.787 mg kg^{-1}), 4. hafta (0.894 mg kg^{-1}), 3. hafta (0.955 mg kg^{-1}) ve 2. hafta (1.007 mg kg^{-1}) farklı ara grupları teşkil etmiştir (Çizelge 6).

Müşküle üzüm çeşidinde her iki araştırma yılında hasat olgunluğundan (1. hafta) itibaren 6. hafta dâhil olmak üzere alınan örneklerde, toplam fenolik bileşik ve toplam tanen miktarının giderek azalma eğilimi gösterdiği belirlenmiştir (Şekil 1 ve Şekil 2). Üzüm çeşidi ve araştırma yıllarına bağlı olarak olgunluk ilerledikçe toplam fenolik bileşik miktarının da azaldığı, konuyla ilgili literatürde vurgulanmıştır (Deryaoğlu ve Canbaş, 2004; Jin ve ark., 2009; Cangi ve ark., 2011). Ben düşme dönemi öncesinde tane bünyesindeki yüksek tanen miktarının olgunluk ilerledikçe azalmasıyla, toplam

fenolik bileşik miktarında da azalma eğilimi görülmektedir.

Sonuçlar

Bu araştırmada, Müşküle üzüm çeşidi salkımlarının hasat olgunluğundan itibaren altı hafta boyunca omcalar üzerinde bekletilmesinin tane kalitesi ve biyokimyasal özellikler üzerindeki etkileri değerlendirilmiştir. Bulgular, SÇKM ve olgunluk indisinde artış, asitlik ve toplam fenolik bileşik miktarında azalma ile olgunlaşma sürecinin karakteristik değişimlerini ortaya koymuştur. Tane ağırlığı, boyut ve kabuk kalınlığı gibi fiziksel özelliklerde hafif dalgalanmalar görülmüş, ancak genel olarak bu parametreler kaliteyi olumsuz etkilemeyecek şekilde korunmuştur. Tane renginde ise parlaklığın ve renk canlılığının azalarak yeşilimsi–sarıdan daha sarı tonlara geçiş olduğu tespit edilmiştir. Toplam tanen ve fenolik bileşik miktarındaki azalmalar, olgunlaşma sürecinin biyokimyasal özellikler üzerindeki etkilerini yansıtmaktadır.

Bu araştırma, erken sonbahar donlarının görülmediği yıllarda Çanakkale gibi ekolojilerde, üzüm salkımlarının pazarlama değerinin yüksek olduğu bir dönemde satışa sunulabilmesi için omcalar üzerinde bekletilmesinin ekonomik avantajlar sağlayacağını ortaya koymaktadır.

Çıkar çatışması: Beyanı Makale yazarları aralarında herhangi bir çıkar çatışması yoktur.

Yazar katkısı: Araştırmanın arazi ve laboratuvar kısmı, verilerin hesaplanması, istatistiki analizi ile değerlendirilmesi kısmında Alper Dardeniz ve Esra Şahin, makalenin yazımı ve literatür katkısı kısmında ise bütün yazarlar eşit oranda katkı sağlamışlardır.

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Araştırma Makalesi/Research Article

Karpuz (*Citrullus lanatus* L.) fidesi gelişim parametreleri ve klorofil içeriği üzerine *Azospirillum lipoferum* ve deniz yosunu uygulamalarının etkileri

Effects of Azospirillum lipoferum and seaweed applications on watermelon (Citrullus lanatus L.) seedling growth parameters and chlorophyll content

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ÖZ

Azospirillum lipoferum (AzL) ve deniz yosunu (DY) uygulamalarının karpuz fidelerinin büyüme parametreleri ve klorofil içeriği üzerindeki etkilerini incelemek amacıyla yürütülen bu çalışma, Türkiye'deki sürdürülebilir fidecilik uygulamalarına katkı sağlamayı hedeflemiştir. Araştırma, Van Yüzüncü Yıl Üniversitesi, Ziraat Fakültesi'nde kontrollü çevre koşullarında gerçekleştirilmiş ve 'Crimson Sweet' karpuz çeşidi kullanılmıştır. Toplamda 10 farklı uygulama grubu (AzL ve DY'nin üçer dozu ile kombinasyonları ve kontrol grubu) değerlendirilmiştir. Sonuçlar, *Azospirillum*'un düşük dozunun (1.25 mL L^{-1}) kök yaş ağırlığı ve klorofil miktarı gibi parametrelerde daha yüksek performans sağladığını, ancak yüksek dozlarda büyümeyi olumsuz etkileyebileceğini göstermiştir. Deniz yosunu özünün yüksek dozu (5 mL L^{-1}), yaprak sayısı, yaprak yaş ve kuru ağırlığı ile klorofil miktarında en iyi sonuçları sağlamıştır. Kombinasyon uygulamaları, özellikle orta dozlarda (2.5 mL L^{-1}), klorofil miktarı ve yaprak yaş ağırlığı gibi fotosentezle ilişkili parametrelerde sinerjik etkiler göstermiştir. Çalışma, *Azospirillum*'un rizosferde besin alımını artırma kapasitesi ile deniz yosununun biyolojik aktif bileşenlerinin birleşiminin bitki gelişimini desteklediğini ortaya koymaktadır. Gelecekte bu biyostimülantların; doz, yöntem ve çevresel koşullara göre optimize edilmesi, etkilerinin moleküler düzeyde incelenmesi ve saha denemeleri ile ekonomik analizlerinin yapılması önerilmektedir. Bu çalışmadan elde edilen sonuçların, sürdürülebilir fidecilik uygulamalarının yaygınlaşmasına katkı sağlayabileceği düşünülmektedir.

Anahtar Kelimeler: *Citrullus lanatus* L., *Azospirillum lipoferum*, Deniz yosunu, Sürdürülebilir Tarım, Fide Yetiştiriciliği

ABSTRACT

This study aimed to investigate the effects of *Azospirillum lipoferum* (AzL) and seaweed (Seaweed) treatments on growth parameters and chlorophyll content of watermelon seedlings and aimed to contribute to sustainable seedling practices in Turkey. The experiment was conducted at Van Yuzuncu Yil University, Faculty of Agriculture, under controlled environmental conditions, using the 'Crimson Sweet' watermelon cultivar. A total of 10 different treatment groups (three doses and combinations of AzL and DY and control group) were evaluated. The results indicated that a low dose of *Azospirillum* (1.25 mL L^{-1}) enhanced parameters such as root fresh weight and chlorophyll content, while high doses appeared to negatively affect growth. The high dose of seaweed extract (5 mL L^{-1}) provided the best results in leaf number, leaf fresh and dry weight and chlorophyll content. Combination treatments, especially at medium doses (2.5 mL L^{-1}), showed synergistic effects on photosynthesis-related parameters including chlorophyll content and leaf fresh weight.

The study reveals that the combination of the capacity of *Azospirillum* to enhance nutrient uptake in the rhizosphere and the biologically active components of seaweed promotes plant growth. Future studies should focus on optimizing these biostimulants based on dose, application method, and environmental conditions, as well as investigating their molecular effects and conducting field trials and economic analyses. The results obtained from this study are thought to contribute to the widespread adoption of sustainable seedling practices.

Key Words: *Citrullus lanatus* L., *Azospirillum lipoferum*, Seaweed, Sustainable Agriculture, Seedling Cultivation

Giriş

Dünya nüfusundaki hızlı artış ve sağlıklı beslenmeye yönelik bilincin yaygınlaşması, tarımsal üretimde sürdürülebilir yöntemlere olan ihtiyacı artırmaktadır. Gıda güvenliğinin sağlanması yalnızca yeterli miktarda gıda sağlamayı değil, aynı zamanda sağlıklı ve dengeli beslenmeyi destekleyen besin çeşitliliği ve kalitesini de gerektirmektedir (FAO, 2020; Benchlih ve ark., 2023; Aoudi ve ark., 2024; Hasan ve ark., 2024). Sebzeler içerdiği vitamin, mineral ve liflerle sağlıklı beslenmede önemli bir rol oynamaktadır. Bu nedenle sebze üretiminde sürdürülebilir yöntemlerin geliştirilmesi kritik bir gereklilik haline gelmiştir (WHO, 2024). FAO verilerine göre, dünya genelinde sebze üretimi 1.128 milyar tona ulaşmış, üretimin %55'i Çin, %11'i Hindistan ve %2.5'i Türkiye tarafından gerçekleştirilmiştir (FAO, 2024). Türkiye, özellikle karpuz (*Citrullus lanatus* L.) üretiminde önemli bir konuma sahiptir. FAO verilerine göre, Türkiye karpuz üretiminde Çin'in ardından ikinci sırada yer almaktadır. Türkiye'de karpuz yetiştiriciliği daha çok Akdeniz ve Güneydoğu Anadolu bölgelerinde yoğunlaşmakla beraber Adana, Antalya ve Diyarbakır illeri önde gelen üretim merkezleri olarak belirlenmiştir (TÜİK, 2024).

Sebze yetiştiriciliği, kaliteli fide materyaline duyulan ihtiyacı artırmaktadır. Bu noktada fidecilik tarımsal üretimin temelini oluşturarak kritik bir rol üstlenmektedir. Ancak artan fide talebi, genellikle kimyasal gübre ve pestisitlerin yaygın kullanımına yol açmaktadır. Bu uygulamalar, kısa vadede verimlilik sağlasa da uzun vadede toprak sağlığını olumsuz etkileyebilmektedir (Dohroo ve Thakur, 2024; Jain ve ark., 2024). Türkiye'de tarımsal ilaç ve gübre kullanımı son yıllarda artış gösterirken, bu kimyasalların çevresel etkilerinin sürdürülebilir tarım için engel teşkil ettiği belirtilmektedir (Gaytancıoğlu ve Yılmaz, 2024; TÜİK, 2024). Fidecilikte organik tarım prensipleriyle uyumlu ve çevre dostu uygulamaların benimsenmesi önem kazanmıştır. Biyostimülantlar ve mikrobiyal gübreler bu kapsamda önemli bir alternatif sunmaktadır. PGPR (Bitki Gelişimini Teşvik Edici Rizobakteriler) ve deniz yosunu gibi biyostimülantların, fidelerin kök ve genel gelişimini desteklediği rapor edilmiştir. Aynı zamanda çevresel streslere karşı dayanıklılığı artırdığı da belirtilmektedir (Rocha ve ark., 2022; Perisoara ve ark., 2022; Kumar ve ark., 2022). *Azospirillum lipoferum*'un, bitkilerin besin alımı ve kök gelişimini desteklediği; deniz yosunu özünün ise içerdiği vitamin, amino asit ve hormonlarla bitki gelişimi üzerindeki olumlu etkileri yapılmış olan çalışmalarda ifade edilmektedir (Khan ve ark., 2020; Shahrajabian ve ark., 2023; Rouphael ve Colla, 2020; Melini ve ark., 2023).

Çalışma, Türkiye'de karpuz fidelerinde *Azospirillum lipoferum* ve deniz yosunu özünün farklı doz ve kombinasyonlarının fide gelişimi üzerindeki etkilerini incelemeyi amaçlamaktadır. Elde edilen bulguların, çevre dostu ve organik tarım uygulamalarının fidecilik sektöründe yaygınlaşmasına katkı sağlaması ve kimyasal gübre bağımlılığının azaltılmasıyla sürdürülebilir tarımsal üretimi desteklemesi beklenmektedir.

Materyal ve Metot

Araştırma, Van Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümü Fizyoloji Laboratuvarı'nda, kontrollü iklim koşullarında gerçekleştirilmiş ve deneme materyali olarak *Citrullus lanatus* L. türüne ait 'Crimson Sweet' çeşidi kullanılmıştır. Çalışma, kontrollü çevre koşullarında gerçekleştirilmiş, sıcaklık 23 °C ± 2 °C, bağıl nem %60-70 arasında sabit tutulmuş ve aydınlatma periyodu 16 saat ışık, 8 saat karanlık olarak ayarlanmıştır. Bitkiler için yetiştirme ortamı

torf ve perlitin 3:1 oranında karıştırılmasıyla hazırlanarak viyollere yerleştirilmiştir. Tohumlar, viyollere eşit derinlikte ekilmiş (6 Mart 2024) ve çimlenme sürecinde saf su ile sulama yapılmış, nem kaybını önlemek için viyollerin üzeri kâğıtla örtülmüştür. Çimlenmenin tamamlanmasının ardından kâğıtlar kaldırılmış, bitkiler büyüme süreçlerini iklim odasında sürdürmüştür. İlk gerçek yaprakların gözlemlendiği 26 Mart 2024 tarihinde, büyümeyi destekleyici çeşitli uygulamalar gerçekleştirilmiştir. Çalışmada, toplam 10 farklı uygulama grubu değerlendirilmiştir, bu uygulamalar; AzL-1, AzL-2, AzL-3, DY-1, DY-2, DY-3, AzL+DY-1, AzL+DY-2, AzL+DY-3 ve kontrol grubudur. Deneme, tesadüf parselleri deseninde planlanmış, her grup üç tekerrürden oluşacak şekilde yürütülmüş ve toplamda 90 bitki üzerinde uygulanmıştır. Farklı dozlarda gerçekleştirilen uygulamalarda, *Azospirillum lipoferum* (AzL) grupları için AzL-1 (1.25 mL L^{-1}), AzL-2 (2.5 mL L^{-1}) ve AzL-3 (5 mL L^{-1}); deniz yosunu özü (DY) grupları için ise DY-1 (1.25 mL L^{-1}), DY-2 (2.5 mL L^{-1}) ve DY-3 (5 mL L^{-1}) dozları uygulanmıştır. Kombinasyon gruplarında *Azospirillum lipoferum* ve deniz yosunu özü eşit oranlarda birleştirilmiştir. Buna göre, AzL+DY-1 grubu 1.25 mL L^{-1} AzL ve 1.25 mL L^{-1} DY; AzL+DY-2 grubu 2.5 mL L^{-1} AzL ve 2.5 mL L^{-1} DY; AzL+DY-3 grubu ise 5 mL L^{-1} AzL ve 5 mL L^{-1} DY dozlarıyla hazırlanmıştır. Kontrol grubuna yalnızca saf su uygulanmıştır. Dozlar, firmaların önerileri ve literatürdeki çalışmalar dikkate alınarak belirlenmiştir (Al Sahatri, 2018; Ibrahim, 2018; Abad ve ark., 2019; Silva ve ark., 2022; da Silva Oliveira ve ark., 2023). Biyostimülantlar, saf su ile karıştırılarak homojen çözeltiler oluşturulmuş ve bitki gelişim ortamına uygulanmıştır. Bu uygulamalar, birer hafta arayla iki kez gerçekleştirilmiştir (26 Mart 2024 ve 2 Nisan 2024). Deneme süresince bitkiler, her 2-3 günde bir düzenli olarak eşit miktarda saf su ile sulanmıştır. Araştırmada kullanılan *Azospirillum lipoferum* bakterisi ($1x10^6$ kob mL^{-1}) Agrobest firması tarafından temin edilmiş; deniz yosunu özü ise Timac Agro'nun Fertileader Vital ürünü olarak sağlanmıştır. Fertileader Vital, %9 N, %5 P, %4 K, %0.05 B, %0.02 Cu, %0.02 Fe, %0.1 Mn, %0.01 Mo ve %0.05 Zn içeriğinin yanı sıra %1.16 g mL^{-1} yoğunluğa ve Seactiv™ kompleksi (ısopentyl adenin, glisin-betain, bitkisel kaynaklı amino asitler) formülasyonuna sahiptir. Çalışmanın sonunda, dikim olgunluğuna ulaşan fidelerde (8 Nisan 2024) çeşitli büyüme parametreleri ile klorofil miktarı ölçülmüştür. Gövde uzunluğu, kök boğazından büyüme ucuna kadar cetvel yardımıyla belirlenmiş; gövde çapı ise dijital kumpas kullanılarak ölçülmüştür. Yaprak sayıları kaydedilmiş, boğum arası mesafe 2. ve 3. boğum arasından dijital kumpas yardımıyla tespit edilmiştir. Kök uzunluğu cetvelle ölçülmüş, yeşil aksam ve köklerin yaş ağırlıkları hassas terazide tartılmıştır. Kuru ağırlık ölçümleri için bitkiler 70 °C'de 72 saat boyunca etüvde kurutulduktan sonra hassas terazide tartılmıştır. Klorofil miktarı SPAD metre kullanılarak belirlenmiştir. Toplanan veriler, Statgraphics istatistik analiz yazılımı ile analiz edilmiş; varyans analizi uygulanmış ve sonuçlar %5 anlamlılık düzeyinde Duncan testi ile gruplandırılmıştır. Çalışmada istatistiksel analizler sonucunda elde edilen gruplar harflerle ifade edilmiştir. Tabloların daha sade ve anlaşılır olması amacıyla 'aralık gösterimi' kullanılmıştır. Örneğin, 'A-D' ifadesi, A, B, C ve D harflerini içeren grupların tamamını temsil etmektedir.

Araştırma Bulguları ve Tartışma

Çalışma sonucu elde edilen gövde ve boğum arası mesafe parametreleri Çizelge 1'de, kök parametreleri Çizelge 2'de, yaprak parametreleri ve klorofil değeri Çizelge 3'te, toplam yeşil aksam yaş ve kuru ağırlıkları ise Çizelge 4'te belirtilmiştir.

Çizelge 1. Uygulamaların gövde ve boğum arası parametreleri üzerine etkileri
Table 1. Effects of applications on stem and internode parameters

Uygulamalar *Applications*	Gövde boyu (cm) *Stem length (cm)*	Gövde çapı (mm) *Stem diameter (mm)*	Gövde yaş ağırlığı (g) *Stem fresh weight (g)*	Gövde kuru ağırlığı (g) *Stem dry weight (g)*	Boğum arası (mm) *Internode length (mm)*
Kontrol	5.937±1.06 A	2.131±0.18	0.415±0.09	0.027±0.00	2.417±0.44
AzL-1	4.762±0.62 BC	2.170±0.32	0.378±0.07	0.026±0.00	3.133±1.00
AzL-2	4.875±0.76 BC	1.801±0.44	0.327±0.06	0.018±0.00	2.520±0.46
AzL-3	4.187±0.30 C	2.066±0.17	0.290±0.03	0.019±0.00	2.615±0.63
DY-1	5.037±0.71 A-C	2.191±0.43	0.458±0.12	0.021±0.00	2.971±0.79
DY-2	4.187±1.12 C	2.140±0.48	0.368±0.16	0.017±0.00	2.711±1.21
DY-3	5.287±0.87 AB	1.802±0.40	0.407±0.11	0.018±0.00	3.446±2.21
AzL+DY-1	5.112±1.01 A-C	2.001±0.25	0.392±0.10	0.021±0.00	2.335±0.69
AzL+DY-2	5.575±1.19 AB	2.160±0.45	0.435±0.10	0.023±0.01	3.290±1.21
AzL+DY-3	5.112±0.75 A-C	1.813±0.27	0.362±0.10	0.017±0.00	3.295±0.39
P Değeri	0.0034	NS	NS	NS	NS

*Aynı sütunda farklı harfi alan ortalamalar arasındaki farklılık önemlidir (p≤0.05).
*Değerler (ortalama ± standart sapma) olarak verilmiştir.
*NS (Not Significant): İstatistiksel olarak anlamlı fark bulunmamıştır (p > 0.05). Harflendirme yalnızca p < 0.05 olan parametrelerde gösterilmiştir.

Karpuz bitkisi gövde boyu parametresi açısından uygulamalar arasında istatistiksel olarak anlamlı farklılıklar tespit edilmiştir (p < 0.05). Kontrol grubu (5.937 cm) ile karşılaştırıldığında, AzL-1 (4.762 cm), AzL-2 (4.875 cm), AzL-3 (4.187 cm) ve DY-2 (4.187 cm) uygulamalarında gövde boyunda meydana gelen azalmaların istatistiksel olarak anlamlı olduğu belirlenmiştir. AzL grubunda en yüksek gövde boyu AzL-2 (4.875 cm), DY grubunda DY-3 (5.287 cm), AzL+DY grubunda ise AzL+DY-2 (5.575 cm) uygulamasında gözlemlenmiştir. Gövde çapı, gövde yaş ağırlığı, gövde kuru ağırlığı ve boğum arası mesafe parametreleri açısından uygulamalar arasında istatistiksel olarak anlamlı bir fark bulunmamıştır (p > 0.05).

Çizelge 2. Uygulamaların kök parametreleri üzerine etkileri
Table 2. Effects of applications on root parameters

Uygulamalar *Applications*	Kök yaş ağırlığı (g) *Root fresh weight (g)*	Kök kuru ağırlığı (g) *Root dry weight (g)*	Kök uzunluğu (cm) *Root length (cm)*
Kontrol	0.406±0.06	0.019±0.00 A	17.037±6.59 AB
AzL-1	0.420±0.11	0.014±0.00 B	13.775±2.34 BC
AzL-2	0.338±0.08	0.011±0.00 BC	17.112±4.43 AB
AzL-3	0.357±0.07	0.012±0.00 BC	20.125±6.95 A
DY-1	0.290±0.07	0.010±0.00 BC	10.862±3.02 C
DY-2	0.296±0.12	0.010±0.00 BC	9.875±4.37 C
DY-3	0.321±0.05	0.011±0.00 BC	13.675±4.15 BC
AzL+DY-1	0.357±0.08	0.011±0.00 BC	13.525±3.19 BC
AzL+DY-2	0.402±0.11	0.013±0.00 BC	16.262±5.27 AB
AzL+DY-3	0.310±0.15	0.008±0.00 C	17.050±3.49 AB
P Değeri	NS	0.0011	0.0007

*Aynı sütunda farklı harfi alan ortalamalar arasındaki farklılık önemlidir (p≤0.05).
*Değerler (ortalama ± standart sapma) olarak verilmiştir.
*NS (Not Significant): İstatistiksel olarak anlamlı fark bulunmamıştır (p > 0.05). Harflendirme yalnızca p < 0.05 olan parametrelerde gösterilmiştir.

Karpuz bitkisi kök yaş ağırlığı parametresi açısından uygulamalar arasında istatistiksel olarak anlamlı bir fark bulunmamıştır (p > 0.05). Kök kuru ağırlığı parametresi açısından uygulamalar arasında istatistiksel olarak anlamlı farklılıklar belirlenmiştir (p < 0.05). Kontrol grubu (0.019 g) en yüksek değeri gösterirken, en düşük kök kuru ağırlığı AzL+DY-3 (0.008 g) uygulamasında

gözlemlenmiştir. Kök uzunluğu parametresi açısından uygulamalar arasında istatistiksel olarak anlamlı farklılıklar belirlenmiştir ($p < 0.05$). En uzun kök AzL-3 (20.125 cm) uygulamasında, en kısa kök ise DY-2 (9.875 cm) uygulamasında tespit edilmiştir.

Çizelge 3. Uygulamaların yaprak ve klorofil değeri üzerine etkileri
Table 3. Effects of applications on leaf and chlorophyll values

Uygulamalar *Applications*	Yaprak sayısı (adet) *Number of leaves (count)*	Yaprak yaş ağırlığı (g) *Leaf fresh weight (g)*	Yaprak kuru ağırlığı (g) *Leaf dry weight (g)*	Klorofil (SPAD) *Chlorophyll value (SPAD)*
Kontrol	3.875±0.35 A	0.788±0.17 B-D	0.076±0.018 AB	42.375±1.66 BC
AzL-1	3.375±0.51 A-C	0.751±0.14 CD	0.071±0.01 AB	43.887±1.90 AB
AzL-2	3.000±0.00 C	0.605±0.10 D	0.054±0.01 B	42.450±1.01 BC
AzL-3	3.375±0.51 A-C	0.610±0.16 D	0.051±0.01 B	41.475±1.23 C
DY-1	3.750±0.46 AB	1.152±0.35 AB	0.080±0.04 AB	45.237±1.49 A
DY-2	3.500±0.75 A-C	1.083±0.64 A-C	0.079±0.06 AB	43.950±1.67 AB
DY-3	3.750±0.46 AB	1.172±0.41 A	0.098±0.02 A	45.687±1.07 A
AzL+DY-1	3.625±0.51 AB	0.972±0.29 A-D	0.097±0.02 A	44.125±1.47 AB
AzL+DY-2	3.250±0.70 BC	1.028±0.29 A-C	0.090±0.03 A	45.662±1.38 A
AzL+DY-3	3.625±0.51 AB	1.052±0.35 A-C	0.080±0.02 AB	45.150±2.74 A
P Değeri	0.0402	0.0018	0.0432	0.0000

*Aynı sütunda farklı harfi alan ortalamalar arasındaki farklılık önemlidir ($p \leq 0.05$).
*Değerler (ortalama ± standart sapma) olarak verilmiştir.
*NS (Not Significant): İstatistiksel olarak anlamlı fark bulunmamıştır ($p > 0.05$). Harflendirme yalnızca $p < 0.05$ olan parametrelerde gösterilmiştir.

Karpuz bitkisi yaprak sayısı parametresi açısından uygulamalar arasında istatistiksel olarak anlamlı farklılıklar belirlenmiştir ($p < 0.05$). En yüksek yaprak sayısı kontrol (3.875 adet) ve DY-1, DY-3 uygulamalarında (3.750 adet) gözlemlenirken, en düşük yaprak sayısı AzL-2 (3.000 adet) uygulamasında tespit edilmiştir. Yaprak yaş ağırlığı parametresi açısından uygulamalar arasında istatistiksel olarak anlamlı farklılıklar belirlenmiştir ($p < 0.05$). En yüksek yaprak yaş ağırlığı DY-3 (1.172 g) uygulamasında, en düşük yaprak yaş ağırlığı ise AzL-2 (0.605 g) uygulamasında tespit edilmiştir. Yaprak kuru ağırlığı parametresi açısından uygulamalar arasında istatistiksel olarak anlamlı farklılıklar belirlenmiştir ($p < 0.05$). En yüksek yaprak kuru ağırlığı DY-3 (0.098 g) ve AzL+DY-1 (0.097 g) uygulamalarında, en düşük yaprak kuru ağırlığı ise AzL-3 (0.051 g) uygulamasında gözlemlenmiştir. Klorofil (SPAD) değeri açısından uygulamalar arasında istatistiksel olarak anlamlı farklılıklar belirlenmiştir ($p < 0.05$). En yüksek klorofil değeri DY-3 (45.687 SPAD) ve AzL+DY-2 (45.662 SPAD) uygulamalarında, en düşük klorofil değeri ise AzL-3 (41.475 SPAD) uygulamasında gözlemlenmiştir.

Çizelge. Uygulamaların toplam yeşil aksam yaş ve kuru ağırlık değerlerine etkisi
Table 4. Effects of applications on total fresh and dry weight of vegetative parts

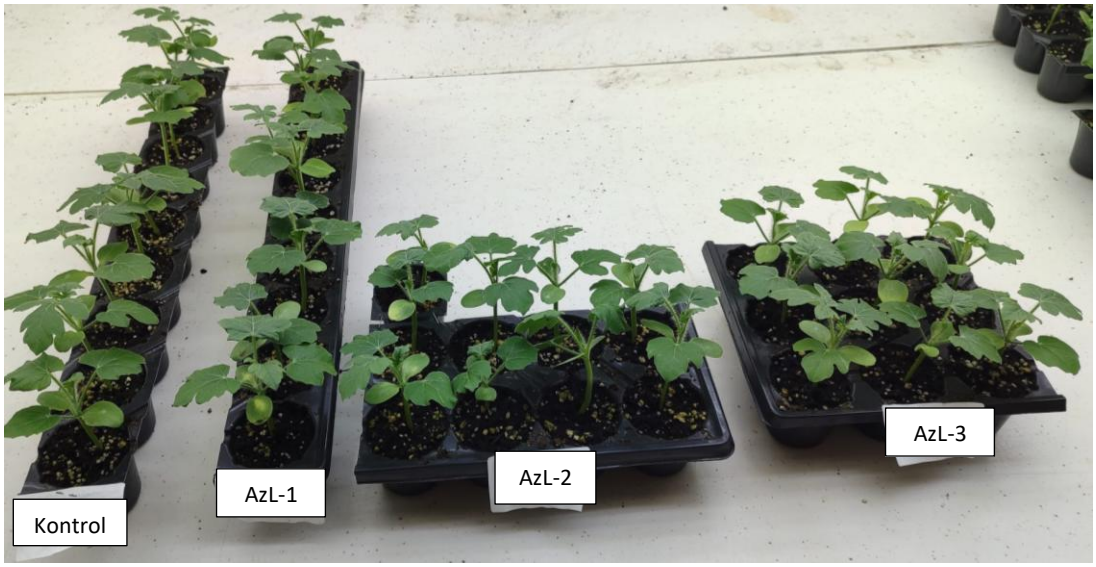
Uygulamalar *Applications*	Toplam yeşil aksam yaş ağırlığı (g) *Total fresh weight of vegetative parts (g)*	Toplam yeşil aksam kuru ağırlığı (g) *Total dry weight of vegetative parts (g)*
Kontrol	1.203±0.23 AB	0.103±0.02
AzL-1	1.130±0.17 AB	0.097±0.02
AzL-2	0.932±0.14 B	0.072±0.01
AzL-3	0.900±0.18 B	0.070±0.01
DY-1	1.611±0.46 A	0.101±0.04
DY-2	1.452±0.80 A	0.096±0.06
DY-3	1.580±0.53 A	0.116±0.03
AzL+DY-1	1.365±0.37 AB	0.118±0.03
AzL+DY-2	1.402±0.43 A	0.114±0.03
AzL+DY-3	1.415±0.42 A	0.097±0.02
P Değeri	0.0078	NS

*Aynı sütunda farklı harfi alan ortalamalar arasındaki farklılık önemlidir (p≤0.05).
*Değerler (ortalama ± standart sapma) olarak verilmiştir.
*NS (Not Significant): İstatistiksel olarak anlamlı fark bulunmamıştır (p > 0.05). Harflendirme yalnızca p < 0.05 olan parametrelerde gösterilmiştir.

Karpuz bitkisi toplam yeşil aksam yaş ağırlığı parametresi açısından uygulamalar arasında istatistiksel olarak anlamlı farklılıklar belirlenmiştir ($p < 0.05$). En yüksek toplam yeşil aksam yaş ağırlığı DY-1 (1.611 g) uygulamasında, en düşük değer ise AzL-3 (0.900 g) uygulamasında tespit edilmiştir. Toplam yeşil aksam kuru ağırlığı parametresi açısından uygulamalar arasında istatistiksel olarak anlamlı bir fark bulunmamıştır ($p > 0.05$).

Azospirillum lipoferum (AzL), deniz yosunu (DY) ve bu iki uygulamanın kombinasyonu (AzL+DY), karpuz fidelerinin büyüme ve biyokütle parametrelerinde belirgin değişiklikler oluşturmuştur. İncelenen AzL dozları arasında, genel olarak AzL-1 dozunun diğer dozlara kıyasla daha yüksek performans sergilediği görülmüştür. Özellikle kök yaş ağırlığı parametresinde, tüm uygulama ve dozlarla karşılaştırıldığında AzL-1 dozu öne çıkmıştır. Bununla birlikte, kök uzunluğu açısından en yüksek değer AzL-3 dozu ile elde edilmiştir. Ancak, AzL-3 dozunun bazı parametreler üzerinde olumsuz etkiler yarattığı da tespit edilmiştir. Literatürde *Azospirillum*'un rizosferde kolonizasyon sağlayarak kök büyümesini ve besin alımını teşvik ettiği, aynı zamanda bitkide hormonal dengeyi düzenlediği belirtilmiştir (Bernados ve ark., 2024). Özellikle indol-3-asetik asit (IAA) üretimi kök büyümesini teşvik etme potansiyeline sahip olmakla birlikte, yüksek dozlarda bu hormonun aşırı üretimi bitki metabolizmasında stres oluşturarak olumsuz etkiler yaratabilmektedir (Dimkpa ve ark., 2012; Koul ve ark., 2015; Coniglio ve ark., 2024). Çalışmada AzL'nin yüksek dozunun büyüme üzerindeki sınırlı etkileri, hormonal dengenin bozulması veya yüksek mikrobiyal aktivitenin rizosferde oksijen tüketimini artırması ile açıklanabilir. Fotosentetik performans açısından, AzL-1 dozu klorofil değerinde hem kontrol grubuna göre hem de diğer AzL dozlarına göre daha yüksek sonuçlar sağlamıştır. Bu durum, düşük dozda *Azospirillum* uygulamasının klorofil sentezine katkıda bulunduğunu göstermektedir. *Azospirillum*'un bitki kökleri ile simbiyotik ilişkisi sonucu demir alımını artırabileceği ve klorofil sentezini destekleyebileceği literatürde de belirtilmektedir (Scott ve ark., 2020; Housh ve ark., 2021; 2022). Ancak, gövde boyu ve yaprak sayısı gibi bazı parametrelerde AzL'nin kontrol grubuna göre üstünlük sağlayamaması, bu biyostimülantın tek başına büyüme performansını artırmada yeterli olmadığını göstermektedir. Ali ve ark. (2019) tarafından gerçekleştirilen çalışmada, *Azospirillum brasilense* ve *Azotobacter chroococcum* biyogübrelerinin kimyasal gübre ile birlikte kullanımının etkileri incelenmiştir. Araştırmada, biyogübrelerin azot fiksasyonu, fitohormon üretimi ve kök gelişimini artırarak su ve besin alımını teşvik ettiği belirtilmiştir.

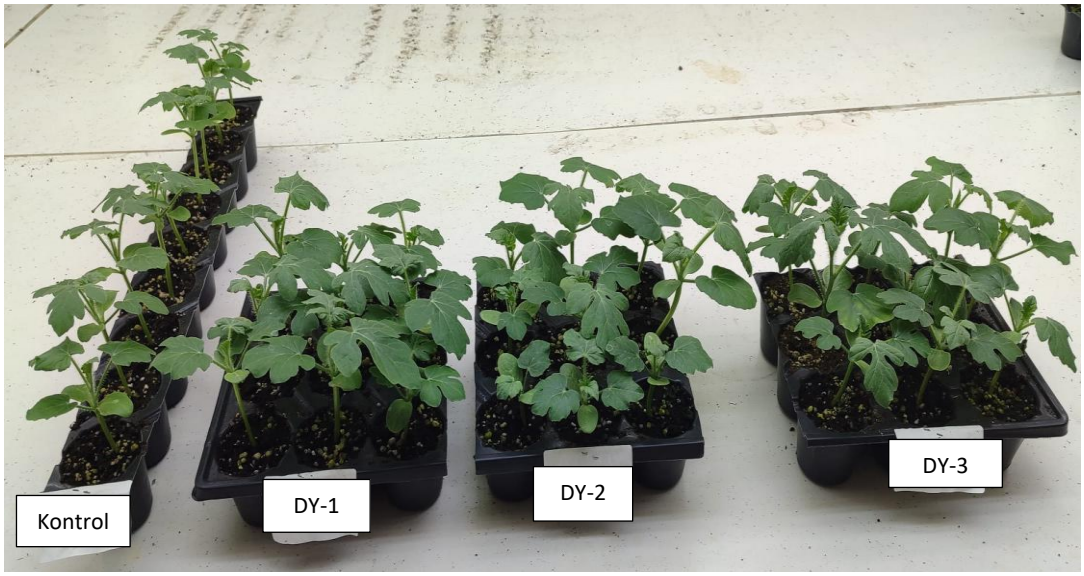
Azospirillum tarafından sentezlenen fitohormonların kök gelişimini iyileştirdiği ve *Azotobacter* tarafından salgılanan büyüme düzenleyici hormonların bitki gelişimini desteklediği ifade edilmiştir. Bu durum, *Azospirillum*'un tek başına bazı büyüme parametrelerinde yeterli etki gösteremeyebileceğini, ancak farklı biyogübreler veya besin çözeltileriyle birlikte kullanıldığında büyüme ve verim üzerinde daha güçlü bir etki yaratabileceğini düşündürmektedir. Benzer şekilde, Vendruscolo ve ark. (2023) çalışmasında, *Azospirillum brasilense*'nin Cantaloupe kavunu (*Cucumis melo* var. *cantalupensis*) üzerindeki etkileri incelenmiş ve bakterinin ilk azot gübresiyle birlikte uygulanmasının bitki gelişimini teşvik ettiği belirtilmiştir. *Azospirillum*'un fitohormon üretimi (IAA, GA_3 ve sitokinin) ve biyolojik azot fiksasyonu mekanizmaları sayesinde büyümeyi desteklediği, ayrıca meyve taze ağırlığı, kabuk ve et kalınlığı gibi biyometrik özellikler ile çözünür katı madde içeriği ve asitlik gibi duyusal niteliklerde iyileşmeler sağladığı rapor edilmiştir. Bu çalışmalar, *Azospirillum*'un tek başına bazı büyüme parametrelerinde sınırlı etki gösterebileceğini, ancak besin yönetimi veya diğer biyostimülantlarla birlikte uygulandığında daha güçlü bir büyüme ve kalite artışı sağlayabileceğini göstermektedir.



Şekil 1. *Azospirillum lipoferum* uygulama dozlarının bitki gelişimi üzerindeki etkileri
Figure 1. Effects of Azospirillum lipoferum application doses on plant growth

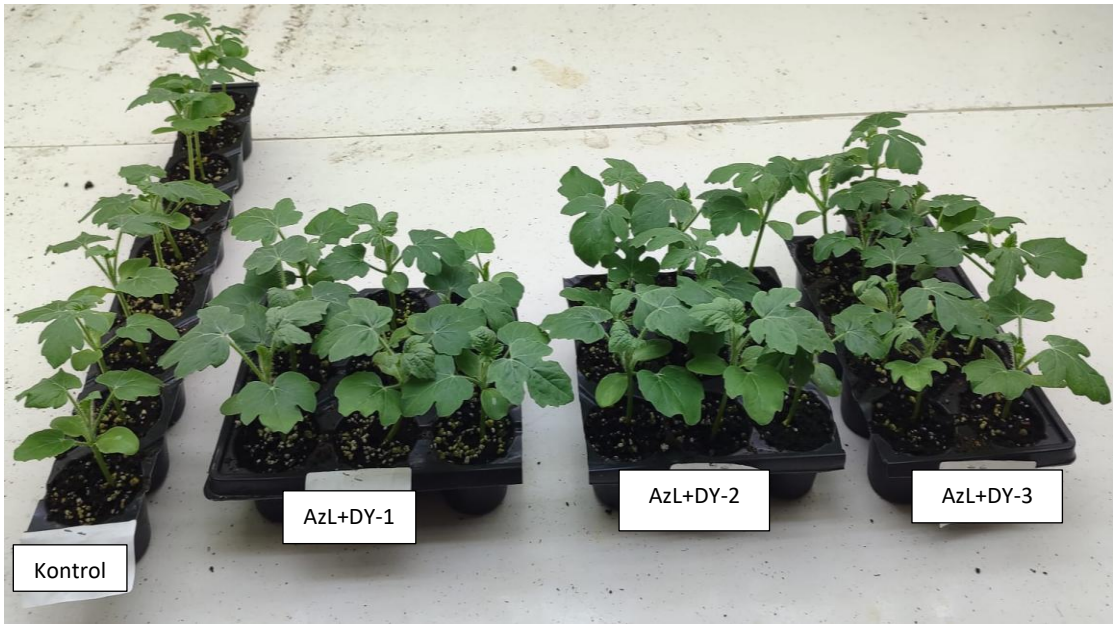
DY uygulamalarında en belirgin olumlu etkiler, yaprak yaş ve kuru ağırlığı, klorofil miktarı gibi fotosentezle ilişkili parametrelerde gözlenmiştir. Özellikle DY-3 (5 mL L^{-1}) dozu, bu parametrelerde en yüksek değerleri sağlamış ve deniz yosununun fotosentez pigmentlerini artırıcı etkisini göstermiştir. Deniz yosunlarının, içeriğinde yer alan sitokininler, gibberellinler ve betain gibi biyolojik aktif bileşiklerle bitki büyümesini ve stres toleransını artırdığı yapılan çalışmalarda da belirtilmektedir (Shil ve ark., 2023; Tejasree ve ark., 2024). DY'nin yüksek dozunun klorofil değerlerini artırması, klorofil pigmentlerinin birikimini teşvik ederek fotosentetik kapasiteyi geliştirdiği düşüncesine yönlendirmektedir. Yaprak yaş ağırlığındaki artış ise, deniz yosununun yaprak dokularında su tutma kapasitesini artırma potansiyeline bağlanabilir (Tahmaz ve ark., 2024). Bu etki, deniz yosunundaki osmotik düzenleyicilerin hücre içi su dengesini koruyarak yaprak büyümesini desteklemesiyle açıklanabilir (Aliko ve ark., 2017; Chaturvedi ve ark., 2022). Ancak, DY uygulamalarının gövde boyu gibi bazı parametrelerde kontrol grubuna üstünlük sağlayamaması, deniz yosununun etkisinin daha çok fizyolojik süreçlere odaklandığını ve büyüme performansının tamamını kapsayamadığını düşündürmektedir (Doğan, 2024). Deniz yosunu özleri, hem bitki büyümesini destekleme hem de stres koşullarında dayanıklılığı artırma

potansiyeline sahip olduğu yapılan çalışmalar ile desteklenmektedir. Örneğin; de Mendonça Júnior ve ark. (2019) ve Radwan ve ark. (2023) çalışmalarında, bu özlerin içerdiği büyüme hormonları ve biyolojik aktif bileşenlerin etkilerini incelemişlerdir. de Mendonça Júnior ve ark., *Ascophyllum nodosum* özünün uygun dozlarda uygulanmasının karpuz fidelerinde gövde ve kök gelişimini teşvik ettiğini, ancak yüksek dozların kök büyümesini olumsuz etkileyebileceğini belirtmiştir. Çalışmamızda ise deniz yosunu özünün artan dozlarının kök büyümesi üzerinde belirgin bir olumsuz etki göstermediği, ancak gövde büyüme parametrelerinde azalmaya neden olduğu gözlemlenmiştir. Radwan ve ark. ise *Ulva lactuca* özünün, tuz stresi altındaki bitkilerde büyüme parametrelerini iyileştirdiğini ve antioksidan savunmayı güçlendirdiğini belirtmiştir. Bu durum, deniz yosunu özlerinin bitki büyümesini doğrudan teşvik etmekten çok, stres toleransını artıran fizyolojik değişiklikleri tetikleyerek dolaylı bir etki sunduğunu göstermektedir. Her iki çalışma, deniz yosunu özlerinin bitki gelişimi ve çevresel stres yönetiminde etkili bir araç olduğunu, ancak uygulamanın doz ve koşullara bağlı olarak optimize edilmesi gerektiğini vurgulamaktadır.



Şekil 2. Deniz yosunu uygulama dozlarının bitki gelişimi üzerindeki etkileri
Figure 2. Effects of seaweed application doses on plant growth

AzL ve DY kombinasyon grubunda yaprak yaş ağırlığında AzL+DY-3, yaprak kuru ağırlığında AzL+DY-1 ve Klorofil değerinde AzL+DY-2 uygulamaları, en iyi sonuçları vermiştir. Kombinasyon uygulamalarının etkili olmasının temelinde, Doğan ve ark. (2024) çalışmasında belirtildiği üzere, AzL'nin rizosferde bitki besin elementlerinin alımını artırma kapasitesi ve DY'nin yapısal gelişimi teşvik etme potansiyeli yer almaktadır. Bu sinerji, büyüme ve fizyolojik süreçlerin farklı mekanizmalarla desteklenmesini sağlamış olabileceğini düşündürmektedir. Kök uzunluğu açısından, AzL+DY-3 kombinasyonu, AzL+DY'nin diğer dozlarına kıyasla en yüksek değeri sağlamış ve yüksek dozun kök gelişimini teşvik edebileceğini göstermiştir. Bununla birlikte, gövde boyu gibi parametrelerde kombinasyonların kontrol grubuna üstünlük sağlayamaması, uygulamanın belirli parametrelerde sınırlı etki gösterebileceğine işaret etmektedir.



Şekil 3. *Azospirillum lipoferum*+deniz yosunu uygulama dozlarının bitki gelişimi üzerindeki etkileri
Figure 3. Effects of Azospirillum lipoferum + seaweed application doses on plant growth

Sonuçlar

Bu çalışma, *Azospirillum lipoferum* ve deniz yosunu özlerinin farklı doz ve kombinasyonlarının karpuz (*Citrullus lanatus* L.) fidelerinin büyüme parametreleri ve klorofil içeriği üzerindeki etkilerini belirlemek amacıyla gerçekleştirilmiştir. Araştırma sonuçları, bu biyostimülantların fide gelişimi üzerinde belirgin etkiler gösterdiğini ve özellikle düşük ile orta doz seviyelerinde uygulandığında büyümeyi desteklediğini ortaya koymaktadır. *Azospirillum lipoferum* uygulamalarında kök uzunluğu açısından en yüksek değer AzL-3 (5 mL L^{-1}) dozu ile elde edilmiş, ancak kök kuru ağırlığında ve bazı büyüme parametrelerinde yüksek dozların olumsuz etkileri olduğu belirlenmiştir. AzL-1 (1.25 mL L^{-1}) dozu, özellikle kök yaş ağırlığı ve klorofil içeriği gibi parametreler açısından daha olumlu sonuçlar vermiştir. Deniz yosunu özü uygulamalarında ise yüksek dozun (5 mL L^{-1}) yaprak sayısı, yaprak yaş ve kuru ağırlığı ile klorofil içeriğini artırdığı belirlenmiştir. *Azospirillum lipoferum* ve deniz yosunu özünün kombinasyon halinde uygulanması, özellikle yaprak sayısı, yaprak yaş ağırlığı, yaprak kuru ağırlığı ve klorofil içeriği gibi fotosentez kapasitesini doğrudan etkileyen parametreler üzerinde sinerjik bir etki göstermiştir. Elde edilen bulgular, biyostimülantların uygulama dozlarının, yöntemlerinin ve çevresel faktörlerin dikkate alınarak optimize edilmesi gerektiğini ortaya koymaktadır. Bununla birlikte, bu biyostimülantların bitki gelişimi üzerindeki etkilerinin moleküler ve fizyolojik düzeyde daha ayrıntılı araştırılması, mekanizmalarının daha iyi anlaşılmasını sağlayacaktır. Ayrıca, bu tür biyostimülantların saha koşullarında uygulanabilirliğini test eden uzun vadeli çalışmaların yürütülmesi, sürdürülebilir fidecilik uygulamaları açısından büyük önem taşımaktadır. Sonuç olarak, bu araştırma, *Azospirillum lipoferum* ve deniz yosunu özünün karpuz fidelerinde büyüme ve fotosentez performansını artırarak sürdürülebilir fide yetiştiriciliğinde önemli bir biyoteknolojik araç olabileceğini göstermektedir. Bu biyostimülantların kullanımı, kimyasal gübre bağımlılığını azaltma, çevresel sürdürülebilirliği destekleme ve tarımsal üretimde verimliliği artırma açısından önemli bir potansiyel sunabileceği düşünülmektedir.

Çıkar Çatışması

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

Yazar Katkısı

YLD ve ÖÖ: Çalışmanın yürütülmesini sağlamışlardır.
YLD: Çalışmanın yazım sürecini gerçekleştirmiştir.

ÖÜ: Çalışmanın istatistiksel analizlerini ve yazım kontrolünü gerçekleştirmiştir.

FY: Çalışmanın planlanması ve programlanmasından sorumlu olmuştur.

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Araştırma Makalesi/Research Article

Şanlıurfa ilinde antepfıstığı üretiminde tarım sigortası yapılmasını etkileyen faktörlerin belirlenmesi

Determination of factors affecting agricultural insurance in the production of pistachio of Şanlıurfa

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ÖZ

Bu çalışma da Şanlıurfa ilinde antepfıstığı üretimi yapan işletmelerin tarım sigortası yapmalarında etkili olan faktörlerin belirlenmesi ve antepfıstığı bitkisi için en uygun prim miktarının tespit edilmesi amaçlanmıştır. Çalışmanın amacına ulaşması için, Şanlıurfa ilinde örnekleme yoluyla seçilen 300 antepfıstığı üretimi yapan işletmeyle yüz yüze görüşülerek anket çalışması gerçekleştirilmiştir. Bu bağlamda görüşülen antepfıstığı üretimi yapan işletmelerden 51'inin tarım sigortası yaptırdığı, 249'unun ise tarım sigortası yaptırmadığı tespit edilmiştir. Elde edilen verilerin istatistiki analizinde, lojistik regresyon analizi kullanılmıştır. Ayrıca verilerin analizinde t-testi ve khi-kare testlerinden de yararlanılmıştır. Lojistik regresyon analizinden elde edilen sonuçlara göre, antepfıstığı üretimi yapan işletmelerin tarım sigortası yapmalarında etkili olan faktörlerin; üreticilerin yaşı, eğitim durumları ve üreticilerin yıllık toplam gelir miktarı olduğu tespit edilmiştir. Test sonuçlarından elde edilen sonuçlara göre üreticilerin gelir düzeylerinin, işletmelerin arazi büyüklüklerinin ve çiftçi örgütlerine üyelik durumlarının, işletmelerin tarım sigortası yapmalarını etkilediği belirlenmiştir. Yapılan diğer analizler sonucunda ise antepfıstığı üretimi yapan işletmelerin mevcut prim miktarını yüksek bulduğu ve devlet desteğinin olmasının işletmelerin tarım sigortası yapmalarında etkili olduğu belirlenmiştir.

Anahtar Kelimeler: Tarım sigortası, risk, lojistik regresyon, antepfıstığı, Şanlıurfa

ABSTRACT

This study aims to determine the factors that are effective in making agricultural insurance for pistachio producing enterprises in Şanlıurfa province and to determine the most appropriate premium amount for pistachio plants. In order to achieve the aim of the study, a survey was conducted by face-to-face interviews with 300 pistachio producing enterprises selected through sampling in Şanlıurfa province. In this context, it was determined that 51 of the pistachio producing enterprises interviewed had agricultural insurance, while 249 did not have agricultural insurance. Logistic regression analysis was used in the statistical analysis of the obtained data. Additionally, t-test and chi-square tests were used in the analysis of the data. According to the results obtained from the logistic regression analysis, it was determined that the factors that are effective in making agricultural insurance in pistachio producing enterprises are the age of

the producers, their education level and the annual total income of the producers. According to the results obtained from the test results, it was determined that the income levels of the producers, the land size of the enterprises and their membership status in farmer organizations affect the agricultural insurance of the enterprises. As a result of other analyses, it was determined that pistachio producing enterprises found the current premium amount high and that the presence of state support was effective in enterprises agricultural insurance.

Key Words: Agricultural insurance, risk, logistic regression, pistachio, Şanlıurfa

Giriş

Tarım ülke nüfusunun gıda maddeleri ihtiyacını karşılaması, tarıma dayalı sanayinin hammaddesi olması, ülkeler için önemli istihdam imkânı sağlaması, dış ticarete doğrudan ve dolaylı olarak katkıda bulunması, milli gelirde önemli bir yer edinmesi sebebiyle sosyal ve ekonomik açıdan büyük öneme sahiptir (Keskinkılıç, 2013).

Tarım sektörü, kendine özgü özelliklerinden dolayı risk ve belirsizliklerle sürekli karşı karşıyadır. Tarım sektöründe üreticiler, en çok doğal risklerden etkilenmektedir. Doğal riskler; dolu, don, kuraklık, fırtına gibi üreticilerin kontrolünde olmayan hava koşullarına bağlı oluşan risklerdir. Bu bağlamda tarımsal faaliyet "üstü açık fabrika" olarak da nitelendirilmektedir (Alay, 2012). Aynı zamanda tarım sektörü doğal riskler başta olmak üzere ekonomik, sosyal ve politik risklerden en çok etkilenen sektörlerdendir.

Tarım sektörü tüm dünyada hem stratejik hem de ekonomik olarak büyük öneme sahip olup desteklenmesi gereken bir sektör olduğundan, sektörün bu risk ve belirsizliklere karşı korunması gerekmektedir. Gelişmiş ülkeler maruz kaldıkları doğal, sosyal, ekonomik, kişisel, politik riskler sebebiyle, yıllardır teknolojinin de yardımıyla teknik koruma önlemleri geliştirmişlerdir. Ancak bu gelişmiş teknik koruma önlemleri de sektörün maruz kaldığı risklerin olumsuz etkilerinin giderilmesinde yeterli olamamıştır. Tarım sektöründe karşılaşılan risklerin, özellikle de doğal karakterli risklerin etkilerinin minimuma indirilebilmesi ancak etkin bir risk yönetiminin uygulanması ile sağlanabilir. Günümüzde dünyada tarım sektörü için kullanılan en önemli ve etkili risk yönetim aracı "Tarım Sigortası"dır (Sevim, 2010).

Tarım sigortası; tarım sektöründe risk ve belirsizliklerden oluşabilecek hasarların ödenmesini temel alarak, üreticilerin emeğini güvence altına alan bir sistemdir (İsel, 2010). Bu sigorta sisteminde amaç, üreticilerin risk ve belirsizlikler sebebiyle ürünlerinde oluşabilecek zarar ve kayıpları kısmen de olsa güvence altına almak ve sigorta primlerinin belli bir kısmının devlet tarafından ödenerek, üreticileri uzun vadede gelir istikrarına kavuşturup, bu sayede tarımsal üretimde sürdürülebilirliğin sağlanmasıdır (Perçin, 2011). Tarım sigortasının diğer bir amacı aynı risk ve belirsizliklerle uğraşan üreticilerin ödemiş oldukları primlerle oluşturulacak, fonlarla hasara uğrayan üreticilerin hasarlarının karşılanmasıdır (Dinler ve ark., 2005).

Dünyada modern anlamda tarımsal sigorta uygulamaları İrlanda'da kooperatifler tarafından hayvan hayat sigortalarının yapılmasıyla birlikte 18. yüzyılın sonlarına doğru başlamıştır. İrlanda'da uygulanan hayvan hayat sigortaları kooperatiflerinin yeterli deneyim ve bilgiye sahip olmamaları, sigorta yaptıran sayısının oransal olarak az olması ve prim miktarlarının düşük olması nedeniyle başarılı olamamıştır (Barış, 2007). 19. ve 20. yüzyılda Almanya, Fransa, İsviçre gibi Avrupa ülkeleri başta olmak üzere ABD ve Japonya gibi ülkelerde daha geniş kapsamlı tarım sigortası uygulamalarına başlanmıştır (Keskinkılıç, 2013). Dünyada bazı ülkelerin uyguladığı tarım sigortası modelleri ilgi çekmiştir ve diğer ülkeler tarafından da benimsenmiştir. Örneğin İspanya'da uygulanan tarım sigortası modeli, birçok ülkenin tarım sigortası modelini etkilemiştir. Türkiye'de günümüzde uygulanmakta olan tarım sigortası modeli de İspanya'nın tarım sigortası modelinden etkilenip oluşturulmuş bir modeldir.

Türkiye'de tarım sigortası ile ilgili ilk çalışmalar Cumhuriyet'in ilk yıllarında yapılmıştır. Daha sonra

bazı kurum ve kuruluşlar konu ile yakından ilgilenmiş ve tarım sigortası ile ilgili çeşitli çalışmalar yapmışlardır. T.C. Ziraat Bankası tarım sigortası ile ilgili çalışmalar yapan ilk kurum olmuştur. Bu kapsamda 1937 tarihli ve 3202 sayılı T.C. Ziraat Bankası Yasası'nda tarım sigortaları konusuna yer verilmiştir (Güngör, 2006).

Türkiye'de tarım sigortaları uygulamaları, özel sigorta şirketlerinin sadece bitkisel ürünlerde dolu riskine karşı sigorta yapmalarıyla birlikte başlamıştır. İlk olarak 1957 yılında Şeker Sigorta, şeker pancarı üreticilerine bitkisel ürünlerde dolu sigortası uygulamasına başlamıştır. Daha sonra Başak Sigorta'nın 1960 yılında bitkisel ürün (dolu) ve hayvan hayat sigortası faaliyetleri ile uygulamalar sürmüştür. 1995 yılında tarım branşında çalışma yapan sigorta şirketlerinin birleşmesi ile Tarım Sigortaları Vakfı (TSV) kurulmuştur. Türkiye'de, TSV 2006 yılına kadar risk inceleme işlemlerini ve hasar tespit organizasyonunu tek elden sağlamıştır. Tarım Sigortaları Havuzu'nun kurulması ile birlikte TSV, 2006 yılında bütün veri ve sistemlerini işletici şirkete (TARSİM) devretmiştir (Tümer, 2004).

Türkiye'de 14/06/2005 tarihinde 5363 sayılı "Tarım Sigortaları Kanunu" çıkarılarak sigorta mekanizması devreye sokulmuş ve bu sayede tarım sektörünü tehdit eden risk ve belirsizliklerin teminat altına alınması amaçlanmıştır. Çıkarılan bu kanun ile birlikte aynı zamanda meydana gelecek hasarlardan tazminatın tek merkezden ödenmesinin sağlanması ve tarım sigortalarının yaygınlaştırılması ve geliştirilmesi amacına yönelik bir "Sigorta Havuzu" kurulmuştur. Sigorta Havuzuna ilişkin bütün işlemler, havuza katılan sigorta şirketlerinin eşit paylarla ortak oldukları Tarım Sigortaları Havuz İşletmesi A.Ş. (TARSİM) tarafından sağlanmaktadır (Perçin, 2011).

Türkiye'de her yıl tarım sigortası kapsamında devlet tarafından sağlanan sigorta prim desteği miktarı, Cumhurbaşkanı tarafından belirlenmektedir. 2019 yılından itibaren bütün ürünlerde ödenmesi gereken sigorta priminin %50'si (don teminatında, 2/3'ü), devlet tarafından karşılıksız destek olarak karşılanmaktadır. Ayrıca "İlçe Bazlı Kuraklık Verim Sigortası'nda" ise primlerin %60'ı devlet tarafından karşılıksız ödenmektedir (Anonim, 2018).

Türkiye'de tarım sigortası sisteminde, sisteme katılım gönüllük esasına bağlıdır. Tarım üreticilerinin sigorta yaptırabilmeleri ve devletin prim desteğinden faydalanabilmeleri için; bitkisel ürünler için, ÇKS'ye, büyükbaş, küçükbaş ve kümes hayvanlarında, TÜRKVET'e, sera için, ÖKS'ye, su ürünleri çiftlikleri için, SKS'ye ve arıcılıkta AKS'ye kayıt yaptırmaları ve yaptırmış oldukları kayıtları, her yıl güncellemeleri gerekmektedir (Anonim, 2018).

Türkiye'de 2023/24 üretim döneminde 4.2 milyon da alanda 176 bin ton antepfıstığı üretimi gerçekleşmiştir. Türkiye 2023/24 üretim döneminde 176 bin ton antepfıstığı üretim miktarıyla Dünyada İran'dan sonra üçüncü sırada yer almaktadır. 2023/24 üretim döneminde antepfıstığı üretim alanında %40'lık paya sahip olan Şanlıurfa 1.6 milyon da üretim alanı ile Türkiye'de en fazla antepfıstığı üretim alanına sahip il olmuştur. Aynı zamanda Şanlıurfa 2023/24 üretim döneminde 60 bin ton antepfıstığı üretim miktarıyla Türkiye'de en fazla antepfıstığı üretimi gerçekleştiren il konumundadır (Anonim, 2024).

Çalışma konusu ile ilgili literatür taraması yapıldığında araştırma bölgesinde antepfıstığı üretiminin önemli bir konumda yer almasına rağmen, antepfıstığı üretimi yapan işletmelerin tarım sigortası yapmalarında etkili olan faktörlerin belirlenmesine yönelik çalışma yapılmadığı tespit edilmiştir. Aynı zamanda çalışmanın birincil verilere dayanması ve kapsamının geniş olması çalışmayı özgün kılmıştır.

Bu kapsamda, bu çalışma Şanlıurfa ilinde antepfıstığı üretimi yapan işletmelerin üretimlerini olumsuz yönde etkileyen risk ve belirsizliklere karşı tarım sigortası yapmalarında etkili olan faktörlerin belirlenmesi ve antepfıstığı bitkisi için en uygun prim miktarının tespit edilmesi amacıyla yapılmıştır. Çalışmadan elde edilen sonuçlar başta "TARSİM" olmak üzere, politika yapıcıların, sigorta şirketlerinin, yayım elemanlarının ve üreticilerin bilgisine sunulmuştur.

Materyal ve Yöntem

Materyal

Araştırmanın ana materyali 2017 yılında Şanlıurfa ili Karaköprü, Haliliye, Bozova, Halfeti, Suruç ve Birecik ilçelerinde faaliyet gösteren 300 antepfıstığı üretimi yapan işletme ile yüz yüze anket yoluyla görüşülerek elde edilmiştir. 2017 yılında Çiftçi Kayıt Sistemine kayıtlı olan antepfıstığı üretimi yapan üretici sayısı, 155020'dir Anket uygulanacak üretici sayısı "Oransal Örnekleme" yöntemi kullanılarak belirlenmiştir (Newbold, 1995).

Oransal Örnekleme Formülü:

$$n = \frac{N * p * q}{(N-1) * \sigma_p^2 + p * q} = \frac{155020 * 0.5 * 0.5}{(155020-1) * (0.0305)^2 + 0.5 * 0.5} \cong 268$$

$$\sigma_p^2 = \left(\frac{r}{Z_{\frac{\alpha}{2}}}\right)^2 = \left(\frac{0.05}{1.64}\right)^2 = 0.0305^2$$

Formülde;

n: Örnek büyüklüğü,

N: Popülâsyondaki işletme sayısı,

σ_p^2: Oranın varyansı,

r: Ortalamadan izin verilen hata payı (%5),

$Z_{\alpha/2}$: z cetvel değeri

p: İncelenen olayın meydana gelme olasılığı olarak ifade edilmektedir.

Örnek büyüklüğünün belirlenmesi için; %5 hata payı ve %90 güven aralığında çalışılmıştır. %90 güven aralığında (z = 1.64) ve ortalamadan izin verilen %5 hata payı ile anket yapılacak işletme sayısı 268 olarak belirlenmiştir. Ancak hesaplanan örnek büyüklüğüyle birlikte yedek anket doldurulması uygun bulunmuştur. Bu nedenle çalışmada yapılan anketlerin %10'u kadar yedek anket doldurulup analizlerde 300 anket kullanılmıştır. Bu bağlamda tarım sigortası yaptıran 51, yaptırmayan 249 olmak üzere toplam 300 antepfıstığı üreticisiyle görüşülmüştür. Bununla birlikte çalışmanın ikincil verileri ise Türkiye İstatistiki Kurumu (TÜİK), Tarım ve Orman Bakanlığı ve TARSİM verilerinden oluşmuştur. Ayrıca konu ile ilgili önceden yapılmış ulusal ve uluslararası araştırma, derleme ve incelemelerden yararlanılmıştır.

Yöntem

Bu çalışmada, Şanlıurfa ilinde antepfıstığı üretimi yapan işletmelere uygulanan anketlerden elde edilen verilerin analizinde çeşitli istatistiki yöntemler kullanılmıştır. Aynı zamanda verilerin analizinde t-testi, khi-kare testi ve lojistik regresyon analizi kullanılmıştır. Lojistik regresyon analizi, özellikle son yıllarda bilimsel çalışmaların çözümlenmesi için sık kullanılmış ve öne çıkan analiz yöntemlerinden biri olmuştur (Yavuz, 2010).

Lojistik regresyon analizi, bağımlı değişken ile bağımsız değişken ya da değişkenler arasındaki matematiksel ilişkiyi analiz etmede çok fazla kullanılan bir yöntemdir (Yazgı, 2017).

Araştırma Bulguları ve Tartışma

Şanlıurfa ilinde antepfıstığı üretimi yapan işletmelerin tarım sigortası yapmalarını etkileyen faktörlerin belirlenmesi için, seçilen bağımsız değişkenler (üreticilerin gelir durumu, işletmelerin arazi büyüklüğü, gübreleme ve bakım masrafları, hasat esnasında yapılan masraflar, girdi maliyetindeki değişme, ürünlerin taşıma ve pazarlama masrafları, işletmelerin borçlanma durumları, hükümetin seçilen ürünlere ilişkin yaptığı politika değişiklikleri) arasında anlamlı bir farklılık olup olmadığını tespit etmek için t-testi kullanılmış ve analiz sonuçları Çizelge 1.'de gösterilmiştir.

Bu bağlamda seçilen bağımsız değişkenlerin, istatistiksel olarak anlamlı oldukları tespit edilmiştir (p<0.05).

Çizelge 1. Şanlıurfa ilinde antepfıstığı üreticilerinin tarım sigortası yapmalarını etkileyen faktörlerde, bağımsız değişkenlere ait t-testi sonucu

Table 1. T-test results of independent variables on factors affecting agricultural insurance of pistachio producers in Şanlıurfa province

Analizler Analyzes	**Örnek Mean**	**N**	**Ortalama Mean**	**Standart Sapma Standart Deviation**	**t**	**p***
Gelir	Tarım sigortası yaptıranlar	51	212156.86	156669.25	-2.78	0.00
	Tarım sigortası yaptırmayanlar	249	152248.99	138651.20		
Arazi büyüklüğü	Tarım sigortası yaptıranlar	51	75.22	59.02	-3.657	0.00
	Tarım sigortası yaptırmayanlar	249	52.03	36.640		
Gübreleme ve bakım masrafları	Tarım sigortası yaptıranlar	51	8911.76	6955.362	-4.383	0.00
	Tarım sigortası yaptırmayanlar	249	6102.01	3339.710		
Hasat esnasında yapılan masraflar	Tarım sigortası yaptıranlar	51	5478.43	5390.893	-3.792	0.00
	Tarım sigortası yaptırmayanlar	249	3608.67	2545.969		
Girdi maliyetindeki değişme	Tarım sigortası yaptıranlar	51	3.37	0.864	-2.724	0.00
	Tarım sigortası yaptırmayanlar	249	3.01	0.848		
Ürünlerin taşıma ve pazarlama masrafları	Tarım sigortası yaptıranlar	51	4208.33	2044.279	-3.730	0.00
	Tarım sigortası yaptırmayanlar	249	3019.33	2008.832		
Borçlanma durumu	Tarım sigortası yaptıranlar	51	3.39	0.874	-2.819	0.00
	Tarım sigortası yaptırmayanlar	249	3.00	0.900		
Hükümetin seçilen ürünlere ilişkin politikalarındaki değişiklikler	Tarım sigortası yaptıranlar	51	4.61	0.532	-3.210	0.00
	Tarım sigortası yaptırmayanlar	249	4.37	0.483		

*p<0.05 olan değerleri içeren sonuçlar istatistiksel açıdan önemlidir.

Şanlıurfa ilinde antepfıstığı üreticilerinin tarım sigortası yapmalarını etkileyen faktörlerde, seçilen bağımsız değişken olan üreticilerin tarımsal amaçlı kooperatiflere üye olma durumlarına bağlı olarak aralarında anlamlı bir farklılık olup olmadığını tespit etmek için khi-kare testi kullanılmıştır. Uygulanan khi-kare testi sonucuna göre, p=0.000<0.05 olduğundan antepfıstığı üreticilerinin tarım sigortası yapmalarıyla tarımsal amaçlı kooperatiflere üye olmaları arasında istatistiksel olarak anlamlılık olduğu belirlenmiştir (Çizelge 2). Tarım sigortası yaptıran üreticilerin %39.2'sinin tarımsal kooperatiflere üye olduğu, yaptırmayan üreticilerin ise %4.4'ünün tarımsal kooperatiflere üye olduğu belirlenmiştir. Bu bağlamda, tarımsal kooperatiflere üyelik durumunun tarım sigortası yapılmasını pozitif yönde etkilediği görülmektedir. Ayrıca, bazı tarımsal kooperatifler üreticilerin tarım sigortası yaptırmalarını zorunlu kıldıkları için, üreticilerin tarımsal kooperatiflere üyelik durumlarının tarım sigortası yapılma

oranını da artırmaktadır.

Çizelge 2. Şanlıurfa ilinde antepfıstığı üreticilerinin tarım sigortası yapmalarını etkileyen faktörlerde, üreticilerin tarımsal amaçlı kooperatiflere üye olma durumuna ait khi-kare testi sonucu

Table 2. The chi-square test result of the factors affecting the agricultural insurance of pistachio producers in Şanlıurfa province, regarding the status of the producers being members of agricultural cooperatives.

Analizler Aanlyzes			**Tarımsal amaçlı kooperatiflere üye olma durumu**		
			Hayır	Evet	**Toplam**
Tarım sigortası	Hayır	Gözlenen değer	238	11	249
		Beklenen değer	95.6	4.4	100.0
	Evet	Gözlenen değer	31	20	51
		Beklenen değer	60.8	39.2	100.0
Toplam		Gözlenen değer	269	31	300
		Beklenen değer	89.7	10.3	100.0

Khi-kare Testi

	Değer	**Df**	**p* değeri**
Pearson Chi-Square	55.320	1	0.000
Likelihood Ratio	40.961	1	0.000
Linear-by-Linear Association	55.136	1	0.000
N of Valid Cases	300		

*p<0.05 olan değerleri içeren sonuçlar istatistiksel açıdan önemlidir.

Lojistik Regresyon Analizi

Şanlıurfa ilinde antepfıstığı üretimi yapan işletmelerden tarım sigortası yaptıranların, tarım sigortası yapmalarını etkileyen faktörlerin tespit edilmesi amacıyla lojistik regresyon analizi yapılmıştır. Bu bağlamda ikili lojistik (binary lojistik) regresyon analizi kullanılması uygun bulunmuştur. Araştırmada ikili lojistik regresyon analizinde bağımlı değişkeni tarım sigortası yaptıran katılımcılar oluştururken bağımsız değişkenleri ise; üreticilerin yaşı, üreticilerin gelir durumu, üreticilerin eğitim durumu, üretici ailesinin yıllık toplam geliri, üreticilerin deneyimleri ve tarımsal kooperatiflere üyelik durumları oluşturmaktadır. Bağımsız değişkenler, seçilirken dikkat edilen en önemli husus, bağımlı değişkendeki değişimi en iyi şekilde açıklayabilme özellikleridir, bu amaçla en uygun bağımsız değişkenler seçilip modele alınmıştır (Yavuz, 2010).

Çizelge 3. incelendiğinde, modelde kullanılan bağımsız değişkenlerden üreticilerin yaşı (p=0.000) üreticilerin eğitim durumu (p=0.000) ve gelir durumları (p=0.008) ile tarım sigortası yaptıran işletmeler arasında yapılan istatistiki analizde anlamlı bir ilişki olduğu belirlenmiştir. Şanlıurfa ilinde antepfıstığı üreticilerinin tarım sigortası yapmalarını etkileyen faktörlerden, üreticilerin tarım dışında herhangi bir iş ile uğraşmaları ile bağımlı değişken arasında anlamlı bir ilişki bulunmamıştır (p=0.056).

Çizelge 3. Çok değişkenli lojistik regresyon modeli

Table 3. Multivariate logistic regression model

		B	**S.E.**	**Wald**	**df**	**p***	**Exp(B)**
Adım 1	Yaş	1.232	0.353	12.185	1	0.000	3.429
	Eğitim durumu	1.525	0.248	37.688	1	0.000	4.596
	Gelir	0.279	0.106	6.949	1	0.008	1.322
	Tarım dışı, başka işle uğraşma durumu	1.290	0.675	3.645	1	0.056	3.631
	Çiftçilik deneyimi	-0.186	0.183	1.027	1	0.311	0.830
	Sabit	-12.071	1.847	42.699	1	0.000	0.000

*p<0.05 olan değerleri içeren sonuçlar istatistiksel açıdan önemlidir.

Çalışmamızın sonucunda elde edilen veriler, önceki çalışmalarda elde edilen üreticilerin tarım

sigortası yaptırmalarında etkili olan faktörlerin belirlenmesi sonuçları ile karşılaştırıldığında çoğunlukla benzer sonuçlar alındığı görülmektedir. Yavuz (2010), arazi büyüklüğünün üreticilerin tarım sigortası yaptırmalarında etkili olduğu belirlenmiştir. Alay (2012), üreticilerin tarımsal amaçlı kooperatiflere üyelik durumlarının etkili olduğu belirlenmiştir. Ertan ve Gök (2012), çiftçilerin yaşı ve eğitim seviyelerinin önemli bir etken olduğu belirlenmiştir. Aydın vd., (2016), üreticilerin tarımsal gelirlerinin, eğitim seviyelerinin, arazi büyüklüklerinin ve tarımsal kooperatiflere üyelik durumlarının pozitif, ancak tarım dışı işle uğraşma durumlarının negatif yönde etkilediği belirlenmiştir. Yazgı (2017), üreticilerin tarımsal amaçlı kooperatiflere üyelik durumları ve eğitim seviyelerinin etkili olduğu belirlenmiştir. Kızıloğlu (2017), işletme büyüklüğü, üreticilerin ilkokul mezunu olma durumları ve üreticilerin gelir durumlarının etkili olduğu belirlenmiştir. İşceberen (2018), arazi büyüklüğü, üreticilerin gelir durumları, üreticilerin yaşı ve eğitim durumlarının etkili olduğu belirlenmiştir. Tümer vd., (2019), üreticilerin bitkisel ürün sigortası yaptırmalarında, eğitim durumlarının etkili bir faktör olduğu tespit edilmiştir. Ceyhan vd., (2021), risk taşıyıp sigorta yaptırmayan işletmelerin, sigorta yaptırabilecek geliri elde edebilecek arazi büyüklüğüne sahip olmadığı belirlenmiştir. Ayrıca, Tufan vd., (2019), sadece çiftçilikle geçimini sağlayan üreticilerin %44, tarım dışında herhangi bir ek geliri olan üreticilerin %8 oranında tarım sigortası yaptırdığı belirlenmiştir.

Literatür incelendiğinde yukarıda belirtilen çalışmalarda olduğu gibi tarım sigortasıyla ilgili yapılan çalışmaların büyük çoğunluğunun tarımsal bir ürün üzerine olmadığı, çalışma bölgesindeki tarımsal üreticilerin, tarım sigortasına karşı tutumlarının belirlenmesi üzerine yapıldığı görülmektedir. Ancak tarımsal ürünler üzerine yapılan çalışmalarda az da olsa mevcuttur. Çalışma konumuzdan farklı tarımsal ürünler üzerine yapılan çalışmalar incelendiğinde, çalışmamızla benzer sonuçlar elde edildiği dikkat çekmektedir. Kabaoğlu (2017), fındık üreticileri üzerine yapılan çalışmada üreticilerin tarım sigortası yaptırmalarında, üreticilerin gelir durumunun etkili olduğu belirlenmiştir. Hayran vd., (2020), yem bitkisi üreticileriyle yapılan çalışmada, arazi büyüklüğünün ve tarımsal kooperatiflere üyelik durumlarının etkili bir faktör olduğu belirlenmiştir. Tekin ve Karlı (2021), elma üreticileriyle yapılan çalışmada, üreticilerin yaşı, üreticilerin eğitim durumları ve arazi büyüklük durumlarının tarım sigortası yaptırılmasında etkili olduğu belirlenmiştir. Bal ve Özüdoğru (2021), Kalecik karası üzüm üreticileriyle yapılan çalışmada, arazi büyüklüğü ve toplam aylık gelirin etkili olduğu belirlenmiştir.

Sonuçlar

Bu çalışma ile antepfıstığı üretimi yapan işletmelerin tarım sigortası yaptırma süreçlerinde etkili olan faktörlerin belirlenmesi için lojistik regresyon analizinin yardımıyla, üreticilerin sahip olduğu özelliklerden bireysel özellikler incelenip değerlendirilmiştir. Lojistik regresyon analizi uygulanan beş değişkenden üçü istatistiksel olarak anlamlı çıkmıştır. Bu bağlamda üreticilerin yaşı, üreticilerin eğitim durumları ve işletmelerin yıllık toplam gelirinin işletmelerin tarım sigortası yaptırmalarında etkili olduğu tespit edilmiştir. Bununla birlikte antepfıstığı üretimi yapan işletmelerin tarım sigortası yaptırmalarında etkili olan faktörlerin belirlenmesi için khi-kare testi ve t-testinden de yararlanılmıştır. Khi-kare testi sonucuna göre üreticilerin üretici örgütlerine üye olma durumlarının, tarım sigortası yaptırma kararları üzerinde etkili bir özellik olduğu tespit edilmiştir. Yapılan çözümlemeye göre işletmelerin, üretici örgütlerine üye olma durumlarında tarım sigortası yaptırma olasılığını da artıracağı belirlenmiştir.

Ankete katılan işletmelerin %82'si riskleri karşılamada sigortayı etkin bir araç olarak gördüklerini %18'i ise riskleri karşılamada sigortayı etkin bir araç olarak görmediklerini belirtmişlerdir. Katılımcıların %67.3'ü prim miktarı daha düşük olursa sigorta yaptıracaklarını belirtmişlerdir. Tarım sigortası yaptıran katılımcıların, %12.3'ünün mevcut prim

miktarından orta derecede memnun oldukları ve kesinlikle memnunum diyen katılımcının ise olmadığı tespit edilmiştir. Bununla birlikte Şanlıurfa ilinde antepfıstığı üretimi yapan işletmelerin 1 dekar için ödemek istedikleri en uygun prim miktarının ortalama 19.92 TL olduğu tespit edilmiştir. Bu bağlamda, üreticilerin prim miktarını yüksek buldukları ve bu sebeple tarım sigortası yaptırmadıkları tespit edilmiştir.

Çalışmadan elde edilen veriler ve özellikle üreticilerin tarım sigortası yaptırmalarında etkili olduğu tespit edilen değişkenlerden bireysel özellikler (üreticilerin yaşı, tarım dışı herhangi bir ile uğraşma durumu) herhangi bir müdahale ile değiştirilebilecek değişkenler değillerdir. Sonuç olarak bu değişkenlerle ilgili bir öneride bulunmak anlamsız olacaktır. Fakat, TARSİM üreticilerin tarım sigortaları konusunda yeterince bilgilendirilmeleri ve tarım sigortası bilincinin tam olarak oluşması amacıyla gerekli eğitimleri düzenli olarak yapmalıdır. TARSİM 2017 yılında buğday bitkisinde uygulamaya koyduğu kuraklık verim sigortasını geliştirip, diğer gerekli ürünler için de teminat altına almalıdır. Bununla birlikte tarım sigortalarının yaygınlaşması için TARSİM tarafından tanıtımlar ve bilgilendirme toplantıları yapılmalıdır. Ayrıca eksper hizmetlerinin daha iyi yapılmasını sağlayacak kaliteye ulaştırılması, prim ödemelerinde üreticilerin üretim ve hasat dönemlerinin de dikkate alınması ve tarım sigortası kapsamının özellikle de kuraklık riskinde daha da genişletilmesi tarım sigortasının yaygınlaşabilmesine olanak sağlayacaktır.

Ekler

Bu çalışma Harran Üniversitesi Fen Bilimleri Enstitüsü'nde yürütülen "Şanlıurfa İlinde Antepfıstığı Üretiminde Tarım Sigortası Yapılmasını Etkileyen Faktörlerin Belirlenmesi" başlıklı Yüksek lisans tezinden üretilmiştir. Yüksek lisans tez çalışmasını 18175 numaralı proje kodu ile destekleyen Harran Üniversitesi Bilimsel Araştırma Projeleri Yönetim Birimi Başkanlığına teşekkür ederiz.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

Yazarların Katkısı

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan ederler.

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Research Article/Araştırma Makalesi

A comprehensive assessment of the antioxidant capacity and sensory properties of wheat-based cereal breads produced in different formulations

Farklı formülasyonlarda üretilen buğday bazlı tahıl ekmeklerinin antioksidan kapasitelerinin ve duyusal özelliklerinin kapsamlı bir değerlendirmesi

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ABSTRACT

In this study, it was aimed to investigate the physicochemical, sensory and antioxidant properties of some wheat-based cereal bread types that are offered for sale and frequently consumed in the markets in Turkey. Bread types were produced with the help of a home-type bread-making machine, and 100% white wheat flour bread (WB) was used as a control. Sensory evaluations were determined by using the nine-point hedonic scale method. Total phenolic contents were determined by using Folin-Ciocalteau's method. Antioxidant capacities were determined by using ABTS, DPPH, and FRAP assays. Besides, all data were evaluated by using principal component analysis (PCA) to discriminate the properties of bread types. Whole-wheat bread showed the highest antioxidant potentials (200.00µM TEAC for ABTS, 147.50µM TEAC for DPPH, 116.94µM TEAC for FRAP) and the highest amount of phenolic content (64.30mg GAE/100gDW), but a low overall acceptability score (6.00/9.00). As a result, the addition of wheat-based cereal flours affected the antioxidant, sensory, and physical properties of the produced breads ($p<0.05$). Different cereal flours added to the bread formulation positively affected the antioxidant properties of all breads, but only wheat bran and oat flour addition positively affected the overall sensory acceptability of the bread types. Hereby this study, individuals will be made aware of consuming bread types enriched with different cereal flours including important micronutrients instead of WB which is so poor in micronutrients. The study also provides convenience to individuals in the production of these breads in a home-type bread-making machine. This study reveals the various data for functional properties of bread types that are consumed less than WB.

Key Words: Antioxidant activity, Cereals, Bread, Sensory evaluation, PCA

ÖZ

Bu çalışmada, Türkiye'de marketlerde satışa sunulan ve sıklıkla tüketilen bazı buğday bazlı tahıllı ekmek türlerinin fizikokimyasal, duyusal ve antioksidan özelliklerinin araştırılması amaçlamıştır. Ev tipi ekmek yapma makinesi yardımıyla ekmek çeşitleri üretilmiş olup, kontrol olarak %100 beyaz buğday unu ekmeği (WB) kullanılmıştır. Duyusal değerlendirmeler dokuz noktalı hedonik ölçek yöntemi kullanılarak belirlenmiştir. Toplam fenolik madde içerikleri Folin-Ciocalteau yöntemi kullanılarak belirlenmiştir. Antioksidan kapasiteleri ABTS, DPPH ve FRAP testleri kullanılarak belirlendi. Ayrıca, ekmek çeşitlerinin özelliklerini ayırt etmek için tüm veriler temel bileşenler analizi (PCA) kullanılarak değerlendirilmiştir. Tam buğday ekmeği en yüksek antioksidan potansiyelleri (ABTS için 200.00µM TEAC, DPPH için 147.50µM TEAC, FRAP

için 116.94µM TEAC) ve en yüksek fenolik içeriği (64.30mg GAE/100g DW) göstermiş, ancak genel olarak düşük kabul edilebilirlik puanına (6.00/9.00) sahip olduğu saptanmıştır. Sonuç olarak, farklı tahıl unlarının ilavesi üretilen ekmeklerin antioksidan, duyusal ve fiziksel özelliklerini etkilemiştir ($p<0.05$). Ekmek formülasyonuna eklenen farklı tahıl unları, tüm ekmeklerin antioksidan özelliklerini olumlu yönde etkilemiş; ancak yalnızca buğday kepeği ve yulaf unu ilavesi ekmek türlerinin genel duyusal kabul edilebilirliğini olumlu yönde etkilemiştir. Böylelikle bu çalışma ile bireyler, mikrobesinler açısından çok fakir olan WB yerine önemli mikrobesinler içeren farklı tahıl unlarıyla zenginleştirilmiş ekmek çeşitlerini tüketme konusunda bilinçlendirilmiş olacaktır. Çalışma ayrıca bu ekmeklerin ev tipi ekmek yapma makinesinde üretilmesi konusunda da bireylere kolaylık sağlamaktadır. Bu çalışma, WB'den daha az tüketilen ekmek türlerinin fonksiyonel özelliklerine ilişkin çeşitli verileri ortaya koymaktadır.

Anahtar Kelimeler: Antioksidan aktivite, Tahıllar, Ekmek, Duyusal değerlendirme, PCA

Introduction

The fact that consumers are aware that nutrition and health are directly related provided them to expect additional benefits from consumed food besides their nourishing properties (Siró et al. 2008). These developments pioneered the emergence of the "functional food" term as a new approach. A food item can be considered "functional" if it is nutritionally adequate and provides additional benefits to human physiology and metabolic functions (Roberfroid 1999). Functional foods can be obtained by food enrichment and supplement or removing unwanted compounds from the foods in order, to support the diet (Hasler 2002). The Codex Alimentarius defines food enrichment as the addition of micronutrients to foods for the purposes of preventing or correcting a demonstrated deficiency (Dary and Mora 2013). Enriching foods in terms of some nutrients is a public health practice that is made to prevent diseases caused by inadequate consumption of them. In the literature, studies are showing that some food items are effective in reducing the risk of developing type-II diabetes, coronary heart, kidney stones, cardiovascular, gastrointestinal diseases, and cancer (Angulo-López et al. 2023; Younas et al. 2020). It is possible to come across products that have been placed in the food industry. For example, enriched/fortified products such as calcium/iron added to milk, dual-fortified salt with iodine, vitamin-added sugar/margarine/spreadable oils, and micronutrient items added to bread/wheat flour (Akhtar, Anjum, and Anjum 2011; Diosady et al. 2002; Perales et al. 2006; Rosell 2008).

Wheat is the most produced and consumed grain variety in the world and Turkey. It is widely used in human nutrition and especially as a raw material for bread production. Bread is the main part of the daily intake of our diet. In Turkey, bread is the basic ingredient of meals because it is cheaper, and easily accessible compared to other foods. Also, approximately 45% of daily energy is obtained from bread. So, it is a good source of energy and an important part of the daily diet (Wrigley 2009). For these reasons, enrichment studies on bread are becoming even more important. In recent years, researchers are trying to increase the nutritional value of bread with natural additives (Dimov et al. 2018; Prokopov et al. 2018; Torrijos et al. 2021).

Polyphenols are natural substances found in all plants and are called secondary metabolites. They are responsible for the organoleptic properties and health benefits of plant products. They have positive effects on the treatment of many diseases and the immune system (Can et al. 2014; Jiang et al. 2021). The type and amounts of phenolic compounds influence the quality, sensory properties, and acceptability of bread by consumers. (Xu, Wang, and Li 2019).

In Turkish Food Codex, "bread" is defined as the product obtained by respectively kneading, shaping, fermentation, and baking the mixture of wheat flour, water, salt, yeast, sugar (if needed), enzymes, and permitted additives. In addition to these components if the bread includes cereal products and natural ingredients called "bread types". In Codex Alimentarius bread types are called "bread-derived products".

Although people consume large amounts of bread every day, they are not aware of the

bioactive effects. Up till today, there isn't any performed work about the phenolic content and antioxidant activities of these "bread and bread types" that are defined in the Turkish Food Codex. Therefore, the purpose of this study was to investigate the phenolic contents and antioxidant activities of the bread and bread types mentioned in the “Turkish Food Codex” to contribute to the consumer's nutritional balance. Also, by using the bread-making machine, each bread could produce in the same conditions; comparable accurately and easily. Besides, with the help of a bread-making machine, and the results of the analysis of the bread types, novel bread formulations can be developed under the name “functional bread”. Thus, in this study, the total phenolic contents and antioxidant activities of the white flour bread called bread (WB, the most being consumed traditional bread in Turkey) were compared to the bread types. Furthermore, the sensory properties and acceptability of the bread and bread types were examined to inform consumers who have never tasted the bread types. Moreover, all data were evaluated by using principal component analysis (PCA) to discriminate the properties of bread types.

Materials and Methods

Experimental setup

Firstly, the breads scoped in the “Bread and Bread Types Communiqué” were researched. (B-1: whole wheat flour bread (100%), B-2: whole wheat flour bread (60%), B-3: white bread (100%), B-4: wheat bran bread (10%), B-5: rye flour bread (30%), B-6: maize flour bread (20%), B-7: oat flour bread (15%), B-8: multi-grain bread (5% rye, 5% maize, 5% oat, 5% barley flour)). The amounts of their ingredients were calculated, and they were adapted to the bread making machine (Table 1). So, eight different commercial breads were prepared and used as samples.

Materials used in bread making

White wheat flour (amount of ash 0.65-1.1%, 14.3% moisture, 11.3% protein, 60% water absorption) and wheat bran were procured from Cesur Mills Company (Trabzon, Turkey). The iodized table salt (Billur Salt Company, İzmir) and instant active dry yeast (Dr. Oetker Company, İzmir) were obtained from a supermarket. Whole wheat flour (max. 1.4% ash, 14.5% moisture, 7.0-12.4% protein, 2.6% lipid, 59.8% carbohydrate, ‰ 0.03 sodium, ‰ 0.32 calcium), oat flour (max. 1.3% ash, 10.5% fiber, 7.0-13.0% protein, 3.6% lipid, 49.6% carbohydrate), rye flour (max. 0.7% ash, 8.15% fiber, 11.65% protein, 0.27% lipid, 36.06% carbohydrate, ‰ 2.82 sodium), maize flour (9.6% fiber, 9.3% protein, 3.8% lipid, 63.1% carbohydrate) and barley flour (max. 1.2% ash, 4.80% fiber, 7.0-12.0% protein, 1.3% lipid, 56.05% carbohydrate, ‰ 2.82 sodium) were obtained from Doğalsan Company (Yenimahalle, Ankara).

Materials used in chemical analyzes

The chemicals used in the analyzes were either analytical grade purity and were imported by Merck and Sigma-Aldrich.

Instruments used in analyzes

A home type bread making machine (Arçelik K-2715) was used (Figure 1). Laboratory equipments such as homogenizer (Daihan Sci., WiseTis HG-15A), vortex (IKA®, USA), analytical balance (Ohaus, Balances, USA), centrifuge (NF 1200R, Turkey), spectrophotometer (Epoch BioTek Ins., USA), multi-heating magnetic stirrer (Wisestir, Daihan) were used for the preparation of bread samples extracts and analyzes.

Figure 1. (a) A home type bread making machine with double-compartment mold (Arçelik K-2715) and (b) the breads obtained on preliminary testing during program optimization in terms of volume and texture in the machine.

Preparation of breads

The steps fermentation and baking are both essential for the flavor of the wheat bread. So, the bread processing program is important for sensory analysis (Heiniö et al. 2016).

The special program number 11 was selected in the bread-making machine and the method previously optimized was adjusted. Program steps in order: knead 1 (14 min), rise 1 (32 min), knead 2 (8 min), rise 2 (31 min), last fermentation (50 min), bake (62 min, 180°C); total time 3 h 17 min (Burnaz, Ertop, and Karataş 2018). The quantities of water, table salt, yeast, and cereal flours used in making the bread dough were added according to the formulations in Table 1. These amounts are calculated by considering the percentages in the "Bread and Bread Types Communiqué" (in Turkish Food Codex).

Table 1. Bread ingredients (Turkish Food Codex Communiqué on Bread and Bread Types, 04 January 2012- 28163).

Ingredients (g)	**Bread Ingredients (codes*)**							
	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8
White wheat flour	–	120	300	270	210	240	255	240
Whole wheat flour	300	180	–	–	–	–	–	–
Wheat bran	–	–	–	30	–	–	–	–
Rye flour	–	–	–	–	90	–	–	15
Maize flour	–	–	–	–	–	60	–	15
Oat flour	–	–	–	–	–	–	45	15
Barley flour	–	–	–	–	–	–	–	15
Salt	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87
Yeast	3	3	3	3	3	3	3	3
Water	180	180	180	180	180	180	180	180

*B-1: Whole wheat flour (100%) bread, B-2: Whole wheat flour (60%) bread, B-3: White bread (control), B-4: Wheat bran (10%) bread, B-5: Rye flour (30%) bread, B-6: Maize flour (20%) bread, B-7: Oat flour (15%) bread, B-8: Multi-grain bread (5% rye flour, 5% maize flour, 5% oat flour, 5% barley flour).

Determination of specific volumes of breads

After baking, the breads were taken out of the mold and kept at room temperature. At the end of the waiting period, the bread weights (g) and bread volumes (cm^3, by the technique of displacement with rapeseed) were measured (International 2001). In bread, the "specific volume" that is used as a quality parameter was measured. The specific volume is calculated as the ratio of bread volume to bread weight and expressed as cm^3/g (Dogan and Yıldız, 2009). With the help of double-compartment mold, the breads were prepared in duplicate.

Preparation of bread extracts

Five grams of bread samples were weighed, and 50 ml of 80% methanol was added on, then homogenized. Subsequently, the external surfaces of erlens were covered with aluminum foil and the mixtures were stirred at 750 rpm for 2 h in a multi-heating magnetic stirrer at 37°C. Finally, the extracts were transferred to conical centrifuge tubes and centrifuged at 5000 rpm and 20°C for 15 min, filtered through black banded filter paper and the volumes were completed to 50 ml with 80% methanol solution (Burnaz et al. 2018). The extracts were stored at -18°C until use in analyses. In the study, the bread samples were prepared to be four repeats. In analyses, the samples were prepared in triplicate.

Total phenolic contents (TPC)

TPC values of the bread extracts were detected by using the Folin–Ciocalteu method (Burnaz 2021; Singleton and J A Rossi 1965). Gallic acid (GAE) and ferulic acid (FAE) were used as standards. TPC values were calculated as mg "ferulic acid or gallic acid"/100 g bread dry weight (DW).

Ferric reducing antioxidant power (FRAP)

FRAP values, as determined by the previous method (Benzie and Strain 1996) with some modifications was applied and the results were expressed as TEAC (Trolox® equivalent antioxidant capacity) by reference to standard curves (Ilyasoɬlu and Burnaz 2015). Trolox® was used as a standard. Primely, 1450 µl fresh FRAP solution was added to each 50 µl of standard/extracts and vortexed. After 20 min incubation at room temperature, the absorbance was read at 595 nm. The results were calculated as µM TEAC.

DPPH and ABTS radical scavenging activity

The stable DPPH$^{\bullet}$ (2,2 diphenyl-1-picrylhydrazyl) radical was prepared according to a previously described method (Brand-Williams, Cuvelier, and Berset 1995). The stable ABTS$^{\bullet+}$ (2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical was prepared according to a previously described method (Re et al., 1999). The results were calculated as µM TEAC and radical scavenging percentages (%) by the following formula:

$$Scavenging\ \% = [(A_B - A_A)/A_B\ ]x100$$

A_B = absorption of blank Radical solution (t=0 min); A_A = absorption of extract (t=60 min for DPPH, t=20 min for ABTS).

Sensory analysis

Sensory analyses of breads were carried out by 30 semi-trained volunteer panelists from faculty members. Before the study, necessary permissions and approvals were obtained from the ethics committee of our university. Before tasting, a form was established to determine the like/dislike status about the bread samples (Altuğ Onoğur and Elmacı 2011). The panelists evaluated the breads according to shell/inner color, crumbling, texture, appearance, brittleness, brightness, odor, and flavor properties and given scores for overall acceptability. For overall acceptability nine-point hedonic scale sensory analysis form was used, ranging from "1-dislike extremely to 9-like extremely (Lawless and Heymamn 2010). Results were calculated as average scores. If the "acceptability average score" is above 5, the bread is "acceptable" (Torbica, Hadnadev, and Dapčević 2010).

Statistical analysis

A statistical program (SPSS®, Version 20) was used. Significance P values and standard deviations (SD) were calculated by using the related program. The bread sample results were compared by using One-Way ANOVA-Duncan's test (Tables 2 and 3).
In order to visualize the relationship between the results of the analyzes in bread varieties, the data were statistically transformed using PCA with the correlation matrix method (Figure 2). Another statistical software package program (XLSTAT Addinsoft SARL 2019) was used to perform PCA. All analyses were carried out in triplicates and results were given as mean ± standard deviation (SD). ANOVA was used to compare the significant

differences in the mean values at p<0.05. Different letters in the same column are significantly different (p<0.05).

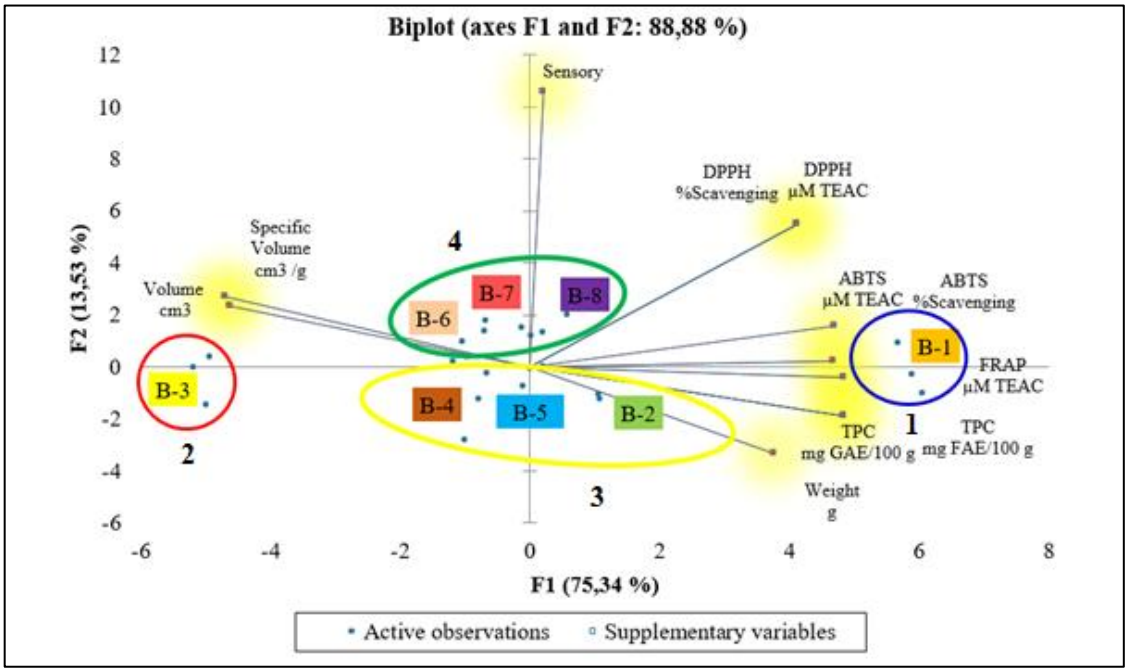

Figure 2. Principal component analysis (PCA) of descriptive sensory, antioxidant and physical analyses of breads

Results and Discussion

Bread quality properties

By using weight and volume measurements, specific volumes (cm^3/g) of breads were calculated. The results of specific volumes of breads are listed as follows: B-3> B-7>B-8>B-6>B-5>B-4>B-2>B-1 (Table 2). The specific volume of the WB (B-3) was found to be the highest (11.07 cm^3/g) and the whole wheat (100%) bread (B-1) was found to be the lowest (26.46 cm^3/g). There was no significant difference between B-6 and B-8 breads in terms of specific volume, but there was a significant difference between the other breads. White flour provides gluten being adequate and high quality, obtaining a strong and elastic dough, excellent gas holding ability, increased bread volume, improved texturization, small and homogeneous pores (Kundakcı and Göçmen 1992). Therefore, since bread made with 100% whole wheat flour without white flour (B-1) cannot form a porous structure, its volume and specific volume are the lowest. While the increase in the proportion of white flour in the formulation positively affects the specific volume, the addition of wheat bran (B-4) has a negative effect. Also, it was seen that different cereal flours (rye, oat, maize, and barley) except the white wheat flour participating in the formulation during the preparation of the bread types affected the amount of gluten in the negative direction. The specific volume, which is the bread quality parameter, and the inner texturing structure of the bread was also adversely affected. The significant reduction in the volume of whole grain-rich bread is primarily due to the dilution of gluten in the flour blends. Similarly, Ragaee et al. (2011) reported a drop in the loaf volume for rye, barley, oat, and cellulose-enriched breads, respectively. In contrary to our work, they found that there were no significant differences in the loaf or specific volume between control white bread and wheat whole grain-enriched bread (Ragaee et al. 2011).

Total phenolic contents

Cereal grains are a good source of natural antioxidant compounds. Phenolic acids are the major antioxidants in cereal grains and have a huge potential to be beneficial for health (Dziki et al. 2014). Whole grains are good sources of dietary fibers, minerals, vitamins, and phytochemicals (Okarter and Liu 2010). The TPC of the WB (B-3)

was found the lowest (15.68 mg GAE/100 g and 12.06 mg FAE/100 g). On the contrary, the TPC of the whole wheat flour bread (B-1) was found the highest (64.31 mg GAE/100 g and 56.27 mg FAE/100 g). It has nearly 4 times higher TPC value than the WB. The TPC values of other bread types were found close to each other. The results of TPC values of breads are listed as follows: B-1>B-2>B-5>B-8>B-4>B-6>B-7>B-3 (Table 2). Zieliński and Kozłowska (2000) have investigated the antioxidant properties of aqueous and methanolic (80%) extracts of raw cereal grains and reported that the highest amounts of phenolics extracted by water from barley, wheat, and rye, respectively. As compared to barley, the oat and buckwheat grain were included about five folds lower total phenolics if extracted by water. In a study on free and bound phenolic fractions of various fiber-enriched breads, compared to the control white bread, oat-grain added bread included the highest level of free phenolics followed by rye-, wheat-, and barley grain added breads, respectively.

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Antioxidant Activities

It is scientifically acknowledged that free radicals and reactive oxygen species formed during cellular metabolism adversely affect the pathogenesis of chronic diseases. Dietary antioxidants fight against free radicals to help reduce the risk of these diseases. In this study, the antioxidant capacities of breads were evaluated based on three different in-vitro antioxidant methods.

The FRAP values of bread extracts are given in Table 2, in terms of µM TEAC values. The FRAP value of the WB was found the lowest (23.06 µM TEAC), and the whole wheat flour bread was found the highest (116.95 µM TEAC), likewise TPC values. The results of FRAP values of breads are listed as follows: B-1>B-2>B-5>B-7>B-6>B-8>B-4>B-3 (Table 2).

In literature, cereal products have important antioxidant potentials. The DPPH results showed that the antioxidant activities of cereals or cereal products are higher than widespread fruits and vegetables but lower than berries (Fogarasi et al. 2015).

Table 2. Changes in the antioxidant capacities and physical properties of the wheat-based homemade cereal breads

Sample no.*	Antioxidant capacities							Physical properties		
	TPC mg GAE/100 g	TPC mg FAE/100 g	DPPH µM TEAC	DPPH %Scavenging	FRAP µM TEAC	ABTS µM TEAC	ABTS %Scavenging	Weight g	Volume cm^3	Specific Volume cm^3 /g
B-1	64.31^{a} ±2.45	56.27^{a} ±2.23	147.50^{a} ±2.52	15.97^{a} ±0.27	116.95^{a} ±5.02	200.00^{a} ±3.57	26.46^{a} ±0.47	444.71^{a} ±6.29	876.33^{f} ±11.02	1.97^{g} ±0.01
B-2	44.71^{b} ±1.63	38.46^{b} ±1.49	85.16^{c} ±1.53	9.22^{c} ±0.16	84.44^{b} ±3.94	150.74^{b} ±1.70	19.94^{b} ±0.22	423.64^{b} ±7.33	983.33^{e} ±22.30	2.32^{f} ±0.02
B-3	15.68^{f} ±1.48	12.06^{f} ±1.34	52.83^{e} ±3.46	5.72^{e} ±0.38	23.06^{f} ±1.27	70.74^{e} ±4.49	11.07^{c} ±3.11	410.31^{d} ±3.69	1276.33^{a} ±18.50	3.11^{a} ±0.02
B-4	35.98^{cd} ±2.84	30.52^{cd} ±2.58	70.83^{d} ±3.46	7.66^{d} ±0.38	49.17^{e} ±1.67	112.96^{d} ±2.80	17.25^{b} ±3.70	421.72^{bc} ±4.44	1048.33^{d} ±10.60	2.49^{e} ±0.01
B-5	41.88^{b} ±1.08	35.88^{b} ±0.99	85.50^{c} ±3.06	9.26^{c} ±0.33	73.05^{c} ±2.92	158.52^{b} ±3.57	18.48^{b} ±1.60	410.65^{cd} ±7.58	1069.00^{cd} ±27.22	2.60^{d} ±0.02
B-6	32.44^{de} ±2.84	27.30^{de} ±2.58	82.83^{c} ±4.58	8.97^{c} ±0.50	64.17^{d} ±3.82	136.30^{c} ±0.64	16.68^{b} ±0.09	416.83^{bcd} ±4.94	1102.00^{c} ±11.53	2.64^{c} ±0.01
B-7	29.37^{e} ±2.04	24.51^{e} ±1.85	117.83^{b} ±6.24	12.75^{b} ±0.68	65.56^{d} ±3.37	140.74^{c} ±4.20	17.28^{b} ±0.57	419.77^{bcd} ±4.61	1157.33^{b} ±15.63	2.76^{b} ±0.01
B-8	37.6 3^{c} ±2.49	32.02^{c} ±2.26	120.16^{b} ±8.14	13.01^{b} ±0.88	63.33^{d} ±2.20	151.85^{b} ±11.88	19.32^{b} ±0.65	411.19^{cd} ±6.65	1096.00^{c} ±24.56	2.67^{c} ±0.02

Note: Results are presented as means; ± standard deviations (n=3). Different superscript letters (a-g) in the same column are significantly different (Duncan's test, $p<0.05$).

* B-1: Whole wheat (100%) bread, B-2: Whole wheat flour (60%) bread, B-3: White bread (control), B-4: Wheat bran (10%) bread, B-5: Rye flour (30%) bread, B-6: Maize flour (20%) bread, B-7: Oat flour (15%) bread, B-8: Multi-grain bread (5% rye flour, 5% maize flour, 5% oat flour, 5% barley flour).

GAE: Gallic acid equivalent, FAE: Ferulic acid equivalent, TEAC: Trolox® equivalent antioxidant capacity.

In current study, DPPH activities of bread types' extracts were calculated as TEAC values and scavenging percentages (%). The DPPH scavenging percentage of WB was found the lowest (5.72%), in common with TPC and FRAP. Besides, the DPPH scavenging percentage of whole wheat bread was found the highest (15.97%). The results of DPPH scavenging percentages of breads are listed as follows: B-1>B-8>B-7>B-5>B-2>B-6>B-4>B-3 (Table 2). It has been determined that the diversification of white bread with different cereal flour/bran, also causes an increase in the DPPH scavenging percentage of breads (Table 2). In a study likewise, Ragaee et al. (2011) reported that the DPPH radical scavenging activity of bread enriched with 30% wholegrain flours was two-fold higher when compared with the control bread. In similar studies, the antioxidant capacities of muffins were measured with the help of DPPH and ABTS scavenging assays. Soong et al. (2014) reported that the whole, barley muffin exhibited the highest free radicals scavenging ability, while rice muffin was the lowest ($p<0.05$). Also, the ABTS and DPPH radical scavenging activities of the wheat, maize, and oat muffins, comparable ($p>0.05$).

The ABTS scavenging percentages and µM TEAC values of bread types' extracts were given in Table 2. DPPH values were significantly lower than ABTS values for all types of bread samples. The ABTS scavenging percentage of the WB was found the lowest (11.07%), in common with other tests. The results of ABTS scavenging percentages of breads are listed as follows: B-1>B-2>B-8>B-5>B-7>B-4>B-6>B-3 (Table 2). The investigated values as means antioxidant functionality of bread types were found better when compared to WB (Table 2). Although their ordering differed slightly, the whole wheat flour bread placed on the top and the WB control bread placed on the last, in all antioxidant analysis. Especially there is a strong correlation between the ranking of results of TPC and ABTS. Although the amount of oat is added in the formula less than maize, the results of the oat flour bread sample in the antioxidant assays were found higher than that of the maize flour bread. In this study, it has been determined that the diversification of white bread with different cereal flour/bran causes an increase in TPC and antioxidant activities of bread.

Generally, the studies show that the antioxidant capacity and TPC of bread change based on the wheat variety, flour type, and color (Masisi, Beta, and Moghadasian 2016).

Sensory evaluations

The average scores that were given by panelists were shown in Table 3. As a result of sensory analysis, the flavor was the most important factor affecting the acceptability of bread. That is, the flavor factor has a linear relationship with the acceptability values of the bread types. Although the TPCs and antioxidant activities of whole wheat bread (B-1) were found to be quite high, the overall acceptability of the whole wheat bread (6.00/9) was evaluated as "like slightly" by panelists. The phenolic content of oat flour bread (B-7) was low, but the antioxidant activity values were moderate, and the overall acceptability of this bread was highest in sensory evaluation (8.27/9), and the overall acceptability was found to be "like very much". Oat is a distinctive cereal grain because of its amount of low starch and high beta-glucan content. It has positive health effects (Heiniö et al. 2016). In a similar study, the addition of 15% wheat bran to wheat bread increased flavor intensity in sourdough baking. Also, the addition of 5% bran to wheat flour had increased the number of free amino acids, dietary fiber, and antioxidant activities of dough compared to white wheat flour, enhanced the flavors (Coda et al. 2014).

Statistical evaluation

With the help of One-Way ANOVA, post-hoc Duncan's test, sensory evaluation, and overall acceptability (Table 3) of breads, and significant differences among the antioxidant tests (Table 2) were compared. The results were presented in Tables 2 and 3 by adding SD (standard deviation). Statistically, there is no significant difference between the overall acceptability of most bread types and control white bread ($p>0.05$), except B-1, B-2, and B-7. In other respects, there is a

significant difference between antioxidant tests (P<0.05).

Table 3. Sensory evaluation of bread types.

Bread type*	Shell color†	Inner color†	Crumbling†	Porous texture†	Flavor†	Odor†	Brittle ness†	Bright ness†	Overall acceptability scores†
B-1	8.26^{a}	7.46^{a}	4.20^{c}	3.60^{e}	6.00^{d}	7.26^{a}	7.20^{a}	3.00^{e}	6.00^{de}
B-2	7.20^{bc}	6.86^{b}	5.80^{b}	5.40^{d}	5.73^{d}	6.43^{b}	6.80^{abc}	4.53^{d}	5.80^{e}
B-3	5.06^{e}	5.26^{d}	7.20^{a}	8.60^{a}	6.87^{bc}	6.36^{b}	2.86^{e}	7.80^{a}	7.00^{bc}
B-4	7.60^{b}	7.33^{a}	4.13^{c}	7.33^{b}	7.27^{ab}	6.40^{b}	5.80^{d}	4.40^{d}	7.33^{b}
B-5	6.66^{c}	6.86^{b}	3.53^{d}	6.73^{c}	6.20^{cd}	5.40^{c}	7.13^{ab}	4.13^{d}	6.27^{d}
B-6	5.80^{d}	5.13^{d}	3.73^{cd}	7.20^{be}	7.23^{ab}	7.20^{a}	6.46^{c}	7.60^{a}	7.00^{bc}
B-7	7.13^{bc}	7.46^{a}	5.80^{b}	6.73^{c}	7.83^{a}	6.40^{b}	6.86^{abc}	5.33^{c}	8.27^{a}
B-8	5.5^{de}	5.80^{c}	4.06^{c}	3.53^{e}	6.40^{cd}	6.86^{ab}	6.66^{bc}	6.26^{b}	6.40^{d}

† Mean values, (N=30). Different superscript letters in the same column are significantly different from each other (Duncan's test, p > 0.05).

* B-1: Whole wheat flour (100%) bread, B-2: Whole wheat flour (60%) bread, B-3: White bread (control), B-4: Wheat bran (10%) bread, B-5: Rye flour (30%) bread, B-6: Maize flour (20%) bread, B-7: Oat flour (15%) bread, B-8: Multi-grain bread (5% rye flour, 5% maize flour, 5% oat flour, 5% barley flour).

Principal component analysis

In order, to determine possible differences between bread samples prepared from different flours, a PCA model was created by using the results of antioxidant, sensory and physical analysis (Figure 2). PCA analysis was performed on the average values of eleven analysis parameters aiming to determine the similarities and differences between the bread samples. PCA data constituted 75.34% for the first component, 13.34% for the second component, 5.13% for the third component, 3.48% for the fourth component, and 1.19% for the fifth component of the total variation of the data. The top five components account for 98.68% of the variances for all data.

In the disclosure of data in PCA analysis; while the parameters with the highest factor coordinate values for PC1 and contributing to the correlations were TPC, DPPH, FRAP, ABTS, weight, volume and specific volume, the contribution of sensory analysis was not in this section. The contribution of sensory analysis has been significant in PC2. The PCA model created four separate clusters based on differences between samples prepared by adding different flours. These clusters are: 1(B-1), 2(B-3), 3 (B-2, B-4, B-5), and 4 (B-6, B-7, B-8). The correlation loads of the first two PCs showed high correlations of all the parameters examined. The clear distinction between the samples pointed to differences in some of the parameters investigated. As seen in the PCA graph, samples B-6, B-7, B-8 had the highest sensory scores, while samples B-2 and B-4 had low sensory properties.

The B-1 sample has the highest value in terms of antioxidant activity, and the B-2 sample ranks second. The B-3 sample has been shown to have the lowest antioxidant capacity. Table 4, which includes loading and score data, gives the rotated loadings and correlations for each analysis. Also in Table 4, the score values for each bread sample and each major ingredient are found. TPC, FRAP, ABTS, DPPH scores and weight results on the first key ingredient were higher in B-1 and B-2 breads than in other breads. These values were found to be lower for B-3 bread. When the second main component was interpreted, bread samples B-7 and B-8 got the highest score in terms of sensory analysis. Sensory results for B-2 and B-4 breads are lower than for other bread types.

Loads are: TPC, FRAP, ABTS, DPPH, and weight in the first component; sensory in the second component; volume and specific volume in the third component.

Table 4. The loadings and the scores of the first five rotated principal components

The loadings	F1	F2	F3	F4	F5
TPC mg GAE/100 g	0.970	-0.160	-0.106	-0.018	-0.051
TPC mg FAE/100 g	0.970	-0.160	-0.106	-0.018	-0.051
DPPH µM TEAC	0.827	0.467	0.287	-0.090	0.025
DPPH %Scavenging	0.827	0.467	0.287	-0.090	0.025
FRAP µM TEAC	0.972	-0.033	-0.016	-0.089	-0.123
ABTS µM TEAC	0.942	0.135	-0.064	-0.220	-0.139
ABTS %Scavenging	0.941	0.020	-0.079	-0.090	0.296
Sensory	0.042	0.900	-0.300	0.310	-0.029
Weight	0.757	-0.284	0.411	0.414	-0.022
Volume	-0.926	0.199	0.285	-0.089	-0.032
Specific Volume	-0.941	0.228	0.164	-0.162	-0.019
The scores	**F1**	**F2**	**F3**	**F4**	**F5**
B-1	5.878	-0.170	0.499	0.362	0.061
B-2	1.184	-1.211	-0.533	0.028	-0.075
B-3	-5.029	-0.402	0.521	0.222	0.070
B-4	-0.929	-0.979	-0.455	0.739	0.301
B-5	0.046	-0.278	-0.717	-0.802	-0.303
B-6	-0.925	0.016	-0.222	0.068	-0.196
B-7	-0.495	1.527	0.878	-0.031	-0.048
B-8	0.268	1.496	0.028	-0.586	0.190

The results show that there is a relationship between the breads produced using different flours in terms of antioxidant, sensory and physical analysis.

Conclusions

In this study, the phenolic contents, antioxidant activities, and sensorial properties of white bread and bread types were investigated. The total phenolic content and antioxidant capacity of the bread are significantly enhanced in the case of different cereal flours used. The ingredients in bread types have contributed to phenolic content and the antioxidant activity. So, bread types can be accepted as functional products in terms of antioxidants.

The results of this research will contribute to the studies on designing and developing bread formulations and production processes for bread production in the bread making machine.

Daclarations

Ethics committee approval: Ethics Board of Gümüşhane University. (date: 05.12.2018; no: 95674917-108.99-E.37457)

Conflict of interest: None.

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Research Article/Araştırma Makalesi

The effect of some fibers and lecithin on phase separation and storage stability of tahini

Bazı liflerin ve lesitinin tahinde faz ayrımı ve depolama stabilitesi üzerine etkisi

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ABSTRACT

The high percentage of oil in the structure of tahini tends to separate from other components during storage. Although oil separation is structurally a natural occurrence, it is undesirable for consumers, leading to the perception of the product as low-quality. Throughout the storage period, a significant portion of the oil accumulates on the surface, and the remaining part below solidifies, making consumption more challenging. The tahini used in the study was brought to the laboratory immediately after production and stored in glass jars with 100 mL each. No additives were added to the sample used as the control group. Three types of additives (sesame fiber, sugar beet fiber, and lecithin) were added to tahini in different proportions (0.5%, 1%, 2%, 3% w/v). The effect of the type of additive, level of addition, and time on preventing the separation of the oil phase from tahini was investigated at different storage times (0th, 30th, 60th, and 90th days). Additionally, its effect on pH, free fatty acidity (FFA), TBA, and conjugated diene values was also studied. According to the results of the analysis of variance, it was determined that the types of additives, levels of addition, and storage time had very significant effects on all parameters ($p<0.01$). According to the results obtained from the study, as the additive levels increased, the amount of oil separated from tahini decreased compared to the control, but no difference was determined between the levels. The main goal of this study is to reduce the percentage of separated oil from tahini, and according to the results of the additives used at different levels, it was determined that the separation of the oil phase was prevented up to 30% compared to the control sample.

Keywords: Tahini, phase separation, fiber, storage stability

ÖZ

Tahinin yapısında bulunan yüksek oranındaki yağ, depolama sırasında diğer yapılardan ayrılmaktadır. Yağ ayrışması yapısal olarak doğal bir olay olmasına rağmen tüketiciler tarafından arzu edilmemekte ve ürün kalitesiz olarak nitelendirilmektedir. Depolama süresi boyunca yağın büyük bir kısmı yüzeyde birikmekte ve altta kalan kısım katılaşarak tüketimi zorlaştırmaktadır. Çalışmada kullanılan sıvı tahin üretimden hemen sonra laboratuvara getirilerek cam kavanozlara 100'er mL dökülerek depolanmıştır. Kontrol grubu olarak kullandığımız örneğin içerisine hiçbir katkı maddesi ilave edilmemiştir. Tahin içerisine üç çeşit katkı (susam lifi, şeker pancarı lifi ve lesitin) ve bu katkılar da farklı oranlarda (% 0.5, % 1, % 2, % 3 (w/v)) ilave edilmiştir. Depolamanın farklı zamanlarında (0., 30., 60. ve 90. gün) katkı çeşidinin, katkı seviyesinin ve zamanın sıvı tahinden yağ fazının ayrılmasının engellenmesi üzerine etkisi araştırılmıştır. Ayrıca pH, serbest yağ asitliği (SYA), TBA ve konjuge dien değerleri üzerine olan etkisi de çalışılmıştır. Varyans analiz sonuçlarına göre bütün parametrelerin üzerine katkı çeşitlerinin, katkı oranlarının ve depolama zamanının

çok önemli etkileri belirlenmiştir ($p<0.01$). Çalışmadan elde edilen sonuçlara göre, katkı seviyeleri arttıkça tahinden ayrılan yağ miktarı kontrole göre azalmış ancak seviyeler arasında fark belirlenmiştir. Çalışmanın en temel amacı sıvı tahinden % ayrılan yağ miktarını azaltmak olup farklı seviyelerde kullanılan katkıların sonuçlarına göre, yağ fazın yapıdan ayrılmasının kontrol örneğine göre % 30 düzeylerine kadar engellediği belirlenmiştir.

Anahtar Kelimeler: Tahin, faz ayrılması, lif, depolama stabilitesi

Introduction

Tahini production from sesame involves several stages, including hulling, drying, roasting, and grinding. By taking advantage of the density difference with salty water, sesame husks are separated, leaving sesame kernels behind. The sesame kernels are then washed to remove the salt, roasted, and sieved before being ground. The ground and roasted sesame kernels constitute the final product, tahini. Throughout the production stages from sesame to the final product, tahini, changes occur in the physical, chemical, and antioxidant properties (Özcan, 1993; Güven et al., 2007).

Sesame seeds undergo a separation process in tahini production, where approximately 15-18% of the seed is separated as husk and bran. The remaining portion is ground to produce tahini. Thus, from 100 kg of sesame seeds, around 82-85 kg of tahini can be obtained (Görgüç, 2018; Yüzer and Gençcelep, 2023). During tahini production, the hulling process leads to a loss of mineral content, particularly calcium. The husk and bran portion has a high level of oxalic acid (> 3%), resulting in an acrid taste due to its calcium and selenium content. Additionally, it forms complexes with metals such as phthalates (present at 1.44% in dry matter) and phytic acid (> 2%), including zinc, iron, and calcium. As a result, these minerals cannot be absorbed in the digestive system. For these reasons, sesame seeds used in tahini production are stripped of their husk (Lokumcu, 2000; Bandyopadhyay et al., 2008; Liu and Chiang, 2008; Cano-Medina et al., 2011).

Tahini is considered a valuable food due to its composition, containing approximately 60% fat, 26% protein, and rich B-complex vitamins, including high-quality protein (such as the essential amino acid methionine). Its raw material, sesame, contributes to its richness in minerals such as calcium, magnesium, iron, zinc, phosphorus, and dietary fiber. Additionally, sesame contains compounds like sesamin and sesamolin, which belong to the lignan group of plant-derived compounds with a polyphenolic structure, making them powerful antioxidants (Özcan, 1993; Karakahya and Yılmaz, 2006; Batu and Elyıldırım, 2009). Sesame, with its phytosterol content resembling the chemical structure of cholesterol, is also rich in phytosterols. It is known that phytosterols obtained through the diet can lower blood cholesterol levels, strengthen the immune system, and reduce the risk of cancer (Güven et al., 2007).

Tahini, high levels of protein and fat, forms a colloidal structure. Essentially, tahini consists of sesame oil and hydrophilic solids dispersed within this oil, creating a suspension. Suspensions are heterogeneous systems with two phases. The external phase (continuous phase) can be a liquid or a semi-solid. The internal phase (dispersed phase) is composed of solid particles that are insoluble in the external phase. The sizes of droplets in the dispersed phase are crucial and typically range from 1 to 10 microns. A dispersion containing droplets below 0.1 micron in size is referred to as a colloidal solution. The small size of particles, especially below 0.1 micron, contributes to the continuous stability of the dispersion, aided by molecular movements in the continuous phase. The merging of droplets in the dispersed phase is also hindered by the increasing viscosity of the liquid in the continuous phase. The close densities of the liquids further reduce the likelihood of gravitational separation. Stabil suspensions ensure the homogeneity of a mixture by keeping the dispersed droplets in the continuous suspension. The tendency of droplets in the continuous phase to merge and coalesce (coalescence, flocculation) contributes to the instability of the suspension. Thickeners and stabilizers are substances that

increase the viscosity of the continuous phase, ensuring the stability of the dispersed phase. Suspension agents are used to slow down settling and increase viscosity. The usage amounts of suspension agents vary between 0.1-10%, depending on the type (Acartürk, 2009).

Tahini, containing 55-60% oil in its structure, undergoes separation from other structures during storage. Although oil separation is a natural occurrence, it is generally undesired by consumers, who may perceive the product as spoiled or of low quality. Throughout the storage period, a significant portion of the oil tends to separate, leaving a solidified bottom layer that makes consumption challenging. The phase separation of the oil, occurring due to particle sedimentation and density differences, is the most prominent characteristic of tahini during prolonged storage (Al-Mahasneh et al., 2017; Evlogimenou et al., 2017; Yüzer and Gençcelep, 2024). Despite its chemical resistance to spoilage reactions, the main challenge during tahini storage is colloidal instability (Çiftçi et al., 2008).

Tahins' storage stability is a primary concern for both producers and consumers. Firstly, during storage, particles in tahini tend to settle, leading to oil separation and sediment formation, adversely affecting consumer acceptability. Secondly, lipid oxidation is one of the most common issues that can develop during storage, resulting in bitterness and an unpleasant taste (Hou et al., 2020). The pH value of the sugar fibers was chosen because it was close to the pH value of tahini. Because, otherwise, the structural differences that may occur due to the acidity difference of the additive added could change the results. Lecithin is one of the most important emulsifiers used in foods. We added it because we thought that oil separation could be reduced by adding an emulsifier. In this study, the aim is to prevent or minimize the phase separation of oil that occurs during the room temperature storage of tahini. This is achieved by increasing the viscosity of the suspension through the addition of certain fibers to hinder the separation of oil from the structure. Additionally, lecithin is introduced to act as an emulsifier, and various changes occurring in the product during this period are examined.

Material and Method

Material

In this study, tahini, sugar beet fiber, sesame fiber, and soy lecithin were used as materials. Tahini was immediately obtained from PROGIDA/SAMSUN, a company based in Samsun, following its production, and it was brought to the laboratory to commence the study. Sugar beet fiber (SBF, Fibrex 600) was purchased from Nordic Sugar in Denmark. Soy lecithin (powdered soy lecithin, Tito Gıda, ISTANBUL) was procured by purchasing it. Sesame fiber was obtained by taking the residue of tahini waste, purchased as bran from PROGIDA, washing it repeatedly with water, and then drying the remaining part in an oven (40°C).

Method

Tahini preparation processes

After receiving freshly produced tahini at the factory, it was transported to the laboratory and poured into glass jars in 100 ml increments. The sample used as the control group had no additives. Additives were introduced into the jars after the samples were placed (% 0.5, % 1, % 2, and % 3 ratios w/v) and mixed at 1000 rpm for 5 minutes using an ultra turrax (IKA Werk Tp 18-10, UpM, Staufen, Germany). Subsequently, the samples were kept at room temperature, and targeted analyses were conducted at specified intervals (0th, 30th, 60th, and 90th days).

Composition analyses

The dry matter content of tahini samples was determined using the drying method. The protein content of the samples was determined based on the Kjeldahl method. The fat content of the samples was determined using the Soxhlet extraction method. The ash content of the samples was determined through incineration (Anonymous, 2000). The surface color of tahini samples was determined using the Minolta Chrometer CR-300 (Japanese). The CIE L* (brightness), a* (redness), and b* (yellowness)

values of the samples were obtained from three randomly selected points on the sample surface.

Determination of water holding capacity

Additives' water-holding capacity was determined according to the method reported by Vioque et al. (2000). For water-holding capacity, 0.5 g of additive samples was mixed with 5 ml of distilled water. The prepared solution was left at room temperature and centrifuged at 3000 g for 30 minutes. The difference between the initially added volume of distilled water and the supernatant volume for the additive samples was determined, and the results were calculated as mL of absorbed water per gram of additive.

Determination of oil holding capacity

The oil-holding capacity of additives was measured according to the method reported by Vioque et al. (2000). For oil-holding capacity, 0.5 g of additive samples was mixed with 5 ml of corn oil for 30 minutes, and then centrifuged at 3000 g for 30 minutes. The volume of separated oil from the additives was measured, and the results were calculated as mL of absorbed oil per gram of additive.

Determination of inflatable capacity

Water binding/swelling capacity analysis was determined according to the method applied by Lecumberri et al. (2007). Initially, 1 g of additive (M) was placed in a graduated cylinder, and its volume (V1) was measured. Then, 10 ml of distilled water was added, and the mixture was shaken until a homogeneous dispersion was formed. The obtained dispersion was left at room temperature (25°C) for 24 hours to allow the powder to fully absorb the water. After 24 hours, the volume of the swollen additive (V2) was measured and recorded. The water absorption capacity (WAC) (ml g-1) was calculated using the formula WAC=(V2-V1)/M.

Water solubility ındex (WSI)

The analysis of water solubility index was conducted according to the method described by Nadeem et al. (2011). A 1% aqueous solution of contributions was prepared and agitated at a constant speed for 1 hour in a shaking water bath (Nüve, Istanbul). The study was conducted at room temperature. The obtained mixture was centrifuged at 3000 g for 10 minutes. The accumulated supernatant on the surface was collected in a Petri dish, and the samples were dried at 105℃ for 18 hours and weighed (S3). The water solubility index (WSI) (%) was calculated using the formula S3/S1×100, where (S1) represents the sample amount.

pH value

Samples were diluted with distilled water at a ratio of 1:10 and homogenized, after which the pH values were measured using a pH meter (Starter 2100, OHAUS). The pH meter was calibrated with buffer solutions of pH 4.00 and 7.00 before conducting the measurements.

Determination of the separated oil ratio

For determining the amount of separated fat in stored jars (100 g), the accumulated fat on the surface was drawn with a syringe, weighed (in grams), and recorded. The weighed amount of fat was then calculated as a percentage relative to the weight of the total tahini.

Free fatty acid (FFA) analysis

The analysis of free fatty acids was determined according to the method of Nas et al. (2001). This analysis was performed on the separated fat that emerged on the surface of the tahini. Five grams of the separated fat from 250 mL of stirred tahini were weighed, and then 50 mL of diethyl ether:ethanol (1:1, v/v) mixture was added. The mixture was shaken for 1 minute to dissolve the fat and fatty acids. Next, 3-4 drops of phenolphthalein were added, and titration was carried out with 0.1 N NaOH in a burette until a permanent light pink color was obtained (at least 15 seconds). The volume of NaOH consumed was recorded. The percentage of free fatty acids was calculated in terms of oleic acid.

Determination of thiobarbituric acid reactive substance (TBARS)

For the determination of TBARS number, 10 g of tahini sample was weighed into a beaker, and then 25 mL of 20% trichloroacetic acid (TCA) and 20 mL of distilled water were added. The mixture was homogenized for 2 minutes using an Ultra Turrax (10,000 rpm). The resulting mixture was filtered through Whatman No:1 filter paper, and 5 mL of the filtrate was transferred to screw-capped tubes. Then, 5 mL of 0.02 M TBA (2-thiobarbituric acid) solution was added, the cap was closed, and the tubes were shaken. After shaking, the tubes were kept in a boiling water bath at 93°C for 30-35 minutes, then cooled for 10 minutes in tap water, and the absorbance value against a blank at 532 nm wavelength was read in a spectrophotometer. The read absorbance values were multiplied by a factor of 7.8 to determine the TBARS number as mg malondialdehyde (MDA) per kg of the sample, following the method described by Lemon (1975).

Determination of conjugated dienes

The conjugated diene numbers of tahini samples were determined according to Juntachote et al. (2007). For this purpose, 3 g of tahini sample was mixed with 30 mL distilled water to create a solution. Then, 0.5 mL of this mixture was taken and mixed with 5 mL hexane: isopropanol (3:1) and centrifuged at 2000 g for 5 minutes. After centrifugation, the absorbance of the upper phase at 233 nm wavelength was measured. The read absorbance was expressed as the conjugated diene value (Juntachote et al., 2007).

Statistical Analysis

The experiments were set up and conducted as two replicates according to a completely randomized experimental design. Some analyses were only performed on fresh products, and the storage factor was not considered in these analyses. Research data were subjected to analysis of variance using a statistical software package, and sources of variation deemed statistically significant were compared with the Duncan multiple comparison test (SPSS, 2020).

Results and Discussion

The results of the composition analyses of tahini and additives used in the study are presented in Table 1 and 2.

Table 1. Tahini composition analysis results (Mean ± standard deviation)

Properties	*Results*
(%) Moisture)	0.14±0.028
(%) Dry matter	99.86±0.028
(%) Oil	51.14±0.89
(%) Protein	24.24±1.28
(%) Ash	2.69±0.026
TBARS Values (*malondialdehyde/Kg)*	5.748±1.32
Conjugated diene	0.765±0.030
pH	5.63±0.084
*L**	27.37±0.001
*a**	1.74±0.002
*b**	9.38±0.004

According to the Tahini Regulation, tahini should have a maximum of 3.2% ash, a minimum of 50% mass fraction of sesame oil, at least 20% protein, a maximum of 1.5% moisture, a maximum of 2.4% acidity (as oleic acid), and a negative value for bitterness (Kreis); additionally, it should not contain foreign substances except for starch (Anonymous, 2015). For example, the obtained tahini in this study has a mass fraction of oil at 51.14%, protein content at 24.24%, dry matter at 99.86%, and ash at 2.69%. Upon examining the data, it is observed that the tahini acquired for the study complies with the regulations. Numerous studies have indicated that tahini typically contains 50-60% fat, 16-28% high-value protein, and B vitamins (Lokumcu Altay and Ak, 2005; Akbulut and Çoklar, 2008; Çiftçi et al., 2008; Hou, 2017; Hou et al., 2018; Tounsi et al., 2019; Yüzer and

Gençcelep, 2024).

In numerous studies, it is stated that sesame seeds have an average protein content ranging from 18% to 25%, while tahini can have protein content reaching up to the 28% range. The protein content of tahini in this study was found to be 24.7%, and these results are consistent with the mentioned values. According to the Tahini Regulation (Anonymous, 2015), the ash content should not exceed 3.2%. In our study, the ash content was determined to be 2.69%, which is in compliance with the values specified in the tahini regulation. Various studies have reported ash content in tahini ranging from 3.00% to 4.05% (Sawaya et al., 1985; Kömez, 2002). Factors such as incomplete hull removal during tahini production, insufficient washing to remove the salt used in hull separation, excessive water use during sesame cultivation, and the variety of sesame can contribute to an increase in ash content in the final tahini product, along with potential adverse effects during processing steps (Özcan, 1993; Güneşer, 2009).

Lokumcu Altay and Ak (2005) found the moisture, protein, fat, and ash contents of tahini to be 0.63%, 26%, 58.8%, and 2.55%, respectively. Akbulut and Çoklar (2008) determined the moisture content of tahini to be 1.86%, protein content at 23.77%, fat content at 55.42%, fiber content at 3.11%, and ash content at 2.78%. Hou (2017) reported that tahini contains 0.12% moisture, 59.71% crude fat, 17% protein, 5.01% ash, 3.78% crude fiber, and 7.70% total carbohydrates. Hou et al. (2018) found that the moisture content of tahini ranged from 0.12% to 1.10%, fat content from 51.80% to 61.56%, protein content from 16.08% to 20.10%, crude fiber content from 2.53% to 3.78%, total ash content from 4.48% to 5.70%, and total carbohydrate content ranged from 6.23% to 18.57%.

In a study on the color values of imported tahini, Kömez (2002) found the a* value to be in the range of 3.50-11.19 and the b* value to be in the range of 23.44-32.04. For domestic tahini, the color values were found to be a* in the range of 3.57-9.42 and b* in the range of 22.53-31.19. Karaman et al. (2017) reported a* and b* values for tahini as 3.12 and 13.88, respectively. When comparing the results obtained in this study, it is observed that the a* value of the tahini used in our study is lower, and the b* value is higher. This difference is presumed to be related to the variety of sesame used.

Tahini is a food substance rich in oil, one of its main components. Tahini is particularly rich in oleic fatty acid, and the second-highest fatty acid present is linoleic acid. Considering the fatty acid composition of the product, it can be said that it contains a high proportion of unsaturated fatty acids. Consequently, the product is considered to be highly susceptible to oxidation. In this regard, the determined TBARS and conjugated diene values in tahini were found to be high.

Three different additives and four different ratios were used in the study, and the physical properties of the additives used in the study are provided in Table 2. The results of the analyses conducted on these additives are believed to be beneficial in interpreting the prevention of oil separation in tahini during storage. Knowing some properties of the additives used in the product is also important in determining the changes they will induce in the product's structure.

In such studies, it is crucial that the additives used do not cause significant differences in certain physical, chemical, and sensory properties of the product. Tahini has a very low water content (Table 1), making it an oil-based product. To prevent possible oil separation, lecithin has been added to the formulation as an emulsifier and stabilizer.

Table 2. Composition analysis results of additives (Mean ± standard deviation)

Properties	*Sugar beet fiber*	*Sesame fiber*	*Lecithin*
(%) Moisture	6.19±0.38	2.64±0.07	2.35±0.01
(%) Ash	5.52±0.01	12.04±0.04	11.13±0.06
(%) Swelling capacity	3.43±0.11	2.46±0.11	0.50±0.00
(%) Water holding capacity	5.37±0.19	6.94±0.21	2.70±0.33
(%) Oil holding capacity	2.88±0.26	3.82±0.25	2.19±0.49
Water solubility index	16.20±1.04	6.16±2.15	43.60±1.15
pH	4.86±0.01	7.38±0.01	7.48±0.02
*L**	74.02±0.95	45.40±1.04	80.63±2.29
*a**	3.08±0.12	4.93±0.14	4.34±0.32
*b**	26.81±0.32	19.09±0.17	40.96±0.95
Dimension	<250µm	<250µm	----

Lesitin has been recognized as "GRAS" (generally recognized as safe) by the Food and Agriculture Organization (FAO), meaning there are no restrictions on the amount of lecithin that can be used in food (Garti, 2001). Lecithins are multifunctional products with broad applications at low levels (0.5-3%), often performing multiple functions in a food product. Their primary function is emulsification, which involves holding two different liquids together to form emulsions of oil in water or water in oil. With their amphoteric properties, lecithins play an indispensable role in food systems (Hui, 1992; Garti, 2001).

Dietary fibers are classified into two groups based on their solubility: soluble and insoluble fibers. Soluble dietary fiber forms a gel and a dense structure by binding water. Insoluble dietary fiber, on the other hand, can absorb up to 20 times its weight in water but does not form a viscous structure (Thebaudin et al., 1997). The nutritional fiber content of sugar beet pulp is 67% insoluble and 21% soluble in dry matter, making up a total of 88%. Insoluble fibers can retain up to 5 times their weight in fat, a feature crucial for preserving lost fat during food processing, as commonly occurs in the production of food items. This characteristic is significant for enhancing the technological properties of food. The high fat-binding capacity of dietary fiber is important for stabilizing fat and water emulsions (Grigelmo-Miguel et al., 1999). It has been determined that the fat-binding capacity of dietary fiber varies with particle size, with larger particles binding more fat. Wheat bran and sugar beet fiber, characterized by their large particles, are noted for their high fat-binding capacities (Thebaudin et al., 1997).

Sesame meal is obtained at the end of sesame oil production. Sesame meal typically contains approximately 40% crude protein and 24% mineral substances (P, K, Ca), as reported by Uğurluay (2002) and Sreedevi and Sivasankar (2009). The main disadvantage of using sesame meal as a food additive is its very low solubility in water, but this drawback can be overcome by modifying the protein (Escamilla-Silva et al., 2003; Radha et al., 2008). Solubility and swelling properties are interconnected. The initial solubility of polysaccharides is associated with swelling. Water moves towards the solid structure, and macromolecules swell until they are completely dispersed, expressing solubility. In contrast, some polysaccharides like cellulose cannot disperse due to their structural characteristics (Thebaudin et al., 1997).

The characteristics of the additives used in the study should be examined to ensure that they do not cause changes in the moisture and ash values of tahini. This is crucial because these values are regulated by regulations. Additionally, the additives should be soluble in the suspension and exhibit good fat-binding properties. It is important that the color values, which significantly influence consumers' purchasing decisions and preferences, do not undergo substantial changes due to the additives.

Protein interactions with water and/or fat are crucial in food systems as they influence properties such as taste and texture. A critical range for water-holding capacity values for viscous foods like soups and sauces, determined by Aletor et al. (2002), is between 1.49 and 4.72 (g/g). Therefore, sesame meal protein hydrolysates, due to their

high water-holding capacity, find applications in the food industry to prevent water loss in bread and cakes, as well as to enhance the utilization of cooked and frozen foods (Vioque et al., 2000; Onsaard et al., 2010). The water-holding capacity of sesame meal hydrolysate has been found to be 3.43 g water/g protein, and the fat-holding capacity is 2.21 ml fat/g protein. Upon examining fat absorption and water-holding capacity, it has been determined that sesame protein exhibits low fat absorption and high water-holding capacity (Demirhan Yılmaz, 2012).

The most crucial feature influencing consumer preferences and decisions is color, and it is one of the essential appearance characteristics of food items (Maskan, 2001). Color is a parameter used in the process control during roasting because as browning and caramelization reactions progress, brown pigments increase (Moss and Otten, 1989).

At the beginning of storage (day 0), after the addition of additives to tahini, the color values for untreated tahini were determined as follows: L* (brightness) value was 27.37, a* (redness) value was 1.74, and b* (yellowness) value was 9.38. In tahini with sugar beet fiber additives, there was no significant change in L* (brightness) and a* (redness) values compared to untreated tahini. However, it was observed that the b* (yellowness) value decreased as the amount of fiber added increased, mainly due to the high b* value of sugar beet fiber (26.81). In tahini with sesame fiber additives, there was no significant change in L* values, while there was a slight decrease in both a* and b* values, especially as the level of additives increased. In tahini with lecithin additives, there was no significant change in L*, a*, and b* values, as the levels of additives were introduced at percentages such as 0.5%, 1%, 2%, and 3%. In general, regardless of the type of additive, the study concluded that the additives used did not cause an excessive color change in tahini, making them suitable for use.

Table 3. Additive addition to tahini, addition rate, storage analysis and analysis results of interactions (Mean ± standard deviation)

	pH	*% Amount of separated oil*	*FFA (% oleic acid)*	*TBA Values* malondialdehyde MA/kg	*Conjugated diene*
Additives (K)					
Sugar beet fiber	5.37 c	4.51 b	0.32 b	7.75 a	0.89 c
Sesame fiber	5.92 b	4.78 a	0.34 a	7.36 c	1.10 b
Lecithin	6.04 a	4.46 b	0.29 c	7.65 b	1.23 a
P<0.01	**	**	**	**	**
Level (S)					
0.0	5.69 c	5.54 a	0.26 d	7.02 e	1.25 a
0.5	5.76 b	4.19 b	0.30 c	7.50 d	0.89 e
1.0	5.80 a	4.42 b	0.32 b	7.61 c	0.93 d
2.0	5.82 a	4.30 b	0.33 a	7.80 b	1.09 c
3.0	5.80 a	4.48 b	0.36 a	8.02 a	1.21 b
P<0.01	**	**	**	**	**
Storage (days) (G)					
0	5.71 d	--	--	5.74 d	0.76 b
30	5.78 b	2.73 c	0.55 a	7.79 c	1.18 a
60	5.75 c	6.49 b	0.35 c	8.05 b	1.18 a
90	5.85 a	9.12 a	0.38 b	8.78 a	1.18 a
P<0.01	**	**	**	**	**
KxS	**	**	**	**	**
KxG	**	**	**	**	**
SxG	**	**	**	**	**
KxSxG	**	**	**	*	**

**P<0.01: There is a very significant difference, *P<0.05: There is a significant difference
a-d: Numbers followed by different letters in a column are significantly different (P<0.05)

The increase in free fatty acidity and pH changes in tahini can occur as a result of microbiological or enzymatic reactions. Osmophilic yeasts are microorganisms capable of producing acid by

utilizing the sugar in the product. However, due to the low water content in the product, the probability of microbiological developments is low, leading to the consideration that this change may be related to enzymatic reactions. In a study conducted by Gamli and Hayoglu (2007) on peanut butter, it was reported that the total acidity value increased with temperature and duration for products stored at 4°C and 20°C, while the pH decreased. In their study, they suggested that the increase in acidity could be attributed to microbiological or enzymatic reactions (Gamli and Hayoglu, 2007).

The addition of sugar beet has led to a decrease in the pH value of tahini. The reason for this is that the pH value of the added additive (4.86, Table 2) is lower than the pH value of tahini. On the other hand, the addition of other additives (sesame fiber and lecithin) resulted in an increase in pH value. This is because the pH values of these additives (7.38 and 7.48, Table 2) are higher than the pH value of tahini. It has been observed that there is not much research on the pH of sesame and its products. As the ratio of added additives to tahini increased, a general increase in pH value was observed, but there was no increase at levels of 1%, 2%, and 3%. It is believed that this difference is the result of the difference in the pH values of the added additives (Table 2). The pH value of sugar beet, sesame fiber, and lecithin (4.86, 7.38, and 7.48, respectively) has resulted in a balance as the levels increase, and the increase has not been statistically determined as different.

In a study conducted by Al-Nabulsi et al. (2014), the pH of tahini was found to be 6.76. In another study on mixtures of tahini, honey, and grape molasses by Karaman et al. (2017), the pH of tahini was determined to be 6.50. The values determined for tahini in this study are lower than those determined in previous studies. It is believed that this difference is attributed to factors such as variety, type, processing method, processing temperature, and compositional variations. Upon examining Table 3, an increase in pH values is observed with the increase in storage time. This increase can be attributed to the breakdown of fatty acids present in the environment, the decomposition of proteins influenced by environmental conditions, and the breakdown of carbohydrates, among other effects.

Tahini has a colloidal structure containing high levels of protein and fat. It is essentially a suspension consisting of sesame oil and hydrophilic solids dispersed in this oil. The fat, constituting about 55-60% of tahini's structure, separates from other structures during storage. One of the most distinctive physical characteristics of tahini is the phase separation of the oil and particle sedimentation due to density differences, which occurs after prolonged storage (Evlogimenou et al., 2017).

One of the physical problems encountered in high-fat foods is fat separation. Fat separation also forms the basis for chemical deterioration because the exposed oil comes into more contact with oxygen and undergoes oxidation more rapidly. The separation of the fat phase relies on the different densities of the components in the product. When the product contains components with both water and fat phases, the use of emulsifiers such as lecithin or stabilizers becomes crucial, and effective mixing processes can also prevent this issue (Muego-Gnanasekharan and Resurreccion, 1992).

As seen in Table 3, lower levels of accumulated surface oil were observed in tahini with added additives at varying ratios compared to the control. However, as the ratio increased, there was no significant change in the amount of separated oil. During storage, oil separation increased, and these values continued to double at the measured time intervals.

Free fatty acidity is a result of the hydrolysis of fat from triglyceride structures due to various factors. While known antioxidants can prevent disruptions caused by oxidative rancidity and oxy-polymerization events, they are not effective for hydrolysis and reversion (Çakmakçı and Gökalp, 1992). Changes in free fatty acidity during storage provide information about the degree of bitterness in the product. It helps determine how far the hydrolysis mechanism has progressed

(Hamilton, 1989).

Evlogimenou et al. (2017) have predicted that oil-rich raw materials' aqueous extraction residues can be included to enhance the stability of tahini against oil separation and particle sedimentation. In the current research, three aqueous extraction residues obtained from hazelnut, corn seed, and sesame seed were converted into powder form and compared as effective physical stabilizers for tahini. As a result, when these powders were included in tahini and stored for an extended period, they increased the stability of tahini against oil separation up to a certain level. The improvement in stability against oil release was reported to be associated with an increase in the number of solid particle interactions within the tahini structure and the strength of these interactions.

In a study investigating the effect of temperature and particle size on tahini to enhance storage durability and address the issue of oil separation, it was reported that in tahinis stored at 20 °C, the oil separation increased as the particle size decreased. However, at a temperature of 30 °C, the storage durability became independent of particle size (Çiftçi et al., 2008). In the same study, statistical analyses revealed that tahini's storage stability was dependent on both temperature and particle size, but the researchers noted that the influence of temperature on stability was more significant than that of particle size. The researchers determined a critical particle size of 5 µm for tahini at all three storage temperature levels (20 °C, 30 °C, and 40 °C).

In concentrated suspensions, the particle size distribution is crucial. In a conducted study, it was determined that 76% of tahini consists of particles smaller than 10 µm, while 14% of the particles are in the size range of 100-500 µm. In the context of unwanted oil separation in tahini, it has been reported that the fraction with smaller particle size is more effective (Lindner and Kinsella, 1991). Although tahini is resistant to chemical degradation reactions in terms of shelf life, colloidal instability during storage is the main problem (Çiftçi et al., 2008).

In their research to prevent phase separation in tahini using natural waxes, Ögütcü et al. (2018) added sunflower (%1 and %3) and beeswax (1.3 and %5) to commercially obtained tahini at specific concentrations. Samples were stored at 25 and 35°C for 21 days. Centrifugation stability, oil leakage, textural properties, viscosity, and consumer tests were analyzed. Samples with added beeswax and sunflower wax exhibited lower oil leakage values compared to plain tahini (control). Viscosity measurement showed that control and samples with added beeswax (%1 and %3) exhibited pseudo-plastic rheological behavior. Textural measurements indicated that tahini prepared with %3 sunflower wax was denser and more adhesive than tahini prepared with %1 sunflower wax and %5 beeswax. Additionally, the textural properties of the samples were significantly influenced by storage temperatures. Furthermore, samples with %1 and %3 sunflower wax and %5 beeswax could be spreadable, while those with %1 and %3 beeswax added became fluid. Consequently, the addition of sunflower and beeswax not only restricted phase separation in tahini but also transformed it into a spreadable form depending on the beeswax concentration.

In the research conducted by Yüzer and Gençcelep (2024), additives were added in order to prevent or minimize oil phase separation, which occurs in sesame paste stored at room temperature and is not desired by consumers. As additives, nanofibers containing sesame proteins produced by the electrospinning method and sesame protein isolates (SPIs) were added. Added additives acted by preventing the separation of the oil phase from the structure, and some changes in the product during storage (0, 30, 60, and 90 days) were investigated. The values obtained showed that the additives reduced oil separation up to 63% compared to the control group. As a result of the study, it was determined that the separation of the oil phase from the structure could be prevented up to 24.73% with the addition of SPI and up to 63.02% with the addition of sesame protein nanofiber (SPINL) compared to the control groups. It has been determined that the addition of SPI to

sesame pastes does not completely prevent the problem of oil separation in sesame paste, but is effective in delaying and reducing it.

According to the results of free fatty acidity analyzed by extracting the accumulated oil on the surface of tahini with the help of an injector (Table 3), the amount of free fatty acids (FFA) increased as the additive levels increased. The analysis results on the 30th day, as shown in Table 3, were higher compared to the 60th and 90th days. This phenomenon can be explained by the further breakdown of fatty acids into advanced decomposition products. In the same samples, the increase in TBARS (Thiobarbituric Acid Reactive Substances) and conjugated diene results on the 30th day (Table 3) was proportionally higher compared to other days.

One of the oldest and most widely used methods for determining lipid oxidation in foods and other biological systems is the 2-thiobarbituric acid (TBA) test. Malondialdehyde is a minor product in the oxidation of polyunsaturated fatty acids (Logani and Davies, 1979; Shahidi et al., 2002).

Upon examining Table 3, it can be observed that the TBARS levels increased with the increasing additive levels. However, it can be noted that these increases were relatively low at low levels of additives. Additionally, for all types of additives at the same level, an increase in TBARS values was observed as the storage time increased. The results suggest that, over time, in addition to oil separation, auto-oxidation within tahini may also increase.

The delay of oxidation is crucial for everyone involved in the food chain, from food producers to consumers. Various techniques can be employed to prevent oxidation, including preventing food contact with oxygen, applying low-temperature processes, inactivating enzymes catalyzing oxidation, reducing oxygen pressure, and using appropriate packaging. The most effective way to protect foods against oxidation is the use of substances with antioxidant properties (Pokorny et al., 2001).

Under normal conditions, lipids are not in radical form but can form radical forms due to the influence of heat, metals, or light (Choe and Min, 2006). The oxidative breakdown of lipids in foods results in the formation of potentially toxic secondary substances, leading to a loss of nutritional quality, rancid odor, and flavor (Choe and Min, 2006). The average conjugated diene values of stored tahini with different levels of additives are provided in Table 3. As shown in Table 3, an increase in both level and storage time led to an increase in conjugated diene values, except for the unsupplemented tahini group (level 0), similar to the TBARS results. It is anticipated that hydroperoxides formed from fatty acids play a role in the gradual conjugation formation and the increase in conjugated diene values. The amount of oil separated increased during the storage period. TBA values increased during the storage period, but the Conjugated diene values did not change after the 30th day.

In conclusion, it was observed that the three types of additives used reduced the separated fat phase from the structure by an average of 25-30%. Moreover, especially for reducing fat separation, it is crucial for the selected additives to mix better with the product and increase phase viscosity, so the particle size should be lower. The presence of protein-based additives in the content of the additives is also essential for holding the fat in the structure and thus preventing flocculation.

Attachments

This study is derived from the master's thesis prepared by Ercan YETKİN and accepted by the Graduate School of Ondokuz Mayıs University.

Conflict of Interest Statement

The authors declare no conflict of interest.

Author contributions

Ercan YETKİN: Investigation, Methodology, Writing original draft, Review & editing, Hüseyin GENÇCELEP: Review & editing, Supervision, Resources.

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Research Article/Araştırma Makalesi

Assessment of in vitro cytotoxicity, anti-Alzheimer, and antidiabetic properties of fenugreek, white mulberry, and nettle leaves

Çemen otu, beyaz dut ve ısırgan otu yapraklarının in vitro sitotoksik, anti-Alzheimer ve antidiabetik özelliklerinin değerlendirilmesi

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ABSTRACT

Leafy plants are known for their rich bioactive profiles and have gained attention for their potential health benefits. This study evaluated the total phenolic content (TPC) using the Folin-Ciocalteu method and antioxidant properties, including ferric reducing antioxidant power (FRAP) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) activities, of ethanolic extracts from fenugreek (FL), white mulberry (WBL), and nettle leaves (NL). It also investigated their inhibitory effects on alpha-amylase, alpha-glucosidase, acetylcholinesterase, and butyrylcholinesterase, and assessed their cytotoxicity on human embryonic kidney cells (HEK-293) and colorectal adenocarcinoma cells (CaCo-2) using MTT assays. The results revealed that the TPC was highest in NL (241.86 mg gallic acid equivalents (GAE) g^{-1} dry weight (DW)), followed by WBL (165.68 mg GAE g^{-1} DW) and FL (72.09 mg GAE g^{-1} DW), with NL also showing the highest FRAP (240.48 µmol Fe^{2+} g^{-1} extract) and ABTS antioxidant activities (19.26 mg trolox equivalents (TE) g^{-1} extract). Moreover, the inhibition of alpha-amylase ranged from 8.85% to 90.39% depending on the extract concentration (62.5–500 µg mL^{-1}), with WBL and NL showing significant inhibitory effects on alpha-glucosidase within the same concentration range. Additionally, NL ethanolic extracts exhibited the highest butyrylcholinesterase inhibitory activity at 38.40% compared to FL (33.87%) and WBL (17.94%) at 2 mg mL^{-1}, while acetylcholinesterase inhibition rates ranged from 23.14% for WBL to 53.35% for NL across all leaf samples. Furthermore, the ethanol extracts from FL, WBL, and NL yielded IC_{50} values of 1159.98, 1235.67, and 972.22 µg mL^{-1}, respectively, on HEK-293 cells, while on CaCo-2 cells, the IC_{50} values were 897.41 µg mL^{-1} for FL, 754.11 µg mL^{-1} for WBL, and 648.80 µg mL^{-1} for NL. These findings underscore the potential of NL, FL, and WBL as valuable natural sources with diverse health benefits and significant therapeutic potential, making them promising candidates for industrial applications as functional ingredients.

Key Words: Fenugreek leaves, mulberry leaves, nettle leaves, cytotoxic activity, enzyme inhibition activity

ÖZ

Yapraklı bitkiler, zengin biyoaktif bileşenler barındırmalarıyla dikkat çeker ve bu bileşiklerin sağlık üzerindeki olumlu etkileri, son yıllarda giderek artan bir ilgiyle

araştırılmalarına yol açmıştır. Bu çalışma, çemen otu (FL), beyaz dut (WBL) ve ısırgan otu yapraklarının (NL) etanol ekstraktlarının toplam fenolik içeriğini (TPC) ve demir indirgeme antioksidan gücü (FRAP) ile 2,2'azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) serbest radikali giderme aktivitelerini içeren antioksidan özelliklerini değerlendirmiştir. Ayrıca, bu ekstraktların alfa-amilaz, alfa-glukozidaz, asetilkolinesteraz ve bütirilkolinesteraz üzerindeki inhibe edici etkileri de araştırılmış ve insan embriyonik böbrek hücreleri (HEK-293) ve kolorektal adenokarsinom (Caco-2) hücre hatlarındaki sitotoksisiteleri değerlendirilmiştir. Sonuçlar, TPC'nin NL'de (241.86 mg GAE g^{-1} DW) en yüksek olduğunu, bunu WBL (165.68 mg GAE g^{-1} DW) ve FL'nin (72.09 mg GAE g^{-1} DW) takip ettiğini ortaya koymuştur. NL ayrıca WBL ve FL'ye kıyasla en yüksek FRAP (240.48 µmol Fe^{2+} g^{-1} ekstrakt) ve ABTS antioksidan aktivitelerini (19.26 mg TE g^{-1} ekstrakt) göstermiştir. Ayrıca, alfa-amilaz inhibisyonu, ekstrakt konsantrasyonuna (62.5–500 µg mL^{-1}) bağlı olarak %8.85 ile %90.39 arasında değişmiş, WBL ve NL aynı konsantrasyon aralığında alfa-glukozidaz üzerinde önemli inhibe edici etkiler göstermiştir. NL etanol ekstraktları, 2 mg mL^{-1} konsantrasyonda FL (%33.87) ve WBL (%17.94) ile karşılaştırıldığında en yüksek bütirilkolinesteraz inhibisyon aktivitesini (%38.40) sergilemiştir. Asetilkolinesteraz inhibisyon oranları ise %23.14 (WBL) - %53.35 (NL) arasında değişmiştir. Ayrıca, FL, WBL ve NL'nin etanol ekstraktlarının IC_{50} değerleri HEK-293 hücrelerinde sırasıyla 1159.98 µg mL^{-1}, 1235.67 µg mL^{-1} ve 972.22 µg mL^{-1} iken, Caco-2 hücrelerinde IC_{50} değerleri FL için 897.41 µg mL^{-1}, WBL için 754.11 µg mL^{-1} ve NL için 648.80 µg mL^{-1} olarak hesaplanmıştır. Bu bulgular, FL, NL ve WBL'nin hem potansiyel sağlık faydalarını hem de dikkate değer terapötik potansiyelini vurgulayarak onları endüstriyel uygulamalar için fonksiyonel bileşen olarak değerlendirilebilecek umut verici doğal kaynaklar haline getirmektedir.

Anahtar Kelimeler: Çemen otu yaprakları, dut yaprakları, ısırgan otu yaprakları, sitotoksik aktivite, enzim inhibitör aktivitesi

Introduction

Herbal remedies, derived from the diverse resources of nature, form a core part of traditional medicine systems in various cultures. For centuries, these natural treatments have been essential in managing health and disease, serving as vital tools for everyday wellness and specialized therapeutic needs across numerous cultures (van Wyk, 2020). Among these valuable remedies are well-known plants such as fenugreek, nettle, and mulberry. For instance, fenugreek, scientifically known as *Trigonella foenum-graecum* L. and belonging to the family Leguminosae, is a medicinal plant traditionally used for both its seeds and leaves. These components are valued not only for their culinary applications but also as crucial ingredients in various traditional medicinal formulations (Verma et al., 2010). Fenugreek has been traditionally used to treat disorders such as high cholesterol, diabetes, gastrointestinal ailments, and wound inflammation. Fenugreek is also acclaimed for its potential anticancer properties, thanks to its advantageous active chemical constituents. Its mechanism of action closely resembles that of various anticancer medications (Alsemari et al., 2014). It contains a variety of active compounds, including saponins (such as protodioscin, dioscin, diosgenin and yamogenin), flavonoids (quercetin, maackiaian, medicarpin, aglycones kaempferol, quercetin, tricin, and narin genin, afroside, luteolin, vitexin, quercitrin, and 7, 4-dimethoxy flavanones), alkaloids (trigonelline), and lignans (secoisolariciresinol) (Nagulapalli et al., 2017; Shadab et al., 2024). Moreover, the genus *Morus* (mulberry) comprises over 150 species, with *Morus alba* L. (white mulberry) standing out as the most significant. Renowned for its powerful therapeutic effects and minimal toxicity, *M. alba* has been extensively utilized in traditional Chinese medicine. Its broad spectrum of health benefits includes antioxidant, antibacterial, antihypertensive, anti-hyperglycemic, neuroprotective, skin tonic, and anti-hyperlipidemic properties (Gryn-Rynko et al., 2016; Zafar et al., 2013). It is abundant in (poly)phenolic compounds, including flavonoids such as quercetin-3-O-glucoside, quercetin-3-O-(6-malonyl)-glucoside, and kaempferol-3-O-(6-malonyl)-glucoside, alongside phenolic acids like caffeic acid and caffeoylquinic acids (Sánchez-Salcedo et al., 2015), as well as flavonols such as rutin, isoquercitrin, and astragalin (Mahboubi, 2019). Furthermore, nettle (*Urtica dioica* L.) is a perennial wild herb from the Urticaceae family, commonly found in temperate regions of Europe, Asia, and America. It typically thrives in untamed

fields, along roadways, on slopes, and in open forested areas, and is distinguished by its light or dark green leaves. Additionally, it demonstrates hypolipidemic, anti-inflammatory, antiviral, antiulcer, antimicrobial, and antioxidant effects, thanks to its active constituents, which include polyphenols, essential amino acids, vitamins (K, B, and C), fatty acids, dietary fibers, carotenes, and terpenes (Kutlu et al., 2020; Kutlu, 2021). It includes hydroxybenzoic acid, gallic acid, quinic acid, syringic acid, vanillic acid, protocatechuic acid, gentisic acid, caffeic acid, and coumaric acid, along with numerous derivatives of quinic and caffeic acids. Additionally, flavonoids such as quercetin, apigenin, catechin, and pelargonidin were detected in both glycosidic and non-glycosidic forms. Furthermore, stinging nettle leaves were found to contain lutein, violaxanthin, neoxanthin, β-carotene, and lycopene (Garcìa et al., 2021; Kregiel et al., 2018). Previous studies have reported on the cytotoxic, anticancer, and enzyme inhibitory activities of fenugreek leaves (Alsemari et al., 2014; Aylanc et al., 2020; Ganeshpurkar et al., 2013; Prithiksha et al., 2022; Ullah et al., 2016; Verma et al., 2010), white mulberry leaves (Chen et al., 2023; Panyatip et al., 2022; Qin et al., 2015), and nettle leaves (Asgharian, 2017; Sharma et al., 2023) were reported in previous studies. However, no research has been concurrently assessed and compared these properties among various plant leaves within a single study. For this reason, the current study sought to (i) evaluate the total phenolic content and antioxidant properties, specifically FRAP and ABTS activities, (ii) investigate enzyme inhibitory activities targeting alpha-amylase, alpha-glucosidase, acetylcholinesterase, and butyrylcholinesterase, and (iii) assess the cytotoxic effects on the human embryonic kidney HEK-293 and human colorectal adenocarcinoma Caco-2 cells, using ethanolic extracts from fenugreek, white mulberry, and nettle leaves.

Materials and Methods

Materials

The plants, specifically *Trigonella foenum graecum* L., *Morus alba* L., and *Urtica dioica* L., were collected in May 2023 from the region located at 39° 20′ 14″ N latitude and 36° 2′ 29″ E longitude in İnkışla, Gemerek district, Sivas province, Türkiye (Figure 1). After collection, the plants were cleaned to remove soil and dust, then dried in a Memmert UF-110 oven (Germany) at 50°C with the fan running at full speed for 18 h. Following drying, the stems and leaves were separated from the roots. The dried plant leaves were ground using a Tefal 8100.31 coffee and spice grinder (France). The ground samples were placed in beakers, sealed with paraffin, and kept in the refrigerator at +4°C for future use.

Figure 1. Images of the leaves used in the study.

Bioactive properties

Extraction

Bioactive extracts were prepared by soaking herb powder samples in absolute ethanol at a ratio of 1:10 (w:v) for 5 h at room temperature, following the method outlined by Kilicli et al. (2023). After extraction, the mixture was centrifuged at 5000 rpm for 5 min at room temperature. The resulting supernatant was then concentrated using a rotary evaporator and

subsequently lyophilized to yield the dry extract.

Total phenolic content (TPC)

TPC of the samples was determined using the method described by Yasar et al. (2022). Specifically, 0.5 mL of extract was combined with 2.5 mL of 0.2 N Folin–Ciocalteu reagent and mixed thoroughly. After 3 min, 2 mL of 7.5% Na_2CO_3 was added to the mixture. Subsequently, the solution was then incubated in the dark at room temperature for half an hour. The absorbance was measured at 760 nm using a UV/VIS spectrophotometer (Shimadzu UV-1800, Japan), and the results were reported as milligrams of gallic acid equivalent (GAE) per gram.

Antioxidant activity assays

ABTS

The ABTS value of leaves was measured using 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid). To perform the analysis, x mL of the sample solution was combined with (4 − x) mL of ethanol, while 4 mL of ethanol alone served as the reagent blank. Subsequently, 1 mL of a 1:10 diluted ABTS radical cation solution was added at 15-sec intervals and mixed thoroughly. The ABTS solution was prepared by mixing a 7 mM ABTS stock solution with 2.45 mM potassium persulfate and allowing the reaction to proceed in the dark at room temperature for 12–16 h. After incubation for 6 min, the absorbance of the mixture was measured using a spectrophotometer (Shimadzu UV-1800, Nippon, Japan) at 734 nm, with ethanol used as the reference. The antioxidant capacity of the tested samples was calculated based on a Trolox calibration curve and recorded as milligrams of Trolox equivalents per g of dry extract (mg TE g^{-1} dry extract) (Erol et al., 2023).

Iron (III) ion reducing antioxidant power (FRAP) assay

To achieve this, 0.1 mL of the herb sample was mixed with 3 mL of freshly prepared FRAP reagent, which consisted of 300 mmol L^{-1} acetate buffer (pH 3.6), 20 mmol L^{-1} ferric chloride, 10 mmol L^{-1} TPTZ, and 25 mmol L^{-1} HCl. The mixture was homogenized using a vortex mixer and incubated at 25°C for 10 min. After incubation, the absorbance of the solution was measured spectrophotometrically at 593 nm using a Shimadzu UV-1800 spectrophotometer (Kutlu, 2024).

Determination of enzyme inhibition activity

To assess the inhibitory activities of the samples against AChE/BChE, an *in vitro* method was utilized following the procedure outlined by Bozkurt et al. (2021). Galantamine was used as positive control, while buffer blanks acted as reference points. The inhibitory effects of the leaf extracts on alpha-glycosidase and alpha-amylase were measured using the approach described by Kutlu (2024), with acarbose serving as the positive control and phosphate buffer as the negative control instead of the sample.

Determination of anticancer activity

CaCo-2 (ATCC, HTB-37) and HEK-293 cell lines (ATCC, CRL-1573) which is a healthy cell line, were sourced from the American Type Culture Collection (Bozkurt et al., 2021). The *in vitro* cytotoxicity assay was carried out with slight adjustments using the MTT method, as described by Kutlu (2024). To assess cytotoxicity, 15 x 10^3 cells per well were seeded in 96-well plates and cultured for 24 h. After removing the culture medium, different concentrations of cell-free filtrate and lyophilized filtrate were added, with noninoculated DMEM F-12 medium serving as a negative control. For CaCo-2 cells, incubation continued for an additional 8 and 24 h. Following this, 100 µL mL^{-1} of MTT solution was added to each well, and after 2 h of incubation at 37°C, 100 µL of dimethyl sulfoxide (DMSO) was used to dissolve the blue crystals. Absorbance was measured at 570 nm using a microplate reader (Bio-Tek, ELX808IU, USA).

Statistical analysis

The study was conducted in triplicate, and quantitative data were presented as mean ± standard deviation. The Shapiro-Wilk W test was employed to evaluate the normality of the data

distribution, where a p-value of ≥ 0.05 was considered indicative of a normal distribution. The results were analyzed using analysis of variance (ANOVA), and the using Tukey's honestly significant difference (HSD) test was employed to assess the significance of differences between the parameters of the tested leaves, with analysis performed using JMP (SAS Institute, Inc., Cary, USA) software.

Results and Discussion

Bioactive properties

Phenolic compounds are crucial for the antioxidant activity of plants, and quantifying their content is vital for assessing their antioxidant potential. These compounds provide multiple benefits, including antioxidant, antimicrobial, and anticancer properties, contributing to the prevention of diabetes, cancer, cardiovascular diseases, and oxidative stress-related conditions (Ghevariya et al., 2023; Hajra & Paul, 2018). In this context, the TPC levels (mg GAE g^{-1} in DW) of various leaf samples were ranked in descending order as follows: NL (241.86) > 165.68 (WBL)> 72.09 (FL) as shown in Table 1.

Table 1. TPC, FRAP and ABTS radical cation scavenging abilities of fenugreek (FL), white mulberry (WBL), and nettle leaves (NL).

Type of leaf	TPC (mg GAE g^{-1} DW)	Antioxidant activities	
		FRAP (µmol Fe^{2+} g^{-1} extract)	ABTS (mg TE g^{-1} extract)
FL	72.09±6.81^{c}	125.66±4.04^{b}	8.51±1.15^{b}
WBL	165.68±22.81^{b}	238.87±2.16^{a}	17.87±2.39^{a}
NL	241.86±20.98^{a}	240.48±4.66^{a}	19.26±2.86^{a}

Statistical differences within the same column are indicated by lower case letters ($^{a-c}$). The differences in TPC and FRAP results were highly significant at the p<0.001 level, while the ABTS activity results demonstrated significance at the p<0.01 level.

A statistically significant difference was found among all samples (p<0.001). In this context, Salam et al. (2023) reported a TPC of 15.27 mg GAE g^{-1} DW in crude extracts from air-dried fenugreek leaves. Similarly, Ghevariya et al. (2023) found the TPC in dried fenugreek leaves to be 53.94 mg GAE/g DW. Moreover, Hafeez et al. (2023) further highlighted that the TPC of different extract fractions (methanol, ethanol, n-hexane, and aqueous) from *T. foenum-graecum* leaves ranged from 5.74 to 6.23 mg GAE 100 g^{-1}. Meanwhile, Wang et al. (2021a) demonstrated that mulberry leaves possess a wide range of phenolic compounds, with their TPC varying between 2.61 and 51.81 mg g^{-1} DW, influenced by cultivar and geographic origin. As well, Iqbal et al. (2012) reported a TPC of 16.21 mg GAE g^{-1} DW in dried mulberry leaves of *M. alba*. Additionally, Shonte et al. (2020) observed TPC values of 118.4 mg GAE g^{-1} DW in fresh stinging NL, 121.5 mg GAE g^{-1} DW in freeze-dried leaves, and 128.7 mg GAE g^{-1} DW in oven-dried leaves.

Antioxidants neutralize free radicals in the body, preventing oxidative damage from pollution, metabolism, and external factors, which can otherwise lead to premature aging, cardiovascular disease, and degenerative conditions such as cancer, Alzheimer's, and cataracts; thus, natural antioxidants in foods like fruits, vegetables, and plant-based diets are essential for disease prevention (Ghevariya et al., 2023). The antioxidant activities of plant samples are known to be influenced by various factors, such as the extraction method, solvent used, and the assay system employed. Consequently, it is important to evaluate antioxidant activity using different assays to account for the various types of antioxidant effects (Wang et al., 2021b). The ABTS radical cation ($ABTS^{\cdot+}$) is produced via enzyme or chemical processes. Due to the ability of $ABTS^{\cdot+}$ to dissolve in both aqueous and organic solvents, it reflects the hydrophilic and lipophilic properties of sample

compounds. In contrast, the ferric reducing antioxidant power (FRAP) assay measures the reduction of the Fe^{3+} complex of tripyridyltriazine $(TPTZ)^{3+}$ to the deeply blue Fe^{2+} complex $(TPTZ)^{2+}$ by antioxidants in an acidic environment (Salam et al., 2023). The use of both FRAP and ABTS tests allows for a more comprehensive assessment of the reaction kinetics and responses of different radicals and phenolic compounds (Iqbal et al., 2012). The ABTS assay was used to evaluate the free radical scavenging activities of the leaves, and the FRAP assay was employed to measure their reducing power. The FRAP and ABTS assay results to assess the antioxidant potential of the leaf extracts for different leaf extracts are presented in Table 1. Accordingly, the ABTS antioxidant activities of the other ethanolic leaf extracts, ranked from highest to lowest, are NL (19.26 mg TE g^{-1} extract) > WBL (17.87 mg TE g^{-1} extract) > FL (8.51 mg TE g^{-1} extract). There is no statistical difference between NL and WBL ($p \geq 0.05$), while there is a statistical difference between FL and the other two ($p < 0.05$). Furthermore, Iqbal et al. (2012) reported that mulberry leaves exhibited ABTS radical cation scavenging activity, with values of 6.12mM Trolox equivalent for *M. alba.* Moreover, ABTS activity in mulberry leaves was 70.30 for the methanolic extract and 82.53 mg TE g^{-1} for the water extract, while FRAP activity was 70.16 for the methanolic extract and 59.54 µM TE g^{-1} for the water extract (Uysal et al., 2016). In addition, Eruygur and Dural (2019) stated that the antioxidant potential of mulberry leaves is primarily attributed to their polyphenolic compounds, including flavonoids and anthocyanins.

Table 1 displays the chelating activity of various leaf extracts on ferrous ions. In this assay, all leaf extracts inhibited the formation of the ferrous-ferrozine complex, indicating their chelating ability to capture ferrous ions before the complex could form. The FRAP activity peaked at 240.48 µmol Fe^{2+} g^{-1} extract in the NL samples, while it was lowest at (125.66 µmol Fe^{2+} g^{-1} extract) in the FL samples. No statistical difference was found between FBL and NL ($p > 0.05$). Wang et al. (2021a) observed that FRAP values ranged from 35.13 µmol Fe^{2+} g^{-1} DW (G1) to 227.8 µmol $Fe^{2+}g^{-1}$ DW, and ABTS values varied between 19.81 µmol TEAC g^{-1} DW and 120.42 µmol TEAC g^{-1} DW. They also found a significant positive correlation between the free phenolic content and antioxidant activity. Wang et al. (2021b) observed that, regardless of the solvent used, the free phenol content showed the strongest relative correlation with ABTS compared to FRAP in mulberry leaves. The FRAP and ABTS values were reported as 258.864 mmol Fe^{2+} g^{-1} and 0.097 mmol g^{-1}, respectively (Zhang et al., 2018). The FRAP activity of NL was reported to range between 9.5 and 75.5 µmol Fe^{2+} g^{-1} DW, depending on the extraction solvent and duration (Vajić et al., 2022). Moreover, the variations in test results may be due to different kinetics of reactions between radicals and phenolic compounds or differing responses of phenolics to various radicals. Therefore, employing a range of antioxidant tests together is important for accurately evaluating the antioxidant capacity of a sample (Iqbal et al., 2012). In this study, a positive correlation between TPC and antioxidant activity values has been identified. Likewise, Salam et al. (2023) identified a linear relationship between TPC and ABTS scavenging activities, as well as FRAP activity. In summary, the high levels of phenolics, combined with substantial FRAP and ABTS radical cation scavenging potential, indicate that NL is superior to other species in terms of its disease-preventive potential.

Enzyme inhibitory properties

Antidiabetic potential of leaf extracts

Phytochemicals have garnered significant interest for diabetes treatment due to their potential benefits, leading many researchers to focus on extracting hypoglycemic agents from medicinal plants. Among these, plant polyphenols and flavonoids are recognized natural antidiabetic agents that inhibit carbohydrate-hydrolyzing enzymes by binding with proteins, thereby helping to reduce postprandial hyperglycemia in diabetes (Ganeshpurkar et al., 2013). In this context, Table 2 presents the antidiabetic potential of leaf extract, revealing that the inhibition of alpha-amylase

ranges from 8.85% to 90.39% depending on extract concentration in the range of 62.5-500 µg mL^{-1}.

Table 2. Antidiabetic activities of fenugreek (FL), white mulberry (WML), and nettle leaves (NL).

Enzyme type	Concentration (µg mL^{-1})	Inhibition percentages					
		62.5 (µg mL^{-1})	125 (µg mL^{-1})	250 (µg mL^{-1})	400 (µg mL^{-1})	500 (µg mL^{-1})	IC_{50} (µg mL^{-1})
Alpha-amylase	FL	8.85±0.56^{De}	20.34±1.00^{Dd}	44.17±1.42^{Dc}	58.17±2.33^{Cb}	62.28±2.65^{Ca}	357.85
	WBL	19.24±0.47^{Ae}	36.29±1.60^{Ad}	64.24±1.46^{Ac}	83.31±3.82^{Ab}	90.39±2.21^{Aa}	213.99
	NL	14.24±0.43^{Be}	32.88±0.78^{Bd}	56.51±1.34^{Bc}	75.14±1.62^{Bb}	86.87±2.45^{Aa}	248.01
	Acarbose	12.53±0.33^{Ce}	28.77±0.21^{Cd}	53.81±0.29^{Cc}	70.89±2.01^{Bb}	79.34±0.53^{Ba}	273.67
Alpha-glucosidase	FL	13.44±0.95^{Be}	25.57±0.72^{Dd}	39.69±0.69^{Dc}	50.91±2.69^{Cb}	58.31±5.11^{Ca}	393.63
	WBL	17.55±1.42^{Ae}	39.36±2.07^{Ad}	66.37±2.62^{Ac}	81.24±3.17^{Ab}	86.73±2.15^{Aa}	213.58
	NL	17.47±1.71^{Ae}	35.20±0.54^{Bd}	59.41±1.24^{Bc}	75.96±3.03^{Ab}	82.06±1.88^{Aa}	239.83
	Acarbose	14.15±1.13^{Be}	32.27±1.76^{Cd}	51.44±2.84^{Cc}	70.09±2.66^{Bb}	74.97±2.27^{Ba}	277.78

Differences among leaf extract concentrations in enzyme inhibition percentages are indicated by different capital letters within the same column, with a significance level of $p<0.01$. Differences in leaf samples within the same row are indicated by different lowercase letters ($p<0.05$).

The inhibitory potential of WBL (IC_{50} = 213.99 µg mL^{-1}) and NL (IC_{50} = 248.01 µg mL^{-1}) was lower than that of acarbose (IC_{50} = 273.67 µg mL^{-1}) but higher than FL (IC_{50} = 357.85 µg mL^{-1}). Our results show that leaf samples WBL and NL exhibited significantly higher *in vitro* alpha-amylase inhibitory activity compared to sample FL, which showed lower activity (see Table 2). Acarbose, a complex oligosaccharide, delays carbohydrate digestion by inhibiting amylase, but synthetic inhibitors can lead to side effects such as abdominal pain, diarrhea, and soft stools (Narkhede, 2012). In this context, Hafeez et al. (2023) found that the inhibition of alpha-amylase by *T. foenum-graecum* leaf extract ranged from 9.43% to 24.95% depending on different extract fractions. Moreover, at a concentration of 250 µg mL^{-1}, the ethyl acetate extract demonstrated significant alpha-amylase inhibition at 64.55% and alpha-glucosidase inhibition at 52.56% (Ganeshpurkar et al., 2013). Alpha-amylase inhibitors act as anti-nutrients by impeding the digestion and absorption of carbohydrates, which can be beneficial in managing obesity and diabetes (Narkhede, 2012). Although the exact mechanisms through which plant protein inhibitors affect alpha-amylase are not fully understood, it is suggested that plant proteins, particularly flavanols, may induce structural changes in the enzyme. Additionally, plant phenolic compounds have been shown to influence carbohydrate breakdown by inhibiting amylase activity. The observed activity in the selected leaf samples may be attributed to the presence of tannins in their ethanol extracts (Narkhede, 2012). These results suggest that the plant may exhibit hypoglycemic activity, likely through the inhibition of alpha-amylase. This effect could be partly attributed to reduced glucose absorption into the bloodstream due to the alpha-amylase inhibitory activity of the plant extract. Additionally, improved glucose tolerance may be linked to other mechanisms such as stimulation of glycogenesis in the liver, increased tissue glucose utilization, and decreased gluconeogenesis. Phytoconstituents such as flavonoids, polyphenols, tannins, alkaloids, glycosides, carbohydrates, and proteins have been shown to possess antidiabetic activity (Bisht et al., 2021). A previous study indicated that oxidative stress may contribute to increased insulin resistance, suggesting that antioxidants could be useful in treating diabetes (Hajra & Paul, 2018). However, in our study, no correlation was found between alpha-amylase and alpha-glucosidase enzyme inhibition and IC_{50} values.

Alpha-glucosidase is an enzyme that breaks down complex carbohydrates into glucose by hydrolyzing the terminal 1,4-glycosidic bonds. This

enzyme is located in the brush-border epithelium of the human intestine. Conventional inhibitors of alpha-glucosidase are less effective at inhibiting this enzyme compared to their impact on alpha-amylase, which is primarily responsible for gastrointestinal discomfort in individuals with diabetes (Wadhawan, et al., 2018). To reduce this side effect, it's essential to investigate alternative herbs and plants as safer sources of alpha-glucosidase inhibitors. In this context, the alpha-glucosidase inhibition activity of WBL (19.24-90.39%) and NL (14.24-86.87%) was observed to be higher than that of the positive control acarbose (12.53-79.39%) as shown in Table 2. Among the samples mentioned, WBL and NL were identified as having a significant inhibitory effect on alpha-glucosidase at the concentration ranges of 62.5-500 µg mL^{-1}. In contrast, FL (13.44-58.31%) did not exhibit significant alpha-glucosidase inhibitory activity. Differences in enzyme inhibitory activity may arise from the varying abilities of solvents to extract specific compounds based on their chemical nature, physicochemical properties such as polarity, and the presence or absence of interfering substances (Sharma et al., 2023). Accordingly, Kim et al. (2011) found that mulberry leaf extract exhibited strong *in vitro* inhibition of intestinal alpha-glucosidase, while its effect on intestinal alpha-amylase was significantly weaker in comparison to acarbose. A recent study has shown that 1-deoxynojirimycin (DNJ) and its derivatives, which are primary constituents of mulberry leaves, effectively inhibit intestinal alpha-glucosidases, thereby slowing down the digestion of carbohydrates (Oku et al., 2016). Additionally, Eruygur and Dural (2019) suggested that the enzyme inhibition activity observed could be linked to the polyhydroxylated alkaloids and specific phenolic compounds found in mulberry extracts from Türkiye. Similar to acarbose, the inhibitory potential of mulberry leaf extracts may delay the metabolism of saccharides, reduce glucose absorption, and thus help manage postprandial blood sugar levels. Moreover, Han et al. (2020) identified alkaloids and flavonoids in mulberry leaf powder as the key agents in combating type 2 diabetes, with alkaloids demonstrating a pronounced inhibition of alpha-glucosidase activity, and flavonoids showing secondary effectiveness. In addition, Habeeb et al. (2012) found that the 60% mulberry alcohol extract had the greatest alpha-glucosidase inhibition at 80.92%, in contrast to the 100% alcohol extract, which had the lowest inhibition of 30%. At a concentration of 1 mg mL^{-1}, the 60% mulberry alcohol extract provided 69.69% inhibition of salivary amylase and 78.77% inhibition of pancreatic amylase, whereas the 100% alcohol extract showed a minimal inhibition of 16.58%. Furthermore, Adisakwattana et al. (2012) found that mulberry leaf extract exhibited the strongest inhibitory effect on intestinal alpha-glucosidase but showed no inhibition of pancreatic alpha-amylase. In this experiment, the leaf extracts demonstrated notable inhibition of alpha-amylase and alpha-glucosidase in a dose-dependent manner.

Anti-Alzheimer potential of leaf extracts

Alzheimer's disease, the most common neurological disorder, currently has limited treatment options. Acetylcholinesterase activity is associated with Alzheimer's disease and can be inhibited by certain medicinal plants. Plant-derived treatments offer neuroprotective benefits that could help manage neurodegenerative conditions (Hafeez et al., 2023). The enzyme inhibitory activities of the leaves against AChE and butyrylcholinesterase (BChE) are summarized in Table 3.

Table 3. Anti-Alzheimer enzyme inhibition activities of various leaves.

Type of wastes	Anti-Alzheimer activity	
	AcHE (%)	BcHE (%)
FL	39.93±1.35^{c}	33.87±3.60^{b}
WBL	23.14±1.74^{d}	17.94±1.40^{c}
NL	53.35±1.93^{b}	38.40±2.62^{b}
Galanthamine	90.28±3.92^{a}	87.12±5.24^{a}

FL: Fenugreek leaf; WBL: White mulberry leaf: WBL; NF: Nettle leaf.
Statistical differences within the same column are indicated by lower case letters ($^{a-d}$) ($p<0.001$).

The AChE inhibition rates for all tested samples of leaves ranged from 23.14% (WBL) to 53.35% (NL). Conversely, the findings reveal that ascorbose achieved a maximum inhibition rate of 90.28% on acetylcholinesterase, whereas none of the leaf extracts demonstrated any inhibition against AChE.

Table 3 illustrates the impact of leaf extracts on inhibiting BChE activity. Among the extracts, NL ethanolic extracts (38.40%) demonstrated the highest BChE inhibitory activity compared to the FL (33.87%) and WBL (17.94%) at 2 mg mL^{-1}. None of the leaf extracts showed inhibition against BChE, as their BChE inhibition percentages were lower than the inhibition percentage of the galanthamine. at 2 mg mL^{-1}. In a related study, Uysal et al. (2016) reported that in mulberry leaves, the AChE and BChE inhibitory activities were higher in ethanol extracts compared to water extracts. BChE activity was observed around 30%, while AChE activity was found to be in the range of 20%.

Cytotoxic properties

Table 4 shows the viability of HEK-293 cells treated with FL, WBL, and NL ethanol extracts, with data presented as percentages normalized to the untreated control. After 24 h of incubation, all leaf ethanol extracts decreased the viability of HEK-293 cells from 100% to 59.33% for FL, from 100% to 61.67% for WBL, and from 100% to 55.33% across the concentration range of 1–1000 µg mL^{-1} (see Table 4). The ethanol extracts from FL, WBL, and NL yielded IC_{50} values of 1159.98 µg mL^{-1}, 1235.67 µg mL^{-1}, and 972.22 µg mL^{-1} on HEK-293 cells, respectively. Notably, the NL extract, with the lowest IC_{50} value, exhibits the strongest inhibitory effect on the HEK-293 cells. This implies that the NL extract may offer a higher level of biological activity compared to the other two extracts.

Table 4 presents the viability percentages and IC_{50} values of leaf extracts for the CaCo-2 cells. The data indicates that the FL extracts exhibited a range of cell viability from 100% to 51.00% across the concentration range of 1–1000 µg mL^{-1}.

Table 4. The percentage of cell viability and IC_{50} values for FL, WBL, and NL.

Name of cells	Sample name	Control	1 (µg mL^{-1})	50 (µg mL^{-1})	100 (µg mL^{-1})	250 (µg mL^{-1})	500 (µg mL^{-1})	1000 (µg mL^{-1})	IC_{50} (µg mL^{-1})
HEK-293	FL	100.00±0.00^{Aa}	100.00±0.00^{Aa}	95.67±1.53^{Aab}	89.28±1.53^{Ab}	82.33±5.86^{Ac}	73.33±1.53^{Ad}	59.33±1.53^{ABe}	1159.98
	WBL	100.00±0.00^{Aa}	100.00±0.00^{Aa}	94.00±1.73^{Ab}	85.26±1.53^{Ab}	77.33±3.51^{ABc}	75.33±0.58^{Ac}	61.67±2.08^{Ad}	1235.67
	NL	100.00±0.00^{Aa}	100.00±0.00^{Aa}	85.33±3.06^{Bb}	86.45±0.58^{Bbc}	73.00±3.61^{Bc}	62.33±2.52^{Bd}	55.33±3.06^{Bd}	972.22
Caco-2	FL	100.00±0.00^{Aa}	100.00±0.00^{Ba}	89.00±2.00^{Ab}	83.67±2.31^{Ac}	72.00±2.65^{Ad}	62.33±1.53^{Ae}	51.00±1.00^{Af}	897.41
	WBL	100.00±0.00^{Aa}	100.00±0.00^{Ba}	86.67±2.08^{Ab}	79.33±1.15^{Bc}	68.00±3.61^{Ad}	53.67±2.08^{Be}	44.67±3.21^{Bf}	754.11
	NL	100.00±0.00^{Aa}	102.00±1.00^{Aa}	81.67±0.58^{Bb}	75.00±2.00^{Cc}	61.67±2.08^{Bd}	44.33±3.06^{Ce}	40.33±1.15^{Cf}	648.08

Fenugreek leaf: FL; White mulberry leaf: WBL; Nettle leaf: NL.
Differences among leaf extract concentrations for the cell viability findings are represented by different capital letters within the same column, with a significance level of $p<0.01$. Differences in leaf samples within the same row are indicated by different lowercase letters ($p<0.05$).

In comparison, WBL extracts showed cell viability values ranging from 100.00% to 44.67% within the same concentration range, while NL extracts resulted in cell viability ranging from 40.33% to 100%. In both cells, an increase in extract concentration resulted in a decrease in cell viability percentage. The IC_{50} values, which reflect the concentration required to inhibit 50% of cell

viability, were calculated as follows: 897.41 µg mL^{-1} for FL, 754.11 µg mL^{-1} for WBL, and 648.80 µg mL^{-1} for NL. These findings suggest that among the leaf extracts tested, NL exhibits the lowest IC_{50} value, indicating the highest potency in reducing cell viability. Conversely, FL and WBL extracts demonstrated higher IC_{50} values, implying relatively lower efficacy compared to NL. The observed differences in IC_{50} values highlight the varying degrees of biological activity among the extracts. NL's lower IC_{50} value suggests it may have a more significant impact on cell viability, potentially making it a more effective candidate for further investigation. In this regard, Salam et al. (2023) found that the highest cytotoxic activity (89.03%) was observed in the crude extract of air-dried fenugreek leaves when tested on the RAW 264.7 cells at a concentration of 100 µg mL^{-1}. Moreover, Wadhawan et al. (2018) observed that the fenugreek microgreen extract was well-tolerated by HepG2 (human liver cancer cell line) cells at a concentration of 20 mg mL^{-1}. However, it was found to be mildly cytotoxic in L6 (rat myoblast) cells at concentrations above 15 mg mL^{-1}. In comparison, fenugreek seed extract has been reported to be toxic at higher concentrations, with an IC_{50} of 1 mg mL^{-1} in HepG2 cells and 2 mg mL^{-1} in L6 cells (Kadan et al., 2013; Khalil et al., 2015). Khoja et al. (2022) found that fenugreek methanolic extracts had dose and time-dependent effects on the viability of MCF-7 cells. Treatment with these extracts resulted in heightened relative mitochondrial DNA damage in the MCF-7 cancer cells, along with reduced metastasis and cell proliferation. Likewise, Yamamoto et al. (2017) investigated DNJ (1-deoxynojirimycin)'s impact from mulberry leaves on male mice with azoxymethane-induced colorectal cancer, revealing that DNJ suppressed tumor growth by promoting apoptosis, potentially through the Bcl-2/Bax signaling pathway. Additionally, Deepa et al. (2013) reported that leaf extracts from *Morus alba* can induce cytotoxic effects in human colon cancer (HCT-15) cells (IC_{50} = 13.8 µg mL^{-1}) and MCF-7 cells (IC_{50} = 9.2 µg mL^{-1}), resulting in significant DNA fragmentation, caspase-3 activation, and morphological changes in the cells, which are characteristics of apoptosis. Moreover, Deepa et al. (2012) found that the anti-proliferative lectin (MLL) derived from *M. alba* leaves caused notable morphological alterations and DNA fragmentation linked to apoptosis in MCF-7 cells. Moreover, Fattahi et al. (2014) noted that BT-474 cell viability was stable at extract concentrations up to 1.5 mg mL^{-1} but decreased to below 50% of the control at 3 mg mL^{-1}, whereas Hela cells showed no significant changes even with 3 mg mL^{-1} of extract over 3 days; furthermore, no significant differences in cell survival rates were observed between consecutive days for both Hela and BT-474 cells ($P>0.05$). Moreover, NL extracts have shown cytotoxic effects against Hep2c, RD, and L2OB cells, with activity varying depending on the extraction technique (16.73-.29.77 µg mL^{-1}) (Zeković et al., 2017). These results indicate that the tested leaf extracts have low toxicity levels on both HEK-293 and CaCo-2 cells, with minimal toxic effects on the cells; furthermore, this effect is lower in the HEK-293 cell.

Conclusions

M. alba L., *T. foenum-graecum* L., and *U. dioica* L. are traditionally recognized for their medicinal and nutritional benefits, being incorporated into diets in the form of herbal teas and other supplements for their health-promoting qualities. This study aimed to assess the total phenolic content (TPC) and antioxidant properties, including FRAP and ABTS activities, of ethanolic extracts from fenugreek (FL), white mulberry (WBL), and nettle leaves (NL). Additionally, it investigated the extracts' inhibitory effects on key enzymes—alpha-amylase, alpha-glucosidase, acetylcholinesterase, and butyrylcholinesterase—and evaluated their cytotoxicity on the HEK-293 and Caco-2 cells. The results indicated that NL exhibited superior disease-preventive potential due to its high phenolic content and strong FRAP and ABTS radical cation scavenging abilities. A positive correlation was found between total phenolic content and antioxidant activity values. Moreover, WBL and NL demonstrated significant

inhibitory properties against both alpha-amylase and alpha-glucosidase. In terms of cytotoxicity, NL showed the highest toxicity and FL the lowest in HEK-293 cells, while in the CaCo-2 cellse, NL again exhibited the highest toxicity and FL showed the lowest. These findings highlight the potential of these leaf extracts in developing functional foods aimed at enhancing the inhibition of intestinal alpha-glucosidase and alpha-amylase. Future research should focus on exploring the *in vivo* effects of these activities to further validate their therapeutic potential. While this study provides valuable insights into the phenolic content, antioxidant activities, and enzyme inhibitory properties of the extracts, it is limited by its *in vitro* nature and the restricted range of cell lines tested. Further *in vivo* studies and a broader exploration of geographical and seasonal variations are needed to confirm these findings.

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Conflict of Interest Statement

The authors declare no conflict of interest.

Author Contributions

Kubra Feyza EROL: Methodology, validation, investigation. **Gozde KUTLU:** Investigation, Writing – original draft, review & editing.

Data Availability Statement

Data will be made available on request upon reasonable request from the corresponding author.

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Research Article/Araştırma Makalesi

The production of ready to drink terebinth coffee and the changes occurring during storage

İçime hazır menengiç kahvesi üretimi ve depolama sırasında meydana gelen değişimler

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ABSTRACT

The purpose of this study was to bring a new product to the food industry and investigate a different application area for ready-to-drink terebinth (*Pistacia terebinthus*) coffee made with water and milk. Accordingly, formulation was determined based on preliminary trials conducted with terebinth coffees. Coffees prepared according to this formulation were processed and preserved using two different methods. In the first method, the products were filled into amber-colored glass bottles using hot filling technique and sterilized, while in the other method, potassium sorbate was added to the products and preserved in amber-colored glass bottles. Samples were stored at room temperature (24°C) and in the refrigerator (+4°C) for 180 days. Dry matter, ash, pH, free fatty acids, fatty acid compositions, color characteristics, antioxidant activity, total phenolic content, and phenolic fractions were investigated in the prepared ready-to-drink menengic coffees. As a result of the analyses, while the fat content remained constant in the coffee samples, an increase in free fatty acids and a decrease in pH were detected. Although there was a slight decrease in antioxidant capacities and phenolic content during the storage period, it was determined that this decrease was not significant. Luteolin, ellagic acid, gallic acid, fumaric acid, and vanillic acid were predominantly found in menengic coffees in terms of phenolic compounds. In milk coffees, it was observed that flocculation occurred due to heat treatment.

Key Words: Pistacia terebinthus, coffee, antioxidant, phenolic, fatty acid

ÖZ

Bu çalışmada, su ve süt ile hazırlanan içime hazır menengiç (*Pistacia terebinthus*) kahvesinin gıda endüstrisinde farklı bir kullanım alanının ortaya çıkarılması ve yeni bir ürünün gıda endüstrisine kazandırılması amaçlanmıştır. Bu amaçla, menengiç kahvesi için yapılan ön denemeler sonucunda bir formülasyon belirlenmiştir. Belirlenen formülasyon doğrultusunda hazırlanan kahveler, iki farklı yöntemle işlenerek saklanmıştır. İlk yöntemde, sıcak dolum tekniği kullanılarak ürünler amber renkli cam şişelere doldurulmuş ve sterilize edilmiştir. Diğer yöntemde ise, ürünlere potasyum sorbat eklenerek amber renkli cam şişelerde muhafaza edilmiştir. Örnekler, cam şişelerde oda sıcaklığında (24℃) ve buzdolabında (+4℃) 180 gün süreyle depolanmıştır. Hazırlanan içime hazır menengiç kahvelerinde kuru madde, kül, pH, serbest yağ asitleri, yağ asitleri kompozisyonları, renk özellikleri, antioksidan aktivite, toplam fenolik madde, fenolik madde fraksiyonları araştırılmıştır. Yapılan analizler sonucunda kahve örneklerindeki yağ miktarı sabit kalırken serbest yağ asitlerinde artış, pH da ise düşüş tespit edilmiştir. Depolama süresi boyunca antioksidan kapasitelerinde ve fenolik madde miktarlarında az da olsa bir azalma olduğu fakat bunun yüksek değerlerde olmadığı belirlenmiştir. Fenolik maddeler açısından menengiç kahvelerinde ağırlıklı olarak luteolin, ellagik asit, gallik asit, fumarik asit ve vanilik asit bulunmuştur. Sütlü kahvelerde ise ısıl işlem etkisiyle floklaşmalar meydana geldiği tespit edilmiştir.

Anahtar Kelimeler: Menengiç, kahve, antioksidan, fenolik, yağ asidi

Introduction

Pistacia terebinthus is a medicinal aromatic plant belonging to the *Anacardiaceae* family, known for containing a high number of bioactive compounds (Özcan, 2004). It has a strong aroma and is rich in oil, protein, and dietary fibers. Pistacia species are also rich in antioxidants and phenolic compounds and possess antimicrobial properties. Additionally, due to its lack of caffeine content and richness in unsaturated fatty acids, menengiç is highly beneficial for health. Terebinth coffee is abundant in vitamins (B and E vitamins) and minerals (sodium, potassium, phosphorus, iron, magnesium, zinc, copper) (Eytemiş, 2016). The fruits of the *Pistacia terebinthus* tree, known as menengiç, are consumed as snacks, either roasted or mashed. Menengiç fruits, harvested from wild trees growing naturally in the mountainous and rural areas of the Mediterranean, Southeastern Anatolia, and Central Anatolia regions, are left to dry under the sun for a few days after being washed. The dried fruits are then roasted in a wide pan until they turn dark brown. After roasting, menengiç coffee is made by grinding them into a paste-like consistency. Menengiç seeds have a high oil content (38-45%), which presents a significant alternative for oil production in the food industry. The oil in the seeds contains a substantial amount of oleic acid (51.6%), palmitic acid (20.9%), and linoleic acid (19.6%). These fatty acids can be included in the groups of oils consumed for their health benefits. The versatile properties of menengiç—such as high protein content, oil, flavor, and aroma—enhance its potential applications in the food industry. Furthermore, the growing demand for natural products increases the consumption of menengiç due to its functional properties (Sidar, 2011). Papageorgiou et al. (1999) identified α-pinene, β-pinene, sabinene, and terpinen-4-ol as the primary constituents in the volatile oil of *P. terebinthus var. chia* (*P. lentiscus var. latifolius*) resin. Küsmenoğlu et al. (1995) determined that α-pinene, terpinolene, and limonene were the main components in the fresh peel essential oil of *Pistacia vera* fruit. According to Özcan et al. (2009), limonene and β-pinene were the primary constituents in the volatile oil of ripe terebinth fruit; however, there were also notable quantities of α-phellandrene, terpinolene, and α-pinene. Geçgel & Arıcı (2008) reported that the significant saturated fatty acids in terebinth were palmitic and stearic acids, while the important unsaturated fatty acids included oleic and linoleic acids. They found that the oleic acid content was notably high (49.26%-52.67%) among the fatty acids. Additionally, the linolenic acid content was found to be below 1% in all samples, and the total trans fatty acid content varied between 0.16% and 0.89%. Somporn et al. (2011) investigated the effects of roasting temperature on several properties of coffee beans, including color values, volatile oils, antioxidant activity, and phenolic compounds. It was determined that as the roasting temperature increased, the color values (L and b) of the coffee beans also increased. Antioxidant activity was found to be higher in lightly roasted coffee beans. Additionally, it was determined that as roasting temperatures increased, the levels of gallic acid, p-coumaric acid, cinnamic acid, and chlorogenic acid in the coffee beans increased.

Pelvan & Demirtaş (2018) examined the oil content, antioxidant activity, and total phenolic content of oils from *Pistacia terebinthus L.* and *Pistacia vera* cultivated in Turkey. The phenolic content of the samples ranged from 3.03 to 4.52 mg GAE/100 g, the oil content from 50.33% to 54.00%, and the antioxidant activity from 371.23 to 736.48 µmol TE/100 g. Hayoğlu et al. (2010) used both roasted and unroasted terebinth in their investigation of the potential application of terebinth in confectionery production. The ash, moisture, and sugar contents of confections made from roasted and unroasted terebinth were found to be 1.83% and 1.23%, 2.67% and 3.78%, and 70% and 60%, respectively. Sensory assessments by panelists revealed that, in terms of flavor and aroma, the roasted terebinth confection was superior. In the study conducted by Amanpour et al. (2015, 2019), a total of 51 volatile components were identified in roasted terebinth, with terpenes

being the most abundant among them. The characteristics of the oil extracted from terebinth seeds were studied by Kaya and Özer (2015). The amounts of fatty acids in the samples were determined to be as follows: oleic acid 45.8%, palmitic acid 24.27%, linoleic acid 23.93%, palmitoleic acid 3.78%, stearic acid 1.7%, and linolenic acid 0.47%. In a study conducted by Köten and Ünsal (2022), it was determined that the protein, ash, fat, phenolic compound, and antioxidant content of the noodles produced by adding roasted terebinth—both in its raw form and roasted at different temperatures before being turned into flour—to wheat flour increased. In this study, some of the ready-to-drink terebinth coffees, prepared with water and milk and formulated through preliminary trials, were sterilized by filling them into amber-colored glass bottles using the hot filling technique, while others were preserved in amber-colored glass bottles with the addition of potassium sorbate. Changes in the products stored at room temperature (24°C) and in the refrigerator (+4°C) for 180 days were examined.

Material and Method

Material

The terebinth coffee used in this study was obtained from the local market in Şanlıurfa. Drinkable quality water was used in the production of the coffee. The milk and/or milk powder, along with sugar, were purchased ready-made from the market.

Method

Terebinth coffee production

The terebinth coffees were prepared according to the traditional method. For this purpose, 30 g of coffee was mixed with 100 ml of water and milk, and this mixture was cooked. The cooked samples were separated from the sediment under suitable conditions. A portion of the prepared product was filled into 200 ml amber-colored glass bottles using the hot filling technique and sterilized at 105-110 °C for 5-10 minutes, while another portion was filled into amber-colored glass bottles after adding potassium sorbate at a ratio of 1:1000. The ready-to-drink products were stored at both refrigerator and room temperatures and subjected to physical, chemical, and sensory analyses at regular intervals. The terebinth coffees were stored in amber-colored glass bottles at room temperature (25±2˚C) and at +4°C for 6 months. Necessary analyses were conducted monthly starting from the beginning of production.

Experimental design

The analyzed samples are coded as follows and will be referred to by their codes throughout the text:

SS4: Coffee prepared with water, sterilized, and stored at 4 ^{0}C

SS24: Coffee prepared with water, sterilized, and stored at 24 ^{0}C

SK4: Coffee prepared with water, preservative added, and stored at 4 ^{0}C

SK24: Coffee prepared with water, preservative added, and stored at 24 ^{0}C

STS4: Coffee prepared with milk, sterilized, and stored at 4 ^{0}C

STS24: Coffee prepared with milk, sterilized, and stored at 24 ^{0}C

STK4: Coffee prepared with milk, preservative added, and stored at 4 ^{0}C

STK24: Coffee prepared with milk, preservative added, and stored at 24 ^{0}C

Analyses made on terebinth coffee

pH, total dry matter (%), moisture (%), ash (%), Protein (%), Cellulose (%), color (L*, a*, b* values) (AOAC, 2005; Cemeroğlu, 2007), fatty acid (Jennings & Akoh, 1999), free fatty acid, Essential Oil (Pirbalouti & Aghaee, 2011) , Antioxidant (Çam et al., 2009), Total Phenolic Substance (Medina-Remon et al., 2009), analyzes were made in the produced terebinth coffees. Trials were made in three replications and two parallels, and the SPSS package program was used in the evaluation ($P \leq 0.05$). The differences between the means in the groups were determined by Duncan test (Curran et al., 1996).

Results and Discussions

Some parameters of terebinth seed

Average values obtained from various analyses performed on terebinth seeds are presented in Table 1. The essential oil composition of terebinth seeds is provided in Table 2.

Table 1. Some analysis values of Terebinth seed (%)

Component		Terebinth seed
Moisture		26.74±0.03
Ash		2.40±0.01
Protein		9.49±0.02
Fat		46.05±0.03
Cellulose		24.56±0.2
Color	L*	30.29±0.01
	a*	-1.55±0.03
	b*	3.98±0.02

Protein, ash, cellulose, antioxidant activity and fat amounts were calculated as % dry matter.

Table 2. The essential oil composition of Terebinth seed (%)

Essential oil components	Terebinth seed
Alpha pinene	31.03±0.02
Limonene	14.45±0.01
Cis-β-osimen	16.04±0.01
Trans-β-osimen	5.74±0.02
Beta Mirisen	6.21±0.03
Alpha Terpinolene	5.33±0.01
Carvacrol	5.25±0.02
Beta Pinene	3.19±0.02
Trans Karyofillen	2.40±0.02
Sabinen	1.76±0.03
Kampen	1.79±0.02
Alpha Thujen	1.27±0.03
Bornilasetat	1.08±0.01
Para Simen	1.10±0.01
4.8-Dimetil-1,3,7-Nonatrien	0.80±0.02
Alfa Terpinen	0.74±0.02
Delta 3 Karen	0.77±0.03
Unrecognized essential oil	1.05±0.02
Elemol	-
Caryophyllene oxide	-
9,12-oktadekadienoik asit, methyl ester	-

Terebinth coffee chemical values

The dry matter values of terebinth coffees are presented in Table 3, ash values in Table 4, pH values in Table 5, and free fatty acid values in Table 6. No change was observed in the total dry matter content of samples prepared with water or milk during storage, and the statistically significant differences were attributed to the very low degree of freedom for error. In the samples prepared with milk and stored at +4°C, no difference was observed in terms of statistical and general total dry matter values until the 120th day. After the 120th day, analyses were terminated due to flocculation in the milk samples. The dry matter values of samples prepared with milk were found to be higher than those of samples prepared with water (Table 3).

No change was observed in the total dry matter content of samples prepared with water or milk during storage, and the statistically significant differences were attributed to the very low degree of freedom for error. In the samples prepared with milk and stored at +4°C, no difference was observed in terms of statistical and general total

dry matter values until the 120th day. After the 120th day, analyses were terminated due to flocculation in the milk samples. The dry matter values of samples prepared with milk were found to be higher than those of samples prepared with water (Table 3).

While no time-dependent change was observed in the ash values of coffees prepared with milk, a decrease in ash values was noted after 6 months in samples prepared with water. Additionally, ash contents were found to be higher in samples prepared with milk (Table 4).

As shown in Table 5, there was a slight, regular decrease in pH values in terebinth coffees prepared with water during the storage period. In the study conducted by Fedai (2018), decreases in pH values over time were also observed. In samples prepared with milk, a decrease in pH was noted over time, albeit irregularly. It is thought that these irregularities are due to flocculation in the structure of the products.

It is believed that there was a significant increase in the free fatty acid values of all samples during the storage period, and this increase may be attributed to chemical reactions occurring in the structure of the samples over time and under varying storage conditions. In the study by Karahan (2017), it was determined that free fatty acid values increased with time (Table 6).

Table 3. Terebinth coffee dry matter values (%)

Days	SS24	SS4	SK24	SK4	STK24	STK4	STS24	STS4
0	43.91±1.09^{aB}	43.91±1.09^{aB}	43.91±1.09^{aB}	43.91±1.09^{aB}	57.07±1,14^{aA}	57.07±1,14^{aA}	57.07±1,14^{aA}	57.07±1,14^{aA}
30	43.86±0.02^{abB}	40.83±0.02^{dC}	43.89±0.05^{aB}	43.94±0.02^{aB}	57.18±0.97^{aA}	56.97±1.32^{aA}	57.22±0.32^{aA}	56.99±1.12^{aA}
60	43.76±0.02^{bB}	43.74±0.00^{bB}	43.81±0.02^{abB}	43.88±0.02^{abB}	56.67±1.01^{aA}	56.93±2.01^{aA}	56.97±0.93^{aA}	56.98±1.43^{aA}
90	43.62±0.07^{cB}	43.63±0.02^{cB}	43.75±0.02^{bB}	43.86±0.04^{abB}	56.28±0.66^{aA}	56.36±0.63^{aA}	56.68±0.86^{aA}	56.55±0.52^{aA}
120	43.65±0.02^{cB}	43.62±0.04^{cB}	43.71±0.04^{bcB}	43.85±0.04^{abB}	56.88±1.71^{aA}	56.86±3.01^{aA}	56.78±1.43^{aA}	56.95±1.01^{aA}
150	43.57±0.03^{cA}	43.61±0.02^{cA}	43.68±0.02^{bcA}	43.81±0.05^{abA}	-	-	-	-
180	43.51±0.05^{dA}	43.56±0.04^{cA}	43.55±0.04^{cA}	43.78±0.02^{bA}	-	-	-	-

The same letters in the same column indicate that the values are statistically insignificant (P>0.05).
The same capital letters in the same row indicate that the values are statistically insignificant (P>0.05).

Table 4. Terebinth coffee ash values (%)

Days	SS24	SS4	SK24	SK4	STK24	STK4	STS24	STS4
0	0.64±0.00^{aB}	0.64±0.00^{aB}	0.64±0.00^{aB}	0.64±0.00^{aB}	0.90±0.00^{aA}	0.90±0.00^{aA}	0.90±0.00^{aA}	0.90±0.00^{aA}
30	0.62±0.01^{bB}	0.63±0.00^{bB}	0.62±0.01^{bB}	0.63±0.02^{aB}	0.91±0.00^{aA}	0.91±0.00^{aA}	0.91±0.02^{aA}	0.90±0.07^{aA}
60	0.62±0.01^{bB}	0.62±0.00^{bB}	0.62±0.02^{bB}	0.62±0.02^{bB}	0.90±0.01^{aA}	0.90±0.01^{aA}	0.90±0.00^{aA}	0.90±0.06^{aA}
90	0.61±0.02^{bcB}	0.61±0.02^{bcB}	0.61±0.01^{bcB}	0.62±0.00^{bB}	0.90±0.02^{aA}	0.91±0.01^{aA}	0.90±0.04^{aA}	0.91±0.03^{aA}
120	0.60±0.01^{cB}	0.61±0.02^{bcB}	0.60±0.01^{cB}	0.62±0.01^{bB}	0.90±0.01^{aA}	0.90±0.02^{aA}	0.91±0.03^{aA}	0.90±0.04^{aA}
150	0.60±0.00^{cA}	0.61±0.00^{bcA}	0.60±0.01^{cA}	0.62±0.00^{bA}	-	-	-	-
180	0.60±0.02^{cA}	0.61±0.03^{bcA}	0.60±0.00^{cA}	0.62±0.01^{bA}	-	-	-	-

The same letters in the same column indicate that the values are statistically insignificant (P>0.05).
The same capital letters in the same row indicate that the values are statistically insignificant (P>0.05).

Table 5. Terebinth coffee pH values

Days	SS24	SS4	SK24	SK4	STK24	STK4	STS24	STS4
0	5.82±0.01^{aA}	5.82±0.01^{aA}	5.82±0.01^{aA}	5.82±0.01^{aA}	5.86±0.03^{aA}	5.86±0.03^{cA}	5.86±0.03^{aA}	5.86±0.03^{cA}
30	5.72±0.00^{bB}	5.80±0.00^{aB}	5.64±0.02^{bBC}	5.80±0.01^{aB}	5.48±0.00^{bC}	6.41±0.00^{aA}	5,72±0.02^{aB}	6.31±0.00^{abA}
60	5.58±0.01^{cB}	5.62±0.03^{bB}	5.53±0.04^{cBC}	5.66±0.02^{bB}	5.51±0.03^{bBC}	6.47±0.01^{aA}	5.22±0.00^{bC}	6.36±0.00^{abA}
90	5.43±0.02^{dB}	5.58±0.01^{bB}	5.42±0.00^{cB}	5.53±0.03^{cB}	4.96±0.02^{cC}	4.96±0.02^{dC}	4.71±0.01^{cD}	6.06±0.00^{bcA}
120	5.36±0.04^{dB}	5.37±0.04^{cB}	5.23±0.04^{dB}	5.38±0.02^{dB}	4.67±0.00^{dC}	4.75±0.01^{eC}	4.58±0.02^{cC}	5.94±0.02^{cA}
150	4.71±0.00^{eD}	5.19±0.01^{dC}	5.12±0.02^{dC}	5.37±0.02^{dB}	5.48±0.00^{bB}	6.53±0.00^{aA}	5.31±0.00^{bB}	6.49±0.00^{aA}
180	4.43±0.02^{D}	5.13±0.04^{dBC}	5.00±0.04^{eBC}	5.22±0.02^{eBC}	5.83±0.07^{aAB}	6.08±0.00^{bA}	5.02±0.02^{bBC}	6.02±0.00^{bcA}

The same letters in the same column indicate that the values are statistically insignificant (P>0.05).
The same capital letters in the same row indicate that the values are statistically insignificant (P>0.05).

Table 6. Terebinth coffee free fatty acids values (%)

Days	SS24	SS4	SK24	SK4	STK24	STK4	STS24	STS4

0	1.92±0.02^{cA}	1.92±0.02^{dA}	1.92±0.02^{eA}	1.92±0.02^{dA}	1.64±0.02^{eB}	1.64±0.02^{dB}	1.64±0.02^{dB}	1.64±0.02^{dB}
30	2.42±0.02^{bA}	2.30±0.00^{cdA}	1.92±0.01^{eB}	2.49±0.00^{cdA}	2.31±0.00^{dA}	1.73±0.04^{dC}	2.03±0.02^{cA}	1.62±0.03^{dC}
60	2.53±0.00^{bB}	2.38±0.02^{cdB}	3.14±0.01^{dA}	2.61±0.04^{cB}	2.42±0.02^{dB}	2.35±0.01^{cB}	2.93±0.04^{bAB}	1.82±0.03^{cC}
90	2.75±0.00^{aB}	2.54±0.02^{cC}	3.31±0.02^{cA}	2.54±0.03^{cC}	2.77±0.03^{cdB}	2.36±0.01^{cC}	2.90±0.00^{bB}	1.95±0.02^{cD}
120	2.81±0.02^{aBC}	2.71±0.01^{cC}	3.42±0.03^{bA}	2.91±0.02^{bB}	2.85±0.03^{cB}	2.52±0.03^{cCD}	2,93±0.02^{bB}	2.24±0.01^{bD}
150	2.84±0.01^{aC}	2.98±0.01^{bC}	3.46±0.01^{bA}	3.60±0.02^{aA}	3,18±0.02^{bB}	3,13±0.02^{bB}	2.95±0.03^{bC}	2.36±0.03^{bD}
180	2.86±0.02^{aD}	3.16±0.00^{aCD}	3.87±0.01^{aB}	3.65±0.00^{aC}	3.44±0.02^{aC}	5.52±0.03^{aA}	3.09±0.07^{aCD}	3.63±0.03^{aC}

The same letters in the same column indicate that the values are statistically insignificant (P>0.05).
The same capital letters in the same row indicate that the values are statistically insignificant (P>0.05).

Terebinth coffee color values

The average L*, a*, and b* values of menengiç coffees are presented in Tables 7, 8, and 9.

The L values in the samples range between 30 and 40, giving the samples a brownish color due to the characteristics of terebinth coffee. Naturally, this is also reflected in the L value. Additionally, while samples prepared with milk exhibited higher L* values at the beginning of storage, they reached almost the same level as samples prepared with water by the end of 6 months of storage (Table 7). It was determined that a value was above 5 in the menengiç coffee samples prepared with both water and milk. An increase in the values recorded on the 30th day was observed in the samples compared to the 0th day, which was attributed to the interaction between menengiç and water and milk. No significant change was observed in the following days (Table 8).

While samples prepared with water initially exhibited significantly lower values than those prepared with milk, by the 30th and 60th days of storage, all samples reached the highest values in their respective series. Although a general decrease in b values was observed from the 60th to the 150th day of storage, an increase was noted again on the 150th day. On the 180th day of storage, a significant decrease was observed in all samples, reaching the lowest values since the beginning of storage (Table 9).

Table 7. Terebinth coffee L* values

Days	SS24	SS4	SK24	SK4	STK24	STK4	STS24	STS4
0	33.17±0.07^{bcB}	33.17±0.07^{bB}	33.17±0.07^{abB}	33.17±0.07^{bcB}	38.55±0.02^{aA}	38.55±0.02^{aA}	38.55±0.02^{aA}	38.55±0.02^{aA}
30	32.91±0.02^{cC}	31.02±0.03^{cD}	34.45±0.02^{abB}	31.59±0.02^{dD}	35.38±0.02^{bAB}	33.43±0.12^{cBC}	36.43±0.07^{bcA}	32.49±0.03^{cC}
60	32.75±0.02^{cBC}	30.47±0.02^{cD}	32.83±0.02^{bBC}	30.35±0.01^{eD}	33.61±0.02^{cB}	32.44±0.07^{dBC}	36.33±0.04^{bcA}	32.00±0.00^{cC}
90	35.83±0.01^{aAB}	35.99±0.03^{aAB}	35.85±0.04^{aAB}	36.65±0.03^{aA}	35.83±0.04^{bAB}	34.32±0.02^{bB}	37.11±0.01^{bA}	34.44±0.04^{bB}
120	32.12±0.03^{cE}	30.17±0.03^{cF}	30.94±1.38^{cF}	33.19± 0.02^{bcD}	35.75±0.02^{bB}	31.10±0.02^{eF}	36.91±0.03^{bcA}	34.31±0.02^{bC}
150	32.09±0.01^{cA}	30.15±0.02^{cB}	31.70±1.69^{bcB}	32.63±0.04^{cdA}	32.52±0.02^{dA}	31.04±0.03^{eB}	32.13±0.02^{eA}	31.86±0.07^{dB}
180	34.23±0.01^{bAB}	33.30±0.00^{bB}	33.56±0.04^{abB}	34.07±0.00^{bAB}	35.17±0.02^{dA}	33.42±0.02^{cB}	33.43±0.04^{dB}	34.44±0.00^{bAB}

The same letters in the same column indicate that the values are statistically insignificant (P>0.05).
The same capital letters in the same row indicate that the values are statistically insignificant (P>0.05).

Table 8. Terebinth coffee a* values

Days	SS24	SS4	SK24	SK4	STK24	STK4	STS24	STS4
0	5,23±0.02^{cB}	5,23±0.02^{cB}	5,23±0.02^{cB}	5,23±0.02^{cB}	6.13±0.02^{bA}	6.13±0.02^{bA}	6.13±0.02^{cA}	6.13±0.02^{bA}
30	7.61±0.01^{aB}	7.23±0.02^{aBC}	7.79±0.01^{aB}	7.04±0.04^{aC}	8.10±0.02^{aA}	7.44±0.00^{aB}	8.07±0.00^{aA}	7.65±0.02^{aB}
60	7.75±0.02^{aA}	7.54±0.01^{aA}	7.84±0.02^{aA}	7.82±0.09^{aA}	7.85±0.01^{aA}	6.05±0.03^{bB}	7.55±0.01^{bA}	7.52±0.03^{aA}
90	6.68±0.09^{bA}	6.76±0.03^{bA}	6.96±0.04^{bA}	6.92±0.02^{bA}	6.52±0.03^{bA}	5.95±0.00^{bB}	6.72±0.02^{cA}	6.67±0.02^{bA}
120	6.52±0.03^{bA}	6.43±0.01^{bA}	6.37±0.03^{bA}	6.37±0.03^{bA}	6.47±0.00^{bA}	5.86±0.02^{bB}	6.50±0.00^{cA}	6.61±0.01^{bA}
150	7.34±0.01^{aA}	5.59±0.00^{cC}	6.22±0.02^{bB}	6.22±0.02^{bB}	6.38±0.00^{bB}	6.14±0.04^{bB}	7.25±0.02^{bA}	6.53±0.03^{bB}
180	6.63±0.04^{bA}	5.66±0.02^{cB}	6.14±0.00^{bA}	6.24±0.02^{bA}	6.44±0.02^{bA}	6.25±0.02^{bA}	6.53±0.01^{cA}	6.61±0.02^{bA}

The same letters in the same column indicate that the values are statistically insignificant (P>0.05).
The same capital letters in the same row indicate that the values are statistically insignificant (P>0.05).

Table 9. Terebinth coffee b* values

Days	SS24	SS4	SK24	SK4	STK24	STK4	STS24	STS4
0	6.76±0.08^{dB}	6.76±0.08^{eB}	6.76±0.08^{fB}	6.76±0.08^{eB}	10,91±0.01^{dA}	10,91±0.01^{bcA}	10,91±0.01^{eA}	10,91±0.01^{cdA}
30	14.22±0.03^{aB}	13.1±0.01^{aC}	14.91±0.01^{aB}	12.19±0.02^{aD}	16.59±0.07^{aA}	14.00±0.05^{aB}	16.06±0.00^{aA}	13.19±0.00^{aC}
60	13.01±0.02^{bB}	12.47±0.00^{bBC}	13.76±0.02^{bB}	11.89±0.04^{aC}	15.15±0.00^{bA}	11.50±0.02^{bC}	15.02±0.03^{bA}	12.33±0.04^{bBC}
90	10.34±0.04^{cB}	10.07±0.04^{cB}	10.19±0.08^{dB}	10.14±0.09^{bB}	12.33±0.04^{cA}	10.11±0.01^{cB}	12.21±0.01^{dA}	10.16±0.02^{cdB}
120	10.09±0.01^{cC}	10.04±0.02^{cC}	11.27±0.00^{cB}	9.32±0.00^{cD}	12.20±0.02^{cA}	9.47±0.04^{dD}	12.11±0.01^{dA}	10.00±0.00^{cdC}
150	13.34±0.07^{bB}	12.11±0.01^{bC}	11.56±0.00^{cD}	10.95±0.01^{bE}	13.45±0.02^{cB}	11.15±0.04^{bD}	14.61±0.01^{cA}	11.25±0.02^{cD}
180	9.69±0.02^{cA}	9.13±0.04^{cA}	8.21±0.02^{eB}	8.76±0.02^{dB}	9.63±0.04^{eA}	9.86±0.02^{cdA}	9.92±0.02^{fA}	9.88±0.20^{dA}

The same letters in the same column indicate that the values are statistically insignificant (P>0.05).
The same capital letters in the same row indicate that the values are statistically insignificant (P>0.05)

Antioxidant capacities and phenolic substance amounts of Terebinth coffees

The antioxidant capacities of menengiç coffees are presented in Table 10, while the amounts of phenolic substances are shown in Table 11. The antioxidant capacities of the samples decreased over the storage period. This decrease is attributed to reactions occurring within the product over time and under varying storage conditions. As Cemeroğlu (2007) stated, antioxidant substances break down over time and temperature, leading to a decrease in their antioxidant capacity. Similarly, Zor (2007) found a decrease in antioxidant capacity in mulberry molasses stored at room temperature, depending on the storage duration (Table 10).

While no significant decrease was observed in the total phenolic substance values of the samples during the initial storage periods, it was determined that the total phenolic substance content decreased in parallel with the storage duration, and this decrease was statistically significant. The total phenolic substance amounts were 361.65 (mg gallic acid/g) in the samples prepared with water and 336.77 (mg gallic acid/g) in the samples prepared with milk at the beginning; however, these values decreased to 308.76 (mg gallic acid/g) and 302.79 (mg gallic acid/g) at the end of storage. Similarly, in the study conducted by Fedai (2018) on beverages, it was reported that there was a decrease in the total phenolic content in beverages prepared according to different formulations in parallel with the storage period (Table 11).

Table 10. Antioxidant capacities of terebinth coffees (mg TEAC/g)

Days	SS24	SS4	SK24	SK4	STK24	STK4	STS24	STS4
0	292.96±3.54^{aA}	292.96±3.54^{aA}	292.96±3.54^{aA}	292.96±3.54^{aA}	294.81±2.26^{aA}	294.81±2.26^{aA}	294.81±2.26^{aA}	294.81±2.26^{aA}
30	283.93±3.55^{bAB}	277.05±0.74^{bB}	287.91±2.19^{aA}	278.75±2.26^{abB}	288.49±3.35^{bA}	285.77±0.51^{bAB}	291.66±4.87^{bcA}	290.49±4.13^{cA}
60	277.52±0.78^{bB}	268.53±0.79^{cC}	283.13±3.62^{abAB}	277.29±4.07^{bB}	283.61±2.82^{cAB}	282.18±2.84^{bAB}	286.37±3.04^{bcA}	285.06±2.47^{cA}
90	265.25±0.55^{cBC}	260.07±0.52^{dcC}	273.77±5.07^{cB}	274.63±3.57^{bB}	279.07±5.31^{bA}	276.3.5±0.51^{cAB}	278.44±3.25^{bAB}	281.73±4.34^{bA}
120	262.71±3.62^{dcC}	252.67±3.49^{deD}	263.37±2.10^{cdC}	272.55±2.18^{bB}	277.88±4.02^{bA}	266.33±1.58^{cC}	276.51±4.22^{bcA}	278.45±3.22^{bA}
150	251.70±2.16^{dcAB}	247.68±2.09^{efB}	251.44±1.10^{deB}	264.76±6.44^{bcA}	-	-	-	-
180	251.76±2.26^{dAB}	241.21±1.42^{fC}	243.77±5.16^{eC}	257.05±3.64^{cA}	-	-	-	-

The same letters in the same column indicate that the values are statistically insignificant (P>0.05).
The same capital letters in the same row indicate that the values are statistically insignificant (P>0.05).

Table 11. Phenolic substance amounts terebinth coffees (mg gallik asit/g)

Days	SS24	SS4	SK24	SK4	STK24	STK4	STS24	STS4
0	361.65±2.19^{aA}	361.65±2.92^{aA}	361.65±2.92^{aA}	361.65±2.92^{aA}	336.77±1.93^{aB}	336.77±1.93^{aB}	336.77±1.93^{aB}	336.77±1.93^{aB}
30	363.57±4.85^{aA}	353.01±3.80^{bAB}	363.88±0.82^{aA}	354.69±2.61^{bAB}	335.92±2.02^{aB}	324.15±1.40^{bB}	331.43±3.07^{abB}	328.49±4.58^{bB}
60	353.16±4.21^{abA}	353.01±3.80^{bA}	353.59±3.71^{abA}	343.16±1.50^{cA}	333.67±1.86^{aC}	322.17±2.75^{bCD}	325.33±2.44^{bCD}	321.26±2.36^{cCD}
90	346.33±0.08^{bA}	336.85±0.95^{cAB}	345.26±2.29^{bA}	334.89±3.62^{dAB}	324.93±3.54^{bB}	311.30±1.64^{cC}	317.19±1.51^{cBC}	318.44±3.88^{cdBC}
120	333.08±3.58^{cA}	329.10±2.29^{cdA}	333.59±3.71^{cA}	327.20±2.77^{eA}	315.75±02.28^{cB}	302.79±3.70^{dC}	309.28±3.03^{dBC}	314.31±4.02^{dB}
150	322.15±2.75^{cA}	328.85±1.27^{cdA}	328.18±1.44^{cA}	318.42±2.85^{fA}	-	-	-	-
180	308.76±1.97^{dBC}	319.14±1.42^{dA}	304.46±2.38^{dC}	314.80±1.50^{fAB}	-	-	-	-

The same letters in the same column indicate that the values are statistically insignificant (P>0.05).
The same capital letters in the same row indicate that the values are statistically insignificant (P>0.05).

Fatty acid compositions of terebinth coffees

The fatty acid compositions of menengiç coffee are presented in Table 12. As seen in the table, the amounts of palmitic acid in all samples decreased slightly by the end of the 180th day of storage compared to the first day, and this decrease was found to be statistically significant. A similar trend was also observed in the amounts of linoleic acid. It was determined that there was an increase in the oleic acid amounts of the samples compared to the raw material, and the same trend was noted between day 0 and day 180. No significant change was observed in the other fatty acids. It is thought that the aforementioned changes are due to the reactions that occur in the structure of the samples over the storage period. Özcan (2004) reported the fatty acid compositions in menengiç fruits as follows: oleic acid 52.3%, palmitic acid 21.3%, linoleic acid 19.7%, palmitoleic acid 3.4%, stearic acid 2.0%, linolenic acid 0.6%, eicosanoic acid 0.1%, myristic acid 0.1%, and lauric acid 0.1%. Kaya (2012) found the fatty acid compositions in menengiç fruits from the Elazığ region to be: oleic acid 45.4%, palmitic acid 24.66%, linoleic acid 24.16%, saturated fatty acids 26.28%, and unsaturated fatty acids 73.72%. Sidar (2011) found that as the fruits of the terebinth ripen, the amount of linolenic acid decreases while the amount of oleic acid increases. The amounts of fatty acids found in raw terebinth fruit were determined as follows: lauric acid 0.1%, myristic acid 0.1%, palmitic acid 21.1%, palmitoleic acid 3.1%, stearic acid 2.0%, oleic acid 55.7%, linoleic acid 16.8%, linolenic acid 0.7%, eicosanoic acid 0.2%, eicosenoic acid 0.3%, and behenic acid 0.1%.

Table 12. Fatty acid composition of terebinth coffee (%)

Fatty acids	Day	SS24	SS4	SK24	SK4
Myristic acid (C14:0)	0	0.077±0.01	0.077±0.01	0.077±0.01	0.077±0.01
	180	0.129±0.02	0.078±0.02	0.073±0.03	0.075±0.01
Palmitic acid (C16:0)	0	22.121±0.02	22.121±0.02	22.121±0.02	22.121±0.02
	180	21.725±0.01	21.879±0.02	21.87±0.02	21.853±0.03
Palmitoleic acid(C16:1)	0	3.022±0.01	3.022±0.01	3.022±0.01	3.022±0.01
	180	2.903±0.01	2.917±0.02	2.944±0.02	3.021±0.01
Heptadecanoic acid (C17:0)	0	0.129±0.03	0.129±0.03	0.129±0.03	0.129±0.03
	180	0.204±0.01	0.124±0.02	0.120±0.02	0.125±0.01
cis-10-Heptadecanoic acid (C17:1)	0	0.077±0.01	0.077±0.01	0.077±0.01	0.077±0.01
	180	0.073±0.01	0.078±0.02	0.072±0.03	0.078±0.03
Stearic acid (C18:0)	0	2.269±0.02	2.269±0.02	2.269±0.02	2.269±0.02
	180	2.599±0.01	2.350±0.01	2.221±0.01	2.472±0.02
Oleic acid(C18:1n9c)	0	48,223±0.03	48,223±0.03	48,223±0.03	48,223±0.03
	180	47.727±0.02	48.843±0.01	48.78±0.02	48.378±0.02
Linoleic acid(C18:2n6c)	0	23.138±0.02	23.138±0.02	23.138±0.02	23.138±0.02
	180	22.8±0.03	22.821±0.02	22.900±0.02	23.005±0.01
Arachidic acid (C20:0)	0	0.051±0.03	0.051±0.03	0.051±0.03	0.051±0.03
	180	0.279±0.03	0.069±0.02	0.073±0.02	0.047±0.03
Cis-11-eicosatrienoic acid (C20:1)	0	0.101±0.01	0.101±0.01	0.101±0.01	0.101±0.01
	180	0	0	0.100±0.01	0.090±0.01
linolenic acid (C18:3n6)	0	0.794±0.02	0.794±0.02	0.794±0.02	0.794±0.02
	180	0.886±0.03	0.100±0.01	0	0.732±0.03
Erucic acid (C22:1n9)	0	0	-	-	-
	180	0.114±0.01	-	-	-
Nervonic acid (C24:1)	0	0	-	-	-
	180	0.496±0.02		-	-
Undecanoic acid (C11:0)	0	0	-	-	-
	180	0.032±0.02		-	-
Tricosanoic acid (C23:0)	0		0	-	-
	180		0.054±0.01	-	-
Caproic acid (C6:0)	0	-	-	0	-
	180	-	-	0.040±0.01	-
Myristoleic acid (C14:1)	0	-	-	0	-
	180	-	-	0.035±0.02	-
Nervonic acid(C24:1)	0	-	-	0[b]	-
	180	-	-	0.065±0.02	-

Phenolic compound composition of terebinth coffees

The phenolic compound compositions of terebinth coffees are provided in Table 13. In these samples, 25 phenolic compounds were detected, with 18 of them identified. Luteolin, ellagic acid, gallic acid, and fumaric acid were predominantly found among the phenolic compounds in the samples. In a study conducted in Elazığ, Dinç (2012) identified the phenolic compounds in Pistacia terebinthus fruit as follows: resveratrol at 373.5 ppm, vanillic acid at 219.167 ppm, caffeic acid at 154.5 ppm, quercetin at 156.83 ppm, and ferulic acid at 9.667 ppm. The phenolic compounds in Pistacia terebinthus coffee were determined to be: resveratrol at 295.41 ppm, vanillic acid at 179.5 ppm, caffeic acid at 101.5 ppm, quercetin at 129.9 ppm, and ferulic acid at 3.5 ppm.

Durak and Uçak (2015) investigated the antioxidant, antimicrobial, fatty acid, and solvent optimization of melon extract. In this research, 12 different samples were used. The total phenolic compound contents of the samples were determined to be 17.629, 12.564, 16.612, 14.184, 18.559, 13.841, 13.627, 16.222, 12.189, 17.330, 26.118, and 36.392 mg GAE/1000 g extract. The antioxidant activities measured were 15.68%, 9.23%, 16.83%, 12.75%, 14.19%, 12.85%, 16.20%, 18.37%, 8.86%, 23.36%, 46.12%, and 64.43% (% DPPH inhibition). As a result of the study, it was determined that antioxidant activity and phenolic compounds were closely related.

Table 13. The phenolic compound compositions of terebinth coffees (ppb)

Phenolic substance	Day	SS24	SS4	SK24	SK4
Catechin hyrate	0	1977.99±0.02	1977.99±0.02	1977.99±0.02	1977.99±0.02
	180	1232.27±0.03	772.73±0.02	434.45±0.02	585.16±0.01
Acetohyroxamicacid	0	31.26±0.01	31.26±0.01	31.26±0.01	31.26±0.01
	180	64.61±0.02	47.11±0.02	51.77±0.02	31.18±0.01
Vanillic acid	0	1449.00±0.01	1449.00±0.01	1449.00±0.01	1449.00±0.01
	180	2541.93±0.03	2375.22±0.03	2054.72±0.03	2320.53±0.02
Resveratrol	0	0	0	-	-
	180	318.18±0.02	321.26±0.01	-	-
Fumaric acid	0	3901.84±0.02	3901.84±0.02	3901.84±0.02	3901.84±0.02
	180	3481.40±0.02	4769.65±0.01	2520.62±0.01	4088.086±0.01
Gallic acid	0	3901.84±0.02	3901.84±0.02	3901.84±0.02	3901.84±0.02
	180	4599.54±0.03	4476.43±0.03	3757.99±0.01	4117.43±0.01
Caffeic	0	54.513±0.01	54.513±0.01	54.513±0.01	54.513±0.01
	180	105.26±0.02	71.37±0.01	63.43±0.03	0
Phloridzindyhrate	0	500.66±0.03	500.66±0.03	500.66±0.03	500.66±0.03
	180	0	476.05±0.02	0	0
Oleuropein	0	-	-	-	-
	180	-			
Hydoxycinamic	0	-	-	1098.78±0.02	-
	180	-		0	
Ellagic acid	0	11806.11±0.02	11806.110±0.02	11806.110±0.02	11806.110±0.02
	180	2674.70±0.01	3968.88±0.03	0	3431.18±0.01
Myricetin	0	-	-	-	-
	180				
Prtcatechuic	0	-	-	-	-

	180				
Silymarin	0	687.62± 0.02	-	-	-
	180	0			
2-hyroxy1,4nph	0	-	-	-	-
	180				
Butein	0	602.08± 0.02	602.08± 0.02	602.08± 0.02	602.08± 0.02
	180	495.86± 0.03	253.39± 0.02	140.47± 0.01	243.53± 0.03
Naringenin	0	-	-	-	-
	180				
Luteolin	0	30921.58±0.01	30921.58±0.01	30921.58±0.01	30921.58±0.01
	180	15857.05±0.03	17457.20±0.02	2563.06 ±0.03	11483.22±0.02
Kaempferol	0	-	-	-	0
	180				1561.65±0.03
Curmin	0	-	-	-	-
	180				
Thymoquinone	0	-	-	-	-
	180				
Alizarin	0	-	-	-	-
	180				
Hydroxyben	0	79.37± 0.02	79.37± 0.02	79.37± 0.02	79.37± 0.02
	180	83.65± 0.01	105.43± 0.01	50.86± 0.02	92.95± 0.00
Salicylic acid	0	6,60± 0.01	-	-	0
	180	0			11.00± 0.01
Quercetin	0	1558,31±0.02	1558,31±0.02	1558,31±0.02	1558,31±0.02
	180	684,05± 0.03	454,25± 0.03	540,79± 0.03	414,65± 0.01

Conclusion and recommendations

During storage, the pH value of the samples decreased while the free fatty acid values increased. These changes did not have any negative effects on the taste characteristics of the samples.

It was determined that the antioxidant capacity of the menengiç coffees was very high due to the presence of menengiç, and although there was a slight decrease in the antioxidant capacity of the prepared coffees during the storage period, the values remained relatively high. Therefore, menengiç coffees could be a good source of antioxidants. It was determined that oleic, linoleic, and palmitic fatty acids were the dominant fatty acids in menengiç coffees, while alpha-pinene, limonene, and β-ocimene were identified as the most abundant essential oils. In light of the obtained data, it was concluded that menengiç coffee, especially when prepared with water, can be consumed and stored as a ready-to-drink product. This practical option can be easily purchased and used by consumers. Furthermore, such a functional product holds significant potential for both introducing a new offering to the food industry and contributing to health benefits.

Ready-to-drink menengiç coffees will be highly appreciated by consumers. However, due to flocculation in coffees prepared with milk, more detailed studies should be conducted on this subject. Considering the recent increase in demand for functional, healthy, and practical products, the production of ready-to-drink menengiç coffee, which is typically difficult to prepare, will not only introduce a new product to the food industry but also add value to the economy. Increasing research in this area will be beneficial for introducing such functional and traditional products to the food industry and offering new flavors to consumers.

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Research Article/Araştırma Makalesi

Pickering emulsions from rice protein-xanthan gum nanoparticles at different oil content: emulsion properties and using producing cake as a fat replacer

Farklı yağ içeriklerinde pirinç proteini-ksantan zamkı nanopartiküllerinden Pickering emülsiyonları: emülsiyon özellikleri ve yağ ikame maddesi olarak kek üretiminde kullanımı

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ABSTRACT

In the present study, Pickering emulsions with different oil content (15%(PE15),30 (PE30), 45(PE45), and 60 (PE60)) were created with nanoparticles produced from rice protein isolate (RPI) and xanthan gum (XG). The aim was to produce cakes with reduced oil content with these emulsions. For this purpose, firstly the emulsion properties were evaluated. Emulsion activity (EAI)-stability indexes (ESI), ζ-potentials, and nanoparticle structures of the emulsions were investigated. The EAI value was determined as 54.14±3.19 m^2/g and 54.15±0.95 m^2/g for the emulsions containing 15% and 30 (w/w) oil, respectively, while the lowest EAI value was determined as 30.12±0.89 m^2/g for the emulsion containing 60% oil. While the ζ-potential value decreased with increasing oil concentration, oil globule diameters increased. Pickering emulsions with 15%, 30, 45, and 60 oil (C-PE15, C-PE30, C-PE45, and C-PE60) and a control sample were produced with oil. The features of cakes made using emulsions with different oil contents were examined, including measuring the viscosity of batter, baking loss, symmetry index, moisture content, ash content, and sensory analysis. When viscosity values are examined, it can be said that the lowest value was generally recorded in the cake batter prepared with PE15. The pH values decreased as the oil content in the cake batter increased. Baking loss and symmetry index did not significantly differ ($p>0.05$) between cakes made with emulsion and control. The decrease in the oil ratio in the emulsion and the increase in the RPI-XG nanoparticle solution ratio increased moisture. As a result, RPI-XG nanoparticles are a suitable material for producing Pickering emulsion. Additionally, cakes can be made with the emulsions that are formed. For product compositions with minimal oil content, the usage of PE15 emulsion can be suggested.

Key Words: rice protein; xanthan gum; nanoparticle; Pickering emulsion; reduced fat cake

ÖZ

Pirinç protein izolatı (RPI) ve ksantan gum (XG) kullanılarak üretilen nanopartiküller ile farklı yağ oranlarına sahip (%15(PE15),30 (PE30), 45(PE45) ve60 (PE60)) Pickering emülsiyonlar oluşturulmuştur. Üretilen bu emülsiyonlar ile yağ oranı düşürülmüş kekler üretilmesi hedeflenmiştir. Bu amaçla ilk olarak emülsiyon özellikleri değerlendirilmiştir. Emülsiyonların emülsiyon aktivite-stabilite indeksleri (EAI-ESI), Zeta(ζ)-potansiyelleri ve partikül yapıları incelenmiştir. EAI değeri %15 va 30 (w/w) yağ içeren PE15 ve PE30

emülsiyonları için sırasıyla 54.14±3.19 m^2/g ve 54.15±0.95 m^2/g olarak belirlenirken en düşük EAI değeri 30.12±0.89 m^2/g olarak %60 yağ içeren PE60 emülsiyon için belirlenmştir. (ζ)-potansiyel değeri artan yağ konsantrasyonu ile birlikte azalırken yağ parçacık çapları artmıştır. Üretilen Pickering emülsiyonları, yağ yerine kek formülasyonunda kullanılmıştır. Bu amaçla, %15, 30, 45 ve 60 yağ içeren Pickering emülsiyonları (C-kontrol, C-PE15, C-PE30, C-PE45 ve C-PE60) kek üretmek için kullanılmıştır ve kontrol olarak sadece yağ içeren kek örneği hazırlanmıştır. Farklı yağ içeriklerine sahip emülsiyonlar kullanılarak yapılan keklerin özellikleri, hamurun viskozitesi, pişirme kaybı, simetri indeksi, nem içeriği ve kül içeriğinin ölçülmesi incelenmiştir. Viskozite değerleri incelendiğinde genel olarak en düşük değerin %15 yağ içeren emülsiyon ile hazırlanan kek hamurunda olduğu söylenebilir. Kek hamuru içerisinde ki yağ oranı arttıkça pH değerlerinin düştüğü görülmüştür. Pişirme kaybı ve simetri indeksi açısından kontrol formülasyonu ile emülsiyon bazlı kekler arasında kayda değer bir fark ($p>0,05$) olmamıştır. Emülsiyondaki yağ oranının azalması ve RPI-XG nanopartikül çözelti oranının artması nem değerlerini artırmıştır. Sonuç olarak, RPI-XG nanopartikülleri Pickering emülsiyonu üretmek için uygun bir malzemedir. Ek olarak, üretilen emülsiyonlar yağı azaltılmış kek üretimi için uygundur. Minimum yağ içeriğine sahip ürün kompozisyonları için PE15 emülsiyonunun kullanımı önerilebilir.

Anahtar Kelimeler: pirinç proteini; ksantan zamkı; nanopartikül; Pickering emülsiyon; yağı azaltılmış kek

Introduction

A solid-particle-stabilized emulsion rather than conventional organic surfactants is known as a Pickering emulsion. Pickering stabilizers made of natural and food-grade polymers are the subject of current research because of their food compatibility and improved resistance to coalescence and separation (Tu, Zhang et al. 2023). Since proteins have unique nutritional value and desired techno-functional qualities, they are used to create stable food-grade Pickering emulsions. Plant protein nanoparticles attracted far more attention than animal protein because of their plentiful and reasonably priced source (Shi, Feng et al. 2020). However, the stabilizing capacity of protein-based particles is limited by their propensity to agglomerate at the interface. The emulsification ability of polysaccharide-based particles is limited due to their high hydrophilia, which is caused by the abundance of hydroxyl groups on their surface (Wu, Tang et al. 2022). Compared with single polysaccharide particles or single protein particles, protein-polysaccharide-formed particles have favorable surface activity, and strong spatial stabilization capacity at the same time (Xu, Li et al. 2023, Li, Wu et al. 2024). Considering this situation, protein-polysaccharide coacervates were formed in the present study to increase the effectiveness of Pickering emulsion. In previous studies, Pickering emulsions were created by obtaining rice protein gum arabic coesarvate (Igartúa, Dichano et al. 2024) and also by obtaining protein-polysaccharide-phenol complex from rice bran (Li, Wu et al. 2024). On the other hand, there are studies examining the effect of protein xanthan gum interaction on Pickering emulsions (Xu, Liu et al. 2023, Li, Wang et al. 2024). However, to the best of the authors' knowledge, there is no study in the literature on emulsions produced with RPI and XG coacervate.

One of the staple foods that is most commonly consumed worldwide is rice (Oryza sativa L.). Approximately 750 million tons of rice are produced worldwide, and 18,000 known kinds are farmed in more than 100 countries (Amagliani, O'Regan et al. 2017, Peanparkdee and Iwamoto 2019). Rice, which is considered the main source of protein, especially in developing and underdeveloped countries, contains approximately 7-9% protein in its endosperm part (Roy, Singh et al. 2023). In the food industry, plant proteins are widely used as emulsifiers in place of animal proteins (Xie, Ouyang et al. 2023). Rice protein is a viable source for the food industry due to its excellent physical-functional qualities, large availability, low production cost, and good quality (Moirangthem, Jenkins et al. 2020). Rice protein, produced from rice and rice by-products low in allergens and rich in nutrients, can be used to create emulsions (Xie, Huang et al. 2021).

The bacteria *Xanthomonas campestris* ferments carbohydrates aerobically to create XG, an anionic heteropolysaccharide (Krstonošić,

Dokić et al. 2015). Because it created a double helix structure. It works well as a stabilizer for emulsions and suspensions to increase system viscosity and stability (Xing, Chitrakar et al. 2022).

It was aimed to in the current study, form nanoparticles by utilizing the interaction between RPI and XG and to produce Pickering emulsions from the newly created solution. Pickering emulsions were produced at different oil concentrations and EAI, ESI, zeta potential values were measured and particle diameters were determined from optical microscope images of the emulsions. In addition, Pickering emulsions containing different oil content were added to the cake formulations in order to produce cakes with reduced oil content, and their effects on the physical properties of the cake batter and cake were investigated.

Material and methods

The rice protein (RPI) was purchased from Türkiye (Vegrano) and xanthan gum was purchased from Sigma Aldrich (St. Louis, MO). Protein isolate is 80% protein by weight. For the analyses, every chemical and chemical reagent used was of analytical grade. Sterilized whole milk was used in the production of cake products. Milk, sunflower oil and corn starch were purchased from a local grocery store.

Preparation of RPI and XG solution

RPI solutions were prepared in accordance with earlier research (Wu, Tang et al. 2022) with some modifications. To create 2% (w/v) RPI dispersions, RPI was first dissolved in distilled water while being constantly stirred at 25 °C for 30 minutes. Then, the protein solution heating were used to cause protein unfolding for 30 minutes of 90 °C. The solutions were then cooled to 25 °C.

The XG solutions were prepared according to previous literature (Matsuyama, Kazuhiro et al. 2021) with some modifications. 0.3% (w/v) XG solutions were heated to 80 °C while being continuously stirred until the solution completely dissolved. To guarantee proper mixing and hydration, the RPI solutions and the XG solutions were constantly mixed at a ratio of 1:1 (v/v) for three hours.

The average particle diameter of the prepared solution was measured using a Zetasizer (ZS90, Malvern Instruments, UK). The particle diameter of the produced solution was found to be 842.2 nm (Figure 1).

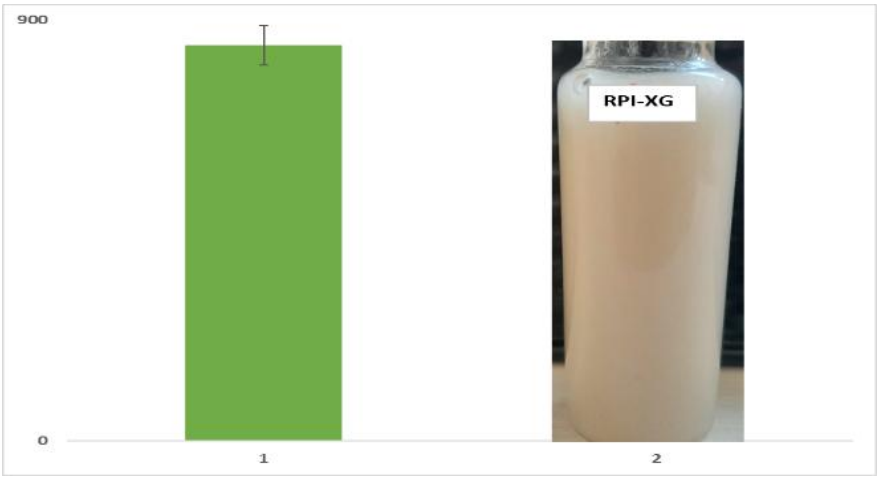


Figure 1. The particle size of the RPI-XG solution

Preparation of Pickering emulsion

The emulsion was prepared according to Xie, Lei et al. (2021) and Abbaszadeh, Aalami et al. (2023) with some modifications. The RPI-XG solutions were mixed with sunflower oil. To investigate the effect of sunflower oil volume fraction in emulsions on emulsifying behavior, oil was added at different ratios (15%, 30, 45, and 60 w/w) to make a total weight of 40 g. The sunflower oil content was determined based on preliminary experiments and literature (Xie, Lei et al. 2021). The mixture was homogenized at 11000

rpm/min for 3 minutes using a homogenizer (IKA-T18, Staufen, Germany). Emulsions are named PE15, PE30, PE45, and PE60 according to their oil content.

Determination of emulsifying properties

Emulsifying Activity Index (EAI) and Emulsion Stability Index (ESI) of emulsions were measured according to Sui, Bi et al. (2017) and Tang, Yang et al. (2024) with slight modifications. The freshly produced emulsions (20 µL) and then 4.98 mL of sodium dodeacyl sulfate (1% w/v) was mixed. The absorbance value of the mixture was measured at 500 nm. Equation following was used to calculate the EAI and ESI based on these measurements:

$$EAI\ \left(\frac{m^2}{g}\right) = \frac{2x2.303xA_oxN}{cx\emptyset xLx10^4} \quad (1)$$

$$\text{ESI (h)} = \frac{\text{A0 x t}}{(\text{A0} - \text{At})} \quad (2)$$

N stands for the dilution factor of emulsions and c (grams per milliliter) for the protein content in the protein aqueous solution. Φ, denotes the oil volume fraction in the original emulsions. L, is the thickness of the cuvette. A0, absorbance immediately after forming the emulsion and At=absorbance 6 h after the emulsion was formed.

ζ-potential

A Zetasizer (ZS90, Malvern Instruments, UK) connected to dynamic light scattering and electrophoresis apparatus was used to assess the ζ-potential of emulsions. The materials were diluted with distilled water (pH 7) before analysis.

Determination of microstructure

The morphological features of the emulsions were characterized using optical microscopy (Leica, DM500, USA) at a 10x magnification, enabling the examination of their structural characteristics on the first day. Next, using Image J software, the sizes of the emulsion droplets were measured in photos. The average droplet diameters and diameter distribution of emulsions were calculated using the Origin program (Thorne, Simkovic et al. 2019).

Cake batter preparation

The pound cake recipe used as reference batter was produced according to (Bedoya-Perales and Steel 2014) (Table 1). A Bosch mixer was used to produce the cake batter. The cakes produced with emulsions containing 15%, 30, 45 and 60 percent oil were named as C-PE15, C-PE30, C-PE45, and, C-PE60, respectively. The formulation is the same in all cakes except for the oil content (Table 2). Also, C-Control produced with only sunflower oil. In the first stage of batter production, eggs, and sugar were mixed at the highest speed of the mixer for 3 minutes in order to cream them and ensure sufficient aeration. In the 2nd stage, other ingredients (milk, emulsion/oil, wheat flour, corn starch, salt, and baking powder) were added and mixed for 2 minutes at the lowest speed. In the 3rd stage, the cakes were baked in the oven at 200 °C for 30 minutes and then cooled.

Table 1. Formulation used in the preparation of cake batter

Ingredients	Grams
Wheat flour	950
Corn starch	50
Whole milk (3% oil)	450
Liquid egg	500
Sugar	787,5
Fat (Sunflower oil, PE15, PE30, PE45, and PE45)	400
Baking powder	25
Salt	5

PE15, PE30, PE45, and, PE60 are Pickering emulsions produced with RPI-XG nanoparticles and 15%, 30%, 45%, and 60% sunflower oil respectively.

Table 2. Sample code and PE-oil content

	control	C-PE15	C-PE30	C-PE45	C-PE60
RP-XG (g)		340	280	220	160
Sunflower oil (g)	400	60	120	180	240

PE15, PE30, PE45, and, PE60 are Pickering emulsions produced with RPI-XG nanoparticles and 15%, 30, 45, and 60 sunflower oil respectively.

Determination of viscosity of cake batter

The viscosity of the cake batter was determined at room temperature (25°C) at varying rpm speeds with a viscometer (Thermo Scientific, HAAKE Viscometer-C, USA) with an L-04 type probe in the C-Control batter and in batters containing Pickering emulsions prepared with different oil ratios (Baltacıoğlu, Temzisoy et al. 2020).

Baking loss

The following equation was used to obtain the baking loss (%) throughout the baking process;

$$Baking\ loss\ (\%) = \frac{Wi-Wf}{Wi} x100 \quad (3)$$

where Wf is the weight of the baked cake after it has cooled to room temperature and Wi is the weight of the batter before baking (Grossi Bovi Karatay, Rebellato et al. 2022).

pH

A pH meter (Hanna Instruments, Italy) was used to measure the pH of the cake. 10 g of cake batter was homogenized in 100 mL of distilled water at 25 °C in order to measure the pH (Bedoya-Perales and Steel 2014, Baltacıoğlu and Uyar 2017).

Symmetry index

The cake symmetry index was computed according to Mustafa, He et al. (2018) with some modifications. To put it briefly, three slices that were positioned at one-quarter (B), one-half (C), and three-quarters (D) of the cake length each had their center height determined. The symmetry index was then determined on the day of production using Eq.4.

$$2xC - B - D \quad (4)$$

Moisture Content

About 3 g of the differently formulated cakes were placed in an oven (IN 160Plus Memmert, Germany) and dried at 105 °C for 3h (Grossi Bovi Karatay, Rebellato et al. 2022).

Sensory Analysis

Thirty semi-trained panelists, including faculty and students from Erciyes University's Food Engineering department, participated in the sensory evaluation of the cakes. White plates containing samples at room temperature were supplied. Each sample's colour, odor, texture, taste, and overall acceptability were assessed by the panelists. A five-point was used to rate the samples: 1 strong dislike,2 dislike, 3 neither a like nor a dislike, 4 like and 5 represented a strong like (Azadfar, Elhami Rad et al. 2023).

Statistical analysis

Using Minitab software (Minitab Ltd., Coventry, England), the ANOVA multiple comparison approach was used to statistically analyze the data collected for this study. Two replicates were studied and Tukey test was used for multiple comparisons of means.

Result and discussion

Emulsifying properties

It is usual practice to use the emulsifying activity index (EAI) and emulsion stability index (ESI) to assess ability to create and maintain an emulsion of protein (Tang, Yang et al. 2024). As seen in Figure 2, as the oil content of the emulsions increased, the EAI value decreased. The difference between the EAI values of PE15 (54.14±3.19 m^2/g) and PE30 (54.15±0.95 m^2/g) samples was found to be statistically insignificant ($p>0.05$). Similarly, there was no significant

difference between PE45 (36.16±3.34 m^2/g) and PE60 (30.12±0.89 m^2/g) samples ($p>0.05$). The highest value was recorded for PE15 and PE30. It's possible that the capacity to expand and disperse of proteins enhances the emulsification process by increasing spatial repulsion and preventing droplet agglomeration and emulsion precipitation (Tang, Yang et al. 2024). Accordingly, it can be said that high protein content improves the emulsion structure.

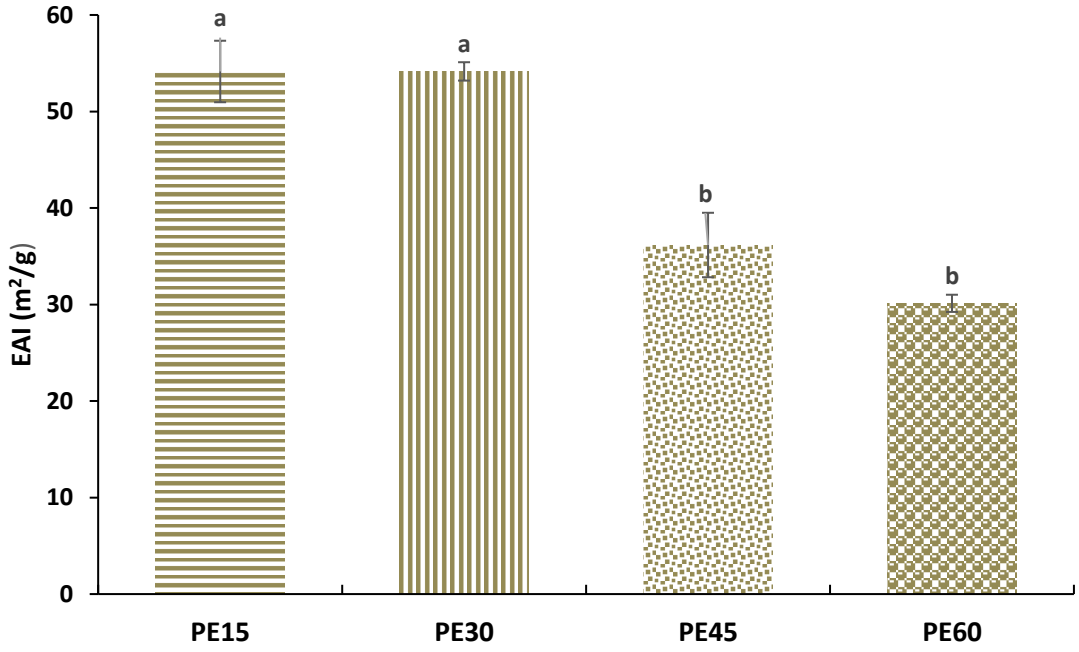


Figure 2. Emulsion activity index (EAI) of Pickering emulsion prepared with RPI-XG nanoparticles
Different letters indicate significant differences ($p\leq 0.05$) between means.
The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %.

ESI value is given in Figure 3. The highest stability index was measured as 31.71±0.87 h for PE60. While no difference ($p>0.05$) was observed between the ESI values of PE15 and PE45 samples, the lowest ESI was determined for PE30. The ESI value was found as 24.88±0.23, 16.06±0.58 h, 26.21±0.02 h, and 31.71±0.87 h for PE15, PE30, PE45, and PE60. The substantial rise in the ESI at 60% oil phase fraction may have resulted from an increase in the rate at which oil droplets accumulated, raising the viscosity of the emulsion and lowering the rate of fat lifting (Sun and Gunasekaran 2009). These findings suggest that the creaming of emulsions is primarily caused by an increase in the oil phase. Therefore, as the oil phase fraction increased, the packing percentage of oil droplets increased as well, improving emulsion stability. (Dickinson and Golding 1997).

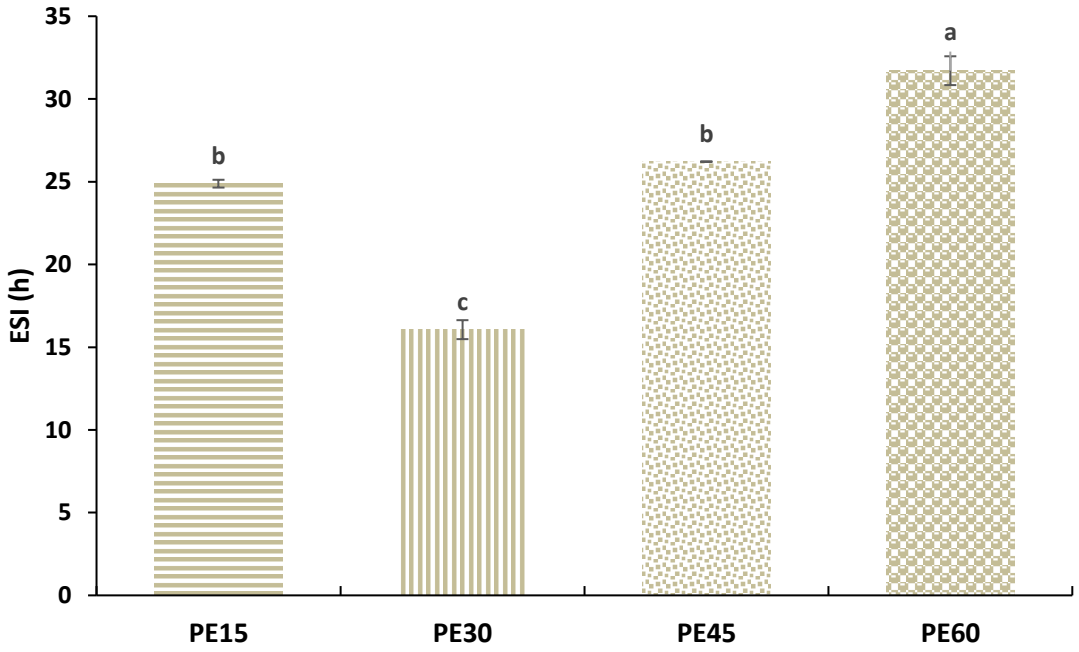


Figure 3. The emulsion stability index (ESI) of Pickering emulsion prepared with RPI-XG nanoparticles
Different letters indicate significant differences (p≤ 0.05) between means.
The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %.

ζ-potential

The electrostatic stability of particles is typically described using the ζ-potential, which represents the net charges on the particle surface and has a direct impact on the functional characteristics of proteins (López-Monterrubio, Lobato-Calleros et al. 2020). Since the pH of the emulsion was greater than the isoelectric point of the RPI, the ζ-potential of every emulsion was negative, allowing the protein to adsorb OH^- (Zhao, Wei et al. 2015). The highest ζ-potential value was recorded for PE15, the sample with the highest protein/oil ratio as -34.10±0.90 mV. A higher oil phase volume fraction necessitates more protein to reduce the surface tension because negatively charged protein can adsorb onto droplet surfaces and the effect of oil phase volume on surface charge is dependent on protein concentration (Sun and Gunasekaran 2009). Accordingly, while the amount of protein remained constant, the increase in the amount of oil decreased the ζ-potential. While the difference between the ζ-potential values of PE15 (34.10±0.90 mV), PE30 (32.88±0.92 mV), and PE45 (31.83±0.99 mV) samples was not significant, the PE60 (27.55±0.33 mV) emulsion was found to be statistically different (p≤0.05) from them.

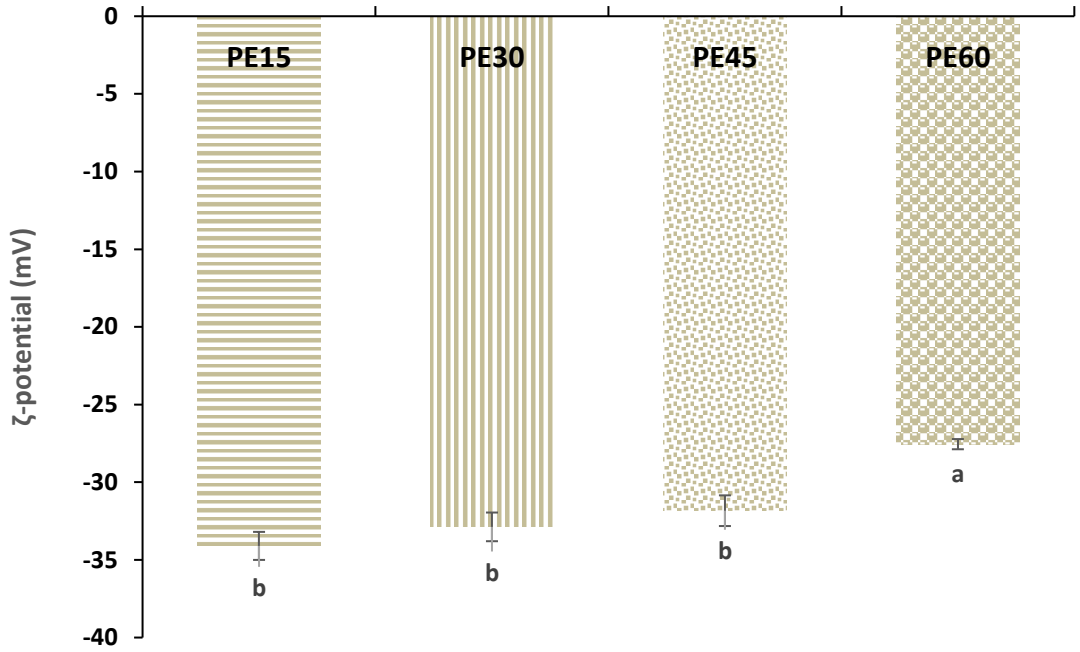


Figure 4. ζ-potential value of the Pickering emulsion prepared with RPI-XG nanoparticles.
Different letters indicate significant differences (p≤ 0.05) between means.
The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %.

Determination of microstructure

Digital photos and optical micrographs of Pickering emulsions stabilized by RPI-XG nanoparticles with varying oil phase volume percentages are displayed in Figure 5. The emulsion droplets had a spherical shape and were typically smaller than 30 µm. All of the emulsions displayed an unimodal droplet size distribution, and the droplet size progressively grew as the oil phase volume percentage increased. As the percentage of sunflower oil rose from 15% to 60%, the size distribution grew. Similar results have been reported by (Xie, Lei et al. 2021). The distribution of globule diameters and the average particle size determined by taking the average of measurements from two different images with the image J program were determined with the origin program. Accordingly, the average particle diameters of PE15, PE30, PE45 and PE60 were found to be 22.63±1.13, 23.18±1.89, 23.41±2.99, and 29.16±1.57 µm, respectively. As a result, it can be said that the size distribution of the emulsion is affected by the sunflower oil ratio. These findings demonstrated that, at a very low and consistent particle concentration, the Pickering emulsion system made with RPI-XG nanoparticles generated a greater oil-water interface as the oil fraction increased. Consequently, there were fewer particles accessible per unit of the oil-water interface, which led to the coalescence of oil droplets and an increase in droplet size (Song, Pei et al. 2015, Tan, Han et al. 2021). It is anticipated that as the oil phase volume fraction rises, the frequency of emulsion droplet collisions would rise as well, leading to faster flocculation (Soleimanpour, Koocheki et al. 2013).

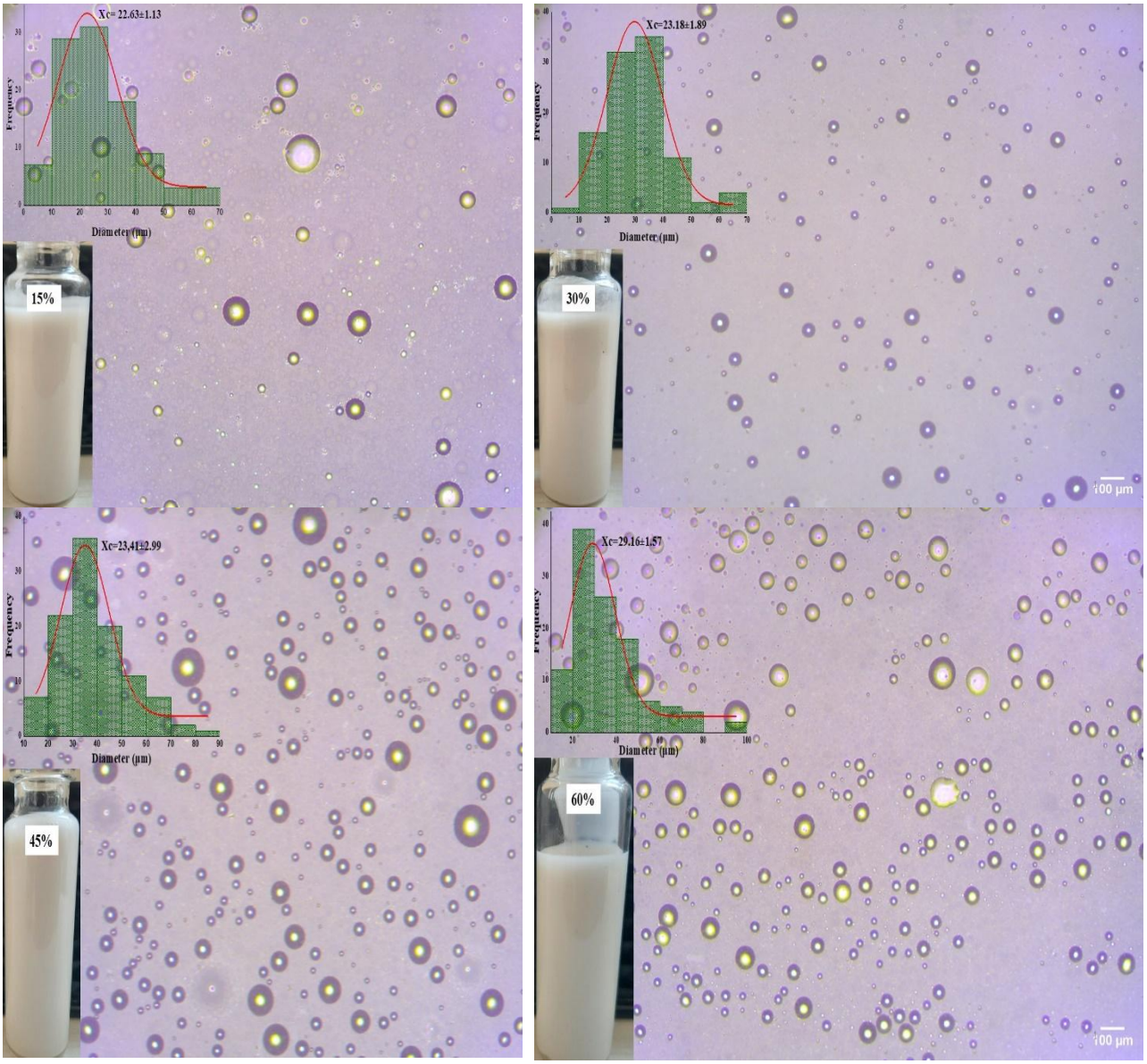


Figure 5. Micrographs of the emulsions stabilized with RPI-XG nanoparticul.
The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %. Inset: digital photos of the corresponding emulsions and the droplet size distribution of fresh emulsions with different oil phase volume fractions.

Cake batter viscosity

Since viscosity affects bubble production and the movement of bubbles upward owing to stability is inversely proportional to viscosity, it is known that the viscosity of the cake batter influences the final cake volume (Handleman, Conn et al. 1961). Viscosity values of cake batters prepared with Pickering emulsions containing different amounts of oil were measured at different rpm (Figure 6). In general, the lowest viscosity values were recorded in the C-PE15 cake batter prepared with PE15 emulsion. It was observed that the viscosity value increased as the amount of oil in the Pickering emulsion increased. Except for the results obtained at 5 rpm, the highest viscosity value was recorded in the control sample (C-control) batter produced using only sunflower oil. Lakshminarayan, Rathinam et al. (2006) reported that when the fat in the formulation was reduced, there was a decrease in batter viscosity and therefore cake volume. Bath, Shelke et al. (1992) said that the retention of leavening gas during baking was related to cake batter viscosity. Incorporating more air bubbles into the batter and preventing them from rising to the top are made possible by higher viscosities in cake batter, which gives the cake greater stability (Kumari, Jeyarani et al. 2011). The results showed that the dough containing 100% sunflower oil was more stable and there was a decrease in dough viscosity with decreasing oil content.

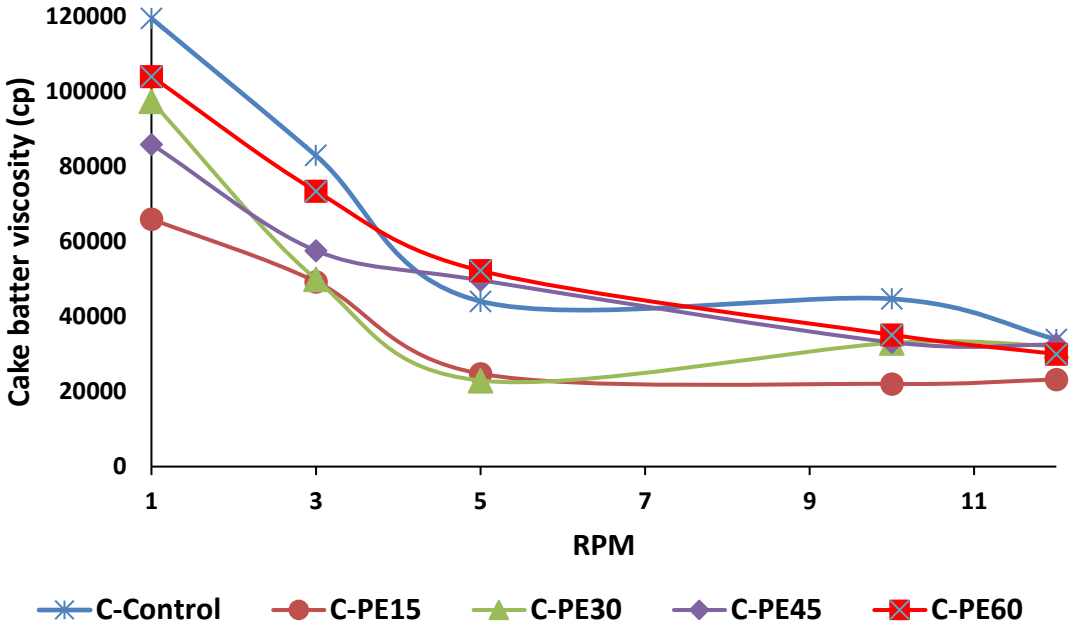


Figure 6. Viscosity of reduced fat cake batter with Pickering emulsions
C-control: the cake produced sunflower oil; C-PE15: the cake produced with PE15; C-PE30: the cake produced with PE30; C-PE45:the cake produced with PE45; C-PE60:the cake produced with PE60. The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %

Cake Properties

Photos taken from the side, top, and middle of cakes made with Pickering emulsions with different fat content are shown in Figure 7. It can be said that the surface color of the control sample is darker, but the bubble distribution is more homogeneous when looking at the internal structure. It was found that when the oil content of the Pickering emulsions added to the cakes increased, the pH value decreased, and the lowest pH value was recorded as 6.62 in the control sample (Table 3). This situation can be attributed to the high pH value of RPI-XG used in emulsion production. As the oil content in the mixture increased, the pH also decreased. In previous studies, it has been reported that cake pH values vary between 6 and 8 (Baik, Marcotte et al. 2000, Masoodi, Sharma et al. 2002). pH values 6.50 to 7.70 are considered good for cake batters because cake texture and color are correlated with pH (Bedoya-Perales and Steel 2014).

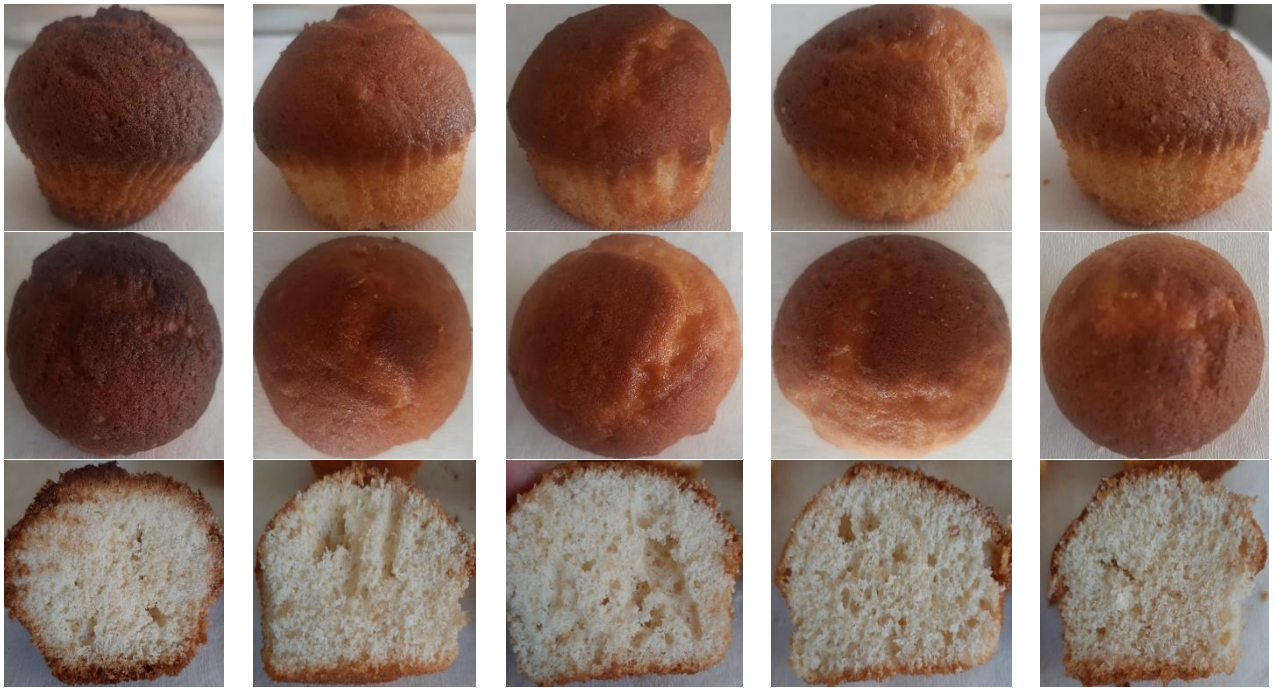
Table 3. Physical properties of reduced fat cakes produced with Pickering emulsion produced with RPI-XG nanoparticles

Sample	pH	Baking Loss (%)	Symmetry Index	Moisture (%)	Ash (%)
C-Control	6.62±0.01^{e}	15.12±1.00^{a}	7.0±1.00^{a}	18.23±0.82^{b}	1.30±0.00^{b}
C-PE15	6.86±0.01^{b}	12.40±2.78^{a}	7.5±0.50^{a}	25.08±2.36^{a}	1.51±0.05^{a}
C-PE30	6.89±0.00^{a}	15.53±3.02^{a}	7.5±0.50^{a}	26.09±3.30^{a}	1.42±0.00^{a}
C-PE45	6.83±0.01^{c}	15.53±3.02^{a}	6.5±0.05^{a}	18.33±1.36^{b}	1.42±0.01^{a}
C-PE60	6.78±0.01^{d}	13.93±2.91^{a}	7.0±1.50^{a}	19.39±0.88^{b}	1.29±0.06^{b}

Different letters indicate significant differences (p≤ 0.05) between means.
The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %. Inset: digital photos of the corresponding emulsions and the droplet size distribution of fresh emulsions with different oil phase volume fractions.

Baking loss, symmetry index, moisture, and ash parameters of cakes made with emulsions containing different oil content (Table 3) were determined. In terms of baking loss and symmetry index, there was no discernible difference (p>0.05) between the control formulation and emulsion-based cakes. When ash and moisture values are examined, it can be said that C-PE15 and C-PE30 cake samples are different from the control, C-PE45, and C-PE60. The decrease in the oil ratio in the emulsion and the increase in the RPI-XG nanoparticle solution ratio increased the moisture and ash values. The strong capacity of the hydrocolloids and the protein in the mixture to bind and retain water may be the cause of this (Azmoon, Saberi et al. 2021). Hydrocolloids and

proteins are thought to have a strong tendency to create hydrogen bonds with water molecules because they contain a large number of hydroxyl groups. As a result, more water molecules would be engaged (Kohajdová, Karovičová et al. 2009).



C- Control C-PE15 C-PE30 C-PE45 C-PE60

Figure 7. Photos of reduced-fat cake samples
C-control: the cake produced sunflower oil; C-PE15: the cake produced with PE15; C-PE30:the cake produced with PE30; C-PE45:the cake produced with PE45; C-PE60:the cake produced with PE60. The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %

Sensory analysis

Sensory acceptability and/or consumer preferences are crucial in assessing success of a product (Zarzycki, Wirkijowska et al. 2022). Figure 8a shows the average sensory evaluations of the control samples and the prepared cakes made by panelists with varying levels of training. Reducing the oil in the PE emulsion affected the taste and overall quality scores due to the overall oily taste of the cake. One of the most important factors affecting first appeal of baked goods is color. C-PE15 cakes enriched with 15% PE had the lowest color scores, while the control sample had the greatest values. As the oil content in the cake samples increased, the color scores also increased. However, the scores of the samples containing PE are very close but lower than the control sample. The C-PE15 cake samples had the lowest odor value. It can be said that there is no difference in odor score between the C-PE30, C-PE45 and C-PE60 samples. Similar situations are valid for texture and taste. The C-PE60 sample had the scores that were closest to the control sample when we looked at general acceptability. Again, C-PE15 received the lowest rating. Semi-trained panelists conducted evaluations on cake samples for overall acceptability (Figure 8b). The C-Control sample received a score of 3.97±1.00, while C-PE60, C-PE45, C-PE30, and C-PE15 samples received scores of 3.73±1.01, 3.87±0.87, 3.8±0.81, and 3.3±1.02, respectively. In terms of general appreciation, the acceptability values of the samples containing PE are close to each other. In general, it is noteworthy that there were no negative ratings in samples in which the oil content was reduced by creating PE, indicating that PE can be a good fat substitute in cakes because it can preserve the sensory properties of these baked goods even when the fat replacer is high.

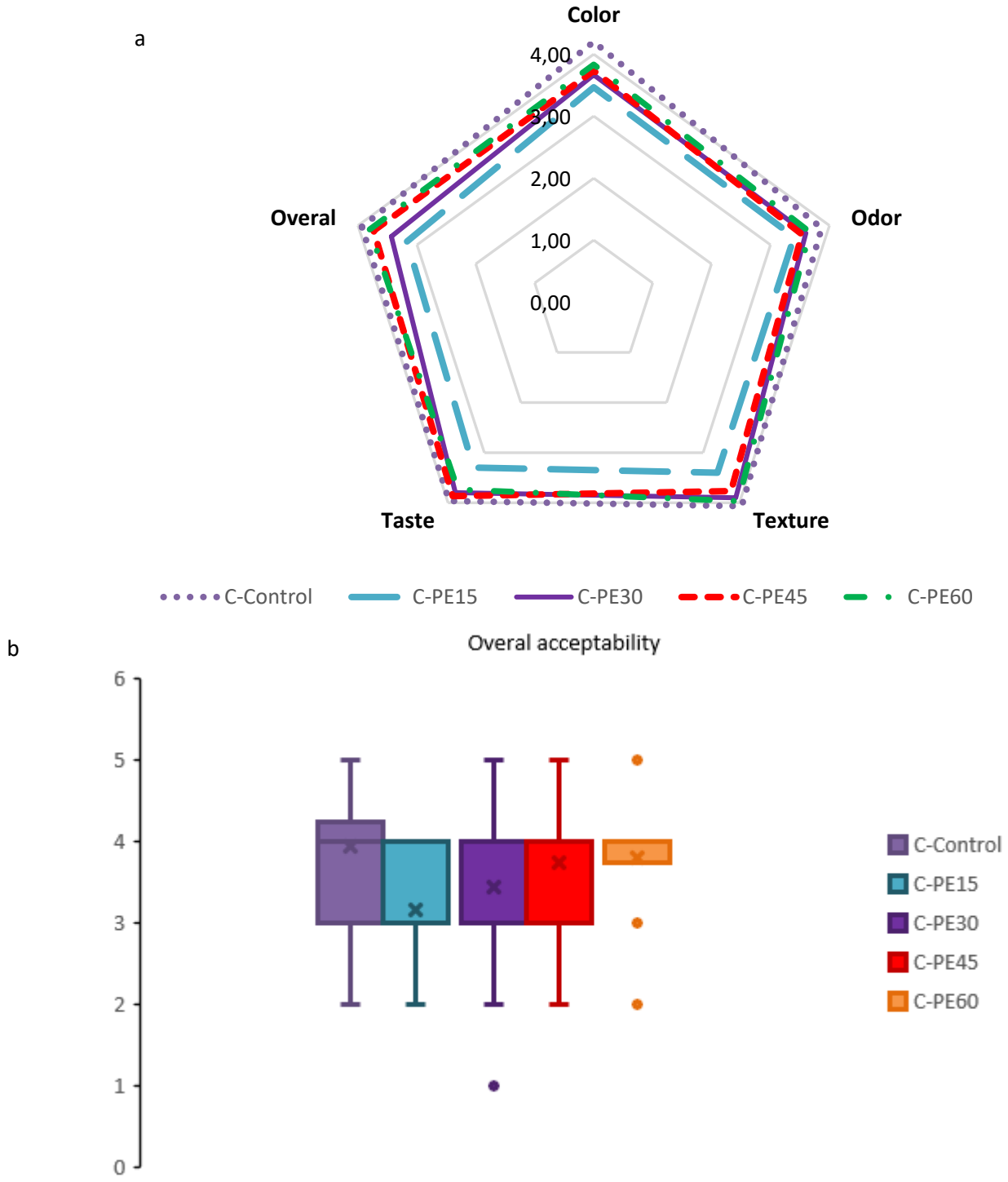


Figure 8. Sensory evaluations and overall acceptability of reduced-fat cake samples
C-control: the cake produced sunflower oil; C-PE15: the cake produced with PE15; C-PE30:the cake produced with PE30; C-PE45:the cake produced with PE45; C-PE60:the cake produced with PE60. The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %

Conclusion

In the present study, it was aimed to produce cake with reduced fat content using emulsions having different oil ratios (15%, 30, 45, and 60) produced from nanoparticles produced with RPI and XG. Firstly, the properties of emulsions produced with different oil ratios were evaluated. For this purpose, EAI-ESI and zeta potential were determined. The particle size of oil globules was measured by examining the microstructure of emulsions with an optical microscope. The highest value was recorded as 54.14±3.19 m^2/g for PE15 for EAI. As the oil content in the emulsion increased, a decrease in the EAI value was observed. ESI value increased with increasing oil phase ratio due to the increase in the packing ratio of oil droplets. The ζ-potential values of the samples were recorded for PE15, PE30, PE45, and PE60 as 34.10±0.90 mV, 32.88±0.92 mV,

31.83±0.99 mV, and 27.55±0.33 mV. As the oil content in Pickering emulsions increased, the potential value decreased. On the other hand, In the images taken with an optical microscope, the globule sizes of all emulsions were measured below 30 µm. In general, it was observed that the PE sample with the lowest oil content was good in terms of emulsion properties.

The Pickering emulsions produced were replaced with oil in the cake formulation. Therefore, cake production was carried out with a control sample containing 100% oil and Pickering emulsions containing 15%, 30, 45, and 60 oil (C-control, C-PE15, C-PE30, C-PE45, and C-PE60). Firstly, the viscosity of the doughs was measured at different rpm and it was observed that the viscosity generally increased with the increase in the fat content. Baking loss, symmetry index, moisture content, and ash characteristics of cakes prepared using emulsions with varying oil contents were investigated. In general, no negative effect of fat reduction on physical properties of cake was observed. In the present study, fat-reduced cake was produced and in addition, fat was replaced with a solution containing valuable nutritional value such as protein. RPI-XG nanoparticles are a suitable example for producing Pickering emulsion. Also, the produced emulsions are suitable for cake production. The use of PE15 emulsion can be recommended for product formulations with low oil content. In future studies, studies can be conducted to evaluate cake shelf life.

Conflict of interest: Concerning the publishing of this work, the authors disclosed no potential conflicts of interest.

Authors' Contribution: Elif Meltem İŞÇİMEN were in charge of choosing the research subject, carrying out the experiments, gathering and analyzing the data, and composing and reviewing the manuscript.

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Research Article/Araştırma Makalesi

Determination of various physical, structural properties and sensory acceptability of Hatay Kömbe cookies produced from Ancestral Seed Kavılca (*Triticum dicoccum* L.)

Ata Tohumu Kavılca (Triticum dicoccum L.) ile üretilen kurabiyelerin çeşitli fiziksel, yapısal özellikleri ve duyusal kabul edilebilirliğinin belirlenmesi

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ABSTRACT

The objective of this study was to investigate the browning index, baking weight loss, texture, fatty acid composition and sensory properties of Hatay Kömbe cookies produced using ancient whole wheat flour Kavılca and white wheat flour. Kavılca, an ancient wheat variety cultivated for thousands of years in the Anatolian region of Turkey, is a substantial source of dietary fibre, contains comparatively low levels of gluten, while being abundant in essential nutrients such as B vitamins, zinc and magnesium. Hatay Kömbe, which has a geographical indication, is a type of sweet cookie that is baked after being shaped in traditionally produced wooden moulds. It is characterised by a distinctive spicy dough composition that pice mixture consists of powder cinnamon, allspice, clove, muscat, ginger, mahaleb and mastic gum. In this study, the physical, functional and nutritional properties of Hatay Kömbe cookies were significantly improved by using whole wheat Kavılca flour. The baking weight loss percentage was ascertained to be 10.34% for HKW and 34.08% for HKK, with no significant variance observed in these values over the storage period of two months. The browning index was determined to be 37.73 for HKW and 123.53 for HKK. The fracturability values of the products recorded as 47.72 for HKW and 48.97 for HKK. In comparison to HKW, HKK exhibited a softer texture and higher fracturability. The sensory characteristics of the production made with Kavılca flour founded to be highly regarded by experts, particularly in relation to its aroma and overall acceptability. Consequently, the utilisation of cookies fortified with ancient whole wheat flour has the potential to yield products that offer enhanced health benefits, particularly when incorporated into grain-based products such as Hatay Kömbe cookies, which is designated with a geographical indication.

Key Words: Kavılca, *Triticum dicoccum* L., Hatay Kömbe, Geographical indication

ÖZ

Bu çalışmanın amacı, antik tam buğday unu Kavılca ve beyaz buğday unu kullanılarak üretilen Hatay Kömbe kurabiyelerinin esmerleşme indeksi, pişirme ağırlık kaybı, dokusu, yağ asidi bileşimi ve duyusal özelliklerini araştırmaktır. Türkiye'nin Anadolu bölgesinde binlerce yıldır yetiştirilen antik bir buğday çeşidi olan Kavılca, önemli bir diyet lifi kaynağıdır, nispeten düşük seviyelerde glüten içerirken, B vitaminleri, çinko ve magnezyum gibi temel besin maddeleri açısından zengindir. Coğrafi işareti bulunan

Hatay Kömbe, geleneksel olarak üretilen ahşap kalıplarda şekillendirilerek pişirilen bir tür tatlı kurabiyedir. Toz tarçın, yenibahar, karanfil, misket, zencefil, mahlep ve sakızdan oluşan parça karışımı ile belirgin bir baharatlı hamur bileşimi ile karakterizedir. Bu çalışmada, tam buğday Kavılca unu kullanılarak Hatay Kömbe kurabiyelerinin fiziksel, fonksiyonel ve besinsel özellikleri önemli ölçüde iyileştirilmiştir. Pişirme ağırlık kaybı yüzdesi HKW için %10.34 ve HKK için %34.08 olarak belirlendi ve iki aylık depolama süresi boyunca bu değerlerde önemli bir değişiklik gözlenmedi. Esmerleşme indeksi HKW için 37.73 ve HKK için 123.53 olarak belirlendi. Ürünlerin kırılabilirlik değerleri HKW için 47.72 ve HKK için 48.97 olarak kaydedildi. HKK kurabiyeleri HKW kurabiyelerine kıyasla daha yumuşak bir doku ve daha yüksek kırılabilirlik sergiledi. Kavılca unu ile yapılan üretimin duyusal özellikleri, özellikle aroması ve genel kabul edilebilirliği açısından uzmanlar tarafından oldukça beğenildi. Sonuç olarak, eski tam buğday unu ile güçlendirilmiş kurabiyelerin kullanımı, özellikle coğrafi işaretle belirlenmiş Hatay Kömbe gibi tahıl bazlı ürünlere dahil edildiğinde, gelişmiş sağlık yararları sunan ürünler üretme potansiyeline sahiptir.

Anahtar Kelimeler: Kavılca, *Triticum dicoccum*, Hatay Kömbe, Coğrafi işaret

Introduction

In recent years there has been a resurgence of interest in ancient seeds, driven by concerns about biodiversity, food security and health. Ancestral seeds, also known as heirloom or heritage seeds, are non-hybridised seeds that have been passed down through generations, often preserving the unique characteristics of crops. Unlike commercially produced seeds, which are bred for uniformity and large-scale cultivation, heirloom seeds maintain genetic diversity and adaptability to local climates (Ray, 2012; Shiva, 2022). This renewed focus is fuelled by the worldwide movement towards sustainable agriculture, slow food and organic farming, as more people recognise the risks of monoculture and genetically modified organisms (GMOs). Ancestral seeds represent not only a cultural heritage but also an agricultural strategy to withstand environmental challenges such as climate change. Their resilience helps to ensure that smallholder farmers, especially in regions vulnerable to environmental changes, can maintain crop yields without relying on chemical inputs (Aistara, 2018). Consumers are also showing an increasing preference for organic and locally sourced food (Mısır and Koç, 2023). This is driving farmers to grow crops from heirloom seeds that are perceived to have superior taste, nutritional value and environmental impact. Seed-saving initiatives, community seed banks and collaborations between scientists and indigenous groups are becoming common practices to protect these vital seeds from extinction (Schmitz et al., 2023). In the main, the increasing focus on ancient seeds is part of a wider trend emphasising sustainability, food sovereignty and biodiversity conservation, reflecting society's desire to reconnect with its agricultural roots and safeguard the future of food production (Petrini, 2003).

Kavılca flour, also known as emmer wheat, is an ancient wheat variety that has been cultivated for thousands of years, especially in the Anatolian region of Turkey. One of the oldest known wheat varieties is distinguished by its sturdy husk and high nutritional value, including fibre, protein and essential vitamins. This grain is respected for its robust, earthy flavor and is often used in the production of bread, pastries and traditional dishes (Speck, 2011; Aydar 2022; Özgören and Işık 2023). Kavılca, produced by traditional methods, is a product with high protein value, these values range from 13.45% to 18.09%, making it a notable source of protein for individuals following a diet high in protein. In addition, the moisture content ranges from 10.7% to 11.8%, while the ash content varies from 1.52% to 3.52%. The carbohydrate content is found to be between 56.91-59.12g/100g, the dietary fibre content between 10.69-13.00g/100g, and the fat content between 1.63-1.67g/100g. Kavılca bulgur contains varying amounts of B vitamins and minerals. Notably, it is particularly abundant in iron, zinc, phosphorus, potassium, and magnesium (Demir, 2020).

Kavılca is also recognised as an important agricultural heritage product for its capacity to thrive in harsh climatic conditions. Kavılca flour is

obtained from Kavılca (*Triticum dicoccom*), one of the oldest wheat varieties in the world and an ancient grain native to the Ardahan region in northeastern Turkey (Dhanavath and Rao 2017; Yüksel 2018; Çetinkaya & Gülbaz 2022). This wheat variety has been cultivated for over 10,000 years and is famous for resisting harsh climatic conditions (Mısır and Alp Baltakesmez, 2024). Kavılca flour is a rich source of dietary fibre, contains low levels of gluten and is a good source of many essential nutrients, including B vitamins, zinc and magnesium (Atak 2017). These characteristics make it a highly nutritious option for use in baking. The distinctive, nutty flavor and dense texture of this grain contribute to the revival of traditional breads and culinary heritage in Turkey. This ancient grain has gained renewed interest due to its role in sustainable agriculture and biodiversity. It is now seen as an important element in the transition to healthier and more environmentally friendly food systems.

Geographically indicated products, also known as Geographical Indication (GI), serve to emphasise the unique qualities of goods linked to specific regions. Facilitating product development in this area is crucial as it supports local economies, improves product differentiation and guarantees the protection of cultural heritage (Checchinato et al., 2024). The development of these products can facilitate increased marketability, tourism attraction and the creation of a distinct identity in global markets. Furthermore, this development ensures quality control, strengthens legal protection and promotes sustainability, thus helping regions to maintain their economic and cultural heritage over time (Barham, 2023).

Hatay Kömbe cookie, which has a geographical indication, is a type of sweet cookie that is baked after being shaped in traditionally produced wooden moulds (İflazoğlu & Aksoy, 2024). It is characterised by a distinctive spice dough composition that pice mixture consists of powder cinnamon, allspice, clove, muscat, ginger, mahaleb and mastic gum (Turkish Patent, 2024). The dough used in the preparation of Hatay Kömbe cookie is characterised by its reduced stickiness and cinnamon in the mixture is responsible for the brown colour of its.

In this study, two products with geographical indications (CGIs) were used. The first of these is Ardahan Kavılca Flour and the other is Hatay Kömbe cookies. The objective of this research was to produce cookies with enhanced physicochemical attributes and sensory properties using Kavılca flour prepared with belong to group of Emmer wheat, and was conducted to assess to evaluate consumer acceptance of the cookies produced from Kavılca flour. For this purpose, the effect of production with Kavılca flour on the properties of cookies such as color, browning index (BI), and baking weight loss (BWL), as well as the changes in textural properties such as hardness and brittleness, which are greatly affected by low gluten content, were examined. In addition, the changes in the fatty acid profile of the cookies at the initially and end the 2 month were determined. Apart from these features, sensory properties of the cookies at the initially, the 1st month and at the end of the 2nd month were determined.

Material and Methods

Raw materials

Kavılca wheat flour used in cookie was obtained from a local flour mill in Ardahan. Other food ingredients used in the production were obtained from the domestic market. The mould used to shape the cookie was obtained from a local business in Hatay. All food materials were stored in Ardahan University Food Microbiology Laboratory. As demonstrated in Table 1 and Table 2, Ardahan Kavılca wheat and white wheat can be distinguished by a number of key characteristics.

Table 1. Quality Characteristics of Ardahan Kavılca Wheat and White Wheat

Characteristic	Ardahan Kavılca Wheat	White Wheat
Hulled hectoliter weight (kg/hL)	47.7–52.5	49.7–54.5
Dehulled hectoliter weight (kg/hL)	74.1–77.4	76.0–81.0
Thousand kernel weight (g)	28.0–31.1	35.0–45.0
Hardness (%)	68.0–74.6	60.0–70.0
Diameter (mm)	2.64–2.79	2.5–3.5
Grain protein content (%)	10.03–12.32	8.0–11.0
Ash content (%)	1.78–1.95	0.55–0.60
Wet gluten (%)	24.4–33.2	27.0–31.0
Dry gluten (%)	8.2–12.3	9.0–11.0
Flour yield (%)	61.9–65.0	70.0–75.0

Table 2. Mineral Contents of Ardahan Kavılca Wheat and White Wheat

Mineral	Ardahan Kavılca Wheat	White Wheat
Zinc (mg/kg)	31.81–39.99	35.2–39.0
Iron (mg/kg)	27.45–30.90	40.6–46.6
Calcium (mg/kg)	309–341	408–450
Magnesium (mg/kg)	1053–1147	1656–1800
Potassium (mg/kg)	3920–4318	4860–5000
Selenium (mg/kg)	0.021–0.077	84.84–90.0

Methods

Cookie Production Process

In the initial stage of the process, the ingredients, namely margarine, oil, milk, water, powder sugar and baking powder, were combined in a large bowl for approximately ten minutes. Subsequently, the flour and spice mixture were incorporated gradually, which was mixed for a further five minutes (Table 3). Subsequently, the dough was refrigerated for 24 hours at a temperature of 4°C. Subsequently, the dough was divided into six equal portions of 60 grams each. The cookies were shaped using a mould, and the shaped of the cookies, referred to as "Kömbe," were dipped in 10 grams sesame seeds and placed on a baking tray. The requisite technological parameters for mixing, resting, and baking are presented in Table 3. The prepared cookies were baked in an oven set to a temperature between 160 and 180˚C for a duration of 25 to 30 minutes. The samples were stored in a cool and dry environment at 21˚C. Each batch of cookies was produced one day before the laboratory and sensory analyses.

Table 3. Spices and quantities used in Hatay Kömbe Cookie (for 100 g)

Spices	Amount (gram)
Mastic Gum	5
Muscat	6
Ginger	6
Mahaleb	6
Powder Cinnamon	23
Allspice	23
Clove	23

The production process of Hatay Kömbe cookies with GI (Registration No: 1173) is outlined in Table 4 and Figure 1 (Turkish Patent, 2024). As illustrated in Table 5 and Table 6, the samples of cookies were labeled (a group with Kavılca) HKK with Kavılca (100%) and (a control group) HKW without Kavılca (0%).

Figure 1. Hatay Kömbe Cookie Production with Kavılca flour (HKK) and White flour (HKW)

Table 4. Ingredients and technological parameters used in the preparation of Hatay Kömbe Cookie

Ingredients(g)	**Kömbe (Cookie) Samples***	
	HKW	**HKK**
Wheat Flour (WF)	100	-
Kavılca Flour (KF)	-	100
Margarine**	35	35
Liquid Oil**	15	15
Powdered Sugar**	25	25
Sodium bicarbonate**	1	1
Milk **	10	10
Water**	10	10
Susame**	10	10
Kömbe (cookie) Spices**	1.8	1.8
Technological Parameters		
Mixing time (min)	15	15
Dough Temperature (˚C)	21˚C	21˚C
Resting time (hour)	24	24
Temperature (˚C)	3-4˚C	3-4˚C
Baking time (min)	25	25
Temperature (˚C)	170˚C	170˚C

Note: * HKW = 0% kömbe (cookie) with 100% WF (control sample); HKK = kömbe (cookie) with 100% KF; ** The auxiliar materials are reported to 100% of flour blends.

Characteristics of Cookie

Physical Parameters

To determine the Hatay Kömbe cookies (HKCs) baking weight loss, the weight of cookies before and after production was measured using a digital scale. The ratio between the weight of the dough and the cooked and cooled cookies was calculated using the equation (1) given by Krupa-Kozak et al (2020).

$$\mathrm{WL}\ (\%) = \frac{(a-c)x\ 100}{a} \quad (1)$$

(a): The weight of batter in the mould before baking (g); (c): The weight of baked and cooled HKC (g).

Colour Analysis

The measurements of the HKCs were made using a Color Spectrophotometer (Hunterlab

Colorquest XE, USA) based on the CIE-LAB system and the results were expressed by the CIELab system, the values were the mean of at least five replicates. The L* (0=black, 100=white), a* (+red, –green) and b* (+yellow, –blue) color coordinates were determined according to the CIELAB coordinate color space system. Also, the browning index (BI), the whiteness index (WI) was calculated using the equation given by Krupa-Kozak et al (2020).

The whiteness index (WI) of HKCs was calculated according to Equation (2):

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (2)$$

The browning index (BI) of the HKCs was calculated according to Equations (3) and (4)

$$\mathrm{BI} = \frac{100\mathrm{x}(\mathrm{X}-0.31)}{0.17} \quad (3)$$

$$\mathrm{X} = \frac{a^*+1.75L^*}{5.645L^*+a^*-3.012b^*} \quad (4)$$

Texture Profile Analysis

The hardness and fracturability values of HKCs were determined 24 hours after baking using a three-point bending probe and a 30 kg load cell on a texture analyzer (TA-XT Plus C, Lloyds Instruments, UK) at Central Research Laboratory Application and Research Center of Ardahan University according to the method of Gül et al., (2021). The test parameters on the texture analyzer were set as pre-test speed: 1.0 mm/s, test speed: 3.0 mm/s, post-test speed: 10 mm/s, distance 5.0 mm and data acquisition speed 500 pps.

Determination of the fatty acid profile

The analysis of fatty acid methyl esters (FAMEs) was conducted at the Central Research Laboratory of Ardahan University's Application and Research Centre, utilising Gas Chromatography-mass spectrometry (Thermo Scientific Trace 1300 equipped with an EGC-YA01 column). This method was employed in accordance with the protocol outlined by Kola et al. (2015). In summary, a total of 150 g of biscuits were reacted with 0.1 mol/L NaOH-MeOH for 5 minutes, followed by reaction with 1.1 mol/L HCl-MeOH for 5 minutes at room temperature. The FAMEs were extracted with isooctane after the addition of water to stop the reaction.

Sensory Evaluation

A panel of six experts (two women and four men), previously selected and trained following ISO guidelines (38 amendment), evaluated the sensory properties of the products 24 hours after cooking. The assessors were already conversant with the Kavılca wheat and were aware of the geographical indications Hatay Kömbe and Ardahan Kavılca Wheat. A sensory analysis form, comprising criteria on appearance, colour, texture, taste, odor and general acceptance, was employed to assess the sensory characteristics of the products. The evaluation was conducted using a 5-point Likert-type scale. The products were assigned alphanumeric identifiers (HKK and HKW) and presented to the evaluators in a randomised order on transparent plates. The sensory evaluation was conducted under standard lighting conditions at room temperature (21˚C) in a sensory laboratory room that met the requirements of ISO standards (41 amendment). It was recommended that bottled mineral water be consumed between each sample evaluation to minimise the effects of residue. The sensory analysis was repeated at 0., 1., and 2 months to determine the shelf life of the products.

Statistical Analysis

The experimental design was completely randomised into a control group (HKW) and a group with Kavılca (HKK). Statistical evaluation of sensory analysis results was performed using SPSS 26.0 (SPSS Inc., Chicago, USA). Sensory data were collected in 3 replicates in shelf-life studies. Sensory data were analysed using SPSS software 29 (IBM Corporation, Somers, NY) and differences were determined by ANOVA at a significance level of $P<0.05$. The results of this test were presented in tabular form.

Results and Discussion

In the current work, various physicochemical properties of HKCs were determined (Table 5). One of these was baking weight loss. BWL, defined as the removal of moisture that affects the texture and staling properties of cooked products, is seen as one of the greatest technological losses and therefore efforts are made to minimize it. A porous structure is formed as a result of the evaporation of water during the cooking process. As a result of this situation, a series of physical and chemical changes occur, such as volume increase (Mondal and Datta 2008; Krupa-Kozak et al., 2020). No statistical significance was observed in the BWL of products within the experimental group. However, a discrepancy was identified between the groups. The mean BWL was determined to be 10.34% for the product obtained from HKW and 34.08% for the product produced using Kavılca flour (HKK). WL is mainly due to the drying process. However, during the initial heating process, water evaporation may occur as the crust formation on the product does not occur immediately (Purlis and Salvadori 2009). This situation has been attributed to the low water-retention capacity of the Kavılca. The values obtained at the end of the second month were examined and although a slight decrease was noted, no significant change was observed in either product.

Table 5. The physicochemical and texture properties of HKCs (initial and after 2 months storage) *

	HKW		HKK	
Parameter	0	2	0	2
Baking Weight Loss (%) (BWL)	10.34 ± 0.02 ^{aA}	09.32 ± 0.03 ^{bA}	34.08 ± 0.01 ^{aB}	32.11 ± 0.02 ^{bB}
Whiteness Index (WI)	116.50 ± 0.06 ^{aA}	115.60 ± 0.05 ^{bA}	34.08 ± 0.04 ^{aB}	34.12 ± 0.06 ^{aB}
Browning Index (BI)	37.73 ± 0.07 ^{aA}	37.23 ± 0.11 ^{bA}	123.52 ± 0.15 ^{aB}	124.12 ± 0.20 ^{bB}
L*	51.26 ± 0.35 ^{aA}	50.06 ± 0.15 ^{bA}	41.87 ± 0.14 ^{aB}	40.27 ± 0.34 ^{bB}
a*	15.84 ± 0.12 ^{aA}	14.64 ± 0.10 ^{bA}	15.61 ± 0.14 ^{aA}	16.11 ± 0.10 ^{bB}
b*	32.49 ± 0.11 ^{aA}	32.19 ± 0.53 ^{aA}	26.93 ± 0.11 ^{aB}	27.03 ± 0.19 ^{aB}
Hardness (g)	850.13 ± 30.79 ^{aB}	1112.70 ± 38.01^{bB}	409.23 ± 47.30 ^{aA}	601.72 ± 4.07 ^{bA}
Fracturability (mm)	47.72 ± 0.12 ^{aA}	47.79 ± 0.40 ^{aA}	48.97 ± 0.68 ^{aA}	49.86 ± 0.58 ^{aA}

Note: * HKW = 0% Hatay Kömbe cookie with 100% WF (control sample); HKK = Hatay Kömbe cookie with 100% FK Values are means of three independent determinations and standard errors.
'a, b' Different letters in the same row indicate differences among the same samples ($P < 0.05$).
'A, B' Different letters in the same row indicate differences among groups ($P < 0.05$).

Colour, which is the first feature by which food is evaluated by the consumer, is used to detect some damage and quality defects in foods. The *L** value determined for the HKK was significantly ($p < 0.05$) lower (*L** value = 41,86) compared with the HKW (51,25). Considering that both products were baked under the same conditions, this result was attributed to the higher protein content of Kavılca flour. Özgören and Işık (2023) compared the color values of commercial white and Kavılca flour in their study, and it was stated that Kavılca was darker in a different way. Also, the same result determined that in a* and b* values of Kavılca flour. In the present study, at the end of production, the a* value representing redness and greenness did not differ between HKK and HKW, while the b* value representing yellowness and blueness was found to be higher in HKW (Table 2).

The browning index is defined as a simple indicator of a chemical change and/or colour change due to oxidation of the surface of a freshly cut fruit or vegetable during storage or drying or baking of bread. For HKW, WI was determined as 116.50 while BI was determined as 37.73. In HKK, WI was determined as 34.08 while BI was determined as 123.53. These results were attributed to; carbonyl groups of reducing sugars produce brown nitrogenous pigments (melanoidins) polymerizing with α-and ε-amino

groups of proteins, peptides or amino acids by the Maillard reaction (Starowicz and Zieliński 2019). There was no significant change for either product when looking at the values at the end of the second month, both colour and BI/WI index.

The textural properties of HKCs were measured using TA-TX Plus C texture analyzer (Lloyds Instruments, UK). Textural profile analysis in all the HKCs was performed to evaluate as hardness, and fracturability (Figure 2). Hardness is one of the important parameters for consumer acceptability, especially in products such as cookies (Mohibbullah et al 2023). The general textural properties of these products are related to starch gelatinization as well as sugar content (Silav-Tuzlu and Tacer-Caba 2021). When the hardness property was evaluated in the study, this value of HKW (850.131) was significantly higher than HKK (409.23). Especially in products like biscuits, texture values are in a very wide range. This situation is attributed by researchers to the substances used in the formulation, their amounts and also the water used (Martinez et al 2018; Gül et al 2021). It is stated that the hardness of biscuits made from gluten-free flour such as buckwheat flour increases throughout the shelf life. Kavılca flour is also one of the flours with low gluten content.

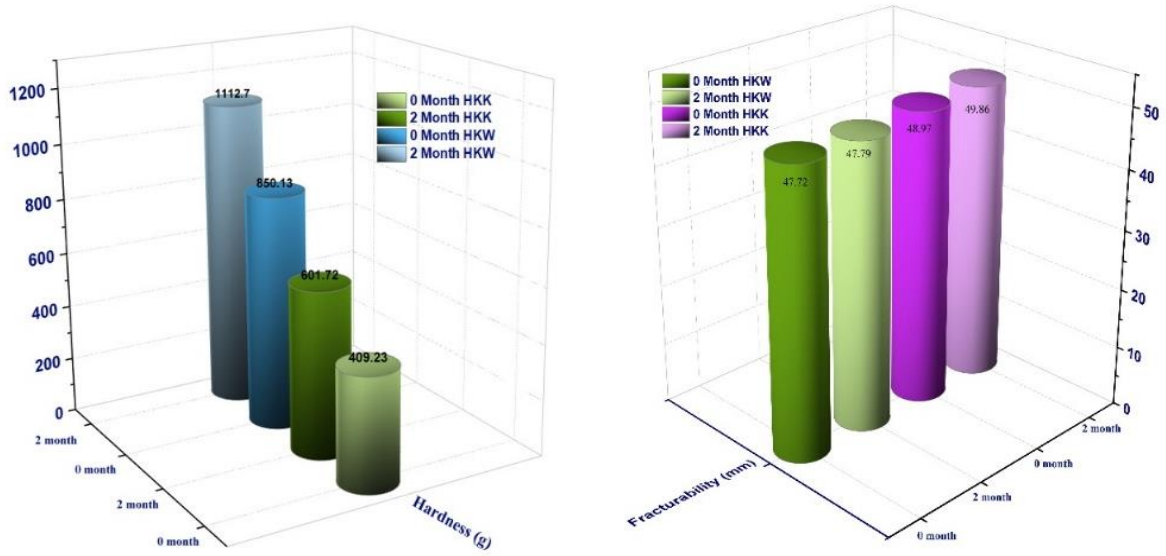

Figure 2. Textural profile in all the HKCs

When the fracturability results of the products were examined; HKW was determined as 47.72 and as HKK 48.97. Gluten is a protein that is important on the main quality and structure of bakery products (Alvarenga et al., 2011), while Kavılca flour is a rich source of dietary fiber but contains low levels of gluten (Atak 2017). These results are more like biscuit varieties made from gluten-free flours (Gül et al 2021; Doğan and Meral 2016; Tüter 2019), and this was attributed to the low gluten protein content of Kavılca. At the end of the 2-month period, it was determined that the hardness value of HKK increased to 601.72, the fracturability value was determined as 49.86. These values for HKW were determined as hardness 1112.70 and fracturability 47.79.

In the study by Arslan-Unal and Ozkaya (2025), various types of flours were utilised in the production of cookies, and a comparative analysis was performed on their respective textural properties. The study concluded that higher proportions of whole flour resulted in cookies with higher hardness. The highest breaking strength (11.9kg) was observed in cookies made from flours with high gluten content, while cookies made from 100% Kavlıca flour exhibited the lowest breaking strength (4.9kg). This was attributed to the dilution of the gluten content available to bind water and the weak gluten structures of ancient wheat. A study was performed to examine the relationship between cookie hardness and protein quality. It was

reported that the higher breaking strength of cookies obtained using 100% whole flour was due to the high-water absorption capacity of bran. Furthermore, it was determined that cookie hardness depends on protein and gluten quality (Hidalgo et al., 2019; Saka et al., 2020). In present study, the HKK exhibited a softer texture and higher fracturability compared to the HKW, and these results are like those of Arslan-Unal and Ozkaya (2025).

An investigation was conducted into the fatty acid profile of cookies produced using Kavılca flour, to provide potential insights into the beneficial health effects. The fatty acid methyl esters and various compounds contents of HKCs after the first production and 2-months are shown in Table 6. The most abundant fatty acid methyl esters were determined as hexadecanoic acid, methyl ester (31.92%) in HKK, followed by 9 octadecenoic acid, methyl ester (33.07%), 9,12 octadecadienoic acid, methyl ester (23.37%), and octadecanoic acid, methyl ester (7.91%). It is important to note that essential fatty acids are those which the human body is unable to synthesize and thus they must be obtained from external sources through the consumption of nutrients.

Table 6. Fatty acids and methyl esters composition of the HKK and HKW cookies*

			HKK		HKW	
			Time (month)			
			0	2	0	2
Compound Name	**Chemical Group**	**Molecular Formula**	**Area %**	**Area %**	**Area %**	**Area %**
Octanoic Acid	Saturated Fatty Acid	C9H18O2	0.09	0.17	0.04	0.05
Hexanoic acid	Saturated Fatty Acid	C7H14O2	0.04	0.03	-	-
Heptanoic Acid	Saturated Fatty Acid	C7H14O2	-	0.03	-	-
Dodecanoic Acid	Saturated Fatty Acid	C12H24O2	0.05	0.05	0.03	0.03
Heptadecanoic Acid	Saturated Fatty Acid	C17H34O2	0.04	0.05	0.02	0.03
Eicosanoic Acid	Saturated Fatty Acid	C20H40O2	0.04	0.04	0.05	0.06
1,2,3-Propanetriol	Glycerine	C3H8O3	-	0.77	-	0.57
Hexanoic Acid, Methyl Ester	Fatty Acid Methyl Ester	C7H14O2	0.03	-	-	-
Phenol, 2-Methoxy-4-(2-Propenyl)- (eugenol)	Phenol	C10H12O2	-	0.07	-	-
Benzoic Acid,3,4,5 Trihydroxy, Propyl Ester	Ester	C10H12O5	-	-	0.02	-
Phenol, 2-Methoxy-5-(1-Propenyl)-, (E)-	Phenol	C10H12O2	-	-	-	0.01
Decanoic Acid, Methyl Ester	Fatty Acid Methyl Ester	C11H22O2	0.16	0.07	0.05	0.03
Dodecanoic Acid, Methyl Ester	Fatty Acid Methyl Ester	C13H26O2	1.82	1.08	0.43	0.36
Heptaethylene Glycol	Ether	C14H30O8	0.50	-	0.30	0.29
Cycloheptasiloxane, Tetradecamethyl	Ether	C14H42O7Si7	-	-	0.10	-
Dodecane, 2,6,10 Trimethyl	Terpenes	C15H32	0.02	-	-	-
Tetradecanoic Acid, Methyl Ester	Fatty Acid Methyl Ester	C15H30O2	2.22	1.26	1.81	1.69
Trans-Caryophyllene	Terpenes	C15H24	-	-	-	0.03
Benzoic Acid,2,4bis[(Trimethylsilyl)Oxy] Trimethylsilyl Ester	Ester	C16H30O4Si3	0.01	-	-	0.02
Hexadecanoic Acid, Methyl Ester	Fatty Acid Methyl Ester	C17H34O2	31.92	27.67	32.32	31.88
Octanoic Acid, Methyl Ester	Fatty Acid Methyl Ester	C18H38O5	0.17	0.09	0.05	0.04
2,2,3,3,4,4 Hexadeutero Octadecanal	Ester	C18H30D6O	0.01	-	-	-
Methyl 9,10 Methylenehexadecanoate	Fatty Acid Methyl Ester	C18H34O2	0.05	-	0.06	-
Heptadecanoic Acid, Methyl Ester	Fatty Acid Methyl Ester	C18H36O2	0.20	-	0.51	-
Dodecachloro-3,4-Benzophenanthrene	Unknown	C18Cl12	-	0.22	-	-
Octadecane, 6 Methyl	Hydrocarbons	C19H40	0.01	-	-	-
Octadecanoic Acid, Methyl Ester	Fatty Acid Methyl Ester	C19H38O2	7.91	0.45	7.88	6.99
9 Octadecenoic Acid, Methyl Ester	Fatty Acid Methyl Ester	C19H36O2	33.07	32.05	30.10	31.73
9,12 Octadecadienoic Acid, Methyl Ester	Fatty Acid Methyl Ester	C19H34O2	23.37	15.56	21.34	20.09
9,12,15 Octadecatrienoic Acid, Methyl Ester,	Fatty Acid Methyl Ester	C19H32O2	0.48	0.25	0.46	0.38
Eicosanoic Acid, Methyl Ester	Fatty Acid Methyl Ester	C21H42O2	0.74	-	0.92	0.62
Cis11eicosenoic Acid, Methyl Ester	Fatty Acid Methyl Ester	C21H40O2	0.40	-	0.39	-
Methyl 9-Eicosenoate	Fatty Acid Methyl Ester	C21H40O2	-	-	-	0.29
Docosane	Hydrocarbons	C22H46	0.01	-	-	-
Octaethylene Glycol Monododecyl Ether	Ether	C28H58O9	0.46	1.44	-	0.76

Note: * HKW = 0% Hatay Kömbe cookie with 100% WF (control sample); HKK = Hatay Kömbe cookie with 100% FK Values are means of three independent determinations and standard errors.

The fatty acids are α-linolenic acid (ALA, 18:3), which has 18 carbons and 3 double bonds, and linoleic acid (LA, 18:2), which contains two double bonds (Çelebi et al., 2017). When LA and ALA are ingested with food, arachidonic acid (20:4 n-6) can be synthesised from linoleic acid, which cannot be synthesised in mammals, by elimination (lengthening of the carbon chain) and desaturation (increase in the number of double bonds). Also, n-3 series fatty acids such as eicosapentaenoic acid (20:5 n-3), docosapentaenoic acid (22:5 n-3) and docosahexaenoic acid (22:6 n-3) can be synthesised from α-linolenic acid (Harris et al 2008; Çelebi et al 2017). Alpha linolenic acid (ALA) is very sensitive to damage from factors such as light, oxygen and heat and is destroyed approximately five times faster than Linoleic acid (LA) (Kaur et al 2014). Omega-6 and omega-3 fatty acids are the form of triglycerides from various food sources digested in the small intestine, absorbed, transported in the blood, and assimilation throughout the body, including the brain, retina, heart and other tissues (Kaur et al., 2014; Paucean et al., 2018).

Fatty acid esters are defined as significant fatty chemicals, which are formed as a result of the reaction of fatty acids with alcohols. Their utilization is highly probable due to their structural properties, and there is a rapid increase in their production and consumption worldwide. Long-chain triacylglycerols (LC-TAGs) are naturally occurring fatty esters that exhibit certain metabolic characteristics (Chiş et al., 2020; Gilbaz 2022). For instance, they are more susceptible to hydrolysis than medium-chain fatty esters (C6 - C12). Additionally, they are utilised in low-calorie diets. In contrast, fatty acid methyl esters (FAMEs) play a critical role in the industry as intermediates, although their direct utilisation remains limited. These esters are increasingly replacing fatty acids as starting materials in various industrial processes (Bogaerts et al., 1990; Kola et al., 2015; Gilbaz 2022).

As demonstrated in Table 6, the analysis revealed that HKK contains higher concentrations of octanoic acid, hexanoic acid, dodecanoic acid, heptadecanoic acid and eicosanoic acid, in addition to a more abundant fatty acid methyl ester, when compared to HKW. Conversely, wheat flour exhibits either an absence or a presence of certain critical fatty acids and esters at minimal levels. In view of these findings, it can be concluded that the use of Kavılca flour during production results in a fatty acid profile that is richer in terms of diversity and concentration. During the shelf life of HKK, losses of hexanoic acid and methyl ester were observed; however, when the detected fatty acids and esters were analysed as a whole, it was determined that they remained relatively stable during storage.

The shelf life of Hatay Kömbe cookie made with white flour (HKW) and Kavılca flour (HKK) was determined according to the results of sensory analysis after 0, 1 and 2 months. It was accepted that the shelf life of both groups was 1 month according to the sensory results. According to this result, it was found that there was a significant decrease in odor, taste, texture and general acceptance in the groups after the first month. With 100% Kavılca flour, the sensory acceptability of the Hatay Kömbe cookies production is quite low. The texture structure of the biscuits produced with Kavılca flour (HKK) was found to be very thin by hand and in the mouth. The taste and aroma of the flour were found to be dominant due to the unique intense nutty taste and smell of the flour. Kavılca flour requires different binders due to the limited gluten content in its structure. The results of the sensory and laboratory analyses support each other. In the study, production with 100% (direct use of the product) Kavılca flour was given priority. However, due to the structure of Kavılca flour, it was found that different mixing processes should be tried during the production of the product (Figure 3 and 4).

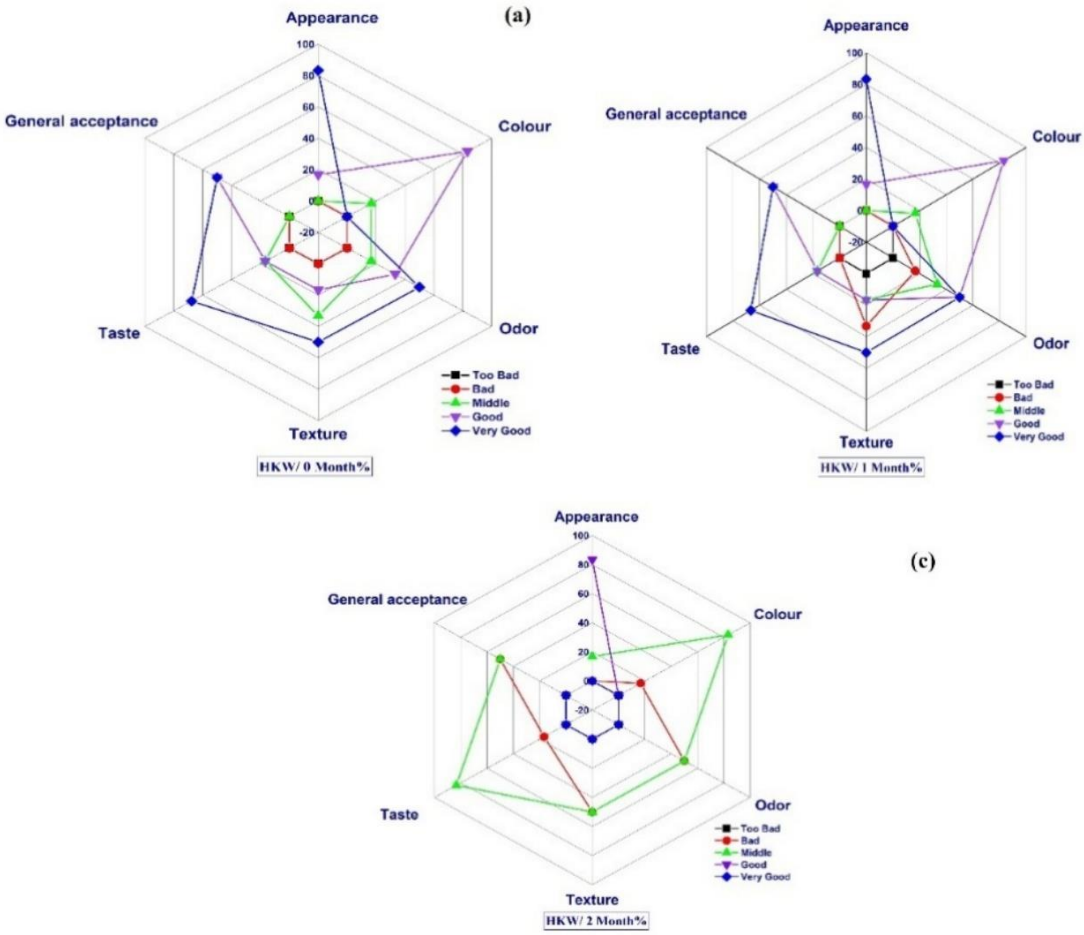

Figure 3. Sensory properties result of the HKW

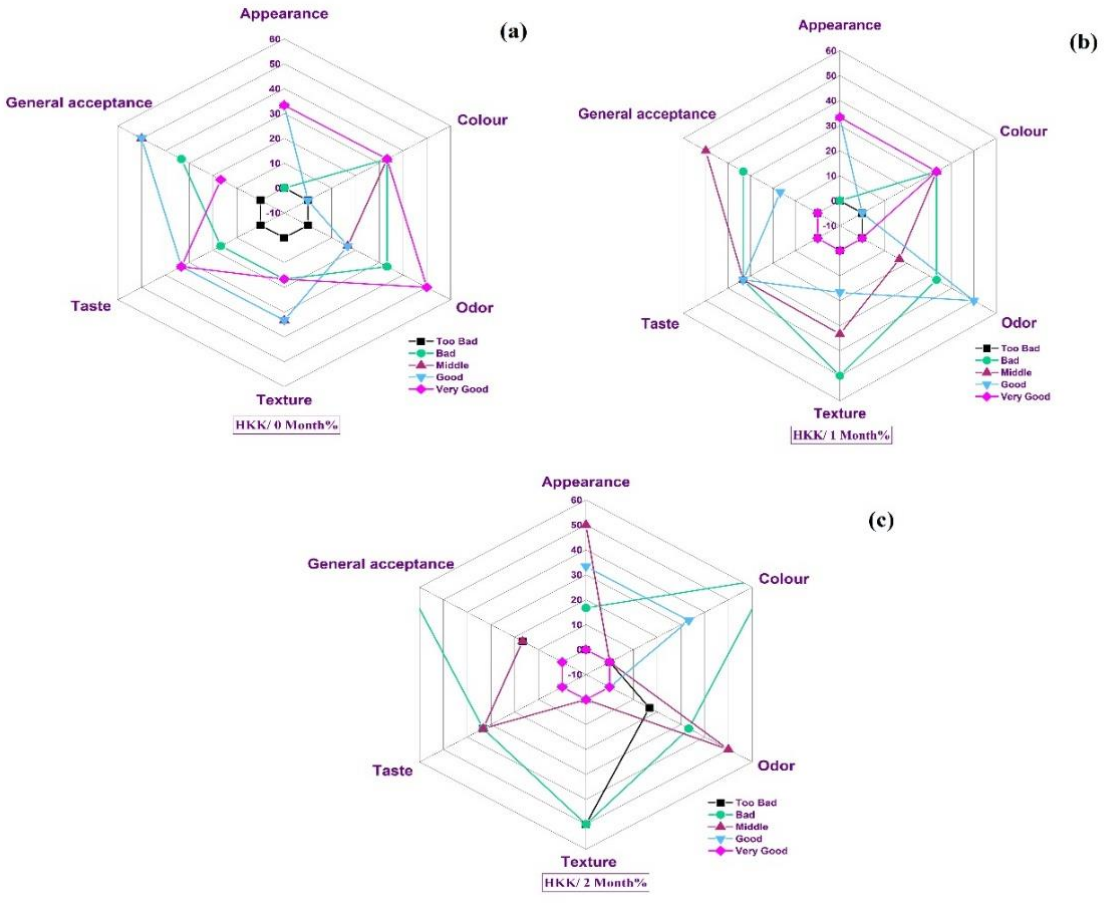

Figure 4. Sensory properties result of the HKK

Conclusion

It is thought that Kavılca wheat, which has low levels of gluten in its structure, will be a natural alternative to new consumption habits. The present study is important in developing and improving the qualities of the products with Kavılca. Kavılca flour, which is rich in terms of nutritional values compared to white flour, has less gluten and is produced from ancestor seed wheat. With these aspects, preparing new recipes with the product is necessary. For this purpose, in the current study, HKCs prepared with various spices and traditional production techniques were prepared with Ardahan Kavılca flour. The results obtained from the analysis of various fatty acids and methyl esters indicated that the cookies (HKK) produced using Kavılca wheat possessed a more substantial composition, thus increasing the nutritional value of the product and contributing to its functionality in terms of health. According to the sensory analysis results, it was determined that HKK was acceptable. However, textural properties affected by gluten content such as hardness were found to be weak. The production of various products with Kavılca flour is a new situation and requires testing different formulations. In this study, Hatay Kömbe cookie was produced with 100% Kavılca flour. However, to improve the textural properties of the product, experiments should be carried out using lower amounts of Kavılca flour.

Declarations

Conflict of interest

The authors declare that there are no conflict of interest.

Author contributions

DAB: Designed study, production of cookies, conducting experiments, data collections and analysis, writing and reviewing the manuscript. **SM**: Designed study, analyzed the data by statistical program, production of cookies, writing and reviewing the manuscript.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Data Availability

The dataset generated during and/or analyzed during in the current study are available from the corresponding author on reasonable request.

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Research Article/Araştırma Makalesi

Extending the shelf life of unsalted white cheese produced for special dietary preferences: role of essential oils and coating

Özel diyet tercihleri için üretilen tuzsuz beyaz peynirin raf ömrünün uzatılması: uçucu yağların ve kaplamanın rolü

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ABSTRACT

Unsalted white cheese is produced for individuals who have health problems and prefer it for special reasons. However, as salt is not used in its production and brining, its shelf life is limited. In this study, edible films prepared with different ratios of whey protein and essential oils were applied as coatings to unsalted white cheese. 5 different experimental groups were prepared and named as group C (control), group 1R (film containing 1% rosemary essential oil), group 3R (film containing 3% rosemary essential oil), group 1L (film containing 1% laurel essential oil), and group 3L (film containing 3% laurel essential oil). The microbiological, chemical, sensory and textural properties of the groups were analyzed after 0, 5, 10, 15, 20 and 25 days of storage at +4^{o}C. On and after the 10th day of the storage period, the C, 1R, 3R, and 1L groups visually deteriorated and the analysis was stopped. However, the 3L group did not deteriorate until the end of the 25th day. In addition, it was determined that the 3L group gave the best results in terms of physical, chemical, microbiological, sensory, and textural analyses.

Key Words: Unsalted white cheese, shelf life, edible films, laurel essential oil, rosemary essential oil, whey protein

ÖZ

Tuzsuz beyaz peynir, sağlık sorunu yaşayan ve özel nedenlerle tercih eden bireyler için üretilmektedir. Ancak üretiminde ve salamurasında tuz kullanılmadığı için raf ömrü sınırlıdır. Bu çalışmada, farklı oranlarda peynir altı suyu proteini ve esansiyel yağlar ile hazırlanan yenilebilir filmler tuzsuz beyaz peynire kaplama olarak uygulanmıştır. 5 farklı deney grubu hazırlanmış ve grup C (kontrol), grup 1R (%1 biberiye uçucu yağı içeren film), grup 3R (%3 biberiye uçucu yağı içeren film), grup 1L (%1 defne uçucu yağı içeren film) ve grup 3L (%3 defne uçucu yağı içeren film) olarak adlandırılmıştır. Grupların mikrobiyolojik, kimyasal, duyusal ve tekstürel özellikleri +4C^{o}'de 0, 5, 10, 15, 20 ve 25 günlük depolama sonrasında analiz edilmiştir. Depolama süresinin 10. günü ve sonrasında C, 1R, 3R ve 1L grupları görsel olarak bozulmuş ve analiz durdurulmuştur. Ancak 3L grubunda 25. günün sonuna kadar bozulma görülmemiştir. Ayrıca 3L grubunun fiziksel, kimyasal, mikrobiyolojik, duyusal ve tekstürel analizler açısından en iyi sonuçları verdiği belirlenmiştir.

Anahtar Kelimeler: Tuzsuz beyaz peynir, raf ömrü, yenilebilir filmler, defne uçucu yağı, biberiye uçucu yağı, peynir altı suyu proteini

Introduction

White cheese is a widely consumed product in Türkiye, Egypt, and Greece, albeit with different names (Feta, Batzos, Halloumi, Domiati, and Beyaz Peynir) and production methods (Albayrak and Duran, 2021; Geronikou et al., 2023). It is a type of cheese that is kept in brine, is usually white in color, and matures with a soft to semi-hard texture (Soltani et al., 2020). In Türkiye, it is among the most consumed cheese varieties (Erkaya-Kotan and Hayaloglu, 2024). Traditional taste habits and the fact that it is a semi-hard cheese type have increased the consumption of white cheese throughout the country. For this reason, it is known that individuals whose salt consumption is limited or prohibited for health reasons prefer to consume classical production white cheese despite all these negativities. On the other hand, white cheese consumption is high, especially in hospitals and elderly care centers, owing to its textural properties.

Salt is one of the most commonly added materials in food formulations, creating sensory-pleasing product forms. In cheese production, salt is used for various purposes, such as providing microbiological safety, flavor, and whey extraction. Although cheese varieties encounter salt at different production points, the most common use is brining (Glass et al., 2024). At this stage, high levels of salt (18-24%) are used especially during brine preparation to ensure that sufficient and safe amount of salt from the brine liquid can pass to the cheese (Lucey, 2021). However, dietary salt intake rates and their effects have been the subject of many studies. Heart diseases, hypertension, and kidney diseases can be prevented by reducing the sodium from salt components (He et al., 2020; Wang et al., 2020). In addition to its negative effects on health, the presence of high salt can negatively affect the presence of probiotic strains in white cheese (Rolim et al., 2020).

Rosemary (*Rosmarinus officinalis*) is a perennial herb that grows in the Mediterranean region and has been used in many areas for many years because of its strong antioxidant and antimicrobial effects. In contrast, it reduces inflammation, cancer, diabetes, and depression (Chen et al., 2024; Eid et al., 2022). Rosemary oil is an important commercial product of this plant that is used as a spice. Rosemary oil, which is used in many fields, can be used in the food sector for purposes such as antimicrobial and antioxidant effects and shelf life extension (Chen et al., 2024; Sirocchi et al., 2017). In contrast, α-pinene and β-pinene hydrocarbons provide rosemary oil with a pungent aroma (Formica et al., 2024). Its dominant aromatic structure may cause sensory limitations in its use as food. Laurel (*Laurus nobilis*) is a plant native to the Mediterranean region, usually used as a spice (Parthasarathy et al., 2008). The antimicrobial and antiseptic properties of laurel leaves and oils are well documented. In particular, their positive effects on foodborne pathogens have been identified (Özogul et al., 2015). Additionally, 1,8-cineole, linalool, α-terpinyl acetate, α-pinene, and β-pinene are the main components of laurel leaves (Stefanova et al., 2020).

Whey protein is a valuable byproduct of milk processing and is known for its ability to increase the stability of the products it is formulated into and its good carrier properties (Kong et al., 2022). In addition to valuable amino acid components, it is also valuable for emulsification and gelling (Wagner et al., 2020). Edible films generally aim to maintain food quality and extend shelf life; protein-based materials and carbohydrates are commonly used in the production of film coatings (Kang et al., 2021). Whey protein is preferred, especially in fat-based edible film coatings, owing to its high gelling and emulsification capacities.

The objective of this study was to investigate the possibility of extending the shelf life of unsalted white cheese, which has become compulsory for consumption for various reasons, especially health problems, and is used extensively, especially in hospital kitchens. In this context, films using rosemary and laurel essential oils and whey protein at acceptable sensory ratios were produced, and white cheeses were coated.

Total psychrophilic bacteria count, coliform group bacteria count, yeast and mold count, lactic acid bacteria count, pH, ash, moisture, titratable acidity, color, texture, and sensory analyses were performed on six different storage days (days 0, 5, 10, 15, 20, and 25).

Materials and Methods

The rosemary and laurel essential oils (Sigma-Aldrich) used in this study were 99% pure. Whey protein (70% protein) (Gemici Tic.), and glycerol (Tekkim Tic.) was obtained from local businesses.

White cheese production

For cheese production, 355 liters of milk was pasteurized using heat treatment. Then, 120 g of $CaCl_2$, 300 g of cheese starter culture, and coagulant rennet were added to the pasteurized milk. After the clot formation started, it was subjected to clot breaking, mixing and heating. After the curdling stage, the whey was separated, and the cheeses were molded and pressed.

Edible film production and coating of cheeses

Whey protein (5% w/v) was dissolved in distilled water and glycerol (5% w/v) was added. 1 N NaOH was added until the pH of the solution was 8 and pH was fixed at 8. The solution was then heated for 30 min until it reached 90±2^{o}C and sodium alginate (0.3% w/v) was added. In the first batch of edible films, 1% and 3% rosemary essential oil were added, and 1% and 3% laurel essential oil were added to the next batch. These ratios were determined based on the initial sensory evaluations and essential oil ratios at acceptable levels for consumers. The edible films were cooled to room temperature.

Unsalted white cheese samples were sliced into 10±0.5 g pieces after the edible film was formed. Approximately 10 g of unsalted white cheese sample was placed in sterile sealed containers. They were immersed in the film solution to cover the top and bottom and maintained for approximately 1.5 hours. After this stage five experimental groups were established and labeled as group C (control), group 1R (film with 1% rosemary essential oil), group 3R (film with 3% rosemary essential oil), group 1L (film with 1% laurel essential oil), and group 3L (film with 3% laurel essential oil). The samples were stored at +4^{o}C until the end of their shelf life for storage analysis. On day 0, analyses were started and samples were analyzed until day 25. This period was determined based on the deterioration time of the samples. 2 replicates were produced for each sample. All analyses were performed in parallel in triplicate.

Physicochemical analysis

The pH of the samples was determined by dissolving 1 g of the sample in 10 ml of distilled water and measuring with a pH meter.

The titration acidity value was calculated by titrating 25 ml of the filtrate obtained by crushing 10 g of cheese sample with distilled water with 0.1 N NaOH. The results determined using the formula expressed the acidity of the cheese in % in terms of lactic acid (AOAC, 2000).

Unsalted White cheeses coated with edible films and control samples were analyzed for moisture and ash contents during storage. For moisture determination, the samples were allowed to reach a constant weight in an oven at 105°C, whereas for ash determination, the samples were incinerated in a ash furnace until white ash residue remained (AOAC, 2000).

The color values of unsalted white cheeses were determined by determining the *L**, *a**, and *b** values using a color analyzer (Minolta Chroma Meter, CR-400).

Microbiological analysis

Unsalted white cheese samples were diluted 10^{-1} with sterilized physiological saline. For the rest of the analysis, the samples were diluted to 10^{-6} and inoculated on Plate Count Agar (PCA) medium for Total Psychrophilic Aerobic Bacteria Count using the pour plate method. The media were incubated at 10°C for 10 days, and the colonies were counted (log CFU/g). Lactic Acid Bacteria enumeration was performed using Man Rogosa Sharpe Agar (MRS)

and M17 media. After inoculation, the medium was incubated at 37°C for 48 hours. Yeast and mold counts were inoculated on Potato Dextrose Agar (PDA) medium using the pour plate method. The medium was incubated at 10°C for 5-7 days. To determine the total coliform count, violet red bile agar (VRBA) medium was inoculated by double pouring. The medium was then incubated at 35°C for 24 h. Colonies were counted (log CFU/g) (Halkman, 2005).

Texture analysis

Texture analysis was performed to determine the effect of biofilm coating on cheese samples during storage. The analysis was performed using a texture meter (Taxt Plus 2-stable macrosystems). P/25 aluminum cylinder probe was used to determine textural properties. Device operating conditions were determined as test speed 1mm/s, pretest speed 5 mm/s, post test speed 1 mm/s and compression 25%. The values of parameters such as hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience were analyzed.

Sensory analysis

All of the unsalted white samples prepared within the scope of the study were sensory analyzed during the specified storage periods. The participants were selected as 25 males and 25 females who were educated in the food engineering department and trained on sensory panel. Sensory analyses were carried out with panelists who did not smoke and did not have any disease. The samples were evaluated in terms of external appearance, internal appearance, structure, odor and taste characteristics by 30 trained panelists (9=most liked, 1=least liked). On the common last day of storage, all samples were compared. Before the analysis, all panelists were informed about the raw materials used in the study and the scope of the study. They were asked whether they were allergic to any component subject to the study and the panel proceeded after approval was obtained.

Statistical analysis

The statistical difference between the sample results was determined by Anova and Tukey tests in Minitab17 package program.

Results and Discussion

Physicochemical analysis

Table 1. pH and titratable acidity of White cheese samples during the storage process

Sample	pH					
	Storage time (day)					
	0	5	10	15	20	25
C	6.64±0.05^{a}	6.36±0.01^{c}	*	*	*	*
1R	6.70±0.01^{a}	6.34±0.02^{c}	6.20±0.01^{d}	5.88±0.04^{d}	*	*
3R	6.74±0.01^{b}	6.17±0.02^{a}	5.90±0.02^{b}	5.80±0.01^{c}	*	*
1L	6.73±0.03^{b}	6.61±0.02^{d}	6.51±0.01^{e}	*	*	*
3L	6.76±0.01^{b}	6.24±0.01^{b}	6.02±0.01^{c}	5.71±0.05^{b}	5.41±0.02^{b}	5.14±0.06^{b}
	Titratable Acidity (%)					
Sample	Storage time (day)					
	0	5	10	15	20	25
C	2.75±0.05^{b}	1.78±0.08^{b}	*	*	*	*
1R	1.80±0.05^{a}	0.95±0.05^{a}	2.48±0.03^{b}	2.97±0.03^{c}	*	*
3R	1.78±0.03^{a}	0.97±0.06^{a}	2.73±0.03^{c}	2.92±0.03^{c}	*	*
1L	2.63±0.19^{b}	1.68±0.03^{b}	2.58±0.10^{b}	*	*	*
3L	2.48±0.42^{b}	1.67±0.08^{b}	2.55±0.05^{b}	2.70±0.05^{b}	2.88±0.03^{b}	3.08±0.13^{b}
C	2.75±0.05^{b}	1.78±0.08^{b}	*	*	*	*

C: Control; 1R: 1% rosemary essential oil; 3R: 3% rosemary essential oil; 1L: 1% laurel essential oil; 3L: 3% laurel essential oil. The difference between samples with different lowercase letters in the same row and samples with different uppercase letters in the same column is significant in itself for each analysis ($p<0.05$)

*samples were not analyzed due to sensory deterioration on the specified storage day

Table 1 shows the pH and titratable acidity values of unsalted white cheese samples. The pH values of the unsalted white cheese samples coated with edible films prepared by adding rosemary and laurel essential oil at different ratios were in the range of 5.14-6.76. When the experimental samples were evaluated between the groups according to the pH value chart, the statistical difference between group C and the other groups on day 0 was significant (P<0.05). When the changes between days were analyzed, a statistically significant difference was found between all days in the 3L sample (P<0.05). The free H+ ion in milk and other ions in equilibrium are defined as the actual acidity in milk and dairy products, and physical, chemical and microbiological changes in cheese production can be explained by changes in pH value (Yerlikaya and Karagözlü, 2014). When the effect of coating change on pH in unsalted white cheese samples coated with edible films prepared using whey powder protein, rosemary, and laurel essential oil was evaluated, a decrease was observed with storage. decrease in pH was observed from day 0 to day 25 of storage. It is thought that acid is released as a result of lactic acid bacteria breaking down lactose in milk; therefore, the pH decreases in the last stages of the storage process as a result of microbial activity. Yangılar (2015) reported that the initial pH values of cheddar cheese samples coated with control, chitosan, and chitosan/whey protein combination-based films was 5.31 and the pH values at 60 days were 5.49, 5.20, and 5.28, respectively. The results of this study were similar to our research results.

The titratable acidity values of the unsalted white cheese samples used in this study were in the range of 0.95-3.08% during the storage period. When the difference between groups was analyzed on day 0, the statistical difference between C, 1L and 3L groups and 1R and 3R groups was found to be significant (P<0.05). During the storage period, titratable acidity values decreased on the 5th day and increased. In the 3L group, the difference was statistically significant (P<0.05). When the titratable acidity values of unsalted white cheese samples coated with whey powder protein using rosemary and laurel essential oil were examined, it was found that the acidity increased towards the last days of storage, but the highest lactic acid value was found in group C. It is thought that the reason why the other groups have lower acid values than group C is due to the buffering properties of the amino acids in the whey. Kavas and Kavas (2014) applied edible film coating to curd cheese and reported that it improved acidity in cheeses and an increase in acidity was observed with the increase in mint essential oil ratio. Wagh et al., (2014) coated cheddar cheese with edible films using casein and whey concentrate and observed that the titration acidity increased in all samples at the end of 30 days of storage compared to the beginning. They reported that the difference between the control and coated groups was statistically significant. In another study, titratable acidity changes in Ricotta cheese coated with edible films containing chitosan and whey proteins were investigated. In the first week of the study, it was determined that titratable acidity increased in film-coated products compared to the control sample (Di Pierro et al., 2011).

Table 2. Moisture and ash content of white cheese samples during the storage process

Sample	Moisture Content (%)					
	Storage time (day)					
	0	5	10	15	20	25
C	59.72±0.45Ca	56.24±0.34Cb	*	*	*	*
1R	60.87±0.31^{Bc}	63.03±0.74^{Bb}	65.23±0.32^{Aa}	65.92±0.14^{Aa}	*	*
3R	62.39±0.55^{Ab}	63.20±0.30^{Bab}	63.95±0.16^{Ba}	64.07±0.21^{Ba}	*	*
1L	62.66±0.29^{Ab}	64.49±0.48^{Aa}	65.02±0.15^{Aa}	*	*	*
3L	62.27±0.30^{Ac}	63.50±0.42^{ABbc}	64.72±0.49^{ABab}	65.11±0.65^{Aa}	65.36±0.53^{a}	65.38±0.49^{a}
	Ash Content (%)					
Sample	Storage time (day)					
	0	5	10	15	20	25
C	1.42±0.01 C^{b}	1.45±0.01 C^{a}	*	*	*	*
1R	1.71±0.01^{ABc}	1.73±0.01^{ABbc}	1.74±0.01^{ABab}	1.75±0.01^{Aa}	*	*
3R	1.72±0.01^{Aa}	1.73±0.02^{ABa}	1.75±0.01^{Aa}	1.75±0.01^{Aa}	*	*
1L	1.68±0.02^{Ba}	1.69±0.01^{Ba}	1.71±0.02^{Ba}	*	*	*
3L	1.70±0.01^{ABa}	1.73±0.01^{Aa}	1.72±0.02^{ABa}	1.76±0.02^{Aa}	1.78±0.03^{a}	1.86±0.04^{a}
C	1.42±0.01 C^{b}	1.45±0.01 C^{a}	*	*	*	*

C: Control; 1R: 1% rosemary essential oil; 3R: 3% rosemary essential oil; 1L: 1% laurel essential oil; 3L: 3% laurel essential oil. The difference between samples with different lowercase letters in the same row and samples with different uppercase letters in the same column is significant in itself for each analysis ($p<0.05$)

*Samples were not analyzed due to sensory deterioration on the specified storage day

The moisture analysis values of unsalted white cheese samples varied between 56.24-65.92% (Table 2). Compared with group C, the moisture values in the other groups increased depending on the storage time. This increase was due to the coating material. An increase in moisture content was observed in the coated groups. According to the results of moisture analysis, when the experimental samples were evaluated between the groups, the statistical difference was found to be significant ($P<0.05$) on day 0 when C and 1R groups were compared with 3R, 1L and 3L groups. The moisture content of sample C was lower than that of the samples with the addition of essential oil to the coating. In the inter-day evaluations, the difference found in the increases on the 0th and 5th days and other storage periods of the 3L sample was found to be statistically significant ($P<0.05$). The moisture content of unsalted white cheeses coated with films prepared with rosemary and laurel essential oils increased during storage. On the other hand, the coated unsalted white cheeses had a higher moisture content than the control group. This may be attributed to the effect of the essential oils added to the coating and barrier properties of the whey protein powder. Whey proteins are known to enhance the water vapor barrier properties of coatings prepared with whey protein (Tang et al., 2003).

The ash analysis values of unsalted white cheese samples varied between 1.42-1.86%. When ash analyses were performed between the experimental sample groups, the statistical difference was found to be significant ($P<0.05$) when the C and 1L groups were compared with the 1R, 3R, and 3L groups on day 0. In all the unsalted white cheese samples prepared within the scope of the study, ash values increased towards the end of the storage period. It is thought that the amount of organic matter in the environment increases as a result of microbial activities, and accordingly, the amount of ash increases. In a similar study, the coating of soft cheese with thyme and green tea extracts and changes in the storage process were investigated. The amount of ash increased during the storage process, and the amount of ash in the samples was between 2.01 and 3.82% (Hazaa and Jassim, 2021).

Microbiological analysis

Table 3. Color properties of white cheese samples during the storage process

Sample	*L**					
	Storage time (day)					
	0	5	10	15	20	25
C	93.40±0.20^{Aa}	93.80±0.13^{Aa}	*	*	*	*
1R	91.81±0.18^{Bc}	92.17±0.05^{BCbc}	93.75±0.82^{Bb}	96.39±0.88^{Ba}	*	*
3R	91.04±0.27^{BCc}	90.29±0.26^{Dd}	93.04±0.23^{Bb}	99.60±0.33^{Aa}	*	*
1L	89.82±0.65^{Db}	91.81±0.28^{Ca}	92.79±0.21^{Ba}	*	*	*
3L	90.71±0.47^{CDe}	92.38±0.25^{Bd}	95.94±0.30^{Ac}	98.19±0.48^{Ab}	98.34±0.19^{b}	99.64±0.18^{a}
	*a**					
Sample	Storage time (day)					
	0	5	10	15	20	25
C	-0.01±0.13^{Ab}	0.13±0.01^{Aa}	*	*	*	*
1R	-0.29±0.10^{Bd}	0.14±0.03^{Ac}	0.33±0.02^{Ab}	1.73±0.07^{Ba}	*	*
3R	0.48±0.01^{BCb}	0.07±0.01^{BCd}	0.18±0.03^{BCc}	3.18±0.03^{Aa}	*	*
1L	-0.59±0.05^{Cc}	0.11±0.02^{ABb}	0.24±0.02^{Ba}	*	*	*
3L	-1.00±0.04^{Dd}	0.05±0.01 ^{Cc}	0.14±0.03^{Cb}	3.27±0.05^{Aa}	3.38±0.19^{a}	4.75±1.71^{a}
	*b**					
Sample	Storage time (day)					
	0	5	10	15	20	25
C	12.59±0.47^{Ca}	12.54±0.02^{Ca}	*	*	*	*
1R	14.66±0.02^{Ab}	14.77±0.08^{Aab}	15.12±0.09^{Ba}	9.80±0.30^{Ac}	*	*
3R	14.91±0.10^{Aa}	11.34±0.21^{Db}	11.84±0.11^{Cb}	9.18±0.85^{Ac}	*	*
1L	13.42±0.37^{Bb}	13.72±0.17^{Bb}	14.64±0.32^{Ba}	*	*	*
3L	13.72±0.25^{Bb}	13.90±0.02^{Bb}	15.65±0.12^{Aa}	6.62±0.58^{Bcd}	6.09±0.14^{d}	7.14±0.23^{c}

C: Control; 1R: 1% rosemary essential oil; 3R: 3% rosemary essential oil; 1L: 1% laurel essential oil; 3L: 3% laurel essential oil. The difference between samples with different lowercase letters in the same row and samples with different uppercase letters in the same column is significant in itself for each analysis ($p<0.05$)

*samples were not analyzed due to sensory deterioration on the specified storage day

Color values of the samples are shown in Table 3. *L** value represents whiteness and brightness. The *L** values of the samples were between 89.82-99.64, indicating that the samples were intensely white and bright. When evaluated between groups, the highest *L** value on day 0 was in group C and the lowest *L**value was in group 1L. When rosemary and laurel essential oil were added to unsalted white cheese samples coated with whey powder protein, the *L** (brightness and whiteness) value increased during storage. This was evaluated as an effect of the glossy structure of the coating material. In other studies, no change in the L value was observed in soft cheese samples during storage (Youssef et al., 2015), while the whiteness of Oaxaca cheese decreased during vacuum storage in the refrigerator (Fuentes et al., 2015). Changes in the preparation process of cheese varieties, ingredients added to the product formulation, and changes in coating materials affect the whiteness values.

*a** value represents redness and greenness indicators. The *a** values of the samples were in the range of -1.00-4.75. On day 0, *a** values were generally negative among the groups, with the lowest value in 3L and the highest value in 3R groups. Negative *a**values represent greenness and positive *a**values represent redness. The increase in *a** values towards the end of storage indicates an increase in redness. *b** value represents yellowness. The *b** values of the samples are in the range of 6.09-14.91. Whey protein powder had a yellowish color, and the yellowness value in cheese samples was high during the first analysis days. When the difference between 1R and 3R and the difference between 1L and 3L were compared between the groups, the statistical difference was found to be significant ($P<0.05$). While the *b** value of group C was the lowest, it increased as the amount of essential oil

increased. The *a** (redness) values of all samples increased during storage. This is because an increase in yeast and mold values was observed during the last days of storage, which also increased the redness values. When the *b** (yellowness) values were examined, the yellowness values of the groups to which essential oils were added were higher than those of the control group. Rosemary and laurel essential oils were found to increase *b**(yellowness) values. In a study conducted by Civelek and Çağrı Mehmetoğlu (2019) on cheddar cheeses, Williopsis Saturnus var. Saturnus in the edible coating against yeasts and molds on the outside of the cheese reported *a** value between -0.74 and - 1.97 towards the end of 56 days of storage, and there was no change in the *b** value.

Table 4. Microbiological properties of White cheese samples during the storage process

Sample	Total Psychrophilic Aerobic Bacteria (log kob/g)					
	Storage time (day)					
	0	5	10	15	20	25
C	<1	3.53±0.38^{Ba}	*	*	*	*
1R	<1	4.18±0.13^{ABa}	4.27±0.36^{Aa}	4.42±0.26^{Aa}	*	*
3R	<1	4.58±0.29^{Aa}	4.31±0.52^{Aa}	4.44±0.48^{Aa}	*	*
1L	<1	4.32±0.45^{ABa}	4.35±0.58^{Aa}	*	*	*
3L	<1	3.48±0.31^{Bab}	4.35±0.58^{Aa}	4.24±2.50^{Aab}	4.69±0.17^{a}	5.26±0.19^{a}
	Total Yeast and Mold Count (log kob/g)					
Sample	Storage time (day)					
	0	5	10	15	20	25
C	4.57±0.38^{Aa}	*	*	*	*	4.57±0.38^{Aa}
1R	4.67±0.19^{Aa}	4.94±0.12^{Aa}	4.97±0.06^{Aa}	*	*	4.67±0.19^{Aa}
3R	4.53±0.38^{Aa}	4.76±0.25^{Aa}	4.93±0.10^{Aa}	*	*	4.53±0.38^{Aa}
1L	4.18±0.05^{ABb}	4.55±0.24^{Aa}	*	*	*	4.18±0.05^{ABb}
3L	3.48±0.22^{Bb}	4.64±0.02^{Aa}	4.74±0.03^{Ba}	4.64±0.11^{a}	4.73±0.13^{a}	3.48±0.22^{Bb}
	Lactic acid bacteria count *(Lactococcus spp.M17-medium)*					
Sample	Storage time (day)					
	0	5	10	15	20	25
C	3.49±0.31^{Aa}	3.28±0.04^{Aa}	*	*	*	*
1R	2.79±0.19^{Ba}	3.01±0.03^{ABa}	2.85±0.04^{ABa}	2.82±0.10^{Aa}	*	*
3R	2.57±0.15^{Ba}	2.49±0.45^{Ba}	2.96±0.05^{Aa}	2.86±0.06^{Aa}	*	*
1L	2.35±0.04^{Ba}	2.58±0.25^{Ba}	2.46±0.10^{cb}	*	*	*
3L	2.46±0.13^{Bb}	2.60±0.19^{Bab}	2.74±0.03^{Ba}	2.81±0.07^{Aa}	2.84±0.03^{a}	2.85±0.04^{a}
	Lactic acid bacteria count *(Lactobacillus spp.MRS medium)*					
Sample	Storage time (day)					
	0	5	10	15	20	25
C	3.27±0.07^{Ab}	3.79±0.03^{Aa}	*	*	*	*
1R	2.25±0.02 ^{Cb}	2.34±0.03^{Cab}	2.38±0.04^{Ba}	2.33±0.08^{Bab}	*	*
3R	2.32±0.08 ^{Ca}	2.34±0.07^{Ca}	2.32±0.03^{Ba}	2.24±0.05^{Ba}	*	*
1L	2.79±0.13^{Ba}	2.45±0.09^{Cb}	2.32±0.04^{Bb}	*	*	*
3L	2.29±0.05^{Cc}	2.65±0.05^{Bb}	2.84±0.08^{Aa}	2.90±0.04^{Aa}	2.86±0.03^{a}	2.85±0.05^{a}

C: Control; 1R: 1% rosemary essential oil; 3R: 3% rosemary essential oil; 1L: 1% laurel essential oil; 3L: 3% laurel essential oil. The difference between samples with different lowercase letters in the same row and samples with different uppercase letters in the same column is significant in itself for each analysis ($p<0.05$)

*Samples were not analyzed due to sensory deterioration on the specified storage day

Table 4 shows the results of microbiological analysis of unsalted white cheese samples. The number of coliform bacteria in the unsalted white cheese samples during 25 days of storage at 4°C was below the detectable level and is therefore not shown in the table. According to the table, the psychrophilic aerobic bacteria values of the unsalted white cheese samples coated with films prepared with different ratios of rosemary and laurel essential oil were found below the

detectable level on day 0 and ranged between 3.48 -5.26 (log_{10}cfu/g) during the storage period. On day 5, the 1R, 3R, and 1L groups were found to be statistically significant compared to the C and 3L groups (P<0.05). On day 5, the lowest value was observed in the 3L group, indicating that the antimicrobial activity of laurel essential oil against psychrophilic microorganisms was effective. In the inter-day evaluations, the difference in the 3L sample between days was not statistically significant (P>0.05). When the effects of rosemary and laurel oil on the number of psychrophilic bacteria in unsalted white cheese samples coated with whey powder protein were examined, the lowest number of psychrophilic bacteria was found in the 3L group on the 5th day of storage. The fact that the number of psychrophilic bacteria in the 3L sample was lower than that in the C sample can be interpreted as an inhibition of the microbial effect of the oil due to the high amount of laurel essential oil in the edible film coating. In addition, the fact that the shelf life of the cheeses produced with all coatings made with essential oils was longer than the control group unsalted white cheese was found valuable in terms of our study. The addition of essential oils to the coating formulation increased the shelf life of unsalted white cheeses coated with whey powder protein during cold storage. Similarly, in another study on essential oils, cheese samples coated with sodium alginate and essential oils showed poor microbial growth (total aerobic mesophilic flora, yeasts, and fecal coliforms) during storage (Mahcene et al., 2020). The addition of Mentha longifolia essential oil and pulegone to edible coatings significantly reduces bacterial growth in cheese (Shahdadi et al., 2023). This was attributed to the superior antimicrobial effects of the essential oils.

The yeast and mold counts of the samples varied between 3.48-4.97 (log_{10}cfu/g) during the storage period. When the experimental samples were evaluated between the groups, the statistical difference was found to be significant (P<0.05) on the 5th day when the C, 1R, 3R, and 1L groups were compared with the 3L group. In the inter-day evaluations, the difference in the 3L sample on the 5th day between the other days was statistically significant (P<0.05). When the effects of rosemary and laurel essential oil on yeast and mold counts in unsalted white cheese samples coated with whey powder protein were examined, an increase in yeast and mold counts was observed as the storage time increased. In the first days of storage, the lowest yeast and mold counts were observed in the 1L and 3L groups. Cheese samples coated with films prepared with essential oils were evaluated in terms of yeast and mold values, and they were determined to have a longer shelf life than uncoated cheese. This result indicates that coating unsalted white cheeses stored in cold storage with films produced with whey protein and laurel essential oil is more effective. The reason for this increase is thought to be that lactose in the environment is broken down into galactose and glucose by the starter culture, and the glucose formed supports the growth and nutrition of yeasts (Liu and Tsao, 2009). On the other hand, the coating applied to curd cheese retains moisture and preserves appearance and color by reducing yeast and mold growth during long-term storage (Mileriene et al., 2020).

The lactic acid bacteria count was analyzed for *Lactococcus spp*. in M17 medium and Lactobacillus spp. in MRS medium. When the experimental samples were evaluated between the groups according to the table of lactic acid bacteria values formed in M17 medium, the statistical difference was found to be significant (P<0.05) when the C, 1R, 3R, and 1L groups were compared with the 3L group on day 0. In the inter-day evaluations, the difference between the values of the 3L sample on day 0 and the other days was statistically significant (P<0.05). The values of lactic acid bacteria in MRS medium of unsalted white cheese samples varied between 2.24 - 3.79 (log_{10}cfu/g) during the storage period. When the experimental samples were evaluated between the groups, the statistical difference between the C and 1R groups and the other groups on day 0 was significant (P<0.05). When the effects of whey and rosemary-laurel essential oils on lactic acid bacteria were examined, it was found that 3L rosemary essential

oil was more effective against lactic acid bacteria. Although the addition of essential oil and the coating process decreased the lactic acid numbers in cheese, this was not in the range to be expressed as inhibition of cheese. Even after the shelf life of the control sample was completed, owing to the prolonged shelf life, lactic acid bacteria ratios in the coated products were detected in valuable ranges. While examining the effects of Melissa officinalis essential oil on microorganisms in cheese, despite its positive effects, an inhibitory effect on lactic acid bacteria was detected, and it was concluded that this essential oil was not suitable for cheese production (Licon et al., 2020). In contrast, thyme essential oil does not negatively affect the growth or metabolic activity of lactic acid bacteria in cheeses coated with thyme (Marcial et al., 2016). The inhibitory effect is thought to be influenced by variables such as the antimicrobial effect of the essential oil and other raw materials used in the coating.

Texture analysis

Table 5 Textural properties of White cheese samples during the storage process

Sample	Hardness					
	Storage time (day)					
	0	5	10	15	20	25
C	1644.90±463.81^{ABa}	1701.92±463.73^{Aa}	*	*	*	*
1R	1139.71±217.10^{ABab}	1167.12±46.20^{Aa}	1020.32±126.19^{Aab}	789.21±85.26^{Ab}	*	*
3R	1743.10±574.92^{ABa}	1610.85±406.20^{ABa}	1159.45±81.00^{ABa}	876.01±77.45^{ABa}	*	*
1L	1936.59±239.83^{Aa}	1827.45±192.04^{Aa}	865.97±222.72^{Ab}	*	*	*
3L	809.49±358.19^{Bab}	1257.98±346.37^{Aa}	825.29±57.00^{Aab}	704.40±381.81^{Aab}	336.90±94.17^{b}	518.95±82.70^{b}
	Adhesiveness					
Sample	Storage time (day)					
	0	5	10	15	20	25
C	-13.73±8.31Cb	-54.92±38.28^{Aa}	*	*	*	*
1R	-5.44±3.97Db	-11.26±5.45C^{a}	-10.58±6.57^{Aa}	-9.78±6.32C^{a}	*	*
3R	-36.32±38.28^{A}b	-41.74±54.50B^{a}	-45.61±2.37^{A}a	-44.69±23.62^{Aa}	*	*
1L	-20.00±12.20Bb	-38.97±43.90B^{a}	-38.48±3.59^{A}a	*	*	*
3L	-13.26±1.34Cb	-13.00±1.70Cb	-24.19±29.30^{Aa}	-23.29±8.81B^{a}	-25.70±1.37^{a}	-26.27±4.68^{a}
	Gumminies					
Sample	Storage time (day)					
	0	5	10	15	20	25
C	1344.68±368.80^{Aa}	1386.31±370.05^{Aa}	*	*	*	*
1R	930.98±169.36^{Ba}	953.80±36.66B^{b}	818.62±89.75^{Bc}	634.01±61.07^{Bd}	*	*
3R	1454.11±467.42^{Aa}	1305.60±358.98^{Ab}	931.37±71.09^{Ac}	718.52±71.52^{Ad}	*	*
1L	1554.00±205.23^{Aa}	1442.61±145.97^{Aa}	703.49±165.94^{Cb}	*	*	*
3L	678.03±300.12^{Cd}	1011.60±260.55^{Ba}	672.74±49.28^{Db}	578.95±316.10^{Cc}	302.76±149.18^{e}	385.78±50.97^{d}
	Chewiness					
Sample	Storage time (day)					
	0	5	10	15	20	25
C	1217.82±361.90^{Aa}	1187.28±366.29^{Aa}	*	*	*	*
1R	846.39±171.73^{Ba}	857.53±55.12^{Ba}	738.22±70.13^{Ab}	570.99±61.40^{Ac}	*	*
3R	1321.90±400.55^{Aa}	1152.47±356.46^{Aa}	874.65±70.33^{Ab}	597.75±84.68^{Ac}	*	*
1L	1400.39±167.16^{Aa}	1296.50±170.72^{Aa}	616.24±125.76^{Bb}	*	*	*
3L	614.81±271.42^{Bb}	902.37±219.34^{Ba}	565.21±90.09^{Bc}	516.91±286.93^{Ac}	223.82±62.52^{d}	283.89±46.18^{d}

C: Control; 1R: 1% rosemary essential oil; 3R: 3% rosemary essential oil; 1L: 1% laurel essential oil; 3L: 3% laurel essential oil. The difference between samples with different lowercase letters in the same row and samples with different uppercase letters in the same column is significant in itself for each analysis ($p<0.05$)

*Samples were not analyzed due to sensory deterioration on the specified storage day

Table 5 presents the texture analysis results of the samples. No statistical difference was found between the springiness, cohesiveness, and resilience values ($P>0.05$); therefore, they are not presented in the table. The hardness value was determined to be in the range of 336.90-1936.59, although it decreased during storage. The adhesiveness values of unsalted white cheeses

varied between -5.44 and -54.92. On day 0, when the difference between the groups was examined, the statistical difference between the C and 3L groups and the 1R, 3R, and 1L groups was found to be significant ($P<0.05$). The group with the highest value in the Gumminies parameter on day 0 was the 1L group, and the values ranged between 385.78 and 1554.00. In contrast, chewiness values of the cheeses ranged between 223.82 and 1400.39. Texture profile analysis of unsalted white cheese samples was performed using seven parameters: when the results were evaluated, the hardness of the samples decreased towards the end of storage. The texture profile analysis values of the samples (cohesiveness, springiness, and adhesiveness) did not vary depending on the concentration of essential oil contained in the sample and did not change the textural properties of the samples. The degree of hardness increased on the first day of storage. The reason for this is thought to be that the enzyme is still active during the ripening stage and the increase is thought to be due to the amount of dry matter. The degree of hardness decreased as the storage period increased. After the 5th day, a significant decrease in hardness was observed. It is believed that the hardness rate decreases as the moisture content increases. According to Akın et al., (2009), in their research on Kashar cheeses, the hardness data increased in the first days, but the hardness rates decreased on the 60th day. Biofilm coating on cheese reduces weight loss and hardness and protects cheese without seriously affecting its organoleptic properties (Mahcene et al., 2020). Edible coatings made from whey protein concentrate and cumin essential oil did not affect the color or texture of the samples during 28 days of storage (Nemati et al., 2023).

Sensory analysis

All unsalted white cheese samples experienced product deterioration at different times and their storage processes were completed. Therefore, a sensory evaluation graph was drawn for the last common storage day, Day 5 (Figure 1). In the sensory evaluation, 3L, 1R, and C received the highest scores for taste, whereas the lowest score was given to sample C for structure. On the other hand, samples coded 1L and 3 B received the lowest scores for odor, while 1L and 1R were the most preferred products in terms of external appearance. Sensory characteristics such as appearance, odor, flavor and texture influence consumer preferences for specialty cheeses (Lawlor and Delahunty, 2000). In the sensory evaluation of unsalted white cheese samples, group C scored the highest in odor and taste. Unsalted white cheese samples containing essential oils were less appreciated because they had a unique heavy and dominant aromatic odor and taste. The exterior appearance, interior appearance, and structure scored higher in the samples containing essential oil than in the C group.

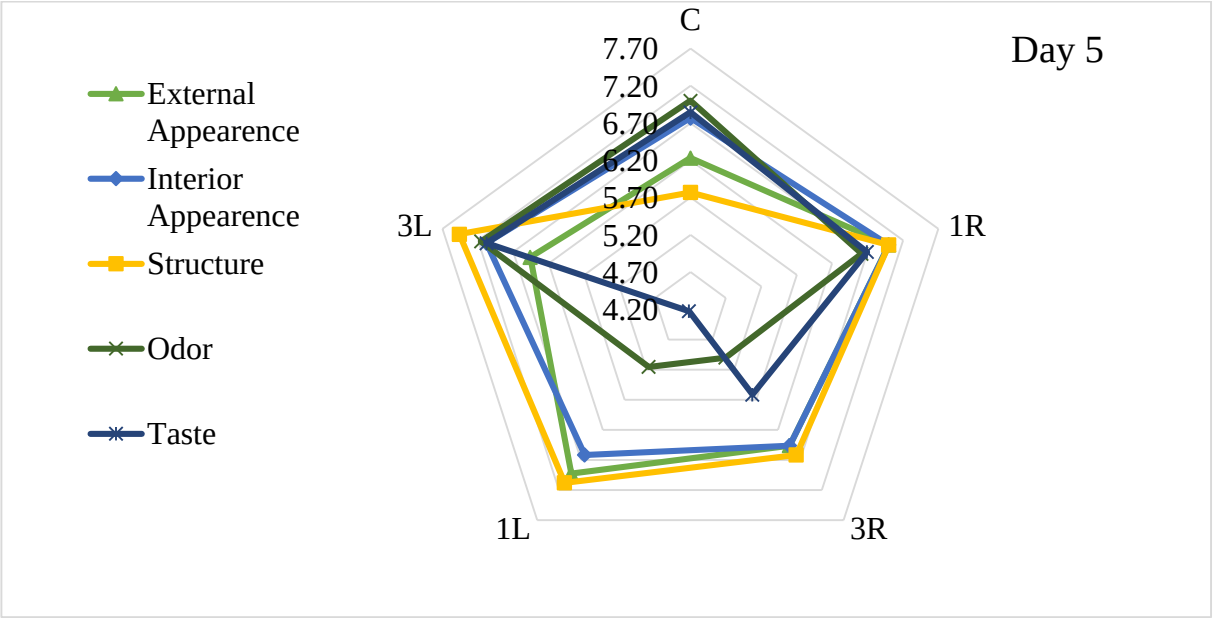

Figure 1. Sensory properties of White cheese samples at 5 day of storage

Conclusions

Based on the microbiological, physical, chemical, and sensory analyses, the 3L group was determined to be the most successful sample in the production of unsalted white cheese. The use of 3% laurel essential oil in whey protein-coated unsalted white cheese samples increases the shelf life and quality of the product. Because the product contains its own aromatic taste and odor, it is difficult to consume; however, the fact that whey protein and 3% laurel essential oil can be used in unsalted white cheese opens a new way to investigate the effects of these essential oils on different types of cheeses.

After this stage, studies can be carried out to obtain the most optimum product formulation by choosing different coating materials in unsalted white cheese. Different film materials can be used. In addition, synergistic effects can be studied with different essential oils. Every study is valuable for unsalted white cheese, which has a very short shelf life, and can support the solution of existing problems.

Availability of Data and Materials:

The data that findings of this study are available from the authors upon reasonable request.

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Author Contributions

ÖPC was responsible for the conception and design of the study. DU performed the experiments. DU and MGS wrote the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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HARRAN TARIM ve GIDA BİLİMLERİ DERGİSİ

YAZIM KURALLARI

1. Makale, **Microsoft Word programında, Calibri** yazı karakterinde, **1.15 satır aralığında**, **12 punto** düz metin ve tek sütun olarak yazılmalıdır.
2. Kenar boşlukları; **sol, sağ, alt ve üst- 3 cm** bırakılarak, her satıra ardışık olarak **satır numarası** verilerek hazırlanmalıdır.
3. Yazar(lar) makalenin ne türde bir yazı **(Araştırma makalesi, derleme, teknik not vb.)** olduğunu belirtmelidir.
4. **Türkçe başlık 14 punto (koyu ve ortalı)** küçük harflerle (Başlığın sadece ilk kelimesinin baş harfi büyük) ve düz yazılmalıdır. **İngilizce başlık 12 punto** ve ortalı yazılmalıdır.
5. Yazar isimleri **Adı SOYADI** kuralına göre Türkçe başlık sonrası **12 punto (koyu, ortalı ve düz)** ve bir boşluk bırakılarak yazılmalı, yazar isimlerinin sonuna adres için üst simge olarak rakam, sorumlu yazarı belirtmek için ise * simgesi verilmelidir. **Adres satırı** yazar isimleri sonrasında 1 boşluk bırakılarak **10 punto (normal, düz ve ortalı)** yazılmalıdır.
6. Adres satırından sonra 1 boşluk bırakılarak yazarların ORCID numaraları yazılmalıdır. ORCID satırının altına, sorumlu yazar e-posta adresi belirtilmelidir.
7. Metin genel olarak;
    - **Öz,**
    - **Abstract**,
    - **Giriş,**
    - **Materyal ve Metot,**
    - **Araştırma Bulguları ve Tartışma,**
    - **Sonuçlar,**
    - **Ekler**
    - **Kaynaklar** şeklinde olmalıdır.
8. Ana başlıkların yazımında koyu olarak kelimelerin sadece baş harfleri büyük yazılmalıdır. İkincil ve üçüncül başlıklarda sadece ilk kelimenin baş harfi büyük, diğer kelimeler küçük, koyu değil ve italik yazılmalıdır. Metin ana başlıkları, metin başlangıcı ve sonunda olmak üzere 1' er boşluk bırakılmalıdır. Alt başlıklardan önce 1 boşluk bırakılmalı, ancak, sonrasında boşluk bırakılmamalıdır. Tüm başlıklar girinti verilmeden sola yaslı olarak yazılmalıdır.
9. **Metin içerisinde kaynak gösterimi (Yazar, yıl)** esasına göre yapılmalıdır. Metin içerisinde iki yazarlı bir kaynağın gösteriminde, metin Türkçe ise **(İlk yazar soyadı ve ikinci yazar soyadı, yıl)** kuralı uygulanmalıdır. İkiden fazla yazarın bulunduğu kaynakların gösteriminde (**İlk yazarın soyadı ve ark., yıl**) kuralı uygulanmalıdır.
   Örneğin; (Mamay, 2020), (İkinci ve Bolat, 2018); (Söylemez ve ark., 2019),
10. Makale İngilizce olarak yazılacaksa **(İlk yazar and ikinci yazar, yıl)** ve (**İlk yazarın soyadı et al., yıl**) kuralı uygulanmalıdır.
    Örneğin; (Söylemez, 2018), (Bolat and Mamay, 2015), (Mamay et al., 2010).
11. Metin içerisinde birden fazla kaynağa aynı anda atıf yapılacak ise; kaynaklar yayınlandıkları yıl dikkate alınarak kronolojik olarak sıralanmalıdır.
12. **ÖZ (ABSTRACT):** Başlık sola yaslı olmalı, 10 punto, koyu, paragraf başında girinti verilmemelidir. Türkçe ve İngilizce metin 300 kelimeyi aşmayacak şekilde, 10 punto ve 1 satır aralığında yazılmalıdır. Öz ile Anahtar Kelimeler ve Abstract ile Key Words arasında tek

satır boşluk (10 punto, düz) bırakılarak metnin hemen altında en fazla 5 adet **Anahtar Kelimeler** (**Key Words**) yazılmalıdır. Key Words ile ana metin (Giriş) arasında iki satır boşluk bırakılmalıdır.

13. Makalelerde fotoğraf, grafik, çizim vb. **"Şekil"** olarak, Tablolar ise **"Çizelge"** olarak ifade edilmelidir.
14. Çizelge ve Şekiller ardışık olarak numaralandırılmalıdır (Şekil 1. veya Çizelge 1.). "Şekil" ve "Çizelge" içerikleri 1 satır aralıklı ve **10 punto** olarak hazırlanmalıdır.
15. Çizelge başlıkları çizelgenin üstünde, şekil başlıkları ise şekillerin altında ilk harf büyük olacak şekilde 1 satır aralıklı **10 punto** olarak yazılmalıdır.
16. **Türkçe yazılmış makalelerde Şekil ve Çizelge başlıklarının İngilizceleri, Türkçe başlığın hemen altında *italik* olarak yazılmalıdır**. (Makale İngilizce olarak yazılmışsa, Şekil ve Çizelge başlıklarının Türkçe karşılıkları yazılmayacaktır)

Şekil 1. Araştırma bahçesinde tespit edilen ortalama sıcaklık, ortalama nispi nem ve aylık yağış miktarı ortalaması değerleri (2007-2011 yılları ortalaması)

Figure 1. The average temperature, average relative humidity and average monthly rainfall data detected in the research garden (average of the years 2007-2011)

Çizelge 2. Şeftali çeşitlerinin 2007 - 2011 yılları arasındaki fenolojik gözlem sonuçları

Table 2. Phenological observation results of peach cultivars for between 2007 and 2011

Türkçe yazılmış makalelerde Çizelge ile Şekillerin içerisinde bulunan parametrelerin İngilizce karşılıkları bu parametrelerin hemen altına ***italik*** olarak yazılmalıdır. (Makale İngilizce olarak yazılmışsa, Şekil ve Çizelgelerin içerisinde belirtilen parametrelerin Türkçe karşılıkları yazılmayacaktır.)

Çizelge 3. Denemede yer alan şeftali çeşitlerinin bazı pomolojik özellikleri

Table 3. Some pomological properties of peach varieties

Çeşitler *Varieties*	Meyve ağırlığı(g) *Fruit weight (g)*	Meyve eni (mm) *Fruit width (mm)*	Meyve boyu(mm) *Fruit length (mm)*	Çekirdek ağırlığı (g) *Kernel weight (g)*
Cardinal	78.19 f	50.73 d	48.48 c	5.06 d
Cresthaven	129.58 b	61.69 bc	59.56 b	8.31 bc

17. Makale metni ve Çizelge-Şekil içerisinde bildirilen ondalık rakamlar, **nokta** ile ayrılmalıdır. (123.87; 0.987 vb.).
18. Çizelge-Şekillerden önce ve sonra **bir satır boşluk** bırakılmalıdır.
19. Makale yazımında **"Uluslararası Birim Sistemi" (SI)**'ye uyulmalıdır. Buna göre; g/l yerine **g l^{-1}**, mg/l yerine **mg l^{-1}** ya da **ppm** kullanılmalıdır. Yüzde ile belirtilen ifadeler açıklayıcı olmalıdır. Örneğin; %3 yerine **%3 (w/v), %3 (v/v), %3 (w/w)** şeklinde belirtilmelidir.
20. **Harran Tarım ve Gıda Bilimleri Dergisi Kaynaklar listesinin bildirişinde APA Formatını kullanmaktadır. Buna göre kaynaklar listesi aşağıdaki kurallar çerçevesinde hazırlanmalıdır.**

1. DERGİ YAYINLARINA ATIF VERME

1.1. Tek yazarlı makale

Mamay, M. (2015). Nar yaprakbiti [*Aphis punicae* Passerini (Hemiptera: Aphididae)]'nin Şanlıurfa ili nar bahçelerindeki bulaşıklık haritası. *Türkiye Entomoloji Bülteni*, *5*(3), 159-166.

1.2. İki yazarlı makale

Soylemez, S., & Pakyurek, A. Y. (2017). Responses of rootstocks to nutrient induced high EC levels on yield and fruit quality of grafted tomato cultivars in greenhouse conditions. *Applied ecology and environmental research, 15*(3), 759-770. DOI: http://dx.doi.org/10.15666/aeer/1503_759770

1.3. İkiden fazla yazarlı makale

Mamay, M., Ünlü, L., Yanık, E., Doğramacı, M., & İkinci, A. (2016). Efficacy of mating disruption technique against carob moth, Apomyelois ceratoniae Zeller (Lepidoptera: Pyralidae) in pomegranate orchards in Southeast Turkey (Şanlıurfa). *International Journal of Pest Management*, *62*(4), 295-299.

Ikinci, A., Mamay, M., Unlu, L., Bolat, I., & Ercisli, S. (2014). Determination of heat requirements and effective heat summations of some pomegranate cultivars grown in Southern Anatolia. Erwerbs-Obstbau, *56*(4), 131-138. DOI: https://doi.org/10.1007/s10341-014-0220-8

2. KİTAPLARI KAYNAK GÖSTERME

2.1. Kaynak kitap ise,

Mohsenin, N. N. (1970). *Physical Properties of Plant and Animal Materials*. New York: Gordon and Breach Science Publishers.

2.2. Kaynak kitaptan bir bölüm ise,

Author, A. A. (Year). Chapter title. In E. E. Editor (Ed.), *Title of book: And subtitle* (pp. pages). Place: Publisher.

2.3. Editörlü kitap

Yeşilyaprak, B. (Ed.). (2003). *Gelişim ve öğrenme psikolojisi*. Ankara: Pegema Yayıncılık.

2.4. Yazarı bilinmeyen kaynakları veya internet kaynaklarını kaynak olarak gösterme;

Anonymous (2005). Tereyağı, diğer süt yağı esaslı sürülebilir ürünler ve sadeyağ tebliği. Türk Gıda Kodeksi, Tebliğ No: 2005/19, Ankara.

FAO, (2015). Statistical data of FAO. Retrieved from: http://faostat.fao.org/site/567/default.asp.

3. YÜKSEK LİSANS ve DOKTORA TEZLERİNE ATIF VERME

Doktora ya da yüksek lisans tezlerine elektronik veri tabanlarından, kurumsal arşivlerden ve kişisel web sayfalarından erişilebilir. Eğer bir teze ProQuest doktora ve yüksek lisans tezleri veri tabanından ya da diğer bir kaynaktan erişildiyse, atıfta bu bilgi verilmelidir. Bir veri tabanı servisinde mevcut olan bir doktora ya da yüksek lisans tezi için aşağıdaki kaynak gösterme biçimi kullanılır:

3.1. Yayımlanmamış tez

Mamay, M. (2013). *Determination of population development and infestation ratio of carob moth [Apomyelois ceratoniae Zell. (Lepidoptera:Pyralidae) in pomegranate orchards in Sanliurfa province and using mating disruption technique for its control* (Yayımlanmamış doktora tezi). Harran Üniversitesi Fen Bilimleri Enstitüsü, Şanlıurfa.

Söylemez, S. (2014). *Effects of nutrient induced salinity levels and rootstocks on plant growing, yield and some fruit quality features at soilless grown grafted tomatoes* (Yayımlanmamış doktora tezi). Harran Üniversitesi Fen Bilimleri Enstitüsü, Şanlıurfa.

3.2. Yayımlanmış tez

May, B. (2007). *A survey of radial velocities in the zodiacal dust cloud*. Bristol, UK: Canopus Publishing.

4. SEMPOZYUM VE TOPLANTI BİLDİRİLERİNE ATIF VERME

Mamay, M. (2017). Population density of overwintering larvae of Carob Moth [*Apomyelois* (=*Ectomyelois*) *ceratoniae* Zell. (Lepidoptera: Pyralidae)] in pomegranate orchards in Southeastern Anatolia. SEAB 2017. *Proceedings of the 3rd International Symposium on EuroAsian Biodiversity*, (pp. 235), 05-08 July 2017, Minsk, Belarus.

Ikinci, A. & Mamay, M. (2017). Effects of fruit thinning on morphological, physico-chemical properties, bioactive compounds, antioxidant activity and pest & disease control in pomegranate fruit (*Punica granatum* L.) *International Conference on Agriculture, Forest, Food Sciences and Technologies*, (pp. 642), 15-17 May 2017, Cappadocia, Turkey.

Sönmez, C., Mamay, M. & Söylemez, S. (2019). Determination of the effect of different hydroponic culture and different NH4:NO3 ratio on the density of aphid [*Aphis* spp. (Hemiptera: Aphididae)] population in greenhouse lettuce. *1st International Gobeklitepe Agriculture Congress* (IGAC-2019), (pp. 599-604), 25-27 November, Şanlıurfa, Turkey.

Not: Yukarıda yer alan kaynak gösterimlerde bulamadığınız farklı materyal veya konu başlıklarındaki kaynak bildirişleri için internetteki APA Kaynak Gösterimi ile ilgili web sayfalarından ya da aşağıdaki linkteki bilgilerden yararlanabilirsiniz.

https://libguides.library.usyd.edu.au/ld.php?content_id=47913440

Şencan, İ., ve Doğan, G. (2017). Bilimsel yayınlarda kaynak gösterme, tablo ve şekil oluşturma rehberi: APA 6 Kuralları. *Türk Kütüphaneciliği Dergisi*, Ankara. https://www.tk.org.tr/APA/apa_2.pdf

HARRAN TARIM ve GIDA BİLİMLERİ DERGİSİ
YAZAR REHBERİ

1. Harran Tarım ve Gıda Bilimleri Dergisi'ne gönderilen makaleler Dergi Yayın Kurulu tarafından belirlenen yazım kurallarına göre yazılmalıdır.

2. Makaleler, **Dergipark Sistemi üzerinden online** olarak yüklenmelidir.

3. Tüm yazarlar tarafından imzalanan T**elif Hakkı Devir Sözleşmesi** ve **Makale Kontrol Listesi** (sorumlu yazar tarafından imzalanacak) makale ile birlikte sisteme yüklenmelidir.

4. **iThenticate Programı Benzerlik Raporu** (%20'yi geçmemelidir) ve gerekli ise **Etik Kurul Kararı** makale ile birlikte sisteme yüklenmelidir.

5. Hazırlanacak olan makale metni genel olarak;

- **Öz,**
- **Abstract,**
- **Giriş,**
- **Materyal ve Metot,**
- **Araştırma Bulguları ve Tartışma,**
- **Sonuçlar,**
- **Ekler,**
- **Beyanlar**
  - **Çıkar Çatışması**
  - **Yazar Katkısı**
- **Kaynaklar** bölümlerinden oluşmalıdır.

6. **Başlık**: Kısa ve açıklayıcı olmalı, **Calibri** yazı karakterinde, **14 punto**, **koyu**, düz, ortalanarak ve küçük harflerle (Başlığın sadece ilk kelimesinin baş harfi büyük) yazılmalıdır. Başlık tercihen 15 kelimeyi geçmemelidir. İngilizce başlık Türkçe başlığı tam olarak karşılamalı, 12 punto ve koyu yazılmalıdır.

7. Harran Tarım ve Gıda Bilimleri Dergisi'ne yayınlanması için makalenin ilk gönderiminde **yazar isimleri, kurum isimleri, adresleri, ORCID numaraları ve e-posta bilgileri yer almamalıdır**.

8. Makalenin hakem değerlendirmesi tamamlandıktan ve makale Yayın Kurulu tarafından kabul edildikten sonra, 7. maddede yer alan yazar isimleri ve diğer bilgiler, hakem önerilerine göre yeniden düzenlenmiş olan makale sayfası üzerine yazıldıktan sonra, Dergi web sayfasında yer alan düzenlenmiş makaleyi gönder sayfasından Dergi sistemine yüklenmelidir. **Kontrol edilmiş veya düzeltilmiş olan makale, yeni bir makale gibi Dergi web sayfasından yüklenmemelidir.**

9. Yazar isimleri **Adı SOYADI** kuralına göre Türkçe başlık sonrası **12 punto (koyu, ortalı ve düz)** ve bir boşluk bırakılarak yazılmalı, yazar isimlerinin sonuna adres için üst simge olarak rakam, sorumlu yazarı belirtmek için ise * simgesi verilmelidir. Adres satırı yazar isimleri sonrasında 1 boşluk bırakılarak **10 punto (normal, düz ve ortalı)** yazılmalıdır. Adres satırından sonra 1 boşluk

bırakılarak yazarların ORCID numaraları yazılmalıdır. ORCID satırının altına sorumlu yazar e-posta adresi belirtilmelidir.

10. **ÖZ**: Çalışmanın yürütüldüğü yer ve zamanını, amacını, yöntemini ve sonuçları içermelidir. Sola yaslı, 10 punto, koyu, paragraf başında girinti verilmemelidir. Türkçe ve İngilizce metin 300 kelimeyi aşmayacak şekilde 10 punto ve 1 satır aralığında yazılmalıdır. Öz ile Anahtar Kelimeler ve Abstract ile Key Words arasında tek satır boşluk (10 punto, düz) bırakılarak, metnin hemen altında en fazla 5 adet **Anahtar Kelimeler (Key Words)** yazılmalıdır. Key Words ile ana metin (Giriş) arasında iki satır boşluk bırakılmalıdır.

11. **Giriş**: Bu bölümde; çalışma konusu, gerekçesi, konu ile doğrudan ilgili önceki çalışmalar ve çalışmanın amacı verilir. Bu bölümde; çalışmanın konusu özetlenmeli, konu hakkındaki mevcut bilgi doğrudan ilişkili önceki çalışmalarla değerlendirilmeli ve bilgi üretimine ihtiyaç duyulan hususlar vurgulanıp çalışma ile ilişkilendirilmelidir. Son olarak çalışmanın amacı net ve açık bir şekilde ifade edilmelidir.

12. **Materyal ve Metot**: Bu bölümde; çalışmada kullanılan canlı ve cansız materyaller, uygulanan yöntemler, değerlendirilen ölçütler, uygulanan deneme desenleri veya örnekleme yöntemleri ile istatistiksel analizler gerektiğinde kaynaklarla da desteklenerek, açık ve net biçimde anlatılmalıdır. Yeni veya değiştirilmiş yöntemler, aynı konuda çalışanlara araştırmayı tekrarlama olanağı verecek nitelikte açıklanmalıdır. Bu amaçla gerektiğinde alt başlık kullanılmalıdır.

13. **Araştırma Bulguları ve Tartışma**: Çalışmada elde edilen bulgular şekil ve çizelgeler yardımıyla ve istatistiksel analizlere dayalı olarak açık ve net bir biçimde verilmelidir. İstatistikî olarak önemli bulunan faktörler, uygulanan istatistik analiz tekniğine uygun karşılaştırma yöntemi ile yorumlanarak ilgili istatistikler üzerinde harflendirme yapılmalıdır. Aynı veriler hem grafik hem de çizelge ile verilmemeli, konuya en uygun araç seçilmeli, anlatımda tekrarlayan cümle ve ifadelerden kaçınılmalıdır. Tartışma kısmında, uyum ve zıtlık açısından önceki çalışmalarla karşılaştırılmalı, doldurduğu bilgi açığı vurgulanmalı, önceki bölümlerdeki ifadelerin olduğu gibi tekrarından kaçınılmalıdır.

14. **Sonuçlar**: Bu bölümde; elde edilen nihai sonuçlar ve varsa öneriler, bilime ve uygulamaya katkısıyla birlikte kısa ve öz olarak verilmelidir.

15. **Ekler**: Çalışmayı destekleyen kurum ve kuruluşlar ile çalışmaya katkı sağlayanlar bu kısımda ifade edilmelidir. Ayrıca, makalenin lisansüstü tezlerden üretilip üretilmediği, abstract olarak kongre ve sempozyumlarda sunulup sunulmadığı da Ekler bölümünde belirtilmelidir.

16. **Beyanlar (Declarations)**

Çıkar Çatışması: Kişiler makalelerin etik ilkeler çerçevesinde değerlendirilebilmesi ve bağımsız bir süreç yürütülebilmesi için olası çıkar çatışmaları ile ilgili olarak yayın kurulunu bilgilendirmelidir. Ekonomik veya kişisel fayda sağlanan durumlar çıkar çatışmasını meydana getirir. Bilimsel sürecin ve yayınlanan makalelerin güvenilirliği; bilimsel çalışmanın planlanması, uygulanması, yazılması, değerlendirilmesi, düzenlenmesi ve yayınlanması sırasında çıkar çatışmalarının objektif bir şekilde ele alınmasıyla doğrudan ilişkilidir. Makale ile ilgili çıkar çatışması söz konusu değilse, "**makale yazarları, aralarında herhangi bir çıkar çatışması olmadığını beyan eder**" ifadesi yazılmalıdır.

Yazar Katkısı: Çalışmanın tasarlanması, planlanması, kurulması, yürütülmesi, verilerin analizi ve

makalenin yazılmasında içeriğe bilimsel açıdan katkı sağlayan her bir yazarın makaleye katkı şekli belirtilmelidir. Yazar katkıları, örnek olarak "**MM çalışmayı tasarlayarak denemeleri kurmuş, MM ve AA çalışmayı yürütmüş, BB verileri analiz etmiş, MM, AA ve BB makaleyi yazmıştır**" şeklinde ifade edilebilir.

17. **Kaynaklar**: Makalede atıfta bulunulan literatürlere Harran Tarım ve Gıda Bilimleri Dergisi Yayın Kurulu tarafından belirlenen **yazım kurallarına göre** yazılmalıdır.

Harran Tarım ve Gıda Bilimleri Dergisi **Yazım Kuralları** için …

18. **Kısaltmalar ve Semboller**: Makale başlığı ve başlıklarda kısaltma kullanılmamalıdır. Gerekli olan kısaltmalar kavramların ilk geçtiği yerde parantez içinde verilmelidir. Kısaltmalarda ve sembollerin kullanımında ilgili alanın evrensel kurallarına uyulması zorunludur.

19. **Formüller**: Makalelerde formüller "Eşitlik" olarak adlandırılmalı ve italik olarak yazılmalıdır. Makalede birden fazla eşitlik varsa numaralandırılmalı, numara formülün yanında sağa dayalı olarak parantez içinde gösterilmelidir.

20. Makaleye ardışık olarak **satır ve sayfa numarası** verilmelidir.

21. **Calibri** karakterinde, **12 punto** ve **1.15 satır** aralıklı yazılan makale **20 sayfayı** geçmemelidir.

22. Yayınlanmasına karar verilen eserler, sadece şekilsel olarak, yukarıda yer alan bilgiler doğrultusunda yeniden düzenlenmeli, yazar(lar)ca herhangi bir eklenti ya da çıkartma yapılmamalıdır.

23. Makale içerisinde, dergi basıldığı haliyle görünen hataların sorumluluğu yazarlara aittir. Yayın Kurulundan kaynaklanan basım hataları için ise düzeltme yayınlanabilir.

24. Harran Tarım ve Gıda Bilimleri Dergisi; yazarlardan makale gönderimi, değerlendirilmesi ve basım aşamalarında herhangi bir basım ücreti almamaktadır.

MANUSCRIPT WRITING RULES

1. The manuscript should be written in Microsoft Word program, in Calibri font, **1.15** line spacing, **12** pt. plain text and a single column.

2. Margins; **Left, right, bottom and top 3 cm** should be left, and each row should be prepared consecutively by giving the line number.

3. Author (s) should indicate the type of manuscript (**Research Manuscript, Review, Technical Note** etc.).

4. **The English title should be written in 14 pt (bold and centered)** lowercase letters (only the first word of the title is capitalized) and in plain text. The Turkish title should be written in 12 font size and centered.

5. Author names should be written in **12 pt. (Bold, centered and plain)** and a space after the title according to the Name SURNAME rule, followed by a number as superscript for the address and a * symbol to indicate the corresponding author. Address line should be written after the author names, leaving **1 space and 10 pt (normal, straight and centered**).

6. Authors' ORCID numbers should be written, leaving 1 space after the address line. Under the ORCID line, the responsible author e-mail address must be specified.

7. The text should generally be in the following form;

- **Abstract**
- **Introduction**
- **Material and Method,**
- **Results and Discussion,**
- **Conclusions**
- **Acknowledgement**
- **References**

8. In the writing of main titles, only the initials of the words should be capitalized in bold. In secondary and tertiary titles, only the first letter of the first word should be capitalized, other words should be in small, not bold and italic. There should be 1 space each, including the main headings of the text, the beginning and the end of the text. 1 space should be left before subtitles, but no spaces should be left after them. All titles should be left justified without indenting.

9. Reference should be cited **in the text based on (Author, year) rule**. In the presentation of a reference with two authors in the text, the rule (**first author's surname and second author's surname, year**) should be applied. In the display of sources with more than two authors (**first author's surname et al., year**) rule must be applied.

For example; (Bilgili, 2020), (Bilgili and vanEs, 2018); (Bilgili et al., 2019).

10. If more than one reference will be cited at the same time in the text; Referencens should be ordered chronologically, considering the year they were published.

11. **ABSTRACT**: Title should be left justified, 10 pt, bold, not indented at the beginning of the paragraph. Turkish and English texts should be written in 10 font size and 1 line spacing, not exceeding 300 words. **A maximum of 5 Key Words** should be written just below the text, leaving a single line space (10 pt., Plain) between Abstract and Keywords, and Öz (Turkish Abstract) and Key Words. Two lines of space should be left between Key Words and the main text.

12. Photographs, graphics, drawings, etc. should be expressed as "Figure" and Tables as "Tables".

13. Tables and Figures should be numbered consecutively (Figure 1. or Table 1.). Contents of "Figure" and "Table" should be prepared with 1 line spacing and 10 pt.

14. Table titles should be written above the table, and figure titles should be written below the figures in 10 pt, 1 line spacing with the first letter capital.

15. Figure and Table titles should be written in italics;

Figure 1. The average temperature, average relative humidity and average monthly rainfall data detected in the research garden (average of the years 2007-2011) Table 2. Phenological observation results of peach cultivars for between 2007 and 2011

16. Decimal numbers in the manuscript text and Table-Figure should be separated by **a period**. (123.87; 0.987 etc.).

17. One blank line should be left before and after the table-figures.

18. Manuscript writing should comply with the "International Unit System" (SI). According to this; Use g l-1 instead of g / l, and mg l-1 or ppm instead of mg / l. Percentages should be descriptive. For example; It should be specified as 3% (w / v), 3% (v / v), 3% (w / w) instead of 3%.

19. Harran Journal of Agriculture and Food Sciences uses **APA Style** in the submission of the sources list. Accordingly, the list of references should be prepared in accordance with the following rules.

19.1. **Citation to journal publications**;

19.1.1. Single author manuscripts;

Mamay, M. (2015). Infestation map of pomegranate aphid [*Aphis punicae* Passerini (Hemiptera: Aphididae)] in Şanlıurfa province pomegranate orchards. Turkey Entomology Bulletin, 5(3), 159-166.

19.1.2. Two-author manuscripts;
Soylemez, S., & Pakyurek, A. Y. (2017). Responses of rootstocks to nutrient induced high EC levels on yield and fruit quality of grafted tomato cultivars in greenhouse conditions. Applied Ecology and Environmental Research, 15(3), 759-770. DOI: http://dx.doi.org/10.15666/aeer/1503_759770

19.1.3. Manuscripts with more than two authors;
İkinci, A., Mamay, M., Unlu, L., Bolat, I., & Ercisli, S. (2014). Determination of heat requirements and effective heat summations of some pomegranate cultivars grown in Southern Anatolia. Erwerbs-Obstbau, *56*(4), 131-138. DOI: https://doi.org/10.1007/s10341-014-0220-8.

19.2. **Referencing Books**;

19.2.1. If the source is a book;
Mohsenin, N. N. (1970). Physical Properties of Plant and Animal Materials. New York: Gordon and Breach Science Publishers.

19.2.2. If it is a chapter from the source book;
Author, A. A. (Year). Chapter title. In E. E. Editor (Ed.), Title of book: And subtitle (pp. pages). Place: Publisher.

19.2.3. Edited book;
Yeşilyaprak, B. (Ed.). (2003). Development and learning psychology. Ankara: Pegema Publishing.

19.3. **Citing sources of unknown author or internet sources**;
Anonymous (2005). Butter, other milk fat-based spreads and plain butter notification. Turkish Food Codex, Communiqué No: 2005/19, Ankara.
FAO, (2015). Statistical data of FAO. Retrieved from: http://faostat.fao.org/site/567/default.asp.

19.4. **Citing Master's and Doctoral theses**;
Doctorate or master theses can be accessed from electronic databases, corporate archives and personal web pages. If a dissertation is accessed from the ProQuest database of doctoral and master's theses or any other source, this information should be provided in the reference. For a doctorate or master thesis available in a database service, the following citation format is used;

Unpublished thesis;
Mamay, M. (2013). *Determination of population development and infestation ratio of carob moth [Apomyelois ceratoniae Zell. (Lepidoptera:Pyralidae) in pomegranate orchards in Sanliurfa province and using mating disruption*

technique for its control (Unpublished doctoral dissertation). Harran University, Graduate School, Şanlıurfa.

Söylemez, S. (2014). *Effects of nutrient induced salinity levels and rootstocks on plant growing, yield and some fruit quality features at soilless grown grafted tomatoes* (Unpublished doctoral dissertation). Harran University, Graduate School, Şanlıurfa.

Published thesis; May, B. (2007). A survey of radial velocities in the zodiacal dust cloud. Bristol, UK: Canopus Publishing.

19.5. **Citing Symposium and Meeting Papers**

Mamay, M. (2017). Population density of overwintering larvae of Carob Moth [*Apomyelois (=Ectomyelois) ceratoniae* Zell. (Lepidoptera: Pyralidae)] in pomegranate orchards in Southeastern Anatolia. SEAB 2017. *Proceedings of the 3rd International Symposium on EuroAsian Biodiversity*, (pp. 235), 05-08 July 2017, Minsk, Belarus.

Ikinci, A. & Mamay, M. (2017). Effects of fruit thinning on morphological, physico-chemical properties, bioactive compounds, antioxidant activity and pest & disease control in pomegranate fruit (*Punica granatum* L.) *International Conference on Agriculture, Forest, Food Sciences and Technologies*, (pp. 642), 15-17 May 2017, Cappadocia, Turkey.

Sönmez, C., Mamay, M. & Söylemez, S. (2019). Determination of the effect of different hydroponic culture and different NH4:NO3 ratio on the density of aphid [Aphis spp. (Hemiptera: Aphididae)] population in greenhouse lettuce. *1st International Gobeklitepe Agriculture Congress (IGAC-2019)*, (pp. 599-604), 25-27 November, Şanlıurfa, Turkey.

Note: You can use the web pages related to **APA Referencing Style** on the internet.

TELİF HAKKI DEVİR SÖZLEŞMESİ

Harran Tarım ve Gıda Bilimleri Dergisi Yayın Kurulu Başkanlığına

Eserin Adı:

Yazar(lar);
a) Sunulan makalenin yazar(lar)ın orijinal çalışması olduğunu;
b) Tüm yazarların bu çalışmaya bireysel olarak katılmış olduklarını ve bu çalışma için her türlü sorumluluğu aldıklarını;
c) Tüm yazarların sunulan makalenin son halini gördüklerini ve onayladıklarını;
d) Makalenin başka bir yerde özet dışında basılmadığını veya basılmak için sunulmadığını;
e) Makalede bulunan metnin, şekillerin ve dokümanların başkalarına ait Telif Haklarını ihlal etmediğini taahhüt ederler.

Bununla birlikte, yazarların veya varsa yazarların işvereninin;
a) Patent hakları;
b) Yazar(lar)ın kitaplarında veya diğer çalışmalarında makalenin tümünü ücret ödemeksizin kullanma hakkı;
c) Makaleyi satmamak koşuluyla kendi amaçları için çoğaltma hakkı gibi fikri mülkiyet hakları saklıdır.
d) Makalenin herhangi bir bölümünün başka bir yayında kullanılmasına Harran Tarım ve Bilimleri Dergisi yayımcı kuruluş olarak belirtilmesi ve Dergiye atıfta bulunulması şartıyla izin verilir.

Ben / Biz, telif hakkı ihlali nedeniyle üçüncü şahıslarca istenecek hak talebi veya açılacak davalarda Harran Tarım ve Gıda Bilimleri Dergisi Editörlerinin hiçbir sorumluluğunun olmadığını, tüm sorumluluğun yazarlara ait olduğunu, ayrıca makalede hiçbir suç unsuru veya kanuna aykırı ifade bulunmadığını, araştırma yapılırken kanuna aykırı herhangi bir malzeme ve yöntem kullanılmadığını taahhüt ederim/ederiz.

1. Yazarın Bilgileri			
Adı SOYADI			
Kurum Adresi			
ORCID Numarası		Tarih	İmza

2. Yazarın Bilgileri			
Adı SOYADI			
Kurum Adresi			
ORCID Numarası		Tarih	İmza

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