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Research Article/Araştırma Makalesi

Determination of the effect of climate change on small cattle milk yield in Iğdır province via machine learning

Iğdır ilinde iklim değişikliğinin küçükbaş süt verimi üzerine etkisinin makine öğrenmesi ile belirlenmesi

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ABSTRACT

This study examines the potential impact of climate change on small cattle livestock and milk productivity in Iğdır province. The study takes into account various factors, including the effects of climate change on animal stress levels, nutrient quality in grazing areas, and the spread of parasites or diseases, which may indirectly affect milk productivity. To evaluate this impact, the study utilizes eXtreme Gradient Boosting (XGBoost) machine learning models with five different climate variables, analyzing the small cattle data from Iğdır province between 2004 and 2023. Two machine learning models were created to investigate the effect of climate variables on milk yield in small cattle in Iğdır province, using a dataset of 10820 rows and 16 columns. The machine learning models revealed that five different climate variables had no significant effect on milk yield. This finding is important for the economic welfare of the region, as cattle farming plays a crucial role in the economy of Iğdır province. The neutral effect of climate change is therefore evaluated positively for Iğdır province. The study suggests that there has been no significant change in milk productivity over the last 20 years due to the constant percentage of sheep that produce milk. It is recommended that farmers in Iğdır province consider increasing the number of lactating sheep to enhance overall cattle milk production.

Key Words: Diary Milk, Machine learning, Milk Yield, Sheep, Goat

ÖZ

Bu çalışma, iklim değişikliğinin Iğdır ilindeki küçükbaş hayvancılık ve süt verimliliği üzerindeki potansiyel etkisini incelemektedir. Çalışmada, iklim değişikliğinin hayvanların stres seviyeleri üzerindeki etkileri, otlatma alanlarındaki besin kalitesi ve süt verimliliğini dolaylı olarak etkileyebilecek parazitlerin veya hastalıkların yayılması gibi çeşitli faktörler dikkate alınmaktadır. Bu etkiyi değerlendirmek için çalışmada beş farklı iklim değişkeni ile eXtreme Gradient Boosting (XGBoost) makine öğrenimi modelleri kullanılmış ve Iğdır ilinin 2004-2023 yılları arasındaki küçükbaş hayvan verileri analiz edilmiştir. Iğdır ilindeki küçükbaş hayvanlarda iklim değişkenlerinin süt verimi üzerindeki etkisini araştırmak için 10820 satır ve 16 sütundan oluşan bir veri seti kullanılarak iki makine öğrenmesi modeli oluşturulmuştur. Makine öğrenimi modelleri, beş farklı iklim değişkeninin süt verimi üzerinde önemli bir etkisi olmadığını ortaya koymuştur. Büyükbaş hayvancılık Iğdır ilinin ekonomisinde önemli bir rol oynadığından, bu bulgu bölgenin ekonomik refahı açısından önemlidir. Dolayısıyla iklim değişikliğinin nötr etkisi Iğdır ili için olumlu olarak değerlendirilmektedir. Çalışma, süt üreten koyun oranının sabit kalması nedeniyle son 20 yılda süt verimliliğinde önemli bir değişiklik olmadığını göstermektedir. Iğdır ilindeki çiftçilere, toplam sığır sütü üretimini artırmak için süt veren koyun sayısını artırmayı düşünmeleri önerilmektedir.

Anahtar Kelimeler: Günlük Süt, Makine öğrenimi, Süt Verimi, Koyun, Keçi

Introduction

Iğdır is a province located in the eastern region of Turkey with a relatively low population and surface area (Koc et al., 2019; Öztürk et al., 2023). The summers are generally hot and dry, while the winters are cold and snowy (Türkeş and Tatli, 2011). These climatic conditions have the potential to affect livestock activities, which in turn can impact milk yield. In Iğdır province, small cattle milk production is primarily obtained from sheep and goats (Yilmaz, 2022). These species are usually domestic and hybrid breeds that can adapt well to the geographical and climatic conditions of the region.

To achieve high milk yield in small cattle, it is crucial to provide them with proper nutrition (Garg et al., 2013). Ensuring that the animals have access to adequate amounts of water and a balanced feeding program not only promotes their health but also increases milk yield. Additionally, it is especially important to feed high-quality feed to promote the growth of calves and milk production in mothers. However, it is important to consider that climate change may lead to warmer and drier conditions, which can result in reduced or inefficient grazing areas. This may have a negative impact on the nutrition of animals, potentially leading to a reduction in milk yield. Additionally, drought, particularly in the summer, can further reduce grazing areas and limit animals' access to natural feed resources, which can further impact the quality and quantity of nutrition, ultimately leading to a reduction in milk yield.

Climate change may have an impact on the spread and distribution of diseases and pests, which may in turn affect animal health and milk yields (Baumgard et al., 2012; Grace et al., 2015). In certain regions, warmer weather conditions may accelerate the spread of parasites and disease-carrying insects, leading to heat stress in animals and reduced milk production. It is important to take into account the potential consequences of climate change on animal agriculture. Changes to temperature and humidity levels have been known to promote the spread of bacteria and fungi, which can have a negative impact on animal health and milk yield.

Additionally, climate variables such as wind, temperature, humidity, and precipitation have been observed to affect milk yield in small cattle (Hill and Wall, 2015; Marumo et al., 2022). It is important to note that strong winds can cause stress in animals, which may lead to reduced milk yield. Temperature conditions can have a notable effect on animal welfare and milk production (Polsky and von Keyserlingk, 2017). Cold winds, for instance, may lower body temperature and cause dehydration, leading to a decrease in milk yield. Therefore, it is crucial to monitor and manage temperature conditions to ensure the best possible milk production.

High humidity can potentially increase the risk of heat stress in animals (Thornton et al., 2021). Sweating efficiency may be reduced in humid environments, which can make it challenging for animals to cool down. This may have a negative impact on animal health and potentially reduce milk yield. Furthermore, high humidity may increase the risk of microbial contamination of milk, which could lead to a decrease in milk quality. In addition, muddy or waterlogged grazing areas during wet weather may impede animal movement and further reduce milk yield. During extended periods of wet weather, it may be necessary for animals to spend more time outside, which could potentially increase the risk of heat stress.

This study examines the correlation between climate change and milk production yield of small cattle in Iğdır province. Meteorological factors, including temperature, precipitation, wind speed, and humidity, were analyzed as climate variables data over the last 20 years to determine the impact of climate change on milk yield in Iğdır province using a machine learning algorithm. There are many different algorithms in machine learning studies. The machine learning algorithms that are most commonly used today are support vector machines, decision trees, linear regression, logistic regression, gradient boosting, and XGBoost (Castro and Ferreira, 2023; Kurani et al., 2023;

Noorunnahar et al., 2023; Soori et al., 2023). Among these algorithms, XGBoost, also known as "extreme gradient boosting," is a highly effective and efficient supervised learning algorithm used in machine learning. XGBoost achieves its results by combining multiple decision trees through an "ensemble" method and correcting the errors of previous trees with each new tree. In addition, XGBoost can provide faster results and more accurate predictions when working with complex data in small data sets compared to other machine learning models. This algorithm was chosen due to its ability to model non-linear relationships, which is particularly important given the non-linear relationships between climate variables and milk yield. By utilizing a tree-based algorithm, the study was able to achieve a more flexible structure and more accurate analysis of the impacts of climate change on milk yield.

Material and Method

Material

To model machine learning, data on small cattle numbers and milk production in Iğdır province was obtained from the Iğdır Provincial Directorate of Agriculture (TOB, 2024). The dataset covers small cattle milk production from 2004 to 2023, including the total number of sheep and goats, as well as the number of sheep and goats that produce milk. The study assessed the milk production efficiency of sheep and goats by calculating the percentages of animals giving milk.

Furthermore, an analysis of climate change was conducted using data on wind speed U and V components, 2-meter temperature values, precipitation amounts, and humidity change. The data set comprised information from 2004-2023 annually for a total of five different climate factors. Data on climate change factors were obtained from the European Union Copernicus Climate Change database (Copernicus, 2024). Upon the integration of all data prior to analysis, a data set comprising 16 columns and 10,820 rows was generated.

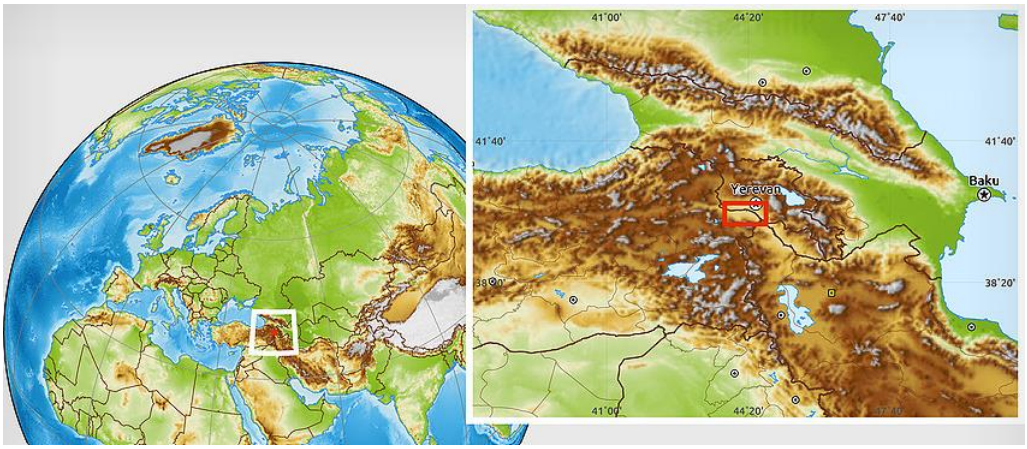

Figure 1. Iğdır province geographical location (Maphill, 2024).

Geographical coordinates of Iğdır province were used to obtain climate change data. Iğdır province coordinates are defined to the system as North 40°, West 43°, South 39°, East 45°. If we define the regions covered by these coordinates in detail; It covers the mountainous region in the north of Tuzluca district in the north of Iğdır province, the Aras River in the west of Aralık district in the west, the Iğdır Plain in the south of Iğdır Central district in the south and the Karasu River in the east of Karakoyunlu district in the east.

Method

The free and open-source RStudio software was used for machine learning modeling. XGBoost algorithm was preferred for the machine learning algorithm. XGBoost (eXtreme Gradient Boosting) is a high-performance version of the Gradient Boosting algorithm that has been optimized with various adjustments(Sahin Demirel, 2024). XGBoost was introduced by Tianqi Chen and Carlos Guestrin in the article "XGBoost: A Scalable Tree Boosting System" published in 2016. With its many

advantages, XGBoost has become one of the most preferred algorithms in machine learning today(Noorunnahar et al., 2023). XGBoost provides high accuracy in data prediction (Anne and Gueye, 2024). Minimizes overlearning through careful editing. Effectively processes missing data. It is one of the best decision tree based algorithms (Anne and Gueye, 2024; Chen and Guestrin, 2016).

The working logic of XGBoost is similar to Gradient Boosting (Bui et al., 2021; Liang et al., 2020; Natekin and Knoll, 2013). With Base Score, an initial estimate of the modeling is made. This estimate is used in subsequent steps to get closer to the correct result. By default, this estimate is 0.5 (Mohamed et al., 2020; Huang et al., 2015). The errors (residual) of the first prediction are analyzed. Errors are the difference between the observed value and the predicted value (Bonavita and Laloyaux, 2020; Shrestha and Solomatine, 2006). As in Gradient Boosting, a decision tree is created that estimates the errors. A similarity score is calculated for each tree branch. This shows how well the data is grouped in the branches (Xie et al. 2019). To determine which tree is better, gain is calculated. As a result, the machine learning model ranks the importance of the variables. In the final stage, it gives the success values of the overall model. The mathematical formula of the XGBoost algorithm forms the basis of this algorithm, which belongs to the Gradient Boosting family (Chen and Guestrin, 2016). Objective Function:

$$\{obj\}(t) = \sum_{i=1}^{n} (y\_i - (y\_i^{(t-1)} + f\_t(x\_i)))^2 + \sum_{i=1}^{t} \omega(f\_i) \quad (1)$$

Where *y_i* is the true target value; $y\_i^{(t-1)}$ is the prediction at step (t-1); *f_t(x_i)* is the new learner (decision tree) prediction at step t; *ω(f_i)* is the regularization term (e.g. tree depth or number of leaf nodes) and {obj}(t) is the objective function at iteration t.

In the XGBoost algorithm, it is also very important to define 7 different parameters in order to make a prediction close to the real values. In this stage, called hyperparameter tunning, value ranges are defined for seven different parameters and the ideal parameter values for the machine learning model are determined in the tunning process(Sahin Demirel, 2024).

The efficacy of XGBoost and other algorithms in making successful predictions has led to the widespread use of machine learning modelling in the literature on milk production and factors affecting milk production. Becker et al. 2021 employed logistic regression, random forest, and Gaussian naïve Bayes algorithms to analyse the levels of animals affected by environmental variables in milk production. In another study by Ji et al. 2022 employed the XGBoost algorithm to investigate the factors influencing milk production in cows. In a similar vein, Ebrahimie et al., 2018 utilised a decision tree algorithm to identify effective milking methods. Furthermore, Kamphuis et al. 2010 employed a decision tree algorithm to investigate the impact of automatic milking and clinical factors on milk production, with promising results. The aforementioned studies demonstrate the efficacy of machine learning algorithms in numerous aspects pertaining to milk production. It is anticipated that the XGBoost algorithm will yield successful outcomes in our study.

Results and Discussion

Small cattle milk production analysis in Iğdır province

Cattle farming on a small scale is a prevalent practice in the region of Iğdır, and milk production plays a crucial role in these activities. In Iğdır, small-scale cattle milk production holds considerable economic significance and plays a vital role in the local economy. The region's milk and dairy products are not only consumed locally but also marketed to neighboring provinces, contributing to the overall economic growth of the region. Figure 2 displays the graphs of small cattle livestock and milk productivity in Iğdır province. Specifically, Figure 2-a illustrates the relationship

between the number of sheep, milk yield, and percentage of dairy sheep from 2004 to 2024. The figure highlights changes in the number of sheep, average milk yield of dairy sheep, and percentage of total sheep herd consisting of dairy sheep over time.

The data shows that the number of sheep was approximately 200,000 in 2004. It increased steadily until 2020, reaching around 600,000. However, from 2021 to 2023, there was a downward trend, and the number decreased to an average of 300,000 in 2023. When evaluating milk yield, it was observed that although there was a variable yield graph, the values were very close to each other. Over a period of 20 years, sheep in Iğdır province produced an average of 11.98 tons of milk annually.

It is important to note that milk productivity remained consistent despite fluctuations in the number of sheep between 2004 and 2023. To gain a better understanding of this complex situation, it is crucial to examine the change in milking sheep. Examining the percentage values of dairy sheep over a 20-year period can help us understand their consistent milk yield. The percentage change of lactating sheep fluctuated only slightly, between 88.9% and 89.0%, during this time. This suggests that the percentage of lactating sheep remained almost constant each year, which may contribute to the consistent milk productivity of sheep.

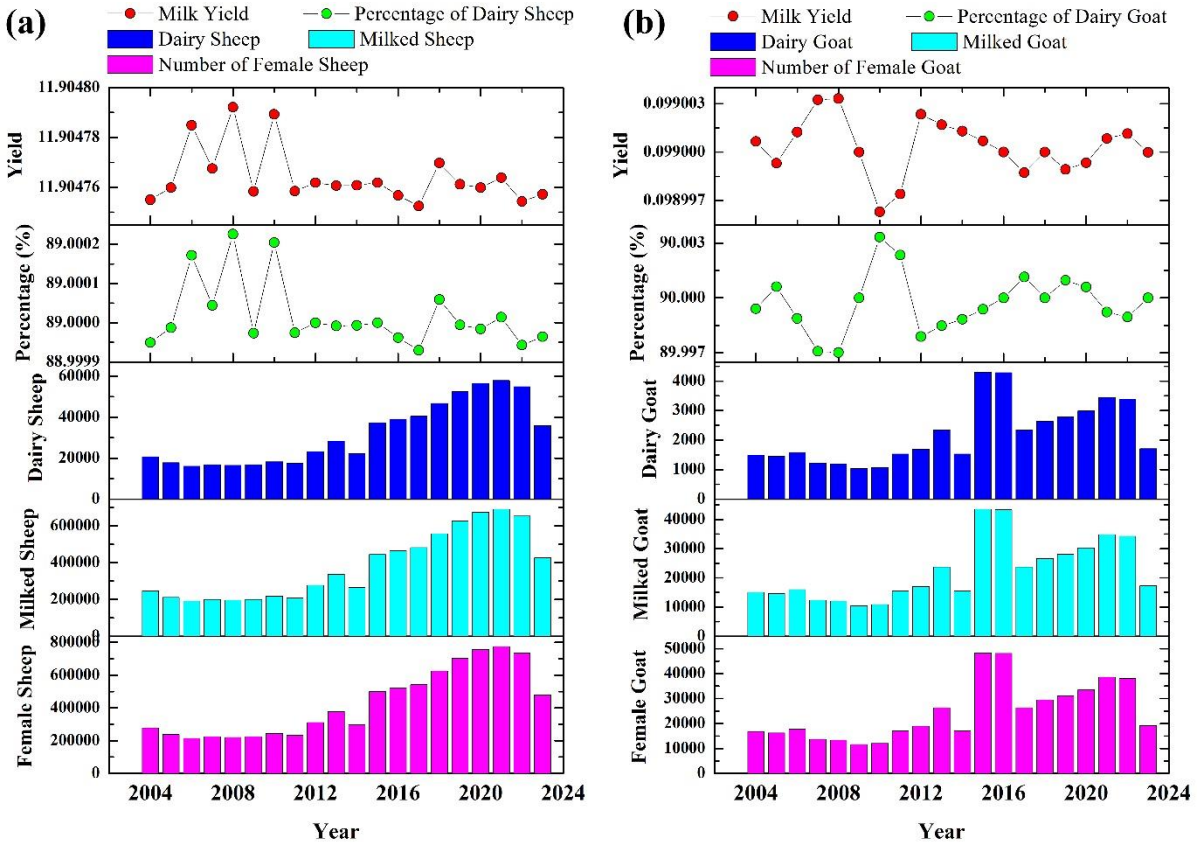

Figure 2. Small cattle milk production performance data for Iğdır province. a) sheep, b) goat

Figure 2-b displays milk production performance data for goats. A similar trend is observed when analyzing the change in the total number of goats, except for 2015 and 2016. It can be interpreted that the tendency of small cattle farmers to feed goats increased in 2015 and 2016. However, it is important to note that enterprises mostly choose sheep for cattle breeding and production. When analyzing the milk production efficiency and percentage values of goats and sheep, a similar trend is observed. However, it is worth noting that goats have a lower annual milk productivity of 0.098 tons compared to sheep's 11 tons. This may explain why some enterprises prefer sheep over goats.

Climate change analysis and machine learning models

Figure 2 shows a consistent trend in milk yield

values. However, it would be beneficial to further investigate the reasons behind the lack of increase or decrease in this trend. Specifically, it is crucial to thoroughly examine the various factors that may affect milk productivity in small cattle in Iğdır province.

It is worth noting that there are differences in milk productivity between sheep and goat breeds when considering the factors that affect milk productivity in small cattle. Milk yield can vary among breeds due to differences in genetic potential (Haenlein, 2007).

It is important to ensure a balanced diet for optimal milk production, as it provides the necessary energy, protein, vitamins, and minerals (Pereira, 2014). In addition, proper grazing areas and feeding programs are essential for maintaining animal health, which directly impacts milk productivity (Hennessy et al., 2020). Parasites, diseases, and infections can have a negative impact on milk production. Therefore, it is recommended to schedule regular veterinary check-ups, vaccinations, and implement appropriate health measures to ensure the well-being of the animal (Perri et al. 2011). Milk yield reaches its highest level during the lactation period of the animal, and it is important to provide proper nutrition and management throughout this period (Gramu, 2019). While milk yield typically increases in the first few months after birth, it may decrease over time. It is important to use correct breastfeeding and milking methods to optimize milk yield. Proper suckling and regular milking are important for optimal milk production in calves. Additionally, environmental factors, such as temperature, humidity, and lighting, can have an impact on milk productivity (Amin Sheikh et al., 2017). Therefore, it is recommended to maintain a comfortable environment for the animals and take appropriate acclimatization measures.

Although there are several factors that can affect milk productivity, the environmental climate factor is considered to be the most significant. This is because the climate has a fundamental impact on various aspects, including possible animal stress, changes in food quality in grazing areas, and the spread of parasites or diseases. Therefore, it is important to take into account the climate change factor when assessing milk productivity in cattle (Becker et al., 2021).

To investigate the impact of climate change on milk production through machine learning, utilized various meteorological parameters such as 10-meter u-wind, 10-meter v-wind, 2-meter temperature, instantaneous moisture flux, and total precipitation values. The performance values and parameters of the machine learning models developed are presented in Table 1.

Table 1. Machine learning Hyperparameters and model performance values.

	Yield for Sheep	**Yield for Goat**
Nrounds	500	1000
Max_depth	3	3
Eta	0.05	0.1
Gamma	0	0
Colsample_bytree	0.7	0.7
Min_child_weight	1	1
Subsample	0.7	0.8
RMSE	0.322	0.0113
MAE	0.322	0.0113
R^2	Not Available	Not Available

Table 1 presents the hyperparameter and the model performance parameter values. The parameter 'Nrounds' refers to the number of rounds the model has been trained on. Increasing the number of rounds can improve the model's generalization ability. In this case, using 1000

rounds resulted in a higher goat yield. The parameter 'Max_depth' was determined to be appropriate for the ideal model in both cases, with a maximum depth of 3 for the trees. Deeper trees may allow the model to learn more complex relationships, but they may also increase the risk of overfitting. The learning rate, or 'Eta', can be adjusted to control how the model updates its weights in each round. A smaller learning rate can result in a slower learning process but a more robust model. In this case, it seems that a smaller learning rate produces better results for sheep yield. Gamma is a parameter that controls the growth decision of the trees. A value of zero indicates that this parameter is not used. 'Colsample_bytree' specifies the proportion of randomly sampled columns for each tree. Using the same values indicates that there is no difference in this parameter. Using the same values indicates that there is no difference in this parameter. 'Min_child_weight' specifies the minimum amount of weight required for a node to be split in the tree. 'Subsample' specifies the random sampling rate of a subset of the training data. It has been observed that utilizing a larger sample size can enhance the model's capacity to generalize. The present case suggests that a larger sample size is associated with improved results for goat yield (Dalal et al., 2022; Putatunda and Rama, 2018).

When evaluating model performance, it is important to consider the RMSE (Root Mean Square Error) which measures the distance between the model's predictions and the actual values (Sahin Demirel, 2024). It is worth noting that the RMSE value for goat yield is significantly smaller than that for sheep yield, indicating better performance for goat yield. MAE (Mean Absolute Error) measures the average distance between the model's predictions and the true values (Sahin Demirel, 2024). Both models performed well, with low MAE values indicating that the predictions are close to the actual values. The model for goat yields outperformed the model for sheep yields, but both were successful.

Finally, analyzing R^2 (R-Square) values reveals a complex situation. It is important to note that R^2 represents the percentage of the variance of the dependent variable explained by the independent variables. An R^2 value approaching 1 indicates a better fit of the model to the data (Sahin Demirel, 2024). However, in this case, R^2 values were not obtained for both models. This may indicate that there is no relationship between the independent variables and the dependent variables. It may be advisable to consider conducting sensitivity analysis for each variable in the machine learning model to help ensure accuracy and reliability.

Table 2. Feature importance and sensitivity analyses for both ML models.

Yield for Sheep			Yield for Goat		
No	**Variable**	**Mean Dropout Loss**	**No**	**Variable**	**Mean Dropout Loss**
1	***Full model***	0.3224164	**1**	***Full model***	0.01133641
2	10 meter u-wind	0.3224164	**2**	10 meter u-wind	0.01133641
3	10 meter v-wind	0.3224164	**3**	10 meter v-wind	0.01133641
4	2-meter Temperature	0.3224164	**4**	2-meter Temperature	0.01133641
5	Humidity	0.3224164	**5**	Humidity	0.01133641
6	Precipitation	0.3224164	**6**	Precipitation	0.01133641
7	***Baseline***	0.3224164	**7**	***Baseline***	0.01133641

Table 2 presents the feature importance and sensitivity analysis values. It is worth noting that all variables have a constant Mean Dropout Loss (MDL) value. Ideally, the MDL values of the variables in this table should differ from each other, resulting in different rankings (Dong et al., 2022). Nevertheless, as there was no correlation between the dependent and independent variables, the MDL values were calculated as shown in Table 2.

The complete model displays the model's performance using all features. Dropout loss measures the effect of removing a feature from the model on its performance. A low dropout loss indicates that a feature has little impact on the model's performance. It appears that a high dropout loss indicates that this feature may be significant to the model's performance. The baseline forecast represents the model's performance without utilizing any features. In this context, the fact that all variables have the same value in both the baseline and the full model means that these variables are ineffective in the model.

When considering Table 1 and Table 2 collectively, it is possible to gather insights on the impact of independent climate variables on milk yield. While the lack of R^2 value calculation under normal conditions may indicate a potential error, the computation of feature importance and sensitivity values provided clarification on why the R^2 value could not be determined. Based on the MDL values, if each variable in the models has the same value, it may suggest that the variables do not significantly affect the model.

However, it is important to note that the low RMSE and MAE values obtained demonstrate the success of the machine learning models. Based on the results of machine learning models, it has been suggested that climatic changes in Iğdır province may not have a significant impact on milk productivity. Furthermore, a correlation analysis was conducted.

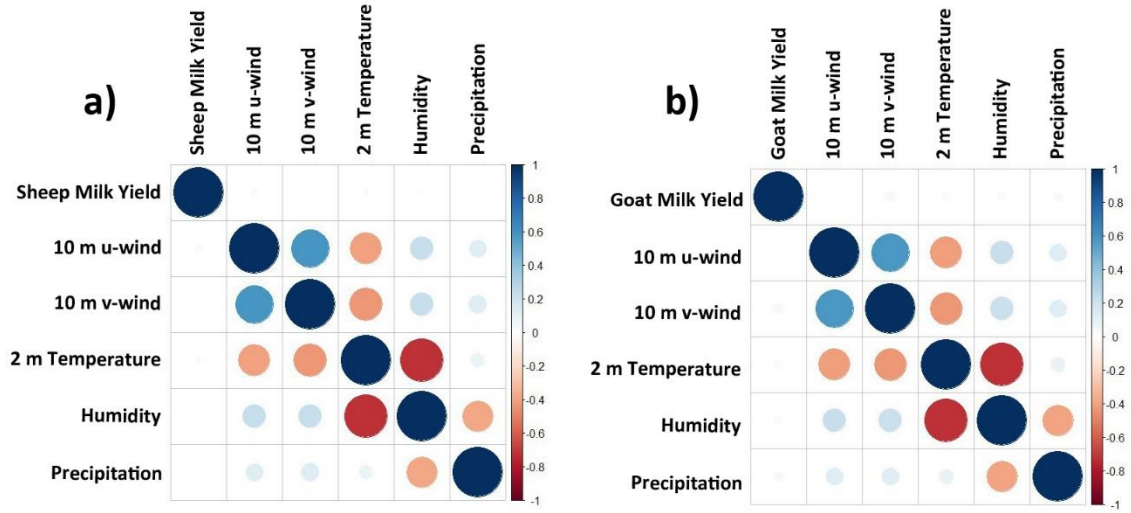

Figure 3. Milk Yield and climate variables correlations. a) for sheep, b) for goat.

Figure 3 displays the correlation matrice graphs, and, the correlation values in Figure 3a and b suggest that there is no correlation between goat and sheep milk data and climate variables, which is supported by machine learning.

This study examines the potential impact of climate change on small cattle livestock and milk productivity in Iğdır province. The results suggest that, at present, there is no significant impact on sheep and goat milk productivity, as indicated by the machine learning models used in the study.

However, it is important to note that these findings do not necessarily imply that climate change has no impact on milk productivity. However, it is important to note that climate change can impact the stress levels of animals, the nutrient quality of grazing areas, and the spread of parasites or diseases, which may indirectly affect milk productivity. It is possible that the models used in the current study did not fully capture these effects.

The study found that milk productivity remained constant over the years because the percentage of sheep giving milk remained almost the same. In conclusion, it is suggested that further research is necessary to develop more comprehensive and precise models to determine the impact of climate change on milk productivity. Additionally, to fully understand the potential impacts of climate change on milk productivity, it is recommended that more comprehensive studies with direct measurements of these impacts

be conducted. This study aims to enhance our comprehension of the consequences of climate change on small-scale cattle farming and milk production. By doing so, we can develop effective strategies to manage these impacts.

Conclusion

This study examines the potential relationship between climate change and small cattle livestock and milk productivity in Iğdır province. The study reveals that climate variables have no significant effect on milk productivity in sheep and goats. While in similar studies conducted for different geographical regions in the literature, it was determined that climatic factors such as temperature, precipitation and humidity affect milk production, this result regarding the neutral effect of five climatic factors for Iğdır province was very remarkable. However, the fact that such a conclusion was reached as a result of the evaluation of only five climatic factors in the study revealed that more research is needed to fully understand the potential effects of climate change on livestock and milk production in the region. The study provides valuable information for farmers and policymakers to better understand the factors affecting milk productivity and develop more effective strategies. It is important to note that milk productivity plays a critical role in the economic welfare of the region. According to this study, the stability of milk productivity can be attributed to the consistent percentage of sheep that produce milk over the years. To improve their flock's overall productivity, farmers may want to consider increasing the number of lactating sheep. This study highlights the benefits of utilizing advanced techniques, including machine learning and sensitivity analysis, to comprehend the intricate interplay between agricultural economics and livestock management. Future studies could focus on conducting long-term research to better understand the long-term impacts of climate change on milk productivity. In order to provide more comprehensive information, several recommendations can be made. It is possible to utilise different machine learning algorithms in order to capture non-linear relationships within a dataset more effectively than linear regression. This can be achieved through the use of algorithms such as Random Forest. Furthermore, polynomial features can be generated in order to capture potential non-linear relationships between variables. In order to enhance the reliability of the findings, the consistency of the findings can be evaluated on distinct data subsets through the application of the cross-validation method. Alternative subsets of data or alternative models can be employed to assess the robustness of the results. Additionally, it would be beneficial to extend the geographical coverage to other regions and integrate improved prediction models with additional variables such as soil quality (for feed quality), diseases, pests and water availability. It is also recommended to investigate the impact on other livestock species, analyse socio-economic factors and develop climate adaptation strategies for livestock management.

Declarations

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

Author Contribution: TE obtained the study data and organized the data set, designed the ANSD study, analyzed the data set, and wrote the manuscript.

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Research Article/Araştırma Makalesi

Determining the impact of the Atatürk Dam on the propagation of meteorological drought by using different drought indices in Sanliurfa Province

Şanlıurfa ilinde Atatürk Barajı'nın meteorolojik kuraklığın yayılımı üzerindeki etkisinin farklı kuraklık indeksleri kullanılarak belirlenmesi

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ABSTRACT

In recent years, the increase in the frequency and severity of natural disasters such as floods, droughts, etc. is evaluated as a sign of climate change. In this context, the study conducted in Sanliurfa province, aimed to determine the spatial and temporal propagation of meteorological drought in two different periods using the De Martonne (I_{DM}), De Martonne-Gottman (I_{DMG}) and Erinc (I_m) methods. Long-term monthly total precipitation (mm), average temperature (^oC) and average maximum temperature (^oC) series obtained from 12 meteorological observation stations were utilized to calculate the annual drought index values for each station. Missing years in the calculated drought index series were completed by correlation and regression analysis. Taking the year 1991, when the Atatürk Dam started to hold water, as the starting year of the 2nd period, the series of stations were divided into 2 different time scales: the 1st period (1961-1990) and the 2nd period (1991-2020). "Sanliurfa Annual Climate Class Maps" for each method were produced for two different periods. Consequently, the spatial and temporal propagation of meteorological drought in Sanliurfa province according to I_{DM}, I_{DMG} and I_m methods is from south to north. The areal average of the drought index values of the methods were represented by 15.6, 7.7 and 18.3 values in the 1st period, while they were 14.5, 7.3 and 17.0 in the 2nd period, respectively. After the first period, when the Atatürk Dam began to hold water, the drought continued to propagate, becoming more severe. The Atatürk Dam is unlikely to prevent the spread of drought from south to north in and around Sanliurfa, and there is no significant difference between the methods in determining drought propagation. If global warming continues at the current rate until the end of this century, Akcakale, Ceylanpinar and Viransehir are likely to experience severe droughts and face desertification.

Key Words: Climate classification, Desertification, De Martonne, De Martonne-Gottman, Erinc

ÖZ

Son yıllarda; sel, kuraklık vb. doğal afetlerin sıklık ve şiddetinde görülen artışlar iklim değişikliğinin bir işareti olarak değerlendirilmektedir. Bu kapsamda Şanlıurfa ilinde yapılan çalışmada; De Martonne (I_{DM}), De Martonne-Gottman (I_{DMG}) ve Erinç (I_m)

yöntemleri kullanılarak iki farklı dönemde meteorolojik kuraklığın alansal ve zamansal yayılımının belirlenmesi amaçlanmıştır. Her bir istasyon için yıllık kuraklık indisi değerlerinin hesaplanmasında 12 meteoroloji gözlem istasyonundan elde edilen uzun dönem aylık toplam yağış (mm), ortalama sıcaklık (^{o}C) ve ortalama maksimum sıcaklık (^{o}C) serileri kullanılmıştır. Hesaplanan kuraklık indisi serilerindeki eksik yıllar korelasyon ve regresyon analizi ile tamamlanmıştır. Atatürk Barajı'nın su tutmaya başladığı 1991 yılı 2. dönemin başlangıç yılı alınarak istasyonlar ait kuraklık indisi serileri; 1. dönem (1961-1990) ve 2. dönem (1991-2020) olmak üzere 2 farklı zaman ölçeğine ayrılmıştır. Her bir yöntem için "Şanlıurfa Yıllık İklim Sınıfı Haritaları" iki farklı dönem için üretilmiştir. Sonuç olarak, I_{DM}, I_{DMG} ve I_m yöntemlerine göre Şanlıurfa ilinde meteorolojik kuraklığın alansal ve zamansal yayılımı güneyden kuzeye doğrudur. Yöntemlere ait kuraklık indisi değerlerinin alansal ortalaması 1. dönemde sırasıyla 15.6, 7.7 ve 18.3 değerleri ile temsil edilirken; 2. dönemde 14.5, 7.3 ve 17.0 olmuştur. Atatürk Barajı'nın su tutmaya başladığı ilk dönemden sonra kuraklık daha da şiddetlenerek yayılmaya devam etmiştir. Atatürk Barajı'nın Şanlıurfa ve çevresinde kuraklığın güneyden kuzeye doğru yayılımını engellemesi pek olası görülmemekle birlikte, yöntemler arasında kuraklığın yayılımının belirlenmesinde anlamlı bir fark yoktur. Küresel ısınmanın bu yüzyılın sonuna kadar mevcut hızda devam etmesi halinde, Akçakale, Ceylanpınar ve Viranşehir'in şiddetli kuraklıklar yaşaması ve çölleşmeyle karşı karşıya kalması muhtemeldir.

Anahtar Kelimeler: İklim sınıflandırması, Çölleşme, De Martonne, De Martonne-Gottman, Erinç

Introduction

In the global climate system, greenhouse gas emissions that exceed normal levels due to human activities cause the sun's rays to be retained more in the atmosphere, resulting in the greenhouse effect, which is considered one of the most important factors of global warming and is counted among the causes of climate change (Kayıkçıoğlu and Okur, 2012; Mikhaylov et al., 2020; Tüzer and Doğan, 2021). While the concentration of the greenhouse gas carbon dioxide in the atmosphere did not exceed 300 ppm until the industrial revolution, today it has reached 412.5 ppm (NASA, 2024; WMO, 2024). If the increase in greenhouse gas emission rates continues on this trend, the global average temperature is expected to rise approximately 2 °C by 2036 (Mann, 2014).

Although there was no significant deviation in the average values of climate elements in a period of 300-500 years in large regions, there are transitions between climate classes in drought studies with 30-year observations (Keskiner and Çetin, 2023a). Uncertainty about the process, severity, duration and impact area of drought, which is defined as a water shortage among natural disasters, creates a multiplier effect and causes more socio-economic damage to people (Özelkan, 2019; Partigöç and Soğancı, 2019). Hence, the United Nations World Water Development Report 2016 (Küçüksakarya and Göçmen, 2019) predicts that 40% of the world could face a water deficit by 2030. In this context, information on the speed (magnitude), severity, frequency and spatial extent of drought is obtained with the help of drought index and drought trend tests; important conclusions are drawn and drought-related damages are prevented (Mishra and Singh, 2010; Keskiner and Çetin, 2023b). Although many methods have been developed for determining drought and climate classes, each method has limitations, strengths and weaknesses due to different climatic conditions. The Köppen, Camargo, Standard Precipitation Index, Thornthwaite, De Martonne, De Martonne-Gottman, Aydeniz, Percent of Normal Index, Exploratory Drought Index, Palmer Drought Severity Index, Erinc Drought Index, Streamflow Drought Index, etc. methods are frequently used in climate classification and drought studies (Gümüş et al., 2016; Aktaş et al., 2018; Aparecido et al., 2020; Özmen, 2022; Keskiner, 2022). However, using different methods in meteorological drought analysis in the same study area is a significant issue in water resources and drought risk management planning. In particular, the use of techniques such as Aydeniz (Keskiner, 2022), Reconnaissance Drought Index (Soydan Oksal and Beden, 2024), etc., which analyse drought by using more variables in calculations, and SPI (Ircan and Duman, 2021), Erinc (Keskiner and Çetin, 2023b), etc., which use fewer variables in calculations, can make planning more realistic by revealing the similarities or differences between the methods.

Turkey, which is under the influence of the "Semi-arid" climate (Oğuz and Akın, 2019) in the eastern Mediterranean basin, is considered one

of the countries that will suffer from climate change (Selek and Pınarlık, 2019; Yüksel Küskü and Söylemezoğlu, 2022). Therefore, it has become imperative to examine long-term climate parameters in order to determine the effects of climate change on drought due to global warming throughout the country, make future projections and take measures against drought. In the climate change projections, the Euphrates-Tigris River Basin is classified among the basins that will be most affected by climate change. In the future, drought is expected to affect agricultural activities and other sectors in the Euphrates-Tigris River basin (Bozkurt, 2013; Birpınar and Tuğaç, 2018; Gümüş et al., 2016; Tutuş and Erdem, 2023). The Southeastern Anatolia Project (GAP), planned in the Euphrates-Tigris Basin region, consists of 13 main projects, 7 in the Euphrates Basin and 6 in the Tigris Basin (Kendal and Sayar, 2013). Sanliurfa, which has 11% of Turkey's economically irrigable area, currently has a total irrigated area of 390 thousand hectares and when GAP is completed, the irrigated area will increase to 940 thousand hectares, which is approximately 50% of the GAP project. Therefore, Sanliurfa is more likely to be affected by drought-induced socio-economic losses (Demircan et al., 2017; Sepetçioğlu et al., 2018; YDO, 2018; Temur et al., 2023). Thus, Keskiner (2022) found in the spatial drought study conducted with the Aydeniz method in the province of Sanliurfa that Akcakale, Harran, Viransehir, Suruc, Ceylanpinar and the city center of Sanliurfa are threatened by meteorological drought starting from the Syrian border. In the point-scale studies conducted by Gümüş et al. (2016) and İrcan and Duman (2021) using the Standard Precipitation Index method in Sanliurfa province, it was determined that there was a significant increase in the number of repetitions of dry months in the last 30 years compared to previous years and significant increases in drought severity, frequency and duration in all stations in the study area, respectively. However, it is noteworthy that the impact of large-scale water resources projects on drought propagation by developing irrigation projects through the construction of large dams such as Atatürk Dam has not been sufficiently evaluated. Furthermore, it is also seen that more than one method is not used in monitoring drought with the help of drought index in the studies (Gümüş et al., 2016; İrcan and Duman, 2021; Keskiner, 2022; Keskiner and Çetin, 2023a). Consequently, determining the spatial and temporal trends of climatic changes in the GAP region by considering drought classes (Keskiner and Çetin, 2023a) is an important prerequisite for better water resource planning and drought risk management. In this context, the aim of the present study conducted in the province of Sanliurfa is twofold:

1. Deriving long-term annual meteorological drought index series for Sanliurfa province by De Martonne (I_{DM}), De Martonne-Gottman (I_{DMG}) and Erinc (I_m) methods,

2. The long-term I_{DM}, I_{DMG} and I_m annual series of the stations were divided into 2 different time scales: the 1st period (1961-1990) and the 2nd period (1991-2020) when Atatürk Dam started to hold water, and it was aimed to determine the effect of Atatrük Dam on the meteorological drought propagation by mapping the spatial and temporal propagation of the meteorological drought trend before and after the construction of Atatürk Dam.

Materials and Methods

Sanliurfa province which has a surface area of 19,242 km² (HGM, 2022), is located in the South Eastern Anatolia region of Turkey between 37˚49'12''- 40˚10'00'' E longitude and 36˚41'28''-37˚57'50'' N latitude. In Sanliurfa province, where continental climate characteristics are dominant and the average elevation is around 500 meters, the topography, shows a decrease in elevation from north to south, with elevation ranging between 348-1800 meters. The low elevation along the Syrian border and in the inland areas from the border to the north exacerbates the occurrence of drought in Sanliurfa from south to north due to extremely hot air masses originating

from the Basra Low-Pressure Center during the summer period. The long-term average temperature in Sanliurfa province is around 18.6 ^{o}C, with long-term total precipitation averages varying between 453 mm in Sanliurfa and 287-300 mm in the Akcakale and Ceylanpinar districts, respectively (İrcan and Duman, 2021; Keskiner and Çetin, 2023b). Within the scope of the research, long-term climate parameters such as average temperature (^{o}C), total precipitation (mm) and average maximum temperature (^{o}C) obtained monthly from the observation stations of the Turkish State Meteorological Service (MGM) were used (Figure 1.).

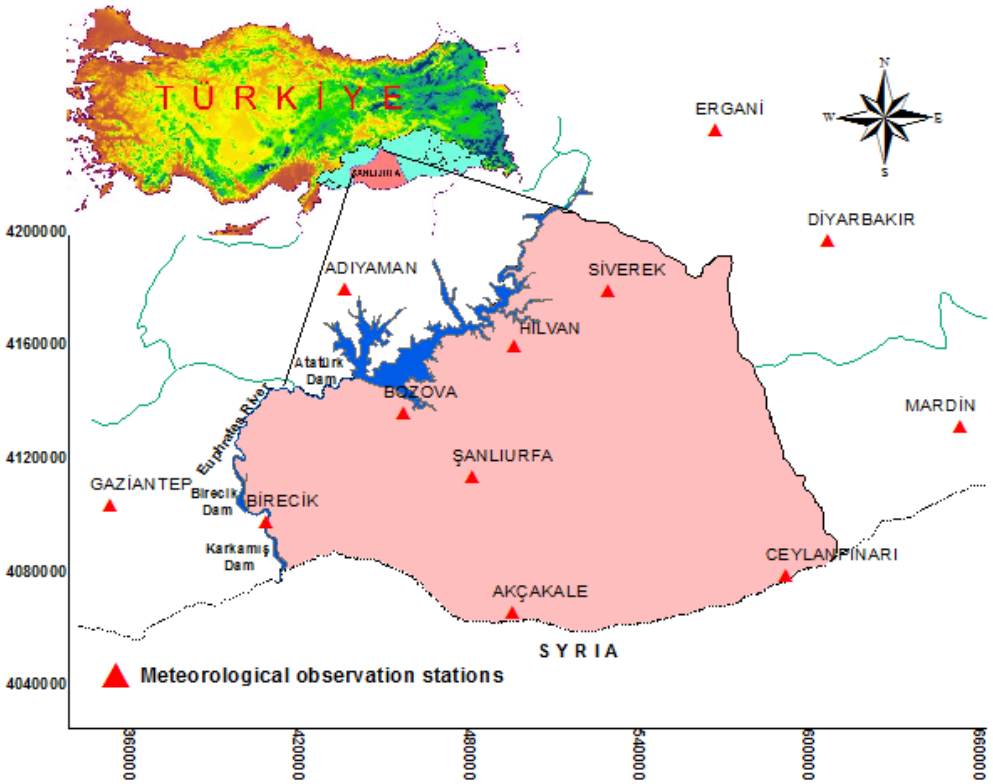

Figure 1. The UTM (Universal Transverse Mercator) coordinates (meter) of the meteorological observation stations used in the study.

Climate parameters consist of the data observed at Sanliurfa, Akcakale, Birecik, Ceylanpinar, Siverek, Adiyaman, Diyarbakir, Ergani, Gaziantep and Mardin meteorological observation stations between 1961-2020 and the data observed at Hilvan and Bozova meteorological observation stations between 1991-2020. The calculation of the locations in a projected coordinate system of the meteorological observation stations was performed according to the reference surface D_WGS_1984_UTM_Zone_37N. Table 1 provides attribute information about the climate elements used in the study and the meteorological observation stations from which these parameters are obtained. Long-term averages of climate elements were calculated by considering the complete observed series. The stations ordered by latitude from north to south show the latitudinal influence on the change in average climate parameters. However, the difference is not solely based on topographical variations in locations. Other variables like aspect, distance from the sea, etc., also play a role, making this difference non-linear. The recording periods of the climate elements obtained from meteorological observation stations were arranged in two periods every 30 years: the 1st period (1961-1990) and the 2nd period (1991-2020). Since the climate elements of Hilvan and Bozova stations did not have sufficient data length before 1991, they were included in the calculations in the second period.

Table 1. Some attributes of climate elements obtained from meteorological observation stations

Stations name	*Latitude (m)*	*Longitude (m)*	*Data period*	*Average annual precipitation (mm)*	*Average annual temperature (C°)*	*Average annual max. temperature (C°)*
Ergani	4235717	567009	1964-2020	744.3	15.9	21.0
Diyarbakir	4196457	606657	1961-2020	492.9	15.9	22.7
Adiyaman	4178911	436356	1963-2020	710.9	17.4	23.1
Siverek	4178373	528991	1964-2020	561.3	16.7	22.3
Hilvan	4159284	495656	1998-2020	432.1	16.9	24.2
Bozova	4135486	456912	2000-2020	401.7	17.3	24.0
Mardin	4130696	653165	1961-2020	661.4	16.2	20.4
Sanliurfa	4112732	481026	1961-2020	453.9	18.6	24.6
Gaziantep	4102634	353385	1961-2020	567.4	15.5	21.9
Birecik	4096909	408503	1964-2020	361.5	17.9	18.0
Ceylanpinar	4077686	591900	1961-2020	300.7	18.3	26.4
Akcakale	4064656	495294	1965-2020	287.8	18.4	25.7

Correlation and regression analysis

Correlation analysis determines the degree and direction of the relationship between variables, while regression analysis mathematically defines this relationship. In order to complete the missing years in the I_{DM}, I_{DMG} and I_m series that could not be calculated at the observation stations due to unobserved climate elements, the stations with statistically significant correlations at the level of 0.05 were identified using the Pearson correlation coefficient (r). A linear regression model with the highest coefficient of determination (R^2) and the smallest standard deviation between meteorological stations was then created at a significance level of α=0.05. The process of supplementing missing years in the drought index series of the stations was described in detail by Kesici and Kocabaş (1998) and Ryan and Cryer (2005).

De Martonne aridity index (I_{DM})

The De Martonne method (I_{DM}) is one of the oldest drought indices that uses annual average temperature and total precipitation values to calculate annual drought index values (Andrade et al., 2021) which can be obtained with the help of equation 1. Table 2. shows the De Martonne index value and climate classification based on the values of I_{DM}) (Hrnjak et al., 2014).

$$I_{DM}=\frac{P}{T+10} \qquad (1)$$

Where I_{DM})is the De Martonne annual drought index, P is the annual total precipitation (mm) and T is the average annual temperature (°C) in a given time series.

Table 2. De Martonne index values and climate classification

Climate classification	*Index values (I_{DM})*
Arid	I_{DM}<10
Semi-arid	10≤I_{DM}<20
Mediterranean	20≤I_{DM}<24
Semi-humid	24≤I_{DM}<28
Humid	28≤I_{DM}<35
Very humid	35≤I_{DM}≤55
Extremely humid	I_{DM}>55

De Martonne–Gottman index (IDMG)

In 1942, De Martonne and Gottman made some modifications to equation 1 (MGM, 2016a). The new equation is as follows (Equation 2).

$$I_{DMG}=\frac{1}{2}\left(\frac{P}{T+10}+\frac{12P_d}{Td+10}\right) \qquad (2)$$

Where I_{DMG})is the De Martonne-Gottman annual drought index, P (mm) and T (˚C) are the annual total precipitation and the average annual temperature (°C) respectively and Pd and Td are the total precipitation and the average temperature of the driest month, respectively. De Martonne-Gottman index values and the climate classes are given in Table 3 (MGM, 2016a ; Dursun and Babalık, 2021).

Table 3. De Martonne - Gottman index values and climate classification

Climate classification	*Index values (I_{DMG})*

Desert	0-5
Semi-arid	5-10
Between Semi-arid and humid	10-20
Semi-humid	20-28
Humid	28-35
Very humid	35-55
Wet	>55
Polar	<0 (T < -5°C)

Erinc's aridity index (I_m)

Erinc's Aridity Index (I_m) developed by Erinc in 1965 expresses the ratio between annual total precipitation and average maximum temperature and is represented by equation 3. Index values are classified as shown in Table 4 (MGM, 2016b; Keskiner and Çetin, 2023a).

$$I_m = P/T_{max\ \_ort} \qquad (3)$$

where I_m is the Erinc's drought index; P and $T_{max\ \_ort}$ are annual total precipitation (mm) and average maximum temperature (°C) observed in a given year, respectively

Table 4. Erinc's classification of climate types

Climate Types	Index Value (I_m)	Vegetation Cover
Severe-arid	<8	Desert
Arid	8-15	Desertification
Semi-arid	15-23	Arid
Sub-humid	23-40	Forest
Humid	40-55	Moist forest
Very humid	>55	Very moist forest

Inverse distance weighted interpolation technique (IDW)

The Inverse Distance Method (IDW) is used to estimate the values of non-sampled points using the values of known sample points (Çetin and Diker, 2003). The basis of this frequently used method is that nearby points on the surface to be interpolated have more influence (weight) on the estimates than those farther away. Mathematical equations and definitions of the Inverse Distance Method are given in detail by Keskiner and Çetin (2023a) and Taylan and Damçayırı (2016). In this study; De Martonne, De Martonne-Gottman and Erinc annual index values were spatially mapped with the inverse distance method in a GIS environment using ArcGIS software.

Results and Discussions

Missing data imputation

De Martonne (I_{DM}), De Martonne Gottman (I_{DMG}) and Erinc (I_m) values of 12 stations used in the study were calculated annually. Index values of the methods could not be calculated for the years without observations of the climate elements used in the methods. It was preferred to complete the drought index series instead of completing missing observations in climate elements. This is because average monthly temperature and monthly total precipitation values are also used in the calculation of annual index values by the De Martonne-Gottman method (Equation 2). The total missing observation period of the De Martonne-Gottman annual drought index values used in the study at all stations is 31 years. However, when Equation 2 is taken into account, the time required to complete the missing observations in monthly precipitation is 372 months and includes consecutive years. Therefore, it was decided that completing the missing years of the annual drought index series would be healthier in terms of the accuracy of the estimation. In this context, the stations with missing years in the index series (Y, dependent variable) and the stations with no missing values in the index series (X, independent variable) which are the closest to the station with missing years were identified (Table 5.).

Table 5. Pearson correlation coefficient (r) values between dependent (Y) and independent (X) variables in De Martonne (I_{DM}), De Martonne Gottman (I_{DMG}) and Erinc (I_m) annual series

De Martonne (I_{DM}) and De Martonne Gottman (I_{DMG})							
X	Diyarbakir	Mardin	Sanliurfa	Gaziantep	Gaziantep	Gaziantep	Siverek
Y	Siverek	Ergani	Akcakale	Bozova	Adiyaman	Birecik	Hilvan
r (I_{DM})	0.87	0.72	0.82	0.72	0.89	0.89	0.89
r (I_{DMG})	0.88	0.72	0.81	0.75	0.76	0.89	0.91
Erinc (I_m)							
X	Diyarbakir	Diyarbakir	Ceylanpinar	Gaziantep	Gaziantep	Adiyaman	Siverek
Y	Siverek	Ergani	Akcakale	Adiyaman	Birecik	Bozova	Hilvan
r (I_m)	0.81	0.71	0.78	0.89	0.89	0.73	0.89

The stations with missing years and the stations with no missing years, which have a significant relationship between the stations were identified using correlation analysis and found to have high correlations (r> 0.7). Particularly, the similarities between the correlated stations and correlation coefficients of the I_{DM} and I_{DMG} methods were remarkable. After identifying the highly correlated stations, these relationships were modeled by regression analysis. As shown in Table 6, the dependent and independent variables of the linear regression models to be used to complete the missing years in the De Martonne (I_{DM}), De Martonne Gottman (I_{DMG}) and Erinc (I_m) annual series as well as the information about the missing years to be completed in the index series.

Table 6. Dependent (Y) and independent (X) variables of linear regression models to predict missing values in the annual series of I_{DM}, I_{DMG} and I_m

Methods	Y	X	Y	
			Non-missing observations	Missing observations
I_{DM} and I_{DMG}	Siverek	Diyarbakir	57 / 1964-2020	3 / 1961-1963
	Ergani	Mardin	57 / 1964-2020	3 / 1961-1963
	Akcakale	Sanliurfa	56 / 1965-2020	4 / 1961-1964
	Bozova	Gaziantep	21 / 2000-2020	9 / 1991-1999
	Adiyaman	Gaziantep	58 / 1963-2020	2 / 1961-1962
	Birecik	Gaziantep	57 / 1964-2020	3 / 1961-1963
	Hilvan	Siverek	23 / 1998-2020	7 / 1991-1997
I_m	Siverek	Diyarbakir	57 / 1964-2020	3 / 1961-1963
	Ergani	Diyarbakir	57 / 1964-2020	3 / 1961-1963
	Akcakale	Ceylanpinar	56 / 1965-2020	4 / 1961-1964
	Adiyaman	Gaziantep	58 / 1963-2020	2 / 1961-1962
	Birecik	Gaziantep	57 / 1964-2020	3 / 1961-1963
	Bozova	Adiyaman	21 / 2000-2020	9 / 1991-1999
	Hilvan	Siverek	23 / 1998-2020	7 / 1991-1997

Linear regression analysis was performed to complete the missing years of the I_{DM}, I_{DMG} and I_m series for the correlated stations listed in Table 6. The missing years in the annual series of I_{DM}, I_{DMG} and I_m for Siverek, Ergani, Akcakale, Bozova, Adiyaman, Birecik and Hilvan meteorological stations were completed using the regression models provided in Table 7.

Table 7. Linear regression models for estimating missing years in I_{DM}, I_{DMG} and I_m series

Y		Regression equation	*Coefficient of determination*	*Standard deviation*
		b_1X+b_0	(%R²)	(STD)
Y(I_{DM})	Siverek	1.092 Diyarbakir + 0.005	76.4	3.03
	Ergani	0.632 Mardin + 12.67	52.3	5.47
	Akcakale	0.577 Sanliurfa + 1.09	66.6	2.20
	Bozova	0.621 Gaziantep + 0.55	52.1	3.34
	Adiyaman	1.145 Gaziantep + 0.43	79.6	3.28
	Birecik	0.602 Gaziantep - 0.52	80.2	1.70
	Hilvan	0.791 Siverek + 0.12	72.9	2.58
Y(I_{DMG})	Siverek	1.101 Diyarbakir - 0.06	77.3	1.48
	Ergani	0.631 Mardin + 6.34	51.9	2.75
	Akcakale	0.576 Sanliurfa + 0.55	66.5	1.1
	Bozova	0.621 Gaziantep + 0.27	52.1	1.67
	Adiyaman	1.242 Gaziantep - 0.68	57.6	3.01
	Birecik	0.603 Gaziantep - 0.27	80.3	0.85
	Hilvan	0.856 Siverek - 0.75	78.2	1.21
Y(I_m)	Siverek	0.900 Diyarbakir + 5.24	46.3	5.95
	Ergani	1.216 Diyarbakir + 8.82	51.1	7.13
	Akcakale	0.790 Ceylanpinar + 2.23	61.5	2.67
	Bozova	0.392 Adiyaman + 4.81	52.5	3.39
	Adiyaman	1.152 Gaziantep + 0.94	80.3	3.94
	Birecik	0.559 Gaziantep - 0.40	79.5	1.98
	Hilvan	0.763 Siverek - 0.79	69.2	3.24

The annual index series of I_{DM}, I_{DMG} and I_m methods were divided into two different time scales based on the year 1991 when the Atatürk Dam started to hold water. The drought index values representing the stations listed in Table 8 were calculated using the median values of each period (Çetin et al., 2001).

Table 8. Median values of I_{DM}, I_{DMG} and I_m annual series of meteorological observation stations for the 1st period (1961-1990) and 2nd period (1991-2020)

Stations	I_{DM_1}	I_{DM_2}	I_{DMG_1}	I_{DMG_2}	I_{m_1}	I_{m_2}
Ergani	30.7	26.9	15.3	13.5	36.2	32.8
Diyarbakir	19.2	18.3	9.6	9.2	22.1	20.8
Adiyaman	24.8	24.6	12.4	12.3	29.6	28.8
Siverek	18.7	20.6	9.3	10.3	22.8	25.3
Hilvan	*NA**	16.2	*NA*	8.0	*NA*	18.2
Bozova	*NA*	14.1	*NA*	7.0	*NA*	16.2
Mardin	28.6	21.2	14.3	10.6	36.8	26.8
Sanliurfa	16.5	13.7	8.2	6.8	19.3	16.5
Gaziantep	21.5	22.1	10.7	11.1	25.0	25.6
Birecik	12.8	12.1	6.4	6.1	13.9	13.2
Ceylanpinar	11.7	8.3	5.8	4.4	12.3	9.0
Akcakale	10.4	8.9	5.2	4.4	11.4	9.8

NA*: Not Available

The methods showed similar behavior and drought severity tended to increase from north to south in all methods. Naturally, this is also seen in the graph drawn considering the index averages (Figure 2.). According to the Erinc method, drought severity tends to increase more from north to south (Siverek-Akcakale) in the study area compared to the De Martonne and De Martonne-Gottman methods. The increasing trend of drought severity in the De Martonne-Gottman method was found to be less than that of the other methods. Considering the trend line

of the linear regression model, it is predicted that in the future, the transitions between the climate classes of each method will occur the fastest in the Erinc method.

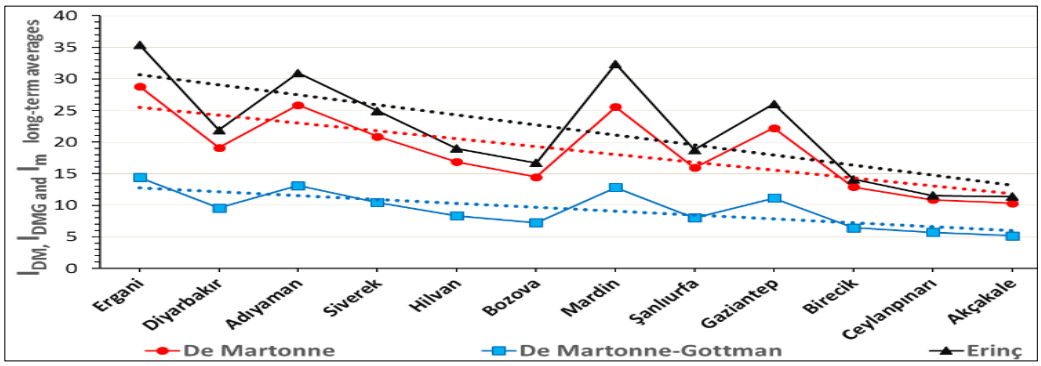

Figure 2. Long-term (1961-2020) averages of De Martonne (I_{DM}), De Martonne-Gottman (I_{DMG}), Erinc (I_m) series and trends represented by the linear regression model

The most comprehensive climate classification study in Turkey using the De Martonne-Gotmann and Erinc methods was conducted by the Turkish State Meteorological Service (MGM, 2016a; MGM, 2016b). The results obtained from the MGM study and this study were evaluated together. In this comparison based on the long-term averages, it was seen that the results significantly overlapped with each other (Table 9.). Within the scope of MGM and this research, it was determined that there were no major changes in the I_{DMG} values obtained from the long-term averages of the periods with different data lengths that would affect the climate class of the stations. According to the I_{DMG} method in both studies; Ergani, Adiyaman, Siverek, Mardin and Gaziantep were represented by 'Between Semi-arid and humid'; Diyarbakir, Birecik, Ceylanpinar and Sanliurfa were represented by 'Semi-arid' climate characteristics. However, while Akcakale station was represented by the 'Desert' climate class with an average I_{DMG} value of 4.6 in the MGM study period (1981-2010\30 years), it was defined by the 'Semi-arid' climate class with an average I_{DMG} value of 5.1 in the study period (1961-2020\60 years) within the scope of this research. In this case, it can be said that the I_{DMG} climate classification threshold value is 5 I_{DMG} may cause the small differences in the averages of the index values calculated at Akcakale station to cause climate class change.

Considering the long-term averages of the index values of MGM and Erinc method within the scope of this research, it was determined that Ergani, Adiyaman, Siverek and Mardin were represented by 'Sub-humid' climate class; Diyarbakir and Sanliurfa stations were represented by 'Semi-arid'; Birecik, Ceylanpinar and Akcakale stations were represented by 'Arid' climate classes in both studies. It was observed that the long-term averages of Erinc method index values in different periods did not create a difference that would cause a climate class change.

Table 9. The averages of the De Martonne-Gotmann and Erinc annual index series obtained within the scope of this research and by the Turkish State Meteorological Service (MGM)

Stations	Time period \ year		I_{DMG}		I_m	
	MGM	This Study	MGM	This Study	MGM	This Study
Ergani	1981-2010\30	1961-2020\60	14.6	13.6	35.2	35.4
Diyarbakir	1981-2010\30	1961-2020\60	9.2	9.5	20.9	21.9
Adiyaman	1981-2010\30	1961-2020\60	12.8	13.1	29.8	30.9
Siverek	1981-2010\30	1961-2020\60	10.7	10.2	25.2	24.9
Mardin	1981-2010\30	1961-2020\60	11.9	12.8	30.2	32.4
Sanliurfa	1981-2010\30	1961-2020\60	7.7	8.0	17.7	18.5
Gaziantep	1981-2010\30	1961-2020\60	11.5	11.1	25.4	26.0
Birecik	1981-2010\30	1961-2020\60	6.4	6.4	13.7	14.1
Ceylanpinar	1981-2010\30	1961-2020\60	5.0	5.7	10.8	11.5
Akcakale	1981-2010\30	1961-2020\60	4.6	5.1	10.2	11.4

Identifying high meteorological drought risk areas

In order to clearly reveal the spatial and temporal distributions of drought classes in Sanliurfa province, the I_{DM}, I_{DMG} and Im annual series were evaluated in two different periods. The year 1991 (DSİ, 2022), representing the period when the Atatürk Dam started to hold water, was taken as a reference within the scope of the study and accepted as the beginning of the second period. The series of stations was divided into two different time scales: period 1 (1961-1990) and period 2 (1991-2020). Since Hilvan and Bozova did not have sufficient observations of the climate elements used in the calculation of the index values in the 1st period, they were included in the calculations in the 2nd period. Using the median values of the drought index series representing the stations (Çetin et al., 2001), I_{DM}, I_{DMG} and I_m annual climate class maps of Sanliurfa were produced for 2 different periods with a resolution of 200x200 m by the Inverse Distance Method (Figure 3.- 5.).

As seen in Figure 3, according to the De Martonne method; in the 1st period (1961-1990), it was determined that the "Mediterranean" climate prevailed in Sanliurfa in a strip along the bed of the Euphrates River extending from Gaziantep to Adiyaman provincial borders and north of Siverek to the Mardin provincial border. The severity of the drought continued to increase from north to south towards the Syrian border and these areas were represented by the "Semi-arid" climate class. In the second period (1991-2020), compared to the first period, there was an increase in drought intensity of 2 "I_{DM}" from south to north. The drought has increased over the 30-year period and has spread northward. Moreover, while Harran, Akcakale and Ceylanpinar were dominated by "Semi-arid" climate characteristics in the 1st period, they were under the influence of "Arid" climate in the 2nd period. This situation is more clearly shown in Table 10, The area represented by the "Arid" climate class within the Sanliurfa province was "0" in the first period, while in the second period the area represented by the "Arid" climate class was 3334.5 km^2, which means shifting towards "Semi-arid" to the "Arid" climate class (Keskiner and Çetin, 2023a). The fact that it was determined in a study conducted by Keskiner and Çetin (2023a) in Sanliurfa that the "Semi-arid" climate type is likely to shift towards the "Arid" climate type in the future coincides with the findings obtained from our research.

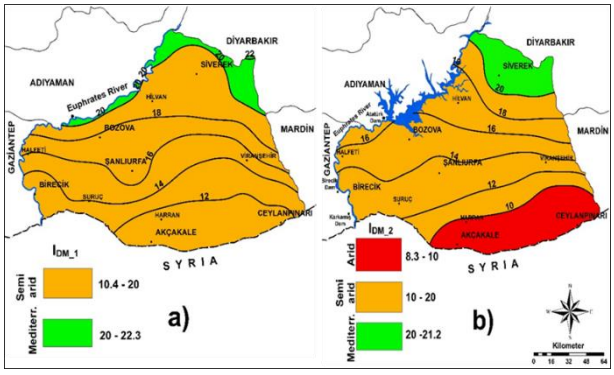

Figure 3. De Martonne (I_{DM_1}) 1st period (a) and 2nd period (b) annual climate class maps

Table 10. Surface area change of climate classes in the first (1961-1990) and second periods (1991-2020) according to the De Martonne (I_{DM}) method

Period	*Climate classes*	*Index values*	*Surface area (km^2)*	*Surface area changing in period 2 compared to period 1*	
				Climate classes changing (km^2)	*I_{DM} (Areal average)*
I_{DM_1}	Arid	<10	0		15.6
	Semi-arid	10.4-20	18034.8		
	Mediterranean	20 - 22.3	1331.5		
I_{DM_2}	Arid	8.3-10	3334.5	3334.5 *(Increase)*	14.5
	Semi-arid	10-20	14339.3	-3695.4 *(Decrease)*	
	Mediterranean	20 -21.2	1692.4	360.9 *(Increase)*	

In the north around Siverek, an area of 360.9 km^2 has spread from the "Semi-arid" climate class to the Mediterranean climate type, which exhibits more humid characteristics. While the areal average of the I_{DM} in the first period was 15.6, it was represented by a value of 14.5 in the second period and it was determined that the drought severity increased after the Atatürk Dam in the study area. According to the results obtained with the De Martonne method, it was determined that the Atatürk Dam could not stop the meteorological drought propagation (Keskiner and Çetin, 2023b) from south to north in Sanliurfa province. In a study conducted by Keskiner and Çetin (2023b) to determine drought trends in Sanliurfa province, it was found that the conclusions that Atatürk, Birecik and Karkamis dams are unlikely to prevent the occurrence of drought from south to north in Sanliurfa and its surroundings except Bozova are compatible with our study.

Figure 4. shows that there is no significant difference between the De Martonne-Gottman and De Martonne methods in terms of drought spread. Indeed, the I_{DMG} method indices are calculated as exactly half of the I_{DM} indices. This is because, as seen in equation 2, the value of the precipitation of the driest month (*Pd*) in arid regions such as Sanliurfa does not have summer precipitation or precipitation that would make a significant difference. When the long-term monthly precipitation of the precipitation series within the scope of the study is analyzed, the Pd value is represented by a zero value in almost all stations. Therefore, there is no significant difference between I_{DMG} and I_{DM} methods in arid regions.

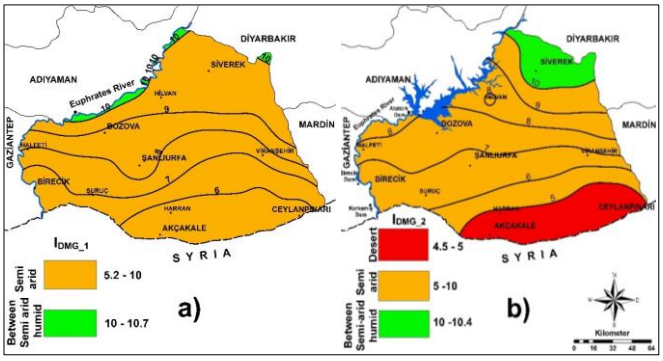

Figure 4. De Martonne-Gottman (I_{DMG_1}) 1st period (a) and 2nd period (b) annual climate class maps

According to the I_{DMG} method, it was identified that the "Between Semi-arid and humid" climate type was observed along the Euphrates river bed extending to the Gaziantep-Adiyaman provincial borders in the 1st period, while the "Semi-arid" climate type affected almost the entire Sanliurfa. The increase in drought severity from the north towards the Syrian border was also evident in the I_{DMG} method. Compared to the first period, there was a gradual increase in drought with 1 "I_{DMG}" in the second period from south to north. Similar to the I_{DM} method, the drought increased in the second period and spread northward during the 30-year period. According to the climate classification of the I_{DMG} method, Harran, Akcakale and Ceylanpinar had "Semi-arid" climate characteristics in the 1st period, while these areas were represented by the "Desert" climate class in the 2nd period. As can be seen in Table 11; while there was no area represented by the "Desert" climate class in the first period, it was determined that an area of 3108.7 km^2 in the second period transitioned from the "Semi-arid" (19005.4 km^2) climate class to the "Desert" climate class, and an

area of 1167.3 km^2 around Siverek to the "Between Semi-arid and humid" climate class. Similar to the I_{DM} method, the areal average of the first and second period I_{DMG} index values decreased from 7.7 "I_{DMG}" to 7.3 "I_{DMG}" values, respectively, and the severity of drought increased in the last 30 years (Ircan and Duman, 2021). The De Martonne-Gottman method, like the De Martonne method, revealed that the Atatürk Dam could not prevent the meteorological drought propagation from south to north in Sanliurfa province.

Table 11. Surface area change of climate classes in the first (1961-1990) and second periods (1991-2020) according to the De Martonne-Gottman (I_{DMG}) method

Periods	*Climate classes*	*Index values*	*Surface area (km^2)*	*Surface area changing in period 2 compared to period 1*	
				Climate classes changing (km^2)	*I_{DMG} (Areal average)*
I_{DMG_1}	Desert	0-5	0		7.7
	Semi-arid	5.2-10	19005.4		
	Between Semi-arid and humid	10-10.7	360.9		
I_{DMG_2}	Desert	4.5-5	3108.7	3108.7 *(Increase)*	7.3
	Semi-arid	5-10	14729.3	4276.0 *(Decrease)*	
	Between Semi-arid and humid	10-10.4	1528.2	1167.3 *(Increase)*	

The Erinc method,which is another method used in the study did not show significant differences in the spatial distribution of the 1st and 2nd period drought classes (Figure 5.). Drought propagation from south to north in Sanliurfa was observed to intensify in the 2nd period similar to the other methods. However, in the Erinc method, it was determined that there was a transition area from"Semi-arid" and "Sub-humid" climate classes to "Arid" climate classes in the 2nd period, with 926.6 km^2 in the south and 862.4 km^2 in the north, respectively (Table 12.). The Erinc method, like the other two methods, found that the Atatürk Dam could not prevent the meteorological drought propagation from south to north in Sanliurfa province. On the other hand, in a study by Keskiner (2022), in which the areas at risk of meteorological drought in Sanliurfa Province were determined using the "Aydeniz Annual Humidity Coefficient ($N_{(hc)annual}$)", the spatial distribution of climate classes, and especially the south of the Suruc-Viransehir line as the regions most exposed to drought severity, is very consistent with the results obtained with the Erinc method used in our research.

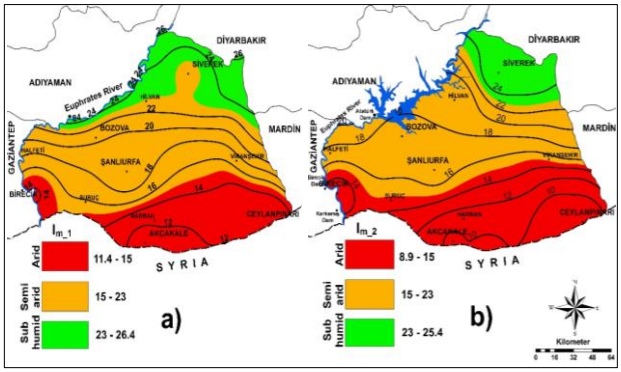

Figure 5. Erinc (I_{m_1}) 1st period (a) and 2nd period (b) annual climate class maps

Table 12. Surface area change of climate classes in the first (1961-1990) and second periods (1991-2020) according to Erinc (I_m) method

Period	*Climate classes*	*Index values*	*Surface area (km^2)*	*Surface area changing in period 2 compared to period 1*	
				Climate classes changing (km^2)	*I_m (Areal average)*
I_{m_1}	Arid	11.4-15	5538.2		18.3
	Semi-arid	15-23	10622.1		
	Sub-humid	23-26.4	3206.0		
I_{m_2}	Arid	8.9-15	7327.1	1789.0 *(Increase)*	17.0
	Semi-arid	15-23	9695.6	-926.6 *(Decrease)*	
	Sub-humid	23-25.4	2343.6	-862.4 *(Decrease)*	

As a result, the spatial averages of the Erinc indices represented by the values of 18.3 "I_m" and 17 "I_m" in the 1st and 2nd periods, respectively, are in agreement with the findings indicating that drought severity increased in the 2nd period. For example, as seen in the areal distribution of index values in Akcakale and its environs (Figure 5); while drought severity was represented by I_m = 12 in the 1st period, it was represented by I_m = 10 in the 2nd period. Drought severity has increased by 2 "I_m" in 30 years. This situation is clearly seen in the calculations made by considering the median values of annual total precipitation and annual average maximum temperature values observed from Akcakale station between 1965-1990 (Table 13.).

Table 13. Changes in drought severity at Akcakale station in the 1st period (1965-1990) and the 2nd period (1991-2020) *according to I_m, I_{DM} and I_{DMG} methods*

Erinc	*Median of the annual total precipitations (mm)*	*Average annual max.temperature (C°)*	I_m	*Changes compared to period 1*			
1.period (1965-1990)	295.2	25.5	11.6	Precip. (%)	Temp. (%)	I_m (%)	I_m (Severity)
2.period (1991-2020)	251.9	25.8	9.8	14.7	1.1	15.6	1.8
De Martonne	*Median of the annual total precipitations (mm)*	*Average annual temperature (C°)*	I_{DM}	*Changes compared to period 1*			
1.period (1965-1990)	295.2	18.3	10.4	Precip. (%)	Temp. (%)	I_{DM} (%)	I_{DM} (Severity)
2.period (1991-2020)	251.9	18.4	8.9	14.7	0.3	14.8	1.5
De Martonne-Gottman	*Median of the annual total precipitations (mm)*	*Average annual temperature (C°)*	I_{DMG}	*Changes compared to period 1*			
1.period (1965-1990)	295.2	18.3	5.2	Precip. (%)	Temp. (%)	I_{DMG} (%)	I_{DMG} (Severity)
2.period (1991-2020)	251.9	18.4	4.4	14.7	0.3	14.8	0.8

When the calculations are taken into consideration, during the thirty years in period 2, the annual total precipitation decreased by 14.7% and the annual average maximum temperature increased by 1.1%. The results obtained from the De Martonne and De Martonne-Gottman methods were similar to the Erinc method. Indeed, Bozkurt (2013) predicts that precipitation in the Euphrates-Tigris basin will decrease by 20-30% and temperatures will increase by 2.1-4.1% by the end of this century. Therefore, the south of Sanliurfa (Birecik, Suruc, Harran, Akcakale, Ceylanpinar and Viransehir) is likely to be under the influence of "Severe arid" climate according to the Erinc method, "Arid" climate according to the De Martonne method and "Desert" climate according to the De Martonne-Gottman method within this century.

Conclusions

Sanliurfa will have approximately 940.000 hectares of irrigated area with the completion of the GAP project. Therefore, it is within the scope of provinces that will be most affected by a possible drought. The De Martonne, De Martonne-Gottman and Erinc annual index series were arranged in two 30-year periods: the 1st period (1961-1990) and the 2nd period (1991-2020) when the Atatürk Dam started to hold water. "Annual Climate Class Maps" of De Martonne, De Martonne-Gottman and Erinc were produced of Sanliurfa for both periods. The areal average of the drought index values of the methods were represented by 15.6, 7.7 and 18.3 values in the 1st period, while they were 14.5, 7.3 and 17.0 in the 2nd period, respectively.

In the second period when the Atatürk Dam started to hold water, the drought continued to propagate more severely. Areas at risk of meteorological drought were determined. In this study, which aims to determine the spatial and temporal propagation of the meteorological drought trend before and after the construction of the Atatürk Dam, the following conclusions can be drawn:

Meteorological drought is more severe in the south of Sanliurfa, while its severity decreases towards the north and there is a risk of meteorological drought in the whole province

Akcakale, Ceylanpinar and Viransehir have been identified as areas that will be primarily affected by drought, and it is predicted that if global warming continues at the current rate until the end of this century, Akcakale, Ceylanpinar and Viransehir are likely to experience severe droughts and face desertification.

While the impact of climate change on drought is revealed by analysing various drought index series for different periods, it seems unlikely that Atatürk Dam in Sanliurfa province will prevent the spread of drought caused by global warming.

In order to reduce the negative impacts of climate change on dry farming and irrigated agricultural lands, it is recommended that afforestation and forest management practices be planned and implemented urgently around Sanliurfa. In drought studies to be carried out in this region; It is important to use drought analysis methods together that utilize different variables in the calculation of drought index values to make water resources and drought risk management plans based on more realistic findings.

Conflict of interest:

The authors declare that there are no personal and financial conflicts of interest within the scope of the study.

Author contributions:

ADK conceptualized the study, developed the methodology and validated the findings. TY performed data analysis and visualized the data. ADK, TY, GİT and MŞ contributed to writing, editing and reviewing the manuscript.

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Research Article/Araştırma Makalesi

Evaluation of heat stress using temperature-humidity index in laying hens in battery cages under Bursa conditions

Bursa Koşullarında Batarya Kafeste Yumurtacı Tavuklarda Sıcaklık Stresinin Sıcaklık-Nem İndeksiyle Değerlendirilmesi

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ABSTRACT

Heat stress has become a more severe threat to the poultry industry with increasing global warming. Poultry is a sensitive animal affected more quickly by environmental conditions than other farm animals. Therefore, they are more easily exposed to heat stress. High temperature and relative humidity are significant environmental factors affecting animal growth, productivity, and welfare by causing heat stress. In laying hens under heat stress, egg production decreases, feed consumption increases, feed efficiency decreases, and deterioration in egg quality occurs. This study aims to determine the heat stress of laying hens using the temperature-humidity index (THI) in a farm where commercial egg production is conducted in battery cages in the Bursa region. According to the study results, the highest THI values occurred in the summer months and were at a critical level for chickens in July (26.0) and August (24.8). There is no statistically significant relationship between egg yields obtained in spring, summer, and autumn and the calculated temperature humidity index values. There is an inverse relationship between indoor temperature and egg production.

Key Words: Bursa, Heat stress, Relative humidity, Outdoor temperature, Laying hen

ÖZ

Artan küresel ısınmayla birlikte ısı stresi kanatlı endüstrisi için daha ciddi bir tehdit haline gelmiştir. Kümes hayvanları diğer çiftlik hayvanlarına kıyasla çevresel koşullardan daha çabuk etkilenen hassas hayvanlardır. Bundan dolayı daha kolay ısı stresine girmektedirler. Yüksek sıcaklık ve bağıl nem, ısı stresine neden olarak hayvanın büyümesini, verimliliğini ve refahını etkileyen başlıca çevresel faktörlerdir. Isı stresine giren yumurta tavuklarında yumurta verimi düşer, yem tüketimi azalır, yemden yararlanma oranı azalır ve yumurta iç dış kalitesinde bozulmalar meydana gelmektedir. Bu çalışmada, Bursa bölgesinde batarya tipi kafeste ticari yumurta üretimi yapılan bir işletmede, yumurta tavuklarında görülen ısı stresinin sıcaklık-nem (THI) indeksiyle belirlenmesi amaçlanmaktadır. Çalışma sonucuna göre, en yüksek THI değerleri yaz aylarında meydana gelmiştir ve Temmuz (26.0) ve Ağustos (24.8) ayında tavuklar için kritik seviyededir. İlkbahar, yaz ve sonbahar aylarında elde edilen yumurta verimleri ile hesaplanan sıcaklık-nem indeksi değerleri arasında istatistiksel olarak anlamlı bir ilişki bulunmamaktadır. Kümes iç ortam sıcaklığı ile yumurta üretimi arasında ters bir ilişki vardır.

Anahtar Kelimeler: Bursa, Çevresel sıcaklık, Isı stresi, Bağıl nem, Yumurta tavuğu

Introduction

Just as humans are affected by environmental factors, animals are also affected by environmental factors. In order to increase the productivity expected from animals, it is necessary to keep the environmental conditions in which they are raised at an optimum level, as well as their genetic capacity. Excessive heat and humidity are the most critical environmental factors in animal production houses. Protecting animal health and ensuring their welfare undoubtedly contributes to animal-derived foods' safety and productivity preservation. Therefore, it is essential to provide conditions that meet the demands of animals in order to achieve the highest efficiency by using resources at an optimum level.

With egg producers focusing so much on house temperatures, relative humidity is often overlooked. However, whether the hens are comfortable in the poultry house is the result of the house's interaction between temperature and relative humidity. As the house's temperature and relative humidity increase, the animal's body temperature begins to increase. When they cannot release heat through sweating or respiration, body temperature increase cannot be prevented, and heat stress begins. In other words, heat stress is the sum of external forces acting on an animal, causing an increase in body temperature and evoking a physiological response (Herbut et al., 2018). Environmental factors such as temperature, relative humidity, air movement, radiation, and precipitation affect heat stress (Narmilan et al., 2021). Animal species and humans may show different sensitivity to these environmental conditions.

The average body temperature of hens is around 41-42°C, and the thermoneutral temperature is between 18-21°C to maximize the growth rate in breeding. Studies have shown that heat stress occurs in poultry at ambient temperatures higher than 25°C (Donkoh, 1989; Kumari and Nath, 2018; Wasti et al., 2020). Physiological changes in hens under heat stress can cause a decrease in feed utilization, changes in body weight, low feed consumption, a decrease in the quality and quantity of egg production, and an increase in the mortality rate (Şentürk et al., 2020).

Heat stress is an essential problem in the poultry industry that affects the health and performance of birds. Heat stress negatively affects the comfort and convenience of hens in the house and suppresses their productivity. In poultry farming, high ambient temperatures and high humidity can devastate hens. Combining these factors produces even more harmful results (Akyuz and Boyacı, 2010). The higher the humidity in the air, the more difficult it is for animals to balance their body temperature. Various indices can be used to estimate the degree of heat stress in farm animals (Akyuz et al., 2010). The temperature-humidity index is widely used to determine the effect of heat stress on animals. The Temperature-Humidity Index (THI) is an indicator to evaluate the stress level caused by high ambient temperature and humidity. By looking at THI values, it is determined whether the animals are in the comfort or stress zone (Bohmanova et al., 2007).

Many studies about the temperature-humidity index in cattle, especially in dairy cattle houses, are in the literature. However, the number of studies on poultry needs to be increased. This study aims to determine the heat stress of laying hens in egg production in battery-type cages in the Bursa region with the temperature-humidity index. In addition, the relationship between heat stress and egg production was tried to be revealed by regression analysis with SPPS statistical software.

Materials and Methods

This study determined the temperature-humidity index for a year in a battery-type laying hen house operating in the Bursa region. There were 3349 hens in the hen house at the beginning

of the study period, and when dead hens were taken into account throughout the study, it was seen that there were 2516 hens. The values of temperature, air velocity and relative humidity were measured in the poultry house with a Testo 435-2 instrument for a period of one year (Figure 1). Measurements were taken continuously for 24 hours throughout the year from December 2020 and data was recorded every 5 minutes. In the study, the dry-bulb temperatures measured indoors and entered into the web-based programme were converted into wet-bulb temperatures (Anonymous, 2023).

Figure 1. Testo 435-2

Temperature humidity index values for laying hens are calculated based on dry and wet bulb temperatures (Gates et al., 1995). Temperature-humidity index values were calculated from the data obtained using the equation (I) given below (Zulovich and DeShazer, 1990; Purswell et al., 2012).

$$THI = 0.6\ T_{db} + 0.4\ T_{wb} \quad (I)$$

THI= Temperature-humidity index

T_{db}= Dry bulb temperature (˚C)

T_{wb}= Wet bulb temperature (˚C)

Figure 2 shows the index card created by combining the effects of temperature and relative humidity for livestock. According to this graph, the white area is the comfort zone, meaning that animals in this region do not experience heat stress (Normal<23). In the yellow area, animals show signs of heat stress, indicating an alarm condition (Alert 24-25.5). The orange zone indicates a dangerous situation for the animals. Serious precautions should be taken, and the animals should be closely monitored (Danger 26-28). Ventilation rates can be increased to increase air movement over the birds to protect flock health in the danger zone. If the humidity in the house is suitable, cooling can be provided through evaporative cooling pads. In addition, feed intake should be carefully monitored. The red zone indicates the most dangerous emergency (emergency >29). Bird transport during daylight hours should be avoided in this area. Animal activity can be reduced by reducing the lighting level, and it is recommended not to feed during hot hours of the day (NFACC, 2013; Yayli and Kilic, 2023)

Temperature		**Relative Humidity (%)**					
˚F	**˚C**	**20**	**30**	**40**	**50**	**60**	**70**
100	**37.8**	26	29	30	31	33	34
98	**36.7**	26	28	29	31	32	33
96	**35.6**	26	27	28	30	31	32
94	**34.4**	26	27	28	29	31	32
92	**33.3**	25	26	27	28	29	30
90	**32.2**	25	26	26	27	28	29
88	**31.1**	24	24	26	27	27	28
86	**30**	23	24	25	26	27	27
84	**28.9**	22	23	24	25	26	27
82	**27.8**	22	23	23	24	25	26
80	**26.7**	21	22	23	23	24	24
78	**25.6**	20	21	22	23	23	24
76	**24.4**	19	21	21	22	22	23

Figure 2. Temperature-humidity index (THI) chart for livestock at spesific temperatures and relative humidity levels (NFACC, 2013)

Results and Discussions

Indoor Climate Conditions

The values of indoor climate parameters obtained from one-year measurements in the examined hen house are given in Table 1. Indoor environmental conditions are mainly within the optimum limits for hens. However, average temperatures remained high in the summer, especially in July and August. Relative humidity and air velocity values were at high values in June.

Table 1. Climatic indoor conditions of the study period in the henhouse

Parameter / Month	Mean Temp (°C)	Min. Temp (°C)	Max. Temp (°C)	Wet Bulp Temp (°C)	Mean RH (%)	Min. RH (%)	Max. RH (%)	Air Velocity (m s^{-1})
Jan	14.65	10.13	21.57	10.28	55.31	41.56	73.14	0.04
Feb	11.99	5.50	21.30	8.16	58.67	27.75	73.30	0.04
March	12.52	6.30	19.70	8.71	60.00	30.60	75.23	0.04
April	16.67	10.35	25.55	12.31	58.30	31.02	78.47	0.05
May	23.22	16.40	30.00	17.42	53.25	30.10	79.70	0.07
June	22.64	16.30	32.50	18.38	65.12	38.20	86.20	0.10
July	27.10	20.40	35.30	21.34	57.35	20.30	81.80	0.07
August	27.13	20.60	33.90	20.75	53.49	28.80	78.60	0.08
Sep	21.93	13.50	36.10	16.84	57.29	31.20	81.10	0.08
Oct	17.49	10.00	25.90	13.18	59.89	34.10	79.30	0.07
Nov	16.03	9.50	26.50	12.37	66.95	42.90	83.30	0.05
Dec	15.68	8.90	21.95	11.49	59.14	38.05	80.55	0.06

Figure 3 shows the relationship between temperature and relative humidity values. Accordingly, there are decreases in relative humidity values in periods when the temperature is rising. The highest relative humidity value was obtained in June.

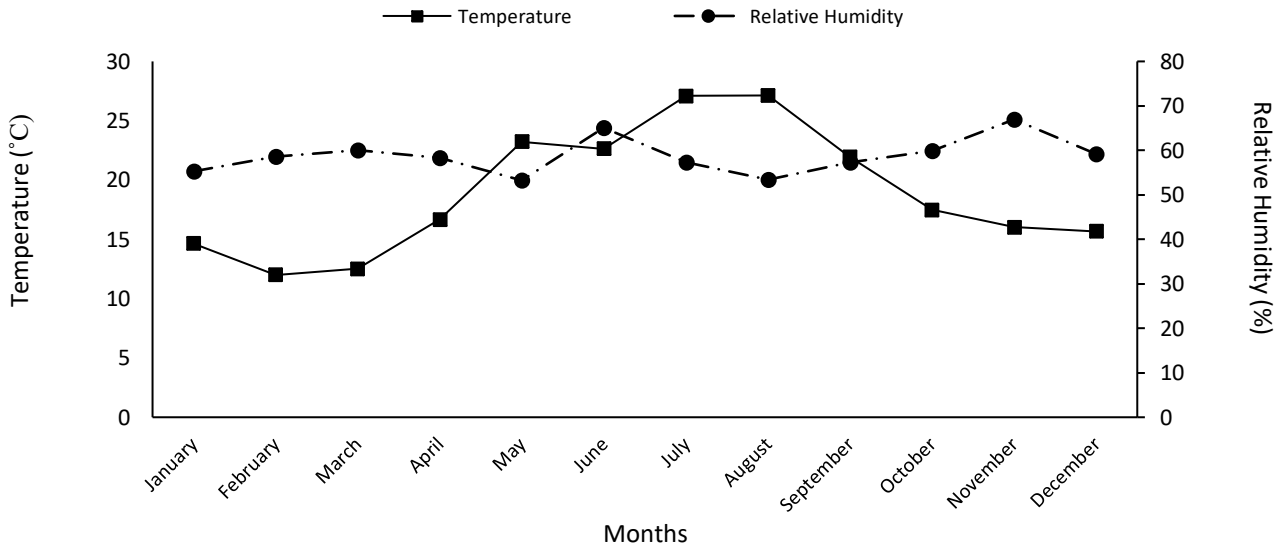

Figure 3. Interaction of between temperature and relative humidity

Temperature-Humidity Index Values

The temperature-humidity index values calculated according to the temperature, humidity, and air velocity measurements in the hen house examined within the scope of the study are given in Table 2. The temperature-humidity index values in the table show the hourly and monthly average values of the temperature-humidity index values obtained during the study period. When the average values are examined, it is seen that the hourly and monthly values are below the danger limit values given for hens. Therefore, the hens in the hen house examined according to hourly and monthly average temperature-humidity index values are

far from the danger zone regarding heat stress.

Table 2. Hourly and monthly averages of temperature-humidity index values

	Parameter	THI
Hourly Basis	Mean	19.4
	Std. Dev.	2.6
	Std. Error	0.1
	Minimum	13.7
	Maximum	25.0
Monthly Basis	Mean	20.0
	Std. Dev.	2.3
	Std. Error	0.0
	Minimum	14.4
	Maximum	26.0

Hourly and Monthly Changes in Temperature-Humidity Index Values

The hourly change in temperature-humidity index values in the hen house examined in the study is given in Table 3. For the temperature-humidity index values, the most critical time for heat stress in the henhouse is 16.00-17.00, when the index values are highest. At these hours, the internal environment of the hen house is in the warning zone in terms of heat stress according to the temperature humidity index graph. However, the calculated maximum values show that the hens are in the danger zone where they can experience heat stress in the temperature humidity index graph of the hen house's internal environment.

Table 3. Hourly change of temperature-humidity index

	Hours	11.00	12.00	13.00	14.00	15.00	16.00	17.00	18.00	19.00
THI	Mean	19.40	19.96	20.49	20.83	21.12	21.30	21.31	21.15	20.79
	Std. Deviation	2.66	2.84	3.02	3.11	3.12	3.10	3.03	2.91	2.72
	Std. Error	0.11	0.11	0.12	0.12	0.12	0.12	0.12	0.12	0.11
	Minimum	13.67	14.00	13.05	13.69	14.94	14.99	15.38	15.35	15.33
	Maximum	25.30	25.86	26.48	28.40	28.43	28.49	28.37	27.91	27.35

Table 4 shows the monthly temperature humidity index values change in the examined henhouse. When the table is examined, it is seen that the highest index values occur in the summer months, as expected. High outdoor temperatures can cause indoor temperatures that can cause heat stress in the hen house. Maximum THI values sometimes indicate that urgent precautions must be taken against heat stress. The obtained THI values indicate that July and August are especially critical for the examined poultry house. During these months, precautions must be taken to reduce the indoor temperature.

Table 4. Monthly change of Temperature-humidity Index

	MONTHS	March	April	May	June	July	August	Sept	Oct	Nov
THI	Mean	14.4	16.5	19.5	22.3	26.0	24.8	20.4	18.4	17.3
	Std. Deviation	1.630	1.822	2.508	2.756	2.861	2.947	2.203	2.025	2.217
	Std. Error	0.024	0.026	0.045	0.040	0.045	0.047	0.034	0.032	0.034
	Minimum	10.38	11.52	13.12	16.22	19.83	18.61	14.88	12.97	11.77
	Maximum	19.56	20.27	25.20	30.69	32.45	31.91	28.55	22.58	22.92

Hourly THI changes for the summer months, which is the critical season for hens in terms of

heat stress, are given in Figure 4. When the figure is examined, it is determined that the most critical hours for THI are between 16.00-18.00 for June and between 15.00-18.00 for July and August. For this reason, growers should take precautions to reduce the indoor temperature, primarily by increasing the ventilation rate and cooling between 15.00 and 18.00, which are critical hours in terms of heat stress in the summer, especially in July and August.

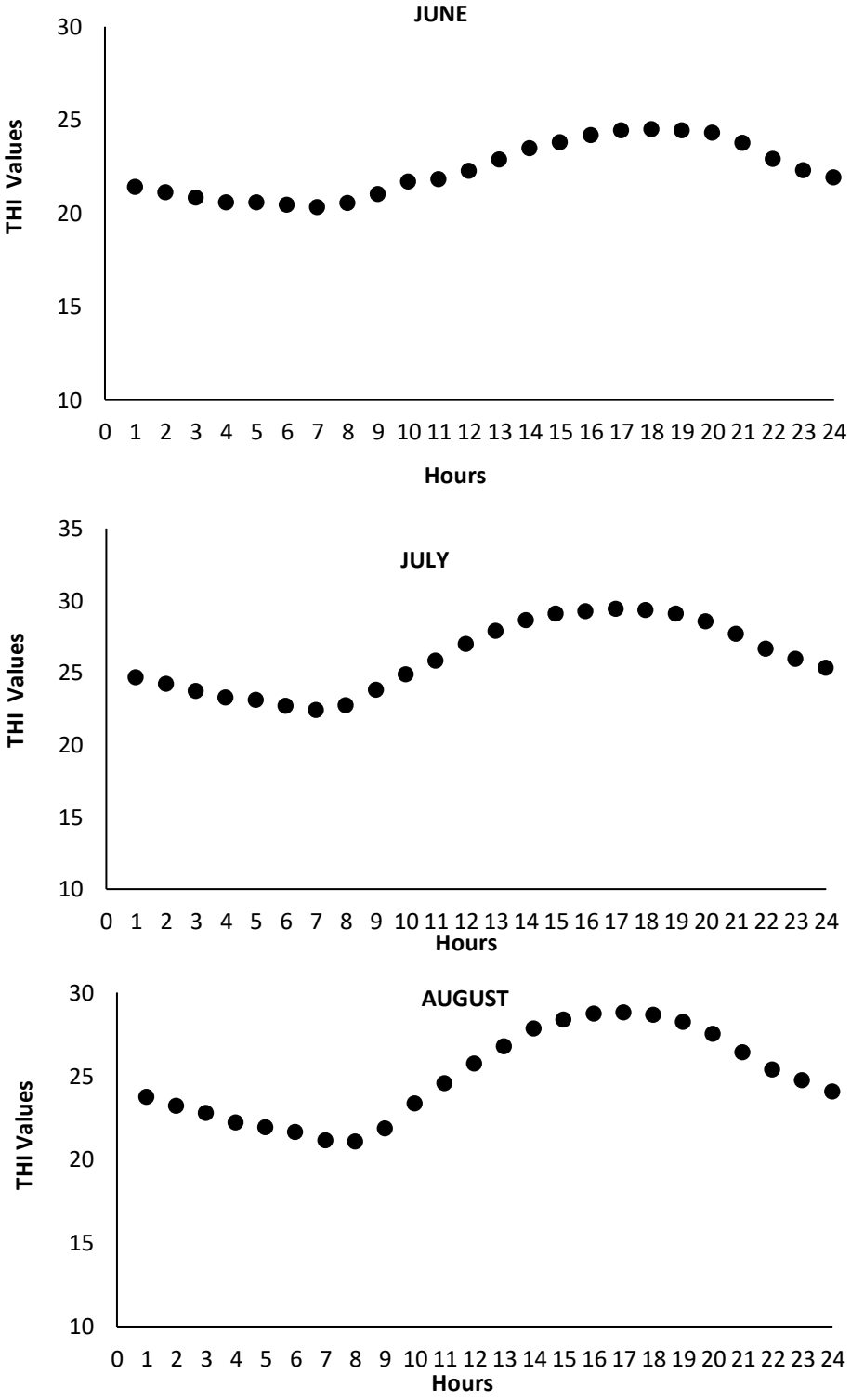

Figure 4. Hourly change of THI in the summer season in the examined hen house

Effect of Temperature-Humidity Index on Egg Production

The temperature-humidity index values calculated using the temperature and relative humidity values measured during the study and the number of eggs produced during the production period are given in Table 5. As can be seen from the table, egg production decreased in the summer season when the temperature-humidity index values are highest. The correlation

between egg production and THI is negative in summer. Egg productivity improved with the decrease in indoor temperature values in spring and autumn. With an increase of approximately 7 points in the temperature humidity index, egg production decreased by 24885 units. This situation emerged as a result of the combined effect of high temperature and relative humidity, causing the hen to undergo heat stress and reduce egg production. After the summer season, towards the end of the production process, poultry began to age and die as production began to be completed. Therefore, the decrease in egg production continued in the autumn season.

Table 5. Relationship between temperature-humidity index and egg production

		Spring	Summer	Autumn
Mean	Temperature-humidity index	16.8	24.4	18.7
	Egg Production (viol)	3349	3232	2516
Standard Deviation	Temperature-humidity index	2.0	2.9	2.1
	Egg Production (viol)	18	15	11
Correlations	Pearson Corelation Coefficients	0.64	-0.953	0.99

Figure 4 shows the relationship between temperature humidity index and egg production. There is no statistically significant relationship between egg yields obtained in spring, summer, and autumn and the calculated temperature humidity index values. Although the study had a positive relationship between temperature humidity index values and egg production, a negative relationship was expected between temperature humidity index and egg production. Because the hens are young, they are less affected by increasing temperature values. In this case, the relationship between egg production and indoor temperature in Figure 5 should be examined. As seen in the figure, there is an inverse relationship between indoor temperature and egg production. Egg production will decrease at high temperatures, which causes hens to undergo heat stress.

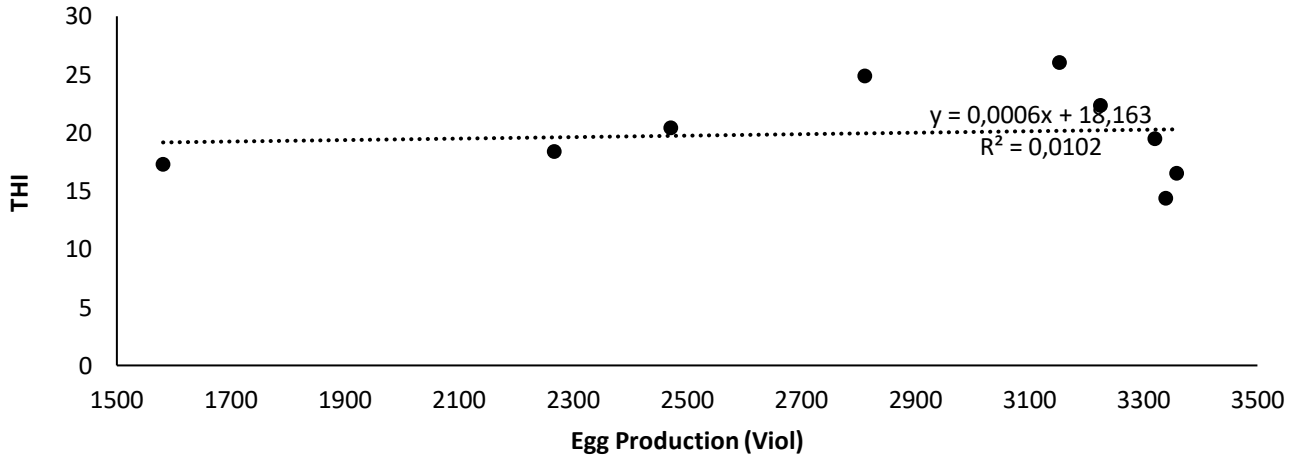

Figure 4. Interaction between THI and Egg Production in Spring, Summer and Autumn

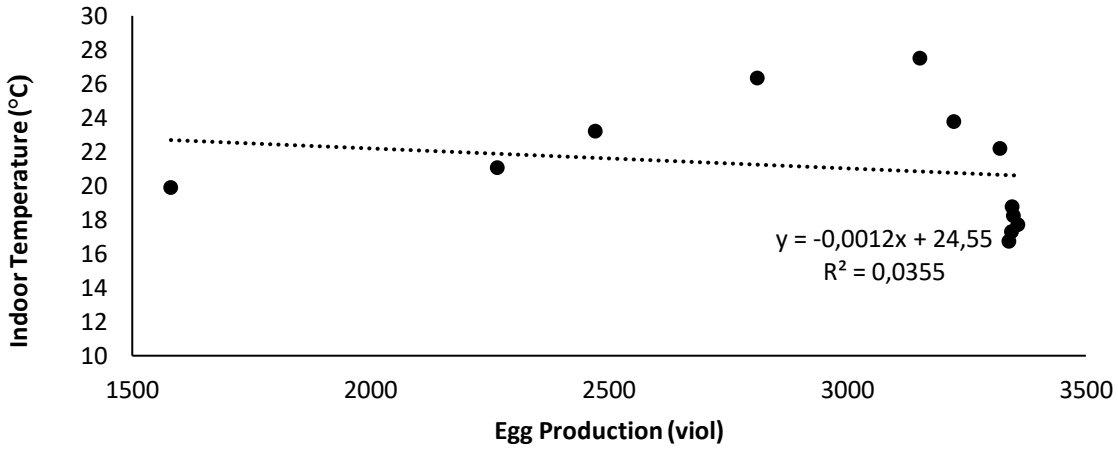

Figure 5. Interaction between egg production and indoor temperature in Spring, Summer and Autumn

Behura et al. (2016), in their study on broiler breeder pullets in hot climatic conditions, stated that the thermal comfort of chickens does not occur, especially in the summer months, and that the energy needs of chickens are significantly affected because they remain above the comfort zone, which harms their performance and welfare.

Cunha et al. (2019) aimed to evaluate a poultry house's distribution of environmental variables and thermal comfort in their study in Brazil. For this purpose, they characterized the indoor environment by calculating temperature-humidity-air velocity index values using air temperature, relative humidity and air velocity. The measured values for the chicken coop are outside the comfort zone and in the alarm zone. It has been stated that air, temperature and relative humidity values are outside the recommended ranges.

In a study by Jongbo (2020), he estimated heat stress in a battery cage chicken coop using the temperature-humidity index. He stated that since chickens spend most of their lives in hot conditions in the coop and the airspeed is very low (0.07 ms-1 to 0.58 ms-1), it may impact their performance. He also stated that the THI value of chickens under heat stress in the chicken coop is higher because they are affected by high relative humidity rather than high temperature.

Yayli and Kilic (2023) determined heat stress in hens using temperature-humidity index values in a laying henhouse with an enriched cage type. According to the study results, it is in the alarm zone during the summer months (July and August) and is inversely proportional to egg production. They stated that chickens are in their comfort zone at other times of the year.

Conclusions

Climatic factors (such as temperature, relative humidity, and air speed) significantly impact the performance and welfare of chickens. High temperatures and relative humidity in the surrounding of hens create thermal stress on animals, stay out them from their comfort zones and causing adverse effects on their metabolic energy and vitality activities and economic losses. Temperature-humidity index one of the most popular heat stress indicators. There are a lot of studies on THI.

Appropriate management strategies and indoor climatic conditions can be maintained at optimum levels in poultry house environments to reduce heat stress. In modern facilities, climatic conditions are more easily controlled by systems such as cooling pads, exhaust fans, air conditioners, and cold perches provided in the hen house design, and they are effective in preventing the adverse effects of heat stress. Providing and operating this equipment can be costly. Therefore, changes to be made in feed formulations (antioxidants such as organic selenium and phytogenic feed additives) and feeding systems may offer a more economical solution for poultry producers to eliminate the adverse effects of heat stress and minimize performance losses.

In the future, the hens' body temperatures and activities will be monitored using remote mechanical and electrical sensing technology, allowing more effective individual monitoring. This will allow more accurate and controlled use of THI, body temperature and other indices in scientific evaluations.

Declarations: The study is not within the scope of any project or thesis. No artificial intelligence tool was used in the study.

Conflict of interest:

The authors declare no conflict of interest.

Author contributions:

All authors contributed equally.

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Research Article/Araştırma Makalesi

Determination of Heavy Metal Content in Imported and Local Red Meat in Northern Iraq (Erbil) Region

Kuzey Irak (Erbil) Bölgesinde İthal ve Yerli Kırmızı Etlerde Ağır Metal Miktarının Belirlenmesi

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ABSTRACT

Red meat is one of the most important and highly demanded foods worldwide. This research investigates the levels of ten elements (Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg, and Pb) in red meat samples from local sources and four countries. The elements evaluated are categorized into three major groups based on their concentration: macro elements (Fe, Zn, Pb), microelements (Hg, As, Cu, Ni), and trace elements (Mn, Cr, Cd). The results, reported in mg Kg^{-1}, showed the following ranges: Fe: 0.113-0.118, Pb: 0.396-1.46, Zn: 1.573-4.689, Hg: 0.238-0.456, As: 1.687-1.886, Cu: 1.177-4.653, Ni: 0.012-3.078, Mn: 0.000-0.001, Cr: 0.000-0.003, Cd: 0.000-0.051. The findings indicate that heavy metal concentrations generally remained below established limits, with variability depending on the meat sample's origin.

Key Words: Meat, heavy metals, Iraq

ÖZ

Kırmızı et, dünyadaki en önemli ve en çok talep gören gıdalardan biridir. Bu araştırmada yerel ve dört farklı ülkeden alınan kırmızı et örneklerinde on elementin (Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg ve Pb) düzeyi incelendi. Araştırmada değerlendirilen elementler konsantrasyonlarına göre makro, mikro elementler ve iz elementler olmak üzere üç ana gruba ayrılmıştır. Birinci grup, Fe, Zn ve Pb gibi Makro veya temel elementlerdir. İkinci grup Hg, As, Cu ve Ni'den oluşan mikro elementlerdir. Üçüncü grup eser elementler ise Mn, Cr ve Cd'dir. Elde edilen sonuçlara göre değerler mg Kg^{-1} olarak sırasıyla, Fe: 0.113-0.118, Pb:0.396-1.460, Zn:1.573-4.689, Hg:0.238-0.456, As:1.687-1.886, Cu:1.177-4.653, Ni:0.012-3.078, Mn:0.000-0.001, Cr:0.000-0.003, Cd:0.000-0.051 arasında bulunmuştur. Elde edilen değerler, genel olarak ağır metal konsantrasyonunun limit değerlerin altında olduğunu ve et örneklerindeki ağır metal miktarının kaynağına göre değişim gösterdiğini ortaya koymuştur.

Anahtar Kelimeler: Et, ağır metaller, Irak

Introduction

Meat is a fundamental component of human diets, providing essential proteins and amino acids for health (Ibrahim, 2002). However, global concerns have escalated regarding the safety of meat products, particularly due to contamination with heavy metals. In regions like Iraq, challenges

such as limited natural pastures and escalating feed prices have amplified dependence on imported meats, raising critical issues of consumer trust in the origin and safety of these products (Dean and Bowen, 1994). Almost 30 of the 92 naturally produced components and metalloids are highly harmful to humans, including Be, Li, Al, Ti, Cr, Mn, Cu, Ni, Cu, As, Se, Sr, Mo, Pd, Ag, Cd, Sn, Sb, Te, Cs, Ba, Pt, Au, Hg, Pb, and Bi. The general name for metallic elements with an atomic weight greater than 40.04 is heavy metal. (Ming-Ho, 2005). Owing to their tendency to persist in human and animal bodies, toxic elements can be quite dangerous even at low concentrations when consumed over a long period. (Ray, 1994; Santhi et al., 2008). Global attention on food safety has intensified due to the potential risks associated with consuming foods contaminated by pollutants such as heavy metals (D'Mello, 2003). Meat, being a primary source of nutrition for many, is not exempt from these concerns. Studies indicate that heavy metals like cadmium, lead, and mercury can accumulate in meat through environmental pollution and agricultural practices, posing significant health risks upon consumption (Badis et al., 2014; Abdallah, 2005). The industrial and agricultural sectors contribute significantly to heavy metal pollution, which enters the food chain through water, soil, and ultimately affects animal products intended for human consumption (Ahmad, 2002). Contaminants can also originate from animal medications, fertilizers, and other chemicals used in agriculture, exacerbating the issue of meat safety (Nkansah and Ansah, 2014). Despite its nutritional richness in proteins, essential amino acids, vitamins (D, B12), and minerals (zinc, iron), meat faces persistent scrutiny due to the potential health implications of heavy metal exposure (Khalafalla et al., 2011). Addressing these concerns necessitates stringent regulatory measures and effective enforcement to ensure food safety standards are upheld throughout production and processing (Pandey and Madhuri, 2014). Advancements in meat processing technologies have undoubtedly improved efficiency but also present challenges in mitigating heavy metal contamination (Lukacova et al., 2014). The environmental impact of heavy metals from meat production extends beyond immediate health concerns, posing risks through bioaccumulation and food chain contamination, thereby affecting ecosystems and human health (Mansour et al., 2009). Studies on the danger of food intake polluted by heavy metals have boosted the growing need for food protection. (Cnossen et al., 2009). Ensuring the quality and safety of meat products demands continuous research efforts and comprehensive monitoring strategies to mitigate the risks associated with heavy metal contamination (Liu et al., 2004; Lasztity, 2009). This study aims to contribute to this field by investigating the levels of heavy metals in locally sourced and imported beef in Erbil, emphasizing the critical need for reliable data to inform regulatory practices and consumer choices.

Materials and Methods

Study area and samples collection

The study used red meat (frozen) imported from four different sources and one local fresh red meat. The samples were obtained by arbitrarily collecting twenty-five samples from different parts of the carcasses in the commercial local markets in the city of Erbil. Each sample is coded as A, B, C, D and E according to its country.

Preparation and treatment of samples

Every group was collected from March to May 2020. The samples were taken in five different positions in the Carcass, afterwards they mixed and minced. Then samples taken from the mixed minced beef, the collected samples were put into clean polythene bags. As demonstrated in the below figure 1, samples were taken from the loin, chuk, rip, plate and hip.

Meat samples were transported to the laboratory, gently washed with distilled water to remove contaminated particles and chopped into small pieces using a clean ceramic knife.

In order to determine the general characteristics of the meat used in the research, pH, water activity

(Majeed et al. 2023), water content, fat, protein and ash analyses were performed on the prepared meat samples (Gökalp et al., 1995). The samples were then prepared for heavy metal analysis.

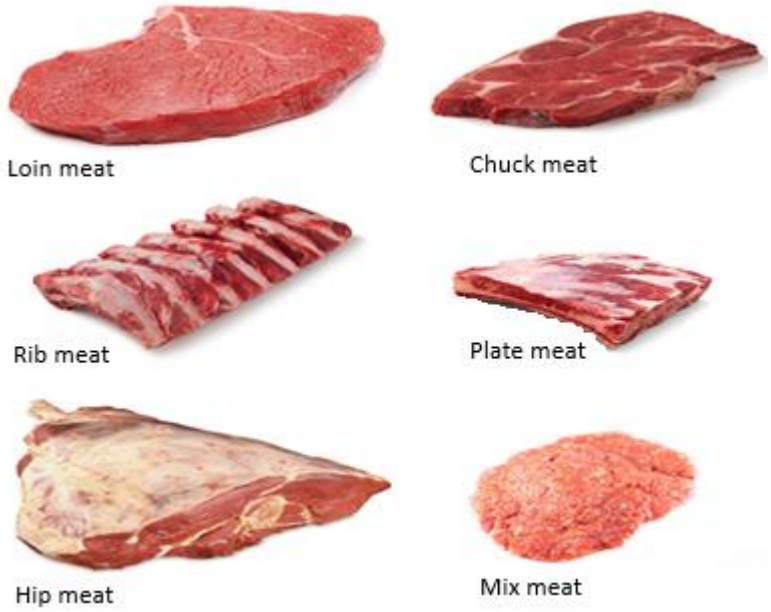

Figure 1. Meat samples used in analysis

Heavy metals determination in meat using ICP-MS

Preparation and treatment of samples

To eradicate any polluted particles, the extracted samples were cleaned with purified water. Then, using a sterile ceramic knife, specimens were cut into small pieces (1 mm thick) and then dried in the oven at 90 ° C until a known mass was collected. The dried samples were ground into fine powder in a ceramic mortar, sieved and then placed in polyethylene bags in the dark in desiccators until they were applied for acid digestion.

For the processing of specimens, the Milestone microwave method was used. The specimens (0.2 g) were weighed and excreted in a microwave digestion method with 10 ml of HNO_3 conc (65 %) and 5 ml of H_2O_2 (digestion requirements are: the first stage with power: 650 W for 10 min, second step with power: 350 W for 10 min) and then distilled with a dual filtered 18 mega Ω cm deionized method. Prodigy Axial high dispersion ICP-OES (Thermo) (RF frequency: 40, RF power: 1.3 K watt, Coolant gas flow rate: 18 L min^{-1}, Auxiliary flow rate: 0.3 L min^{-1}, Carrier gas pressure: 34 psi, Integration time: 4 sec, Sample intake rate: 1.0 ml sec^{-1}) was used to determine the elements in all specimens at the Central Lab for Elementary and Isotopic Analysis in the Nuclear Research Center, Van Yüzüncü Yıl University.

Statistical analysis

The study applied SPSS software (version 25) to analyze the differences between meat samples. Furthermore, the ANOVA test was conducted to determine the level of significance both between and within groups. Significant differences between the means have been determined by Tukey test and $P \leq 0.05$ values accepted as significant.

Results and Discussion

General composition of meat samples provided for heavy metal analysis are given in table 1.

Table 1. Average values of the general characteristics of the meat samples

Meat Sample	Components %					
	pH	Water Content	Water Activity	Fat	Protein	Ash
A	5.70±0.13^{b}	68.40±0.36^{a}	0.92±0.01^{ab}	6.40±0.30^{a}	21.44±0.41^{a}	2.02±0.08^{ab}
B	5.45±0.10^{b}	69.03±0.31^{a}	0.95±0.01^{a}	6.90±0.20^{a}	21.95±0.15^{a}	1.66±0.05^{b}
C	6.16±0.15^{a}	68.00±0.26^{a}	0.92±0.02^{ab}	6.13±0.15^{a}	21.15±0.30^{a}	1.19±0.09^{c}
D	5.74±0.07^{b}	67.20±0.70^{a}	0.88±0.02^{b}	6.27±0.27^{a}	21.10±0.10^{a}	2.17±0.06^{a}
E	5.73±0.06^{b}	68.00±0.36^{a}	0.96±0.01^{a}	6.38±0.34^{a}	21.33±0.33^{a}	2.27±0.07^{a}

The differences between the values shown with the different letters in the same column are significant (P≤ 0.05).

As can be seen from Table 1, the mean pH values of the meat samples varied between 5.45 and 6.16. While the moisture contents of the samples varied between 67.20-69.03%, their water activities were found within the range of 0.88-0.96. Fat ratios of the samples used were determined between 6.13-6.90% and protein ratios were determined between 21.10-21.95%. Ash ratios of meat samples varied between 1.19-2.27%. In general, the values obtained are compatible with the values given in the literature AL-Hussainy and AL-Fadhly (2019), Al-Husseiny (2017), Khoshnaw (2015), Fennema and Carpenter (1984).

This research studied the level of ten elements (Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg, and Pb) in red meat samples from local and four different countries. The evaluated elements in the research are divided three major groups according to their concentration: macro, micro and trace elements.

The first group, the macro or essential elements, such as Fe, Zn and Pb are usually present in high concentrations in red meat in comparison to other elements. The second group is microelements, which consist of Hg, As, Cu and Ni. One of the most important sources of microelements such as Hg, As, Cu and Ni is red meat. The final group of elements in this study includes Mn, Cr and Cd. The mean, standard deviation, minimum and maximum (mg Kg^{-1}) values of red meat samples from five different countries are shown in Table 2.

Table 2. The mean, standard deviation, minimum and maximum values of red meat samples from five different countries (mg Kg^{-1}).

		N	Mean	Std. Deviation	Minimum	Maximum
Fe	A	3.000	0.114	0.003	0.110	0.120
	B	3.000	0.113	0.009	0.100	0.120
	C	3.000	0.117	0.009	0.110	0.120
	D	3.000	0.118	0.007	0.110	0.130
	E	3.000	0.116	0.011	0.110	0.130
Pb	A	3.000	1.460	1.670	0.456	3.400
	B	3.000	0.420	0.126	0.303	0.554
	C	3.000	0.407	0.059	0.356	0.472
	D	3.000	0.436	0.082	0.348	0.510
	E	3.000	0.396	0.180	0.250	0.597
Zn	A	3.000	1.573	0.224	1.360	1.807
	B	3.000	4.656	0.324	4.330	4.978
	C	3.000	3.258	1.982	1.447	5.375
	D	3.000	3.628	1.008	2.660	4.672
	E	3.000	4.689	0.146	4.551	4.842
Hg	A	3.000	0.268	0.108	0.150	0.360
	B	3.000	0.456	0.132	0.330	0.590
	C	3.000	0.297	0.135	0.150	0.420
	D	3.000	0.283	0.155	0.150	0.460
	E	3.000	0.238	0.111	0.160	0.370
As	A	3.000	1.687	0.189	1.520	1.890
	B	3.000	1.753	0.258	1.500	2.020
	C	3.000	1.886	0.216	1.700	2.120
	D	3.000	1.842	0.309	1.590	2.190
	E	3.000	1.857	0.189	1.670	2.050
Cu	A	3.000	1.247	1.101	0.000	2.080
	B	3.000	1.177	1.103	0.000	2.190
	C	3.000	3.713	3.290	0.000	6.270
	D	3.000	1.806	1.743	0.000	3.480
	E	3.000	4.653	1.814	3.160	6.670
Ni	A	3.000	3.078	3.043	0.000	6.090
	B	3.000	0.012	0.012	0.000	0.020
	C	3.000	1.953	1.965	0.000	3.930
	D	3.000	0.717	0.625	0.000	1.150
	E	3.000	2.268	0.592	1.900	2.950
Mn	A	3.000	0.001	0.001	0.000	0.000
	B	3.000	0.000	0.000	0.000	0.000
	C	3.000	0.000	0.000	0.000	0.000
	D	3.000	0.000	0.000	0.000	0.000
	E	3.000	0.001	0.001	0.000	0.000
Cr	A	3.000	0.000	0.000	0.000	0.000
	B	3.000	0.000	0.000	0.000	0.000
	C	3.000	0.001	0.001	0.000	0.000
	D	3.000	0.003	0.006	0.000	0.010
	E	3.000	0.000	0.000	0.000	0.000
Cd	A	3.000	0.051	0.08	0.000	0.130
	B	3.000	0.000	0.000	0.000	0.000
	C	3.000	0.001	0.001	0.000	0.000
	D	3.000	0.000	0.000	0.000	0.000
	E	3.000	0.000	0.000	0.000	0.000

It can be seen in Table 2, there are slight variation in the level of Fe in the red meat samples. While the highest Fe concentration (0.118 mg Kg^{-1}) was in the red meat sample from C, the lowest level (0.113mg Kg^{-1}) of Fe was in the B local meat sample. It is shown that the A sample contain the

highest Pb (1.468 mg Kg^{-1}). However, all the other meat samples contained low level of Pb. The concentrations of Pb were (0.420, 0.407, 0.436 and 0.396 mg Kg^{-1}) in red meat samples of B, C, D and E, respectively. The main source of Pb contamination for livestock is food, water and air according to Halliwell et al. (2000). It is clear that the E sample had the highest Zn concentration (4.689mg Kg^{-1}) compared to the other meat samples. The concentration of Zn in A, B, C and D samples were (1.573, 4.656, 3.258 and 3.628 mg Kg^{-1}), respectively. In general, meat, milk products such as cheese are the main sources of Zn. The result of Zn content of the samples is consistent with the results of Amfo-Otua et al. (2014). While the sample B had the biggest Hg content (0.456) mg Kg^{-1} the smallest amount of Hg (0.238) mg Kg^{-1} was in E meat sample. In addition, the Hg concentrations were (0.268, 0.297, 0.283 and 0.238 mg Kg^{-1}) in A, B, C and E meat samples, respectively. The main sources of Hg exposure are food and air. Life threatening damages to the lungs can occur by the exposure to high concentration of Hg. The data obtained shows that the mercury content of meat samples is within the limits of IEC standards (EC, 2006). The amount of As in red meat from A, B, C and E and B local meat varied. It is shown that the C meat sample had the highest As content (1.886 mg Kg-1) whereas the A meat had the lowest As concentration (1.687 mg Kg^{-1}). Furthermore, the concentrations of As in B, C and E meat samples were (1.753, 1.886 and 1.857 mg Kg^{-1}) respectively. The As usually dispersed in natural water as a result of geological sources. The exposure to as chronically causes high risk of developing skin, liver, bladder, kidney and lung cancer. While the amount of as in meat is given as a maximum of 0.5 mg Kg^{-1} according to FAO standards (FAO, 2008), it is stated as 1 mg Kg^{-1} according to Australian and New Zealand standards (2015). The concentrations of Cu in red meat from A, B, C, D and E were (1.247, 1.177, 3.713, 1.806 and 4.653 mg Kg^{-1}) correspondingly. It is shown that the E meat sample had the biggest Cu content (4.653mg Kg^{-1}) while the B sample had the smallest Cu concentration (1.177mg Kg^{-1}). Health issues like liver and kidney damages might be caused by high level of Cu. The result of Cu content of the samples is consistent with the results of Amfo-Otua et al. (2014). There were variations in the content of Ni red meat from A, C, D and E and B local meat. It is revealed that the A meat sample had the biggest Ni content (3.078mg Kg^{-1}) but the B local meat had the lowest Ni concentration (0.012 mg Kg^{-1}). Moreover, the amount of Ni in C, D and E meat samples were (1.953, 0.717and 2.268 mg Kg^{-1}) separately. General Ni is found in tiny amount in soil, air, food and air. The most extremely poisonous Ni compound is nickel carbonyl. While Mn, Cr and Cd could not be detected in most of the samples, it was determined that the values were below the limit values in some samples.

Comparison the content of elements in red meat samples with other reported values

Table 3 indicates the comparison between the amount of (Mn, Cr and Fe) found in this study and other findings. It is revealed that Cabrera et al. (2010), Schönfeldt et al. (2010), Williams (2007), Williamson et al. (2005) have found slightly higher Mn content compared to the present study. In addition, while the present study found 0.00 amount of Cr in the studied red meat samples, Tinggi et al., (1997) and Jorhem et al. (1989) found 0.049 to 0.3 mg Kg^{-1}. Furthermore, Sivertsen et al. (1995), Jorhem et al. (1989), López-Alonso et al. (2012) and Vikøren et al. (2005) stated that the concentration of Fe was between 0.18- 0.20 mg Kg^{-1}; however, the concentration of Fe in the present study ranged from 0.1134 to 0.1184 mg Kg^{-1}. Briefly, it can be stated that the concentration of Manganese, Iron and Chromium in red meat samples were lower in this research compared to some other literatures.

Table 3. The comparison of the concentration of microelements (Mn, Cr and Fe) in red meat samples in this study with other literatures about red meat samples

Elements	Concentrations (mg Kg^{-1})	References
Mn	Not detected	This study
	<0.01	Cabrera et al., 2010; Schönfeldt et al., 2010; Williams, 2007: Williamson et al., 2005
Cr	Not detected	This study
	0.049- 0.03	Tinggi et al., 1997; Jorhem, L. et al., 1989
Fe	0.1134- 0.1184	This study
	0.18 – 0.20	López-Alonso et al., 2012; Vikøren et al., 2005; Sivertsen et al., 1995; Jorhem et al.,1989;

Table 4 displays the results of the level of zinc (Zn), nickel (Ni), copper (Cu), arsenic (As) in red meat in this study and other research. There is variation between the results of the present study and the other reported values. It can be seen that this research found significantly lower amount of Zn in red meat samples compared to Williams (2007), Sadler et al. (1993), Sinclair et al. (1999). However, AMAP (2002), Mertz, (1980), Mertz (1986), Biehl (1987), McDowell (2003) found significantly higher level of Ni in comparison to the present study. In addition, the difference in Cu concentration the present study and Williams (2007), Sadler (1993), Sinclair et al. (1999) was small. Moreover, AMAP (2002), Mertz (1980), Biehl et al. (1987), McDowell (2003) found considerably bigger amount of as in red meat samples than the present study.

Table 4. Concentration of elements (Zn, Ni and Cu) in red meat samples in this study and some other research

Elements	Concentrations (mg Kg^{-1})	References
Zn	1.573-4.689	This study
	45.55- 46.00	Williams, 2007; Sinclair et al.,1999; Sadler et al.,1993
Ni	0.12- 3.07	This study
	19.7 -101.7	McDowell, 2003; AMAP, 2002; Biehl, 1987; Mertz, 1980
Cu	1.17- 4.65	This study
	1.2- 2.2	Williams, 2007; Sinclair et al.,1999; Sadler et al.,1993
As	1.68- 1.88	This study
	1,3-82.6	McDowell, 2003; AMAP, 2002; Biehl,1987; Mertz, 1980

The concentration of elements such as (Cd, Hg and Pb) in red meat samples from various countries is explained in table 5. There are differences between the findings of this research and the other reported values. The results of this study do not comply with AMAP (2002), Mertz (1980), Biehl, (1987), McDowell (2003) who found considerably higher amount of Cd in red meat samples. In addition, the present study found noticeably lower concentration of Hg in comparison to Williams (2007), Sadler et al. (1993), Sinclair et al. (1999). Nonetheless, AMAP (2002) Mertz (1980), Biehl (1987), McDowell (2003) found close level of Pb in comparison to the present study.

Table 5. Concentration of elements (Cd, Hg and Pb) in red meat samples in this study and some other research

Elements	Concentrations (mg Kg^{-1})	References
Cd	0.00-0.051	This study
	45.55- 46.00	McDowell, 2003; AMAP, 2002; Biehl, 1987; Mertz,1980;
Hg	0.238-0.456	This study
	19.7 -101.7	Fujise and Geiken-Tsushin, 2020; Iwasaki et al., 2002
Pb	0.396-1.468	This study
	1.2- 2.2	McDowell, 2003; AMAP, 2002; Biehl, 1987; Mertz, 1980

Conclusion and Recommendations

Conclusion

There is no significant difference between the nutritional contents of imported and locally produced meat.

There are differences in terms of heavy metal contents and these differences arise from the region where the meat comes from,

It has been determined that heavy metal contents are below international limits.

Recommendations

Implementation of regulatory frameworks and controls on heavy metal levels in meat products will contribute to the protection of consumer health. In order to increase transparency in the meat supply chain, especially in imported products, the implementation of labeling indicating the origin of the product will help consumers make informed choices about their purchases.

It would be beneficial to launch educational campaigns to inform the public about the risks associated with heavy metal exposure resulting from meat consumption. Empowering consumers with information will promote safer dietary practices and facilitate demand for safer products.

Priority should be given to continued research into alternative methods for reducing heavy metal concentrations in livestock farming and improving meat processing technologies.

Facilitating collaboration between government agencies, research institutions and international organizations will promote the sharing of best practices and standards in food safety. This collaborative approach will be beneficial in addressing global concerns about heavy metal contamination in food products.

Implementation of these recommendations will contribute to ensuring the safety and quality of meat products in Erbil, Northern Iraq and other regions, thereby protecting public health and increasing consumer confidence in the food supply chain.

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Research Article/Araştırma Makalesi

Sustainable edible films based on seaweed mucilage enriched with pomegranate peel extract

Nar kabuğu ekstraktı ile zenginleştirilmiş deniz yosunu müsilajı esaslı sürdürülebilir yenilebilir filmler

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ABSTRACT

The objective of this study was to develop sustainable edible films based on seaweed mucilage (*Chondrus crispus*) containing pomegranate peel extract (PPE). For this purpose, films containing different concentrations of PPE (%0, 0.25, 0.50, and 1; w/v) were evaluated for their thickness, mechanical properties, color, opacity, antioxidant capacity, and total phenolic content (TPC). The incorporation of PPE significantly affected the values of elongation at break, color, antioxidant capacity, and TPC ($p<0.05$). However, the effect of PPE incorporation on the thickness, tensile strength, and opacity of the films was not significant ($p>0.05$). The addition of PPE significantly ($p<0.05$) increased the elasticity of the films, with the highest elongation at break observed in the film containing 0.5% PPE (126.90%). The DPPH scavenging activity of the films containing PPE ranged from 0.13 to 0.23 mmol TEAC/g, and the TPC values ranged from 8.39 to 29.95 mg GAE/g film. The antioxidant capacity and TPC values of the films increased significantly ($p<0.05$) with higher concentrations of PPE. The addition of PPE resulted in a decrease in film brightness, but an increase in redness and yellowness. In conclusion, the developed films offer a promising alternative for sustainable edible film production.

Key Words: Edible film, Sustainable film, Seaweed, *Chondrus crispus*, Natural extract

ÖZ

Bu çalışmanın amacı, nar kabuğu ekstraktı (NKE) içeren deniz yosunu müsilajı (*Chondrus crispus*) esaslı sürdürülebilir yenilebilir filmler geliştirmekti. Bu amaçla, farklı konsantrasyonlarda (%0, %0.25, %0.50 ve %1; a/h) NKE içeren filmler kalınlık, mekanik özellikler, renk, opaklık, antioksidan aktivite ve toplam fenolik içeriği (TPC) açısından incelendi. NKE ilavesi filmlerin uzama katsayısı, renk, antioksidan aktivite ve TPC değerleri üzerinde önemli ($p<0.05$) bir etkiye sahipti. Ancak, NKE ilavesinin filmlerin kalınlığı, çekme direnci ve opaklık değerleri üzerindeki etkisi önemli değildi ($p>0.05$). NKE ilavesi filmlerin elastikiyetini önemli ($p<0.05$) düzeyde artırdı ve en yüksek uzama katsayısı değeri %0.5 NKE içeren filmde (% 126.90) belirlendi. NKE içeren filmlerin DPPH süpürme aktivitesi 0.13-0.23 mmol TEAC/g arasında değişirken, TPC değerleri 8.39-29.95 mg GAE/g film aralığında bulundu. Bunlara ek olarak, filmlerin antioksidan aktivite ve TPC değerleri, NKE konsantrasyonunun artmasıyla birlikte belirgin ($p<0.05$) düzeyde arttı. NKE ilavesi filmlerin parlaklığında azalmaya, ancak kırmızılık ve sarılıkta artışa neden oldu. Sonuç olarak, geliştirilen filmler sürdürülebilir yenilebilir film üretimi için umut verici bir alternatif sunmaktadır.

Anahtar Kelimeler: Yenilebilir film, Sürdürülebilir film, Deniz yosunu, *Chondrus crispus*, Doğal ekstrakt

Introduction

The impact of plastic waste is a major concern in the food industry due to the large proportion of traditional packaging materials (Briassoulis & Giannoulis, 2018). Environmental concerns about plastic packaging materials have led to these materials being replaced with bio-based alternatives (Briassoulis & Giannoulis, 2018). Edible bio-based films are emerging as primary alternatives to traditional fossil-based plastic films, especially for applications such as food packaging. Bio-based films exhibit superior properties compared to fossil-based films in terms of carbon footprint, biodegradability, and active properties (Sampaio et al., 2023).

Seaweeds have received considerable attention due to their rich bioactive components such as dietary fibers, minerals, vitamins, unsaturated fatty acids, polyphenols, carotenoids, and tocopherols (Albertos et al., 2019). Bio-based edible films derived from seaweeds represent one of the recent advances in bioplastic production. Seaweeds are considered a promising source for bioplastic production due to their film-forming ability (Lim et al., 2021). Several studies have been conducted on the use of seaweed derivatives or extracts in edible film and coating formulations (Yang et al., 2017; Augusto et al., 2018; Goma et al., 2018; Albertos et al., 2019). Chondrus crispus, like most seaweeds, has typical properties such as antioxidant capacity, vitamins, minerals, and fiber content (Collen et al., 2014). Despite the utilization of C. crispus derivatives or polysaccharides in edible film/coating formulations (Thakur et al., 2018; Daei et al., 2022), there is limited research available on the use of C. crispus mucilage in film formulations.

Food waste is used to produce active packaging materials, contributing to the development of sustainable packaging materials (Khalil et al., 2024). Food waste is used to produce active packaging materials, contributing to the development of sustainable packaging materials (Khalil et al., 2024). Pomegranate (Punica granatum L.) is a nutritious fruit that contains numerous bioactive compounds, particularly in its inedible parts such as the peel and seed. Pomegranate peel serves as a potential source of antioxidant compounds and exhibits antifungal, antimicrobial, and antibacterial activity (Bertolo et al., 2020). Recently, pomegranate peel has attracted the attention of researchers due to its proven therapeutic properties (Cui et al., 2020). Pomegranate peel extract has been incorporated into edible films in numerous studies to produce active films and improve film properties (Alsaggaf et al., 2017; Dai et al., 2022). The addition of pomegranate peel extract to edible films has been reported to increase the phenolic and antioxidant activities of the films (Kumar et al., 2019) and contribute to the improvement of film properties (Bertolo et al., 2020; Kumar et al., 2021; Munir et al., 2019). For all these reasons, the aim of this study was i) to produce sustainable edible films based on C. crispus mucilage containing different concentrations of pomegranate peel extract and ii) to investigate the properties of thickness, mechanical, optical, color, antioxidant, and total phenolic contents the films.

Materials and Methods

Materials

Chondrus crispus and pomegranates (Punica granatum) were supplied from a local market. Glycerol was sourced from Merck (Darmstadt, Germany). Chemicals including sodium thiosulfate, Folin-Ciocalteu reagent, gallic acid, sodium carbonate, methanol, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich.

Preparation of C. crispus mucilage

The extraction of C. crispus mucilage was conducted with modifications to the method proposed by Albertos et al. (2019). For this, the seaweed sample was weighed and washed under running water. It was then diluted 1:10 with distilled water and stirred at a constant speed at 90 °C for 30 min using a magnetic stirrer. This was transferred to Falcon tubes and centrifuged

(Hettich Lab., Universal 32 R centrifuge, Tuttlingen, Germany) at 4500 rpm for 40 min at 4 °C. The mucilage phase was separated, and the extraction process was repeated twice. The obtained mucilage was frozen at -20 °C and then lyophilized (Biobase, Seoul, South Korea). The lyophilized mucilage was ground and stored in a sealed bag at -20 °C until film production.

Preparation of pomegranate peel extract

The pomegranate peel extract (PPE) was obtained through a modification of the methodology proposed by Kanatt et al. (2012). The pomegranates (Hicaz pomegranate) were washed under running water, and the outer peel was separated. The peels were cut into small pieces and dried at room temperature for 4 h in a fume hood. The dried peels were then ground and diluted with distilled water (1:10). This mixture was stirred at a constant speed at 35 °C for 3 h. Subsequently, it was filtered using coarse filter paper at 4 °C for 15 h. To increase extraction yield, this process was repeated twice. The obtained pomegranate peel extract (PPE) was centrifuged at 4500 rpm for 20 min at 4 °C. The moisture from the PPE was removed using a vacuum evaporator at 140 rpm and 75 °C, and the extract was then lyophilized. The lyophilized PPE was stored at -20 °C for film production.

Preparation of edible films

The production of edible films was carried out by modifying the method proposed by Hajivand et al. (2020). Seaweed mucilage (3%, w/v), glycerol (30% of the mucilage, w/w), and distilled water were mixed using a magnetic stirrer for 30 min. Then, PPE was added to the mixture containing 0, 0.25, 0.5, and 1% (w/v) PPE and mixed for 20 min. 8.5 mL of the film-forming solution was transferred to Petri dishes (85 mm diameter). The samples were dried at 40 °C for 16-18 hours. Subsequently, the dry films were peeled off and transferred to zipped bags. The films were conditioned in a desiccator containing silica gel at 25 ± 2 °C for 2 days before thickness, mechanical, color, and opacity analyses.

Determination of the properties of edible films

Thickness

The film thickness was determined using a digital micrometer (Insize 3108-25A, Germany). For this purpose, the thickness was measured at a minimum of 10 random points on the film, and the average film thickness was calculated (Ciurzynska et al., 2024).

Mechanical properties

The mechanical properties of the films were determined using a TA-XT Plus Texture Analyzer (Godalming, Surrey, UK). Samples were placed between AT/G Mini Tensile Grips and stretched to break. The instrument software was used to calculate the tensile strength (TS) and elongation at break (EAB) values of the films (Ceylan and Atasoy, 2022).

Total phenolic content

The total phenolic content (TPC) of the films was determined using the method proposed by Moghadam et al. (2020). First, 0.05 g of the film was mixed with 10 mL of distilled water and allowed to dissolve at ambient temperature for 2 h. The mixture was centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was analyzed using the Folin-Ciocalteu method. For this purpose, 100 µL of the extract (supernatant) was mixed with 2 mL of Folin-Ciocalteu reagent and 2.5 mL of Na_2CO_3 solution (7.5%, w/v), respectively. The mixture was incubated in the dark at ambient temperature for 30 min, and the absorbance was measured at 765 nm using a spectrophotometer (Biochrom Libra, S60, Cambridge, UK). The results were calculated as mg GAE/g film.

Antioxidant properties

0.05 g of the film was mixed with 10 mL of distilled water and allowed to dissolve at ambient temperature for 2 h. The mixture was centrifuged at 4000 rpm and 4 °C for 10 min. The supernatant was used in antioxidant capacity analysis. 100 µL

of extract (supernatant) was added to 3.9 mL of DPPH solution and incubated at ambient temperature (30 min). Subsequently, the absorbance was measured at 517 nm. The results were calculated as mmol TEAC/g (Carpes et al., 2021).

Color and opacity

The color of the films was measured on a white background (L*: 95.17, a*: 3.69, b*: -6.29). The L*, a*, b*, and ΔE values were determined using a colorimeter (Spec HP 200, China) based on the CIELab color measurement system (Khalil et al., 2024). The values were calculated by averaging measurements taken from at least 10 random points on each film. In the calculation of the ΔE value, the color values of the white background were used as a reference.

The opacity of the films was determined using a UV/visible spectrophotometer (Biochrom Libra, S60, Cambridge, UK). For this purpose, the absorbance of the film was recorded at 600 nm. The opacity was calculated by dividing the absorbance at 600 nm by the thickness of the film (Ceylan and Atasoy, 2022).

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) with SPSS software (IBM Corp, Armonk, NY, USA). Differences between films were determined utilizing the Duncan multiple comparison test. Analyzes were conducted in two replicates and two parallels.

Results and Discussions

Thickness

The thickness of edible films is a highly critical characteristic that influences the properties of the film (Kanatt et al., 2012). The thickness values of the films are presented in Table 1. The thickness values of the films were found to be between 0.023-0.026 mm. Moreover, the thickness values of the films containing different concentrations of PPE and the control film were similar ($p>0.05$). Similar findings were reported by Kanatt et al. (2012) for chitosan-polyvinyl alcohol films enriched with natural extracts. Emam-Djomeh et al. (2015) stated that the addition of PPE had no statistically significant effect on the thickness of sodium caseinate-based films. Similarly, Kumar et al. (2021) reported that the incorporation of PPE did not affect the thickness of chitosan-based films.

Contrary to our results, Dai et al. (2022) reported that the addition of PPE increased the thickness in polylactic acid-based composite films. In another study (Hanani et al., 2019), it was reported that increasing the pomegranate peel powder content from 1% to 2% significantly increased the thickness of gelatin-based films.

Mechanical properties

The mechanical properties of edible films play a critical role in ensuring their structural integrity and stability during food processing and storage (He et al., 2019). The maximum resistance to the applied stress on edible films is represented by the tensile strength (TS), while the elongation at break (EAB) is represented by the flexibility (Mushtaq et al., 2018). The TS and EAB values of the films are shown in Table 1. TS values of films containing different concentrations of PPE ranged from 5.45 to 7.22 MPa, while the EAB values ranged from 104.04% to 126.90%.

Table 1. The value of thickness, tensile strength, and elongation at the break of edible films

Films	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)
PPE-1	0.026±0.004[a]	5.45±0.87[a]	111.93±4.71[b]
PPE-0.5	0.022±0.003[a]	7.22±1.10[a]	126.90±2.51[a]
PPE-0.25	0.023±0.004[a]	6.93±0.87[a]	112.62±6.05[b]
PPE-0	0.023±0.005[a]	6.06±0.82[a]	104.04±2.10[c]

Different lowercase letters indicate significant differences between films containing different concentrations of PPE (p<0.05). Values are given as mean ± std deviation. PPE-0: control (containing 0% PPE), PPE-0.25: containing 0.25% (w/v) PPE, PPE-0.5: containing 0.50% (w/v) PPE, PPE-1: containing 1% (w/v) PPE, and PE1.50: containing 1.5% (w/v) PPE. PPE: pomegranate peel extract.

The addition of PPE had a significant (p<0.05) effect on the EAB values of the films, while the effect of PPE on the TS values was not significant (p>0.05). The addition of PPE resulted in a significant (p<0.05) increase in the EAB value of the films. The highest EAB value was observed in the PPE-0.5 film, followed by the PPE-0.25 and PPE-1 films. The increase in film elasticity due to the addition of PPE can be attributed to the interaction between the bioactive components of PPE and the film matrix (Kanatt et al., 2019). Several studies (He et al., 2019; Kumar et al., 2019; Kumar et al., 2021) have indicated that the mechanical properties of films can be influenced by the interaction between natural components and phenolic compounds in plant extracts with the film matrix. Furthermore, the higher elasticity value of the PPE-0.5 film compared to the PPE-1 film can be attributed to the expansion of the stress field in the PPE region due to the higher concentration of PPE (Dai et al., 2022). The results demonstrated that the elasticity of the film was affected by the concentration of PPE, depending on the phenolic components.

Consistent with our findings, He et al. (2019) reported that the addition of PPE increased the elasticity of the film. In similar, Cui et al. (2020) stated that the addition of PPE to zein films significantly increased the film elasticity. The researchers attributed the increase in film elasticity to the plasticizing effect of PPE due to its viscous nature. In contrast to our findings, He et al. (2019) reported that the addition of PPE weakened the mechanical properties of the film, and the highest TS was observed in the control film. Hanani et al. (2019) reported that the addition of 1% pomegranate peel powder (PPP) to the edible film caused a significant increase in the TS value, while 2% and 3% PPP had no significant effect on TS.

Antioxidant capacity and total phenolic content

The values of antioxidant capacity and total phenolic content (TPC) of edible films are provided in Table 2. The antioxidant capacity of the films was evaluated using DPPH scavenging activity. The DPPH scavenging activities of the films containing PPE ranged from 0.23 mmol TEAC/g to 0.13 mmol TEAC/g. The effect of PPE addition on the antioxidant capacity of the films was significant (p<0.05). The results demonstrated that the antioxidant capacity of the film increased with the concentration of PPE. The highest DPPH scavenging activity was observed in the PPE-1 film, while, the control film exhibited no antioxidant activity. Similarly, Mushtaq et al. (2018) reported that zein films without PPE exhibited no antioxidant capacity, whereas films containing PPE showed significant capacity, which increased with higher concentrations of PPE. Consistent with our results, Dai et al. (2022) observed a significant increase in the DPPH scavenging capacity of polyvinyl alcohol film with increasing PPE concentration.

Table 2. The values of antioxidant capacity and total phenolic content of edible films

Films	DPPH TEAC (mmol TEAC/g)	Total phenolic content (mg GAE g^{-1} film)
PPE-1	0.23±0.00[a]	29.95±1.37[a]
PPE-0.5	0.21±0.01[b]	19.16±1.29[b]
PPE-0.25	0.13±0.01[c]	8.39±0.95[c]
PPE-0	-	0.62±0.06[d]

Calibration curves: DPPH (y=-493.11x+ 330.76, R^2: 0.99); TPC (y=92.776x-7.9843, R^2: 0.99).
Different lowercase letters indicate significant differences between films containing different concentrations of PPE (p<0.05). Values are given as mean ± std deviation. PPE-0: control (containing 0% PPE), PPE-0.25: containing 0.25% (w/v) PPE, PPE-0.5: containing 0.50% (w/v) PPE, PPE-1: containing 1% (w/v) PPE, and PE1.50: containing 1.5% (w/v) PPE. PPE: pomegranate peel extract.

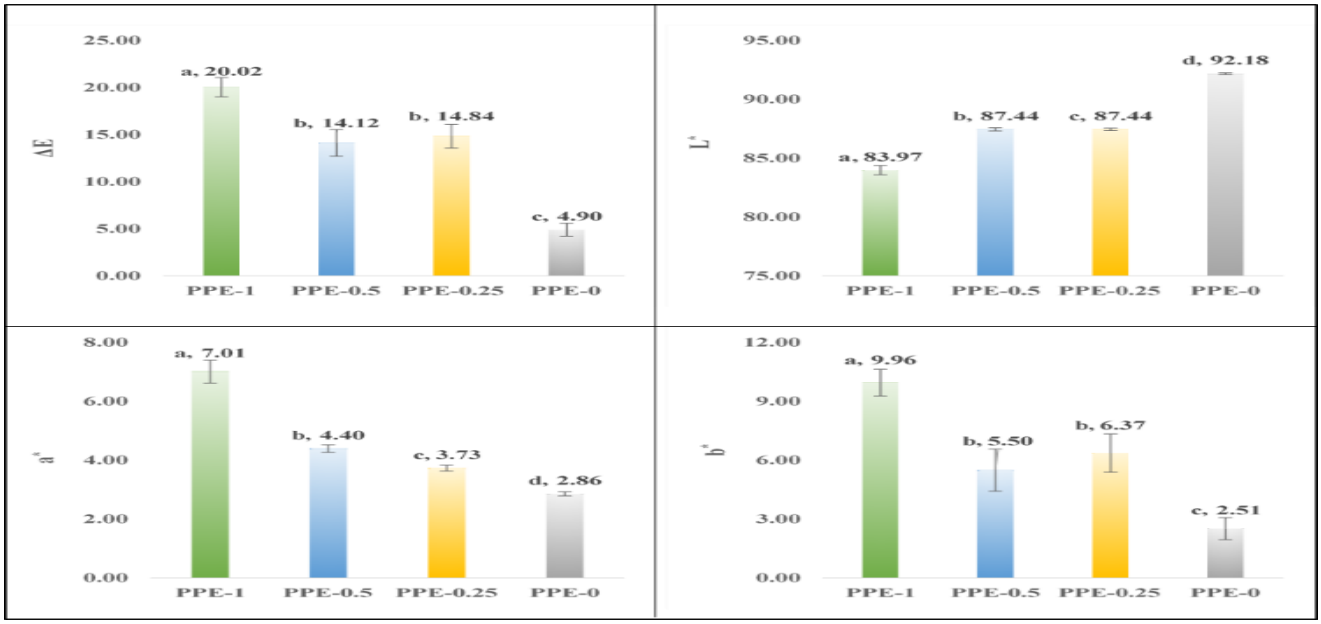

Figure 1**.** Color properties of edible films
Different lowercase letters indicate significant differences between films containing different concentrations of PPE (p<0.05). Values are given as mean ± std deviation. PPE-0: control (containing 0% PPE), PPE-0.25: containing 0.25% (w/v) PPE, PPE-0.5: containing 0.50% (w/v) PPE, PPE-1: containing 1% (w/v) PPE, and PE1.50: containing 1.5% (w/v) PPE. PPE: pomegranate peel extract.

The TPC values of the films ranged from 0.62 to 29.95 mg GAE g-1 film. The effect of PPE incorporation on the TPC values of the films was significant (p<0.05). The results indicated that the TPC in the films increased with the concentration of PPE. The highest TPC was observed in the PPE-1 sample, while the lowest TPC was detected in the control film (PPE-0). Similar findings were reported for zein films by Mushtaq et al. (2018). The antioxidant capacity of the films may be attributed to the phenolic components of PPE. PPE serves as a significant source of bioactive compounds, primarily polyphenols, and functions as a potential reservoir of antioxidant compounds (Bertolo et al., 2020; Vargas-Torrico et al., 2024). The antioxidant capacity and presence of phenolic content are attributed to the presence of compounds such as hydrolyzable tannins, phenolic acids, flavonoids, ellagic acid, citric acid, derivatives of caffeic acid, quinic acid, quercetin-3-O-glucoside, and gallocatechin Vargas-Torrico et al. (2024). In addition to these, tocopherols, carbohydrates, terpenes, carotenoids, vitamin C, and pigments also can contribute to the antioxidant capacity of films (Saberi et al., 2017).

Color and opacity

The color of edible films is an important factor in consumer acceptance (Nayak et al., 2024). L*, a*, b*, and ΔE values of edible films are shown in Figure 1. A high L* value indicates lightness, whereas a low L value indicates darkness. A positive a* value represents redness, while a positive b* value represents yellowness (Nayak et al., 2024). The effect of PPE addition on the color values of the films was significant (p<0.05). As

expected, the control film had significantly ($p<0.05$) higher L* values and lower a* and b* values compared to the films containing PPE. As the amount of PPE increased, the lightness decreased and the intensities of the yellow and red colors increased. In addition, the incorporation of PPE resulted in a significant increase in the ΔE values of the films, indicating an overall difference in the color properties of these films. Similar findings have been reported by Nayak et al. (2024) for films incorporating corn starch/moringa gum with pine cone extract. In line with our findings, Munir et al. (2019) indicated that the control film without PPE had the highest L* value, the lowest a* and b*, and the ΔE value. The observed change in the color of the films with the addition of PPE may be due to the presence of antioxidants and anthocyanin pigments (Kumari et al., 2017). Furthermore, the increase in film redness and yellowness at higher concentrations of PPE is due to the increased concentration of color pigments present in PPE (Munir et al., 2019).

The opacity values of films containing PPE at different concentrations were in the range of 3.27 - 3.67 A/mm (Figure 2). The opacity of edible films increased slightly with the addition of PPE, but this increase was not statistically significant ($p>0.05$). The fact that the addition of PPE did not affect film opacity may be due to the thin nature of the produced films. Another possible reason could be the concentration of PPE. Emam-Djomeh et al. (2015) found that the addition of PPE significantly reduced the transparency of sodium caseinate-based films, but this reduction was not significant at lower extract concentrations. Contrary to our results, Munir et al. (2019) reported that the transparency of films increased with the concentration of plant extracts, which was attributed to the type and level of phenolic compounds. In another study (Dai et al., 2022), it was stated that the addition of PPE increased the transparency of edible films. Vargas-Torrico et al. (2024) reported that the opacity of gelatin-carboxymethylcellulose films increased with increasing PPE concentration.

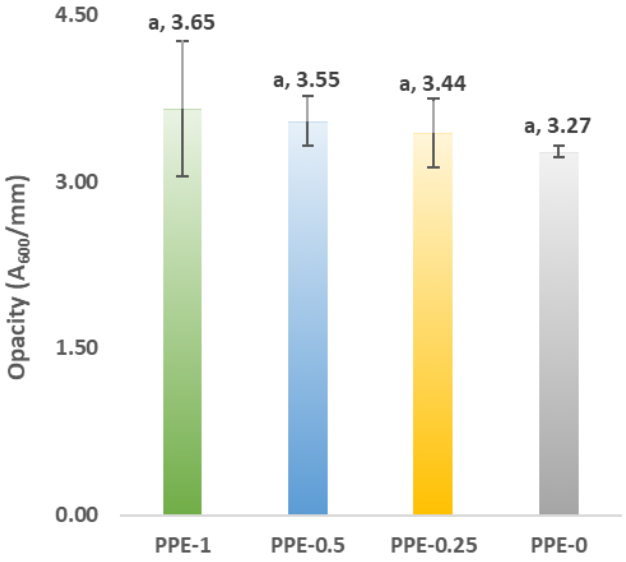

Figure 2. The opacity values of edible films
Different lowercase letters indicate significant differences between films containing different concentrations of PPE ($p<0.05$). Values are given as mean ± std deviation. PPE-0: control (containing 0% PPE), PPE-0.25: containing 0.25% (w/v) PPE, PPE-0.5: containing 0.50% (w/v) PPE, PPE-1: containing 1% (w/v) PPE, and PE1.50: containing 1.5% (w/v) PPE. PPE: pomegranate peel extract.

Conclusions

In this study, edible films containing pomegranate peel extract (PPE) based on C. crispus seaweed mucilage were developed and characterized. The thickness, color, opacity, mechanical properties, antioxidant capacity, and total phenolic content (TPC) of the obtained films were determined. The addition of PPE improved the elasticity of the edible films. The obtained films were a significant source of phenolic compounds and exhibited antioxidant activity. The incorporation of PPE resulted in an increase in the antioxidant activity and TPC of the films. Films containing PPE exhibited darker, red, and yellow color intensities compared to the control film.

The tested films can be considered an important alternative to sustainable edible films. To enhance the water resistance of the obtained films (data not shown), future studies could explore the incorporation of hydrophobic components into the formulation or the development of composite films using different polymers. However, for a comprehensive understanding of the film properties, future

studies should also evaluate the barrier, morphological, and structural properties of the films. Furthermore, investigating the ultraviolet (UV) light transmittance properties of the films could provide valuable insights for their application in light-sensitive food packaging. To better understand their potential as edible packaging materials, it would be beneficial to test these films in food applications.

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Conflict of interest:

The author declares that they have no conflict of interest.

Author contributions:

Huriye Gözde CEYLAN Conceptualization, Investigation, Methodology, Validation, Formal analysis, Writing - Original Draft, Visualization.

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Research Article/Araştırma Makalesi

Determination of the physical quality, structural characteristics, and sensory acceptability of biscuits prepared from einkorn-based lentil composite flours

Siyez bazlı mercimek kompozit unlarından hazırlanan bisküvilerin fiziksel kalitesi, yapısal özellikleri ve duyusal kabul edilebilirliğinin belirlenmesi

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ABSTRACT

Composite flour refers to a blend of flours sourced from tubers, grains, legumes, oilseeds, vegetables, and fruits, utilized in the formulation of bakery, pastry, and complementary food products in addressing protein-energy malnutrition and micronutrient deficiencies, the inclusion of high-protein legume is essential. Hence, the objective of this research was to produce biscuits with enhanced physicochemical attributes and sensory properties using composite flours prepared with einkorn flour-based green, red, and yellow lentil flours. According to the obtained results, all lentil flours except red lentil flour significantly increased the WAC (water absorption capacity) value of einkorn flour-based composite flours ($p < 0.05$). Specifically, composite flour containing red lentil flour exhibited the highest foam capacity at 16.00%, followed by samples formulated with yellow (S2) and green lentil flour (S1), and control (C) samples, respectively. The differences in composite flour formulations had a significant effect ($p < 0.05$) on the specific volume and spread ratio of biscuits. Specific volume measurements for control biscuits (1.70 $cm^3\ g^{-1}$) were notably higher compared to the lower values recorded for composite biscuits (0.92–1.24 $cm^3\ g^{-1}$). According to scanning electron microscope (SEM) results, the control biscuit exhibited a noticeably crumbly texture, unlike the other samples, which had a more cohesive starch-gluten composite network. Panelists showed a preference against biscuits containing all lentil flours together (S4), as evidenced by lower scores in terms of color, odor, brittleness, and taste. Additionally, the results underscored the significance of the formulated products in enhancing dietary variety and addressing food fortification within low-income households.

Key Words: Composite flour, einkorn, biscuit SEM microscopy, biscuit sensory attributes

ÖZ

Kompozit un, fırıncılık, pastacılık ve tamamlayıcı gıda ürünlerinin formülasyonunda kullanılan yumru kökler, tahıllar, baklagiller, yağlı tohumlar, sebzeler ve meyvelerden elde edilen unların bir karışımını ifade eder. Yüksek protein içeriğine sahip baklagillerin kullanımı, protein-enerji yetersizliği ve mikrobesin eksikliklerinin iyileştirilmesinde hayati bir rol oynamaktadır. Bu nedenle, bu çalışmanın amacı, siyez unu bazlı yeşil, kırmızı ve sarımercimek unları ile hazırlanan kompozit unlarla daha iyi fizikokimyasal

özelliklere ve duyusal kabul edilebilirliğe sahip bisküviler geliştirmektir. Elde edilen sonuçlara göre, kırmızı mercimek unu hariç tüm mercimek unları, siyez unu bazlı kompozit unların WAC (su emme kapasitesi) değerini önemli ölçüde artırdığı gözlenmiştir ($p < 0.05$). Özellikle, en yüksek köpük kapasitesi %16.00 ile kırmızı mercimek unu içeren kompozit unda kaydedilmiş ve onu sırasıyla sarımercimek unu (S2) ve yeşil mercimek unu (S1) ile hazırlanan unlar ile kontrol (C) örnekleri izlemiştir. Kompozit un formülasyonlarındaki farklılıklar bisküvilerin yayılma oranı ve özgül hacim değerlerini önemli ($p < 0.05$) ölçüde etkilemiştir. Kontrol bisküvileri için özgül hacim ölçümleri (1.70 $cm^3 g^{-1}$), kompozit bisküviler için kaydedilen daha düşük değerlerle (0.92–1.24 $cm^3 g^{-1}$) karşılaştırıldığında belirgin şekilde daha yüksek bulunmuştur. SEM analiz sonuçları, kontrol bisküvisinin, diğer numunelerde gözlemlenen daha bütünleşik nişasta-gluten ağına kıyasla, belirgin bir kırılgan dokuya sahip olduğunu ortaya koymaktadır. Panelistler, renk, koku, kırılganlık ve tat açısından daha düşük puanlarla ifade edilen, tüm mercimek unlarını içeren (S4) bisküvileri daha az tercih etmiştir. Çalışmanın bulguları, geliştirilen ürünlerin düşük gelirli ailelerin beslenme çeşitliliğini artırmada ve gıdaların besin değerini zenginleştirmede önemli bir katkı sağladığını ortaya koymuştur.

Anahtar Kelimeler: Kompozit un, siyez unu, bisküvi SEM mikroskobu, bisküvi duyusal özellikleri

Introduction

The bakery industry, especially the segment dedicated to biscuits, offers a wide variety of products that are popular due to their convenient consumption and economical value (Arepally, Reddy, Goswami, & Datta, 2020; Misra & Tiwari, 2014). Traditionally, biscuits are made from a basic combination of flour, eggs, butter, and sugar. However, with the increasing health awareness among consumers, there has been a significant shift towards developing innovative food products that offer specific health benefits (Lippolis, Cofano, Caponio, De Nunzio, & Notarnicola, 2023). This trend has opened up opportunities for reformulating biscuits to improve their nutritional profile. One promising approach is the use of composite flours, which are blends of flours from various sources such as tubers, grains, legumes, oilseeds, vegetables, and fruits. Composite flours not only reduce dependency on wheat imports but also promote the use of domestic and underutilized crops while enhancing the nutritional value of food products (Akinola, Pereira, Mabhaudhi, De Bruin, & Rusch, 2020; Hasmadi, Noorfarahzilah, Noraidah, Zainol, & Jahurul, 2020). While the baking industry has traditionally relied on wheat flour for its gluten content, which provides key properties like plasticity, elasticity, and viscosity, its nutritional profile is often lower in minerals and protein compared to legumes (Dewettinck et al., 2008). Therefore, the incorporation of composite flours, rich in protein and fiber, is being advocated by food scientists to improve the nutritional composition of biscuits, reduce production costs, and decrease waste (Ezegbe, Onyeka, & Nkhata, 2023). Consequently, there has been a growing interest in producing biscuits by blending wheat with various non-wheat ingredients, such as common bean and defatted soybean (de Oliveira Silva et al., 2018), groundnut (Dauda, Abiodun, Arise, & Oyeyinka, 2018), chickpea (Lu, He, Liu, Wen, & Xia, 2022), and fluted pumpkin (Melese & Keyata, 2022).

Biscuits commonly found in the market are typically prepared from wheat flour, which often lacks sufficient quality protein due to its limited lysine content, as well as dietary fiber (Chandra, Singh, & Kumari, 2015a). This research utilized lentil (yellow, red, green) flour and einkorn flour, renowned for their exceptional nutritional profiles rich in protein, vitamins, and minerals, in biscuit formulation (Brandolini & Hidalgo, 2011; Romano, Gallo, Ferranti, & Masi, 2021). While the concept of employing composite flour in the baking industry is not novel (Aljahani, 2022; Dahal, Dangal, Pradhananga, Timsina, & Timsina, 2022; Ginindza, Solomon, Shelembe, & Nkambule, 2022; Jukić et al., 2022), extensive data on biscuits produced from blends of einkorn flour and lentil flour are scarce. Existing literature indicates that einkorn flour boasts superior carbohydrate and starch content (Brandolini & Hidalgo, 2011), while lentil flour is distinguished for its high protein content (Romano et al., 2021). The study presents insights into a commercially viable approach aimed at augmenting the protein and fiber content of

biscuits (Cankurtaran-Kömürcü & Bilgiçli, 2023; Chelladurai & Erkinbaev, 2020; Goencue & Çelik, 2020), thereby addressing societal challenges associated with malnutrition and deficiencies in essential macro- and micronutrients. Lentil varieties exhibit a spectrum of colors, including yellow, green, red, brown, and black. Notably, red lentils account for about 80% of global lentil consumption (Asif, Rooney, Ali, & Riaz, 2013). In this study, we focused on red, green, and yellow lentils due to their significant role in pulse production, processing, and marketing in Türkiye. Furthermore, the study sought to enhance the utilization of lentil and einkorn flour by integrating them with wheat flour, forming composite flour, to enhance the quality and characterization of biscuits. This study was conducted to assess the techno-functional attributes of composite flours, as well as to evaluate consumer acceptance of the biscuits produced from these flours.

Materials and Methods

Materials and chemicals

The einkorn flour (63.1% carbohydrate, 2.4% fat, 10.2% dietary fiber, 10.8% protein) used in the preparation of composite flours, along with green lentil (63.62% carbohydrate, 0.92% fat, 8.8% dietary fiber, 23.00% protein), yellow lentil (59.5% carbohydrate, 1.5% fat, 25.99% dietary fiber, 24.94% protein), and red lentil (63.0% carbohydrate, 1.0 fat%, 8.8% dietary fiber, 25.0% protein) flours were purchased from local markets. Butter, salt, sugar, milk, eggs, and vanilla used for biscuit preparation were sourced from local markets located in Kayseri, Türkiye.

Methods

Preparation of composite flour

To produce composite flour, einkorn flour and lentil flours (yellow, red, green) were mixed in different proportions. The mixing ratios of the composite flours used in the study are given in Table 1.

Table 1. The blending ratio of the flours

Samples	EF (%)	GLF (%)	YLF (%)	RLF (%)
C	100	-	-	-
S1	50	50	-	-
S2	50	-	50	-
S3	50	-	-	50
S4	50	16.66	16.66	16.66

C: Control, EF: einkorn flour, GLF: green lentil flour, YLF: yellow lentil flour, RLF: red lentil flour

Techno-functional properties of composite flours

Loose/Tapped Bulk Density and Hausner ratio

Bulk density indicates the mass or weight contained in a unit volume of particulate matter (Oshins, Michel, Louis, Richard, & Rynk, 2022). To conduct loose bulk density analysis, composite flours were carefully poured into 50 mL measuring cylinders without any compression, and their respective weights were recorded. Loose bulk density analyzes were determined by mass/volume ratio calculation (Du, Jiang, Yu, & Jane, 2014b). Tapped bulk density of composite flours was determined according to the method described by Gupta, Parvez, and Sharma (2015). The Hausner ratio (HR) is used to assess flowability, reflecting the resistance encountered in a bed of particles as a result of their interparticle interactions (Gaikwad et al., 2023). The Hausner ratio of the samples was assessed following the method outlined by Mahesh (2018).

Water Absorption Capacity (WAC)

The WAC of the composite flours was assessed using the procedure outlined by Adebowale, Adegoke, Sanni, Adegunwa, and Fetuga (2012). The sample, weighing one gram, was combined with 10 mL of distilled water at room temperature and mixed for 30 seconds. The suspension was left undisturbed for 30 minutes prior to centrifugation. After centrifugation, the centrifuge tube was kept open, and the supernatant was carefully drained.

The tube was reweighed, and the variance between the initial and final weights after draining the supernatant yielded the WAC, expressed as grams of water retained per gram of flour sample.

Oil Absorption Capacity (OAC)

About one gram of the sample was measured into pre-weighed 15 ml centrifuge tubes and mixed extensively with 10 ml of refined corn oil using a vortex mixer. Following mixing, the samples were left to stand for 30 minutes. The centrifuged sample-oil mixture was carefully decanted into a graduated cylinder immediately after centrifugation, and the volume was noted. Subsequently, the oil absorption capacity was computed (Achy, Ekissi, Kouadio, Koné, & Kouamé, 2017).

Swelling Power

One gram of flour sample was precisely weighed and placed into a clean, dry test tube, which was then reweighed for accuracy. About 15 mL of distilled water was added, followed by gentle stirring for 5 minutes at low speed. The suspension underwent heating at 75°C in a thermostatically controlled water bath for 30 minutes, with intermittent stirring to avoid lump formation. The test tube and its contents were quickly cooled to 20°C. Subsequently, the cooled paste underwent centrifugation, and immediately after, the supernatant was transferred into a pre-weighed evaporation dish. The supernatant was then dried at 100°C for approximately 4 hours until a constant weight was achieved. The weight of the residue was measured and reported as the mass after swelling (Tangsrianugul, Wongsagonsup, & Suphantharika, 2019).

Foaming Capacity

The foaming capacity of the samples was assessed following the procedure outlined by Chandra, Singh, and Kumari (2015b). The sample (1 gram) was introduced into 50 ml of distilled water at 30 ± 2 °C within a 100 mL graduated cylinder. Subsequently, the mixture was vigorously stirred and agitated for 5 minutes to induce foam formation. Following 30 seconds of agitation, the foam volume was quantified as the foaming capacity.

Dispersibility

Ten grams of flour sample were measured into a 100 ml measuring cylinder, and water was added until reaching a total volume of 100 ml. The mixture was then vigorously stirred and left to stand for 3 hours. The volume of settled particles was measured, and dispersibility was calculated as the percentage remaining after subtracting it from 100 (AACC, 2000).

Preparation of biscuits

To obtain a doughy consistency, composite flours (200 g) as illustrated in Table 1, salt (0.2 g) and butter (33 g) were mixed by hand for 5 minutes. The milk (15 ml), baking powder (2.0 g), vanilla (1.0 g) sugar (1.25 g) and whole eggs (1.25 ml) were meticulously mixed together. While continuously stirring, 65 ml of water was gradually added until the dough reached a desirable texture, slightly firm in consistency. The dough underwent kneading for 4 minutes on a clean, flat surface. Subsequently, it was manually rolled into sheets and shaped using a stamp cutting method. The resulting dough shapes were then placed onto greased trays and baked in an oven set at 180°C (Melese & Keyata, 2022).

Analysis of biscuits

Spread ratio

The thickness and diameter of each biscuit were measured at three different points using a composing stick (Sentez Teknik, Türkiye), and the average of each was calculated and the spread ratio was computed (Chauhan, Saxena, & Singh, 2015).

Specific volume

The specific volume of the biscuits was assessed via the seed replacement method, conducted 2 hours post-baking. Calculation of specific volume involved dividing the volume of the biscuits by their respective weight (AACC, 2000).

Scanned electron microscopy (SEM)

The morphological and structural characteristics of the biscuits were examined using a scanning electron microscope (Zeiss Gemini 500, Carl Zeiss Microscopy GmbH, Germany). Before imaging, the dry samples were coated with gold under vacuum conditions. Micrographs were obtained for each sample at different magnifications of 1.0kx and 5.0kx.

Sensory analysis

The sensory assessment of the biscuits prepared was carried out with the participation of 30 semi-trained panelists comprising staff and students from the Food Engineering department at Erciyes University. Prior to the evaluation session, the panelists received orientation regarding the sensory evaluation procedure. Subsequently, the panelists were presented with coded samples in a randomized sequence for the assessment of sensory characteristics including color, odor, taste, appearance fragility, and overall acceptability. Ratings were provided using a hedonic scale, ranging from 1 (extremely dislike) to 9 (extremely like).

Statistical analysis

The data obtained in this study were underwent statistical analysis using the ANOVA multiple comparison method via Minitab software (Minitab Ltd., Coventry, England). The Fisher's LSD test was employed to assess differences between means, with statistical significance indicated by $p<0.05$.

Results and Discussion

Powder properties of composite flours

The distinctive attributes of powdered products, encompassing loose bulk and tapped bulk density as well as the Hausner ratio, play a critical role in food formulation, preparation techniques, and storage parameters. Detailed findings elucidating these powder characteristics are presented in Table 2. Noteworthy is the observation that sample S1 exhibited the highest loose bulk density value, indicative of the impact of lentil flour inclusion in composite flours on this specific parameter.

Table 2. Powder properties of composite flours

Samples	Loose Bulk Density (g ml^{-1})	Tapped Bulk Density (g ml^{-1})	Hausner Ratio
C	0.46±0.01^{d}	0.65±0.01^{b}	1.43±0.00^{c}
S1	0.54±0.01^{a}	0.71±0.02^{a}	1.32±0.00^{e}
S2	0.50±0.01^{b,c}	0.74±0.01^{a}	1.47±0.00^{a}
S3	0.49±0.01^{c}	0.73±0.01^{a}	1.47±0.00^{b}
S4	0.52±0.01^{a,b}	0.73±0.01^{a}	1.39±0.00^{d}

* Different letters within a column indicate statistically significant differences between the data ($p < 0.05$).

The bulk density of powdered products holds paramount significance from economic, commercial, and functional perspectives. Ensuring a high bulk density in powder products is crucial for minimizing packaging volume, particularly during long-distance transportation, thereby resulting in savings in packaging material. Additionally, the density of a powder must be carefully considered in relation to container volume, packaging material requirements, and machine selection for processing, as emphasized in previous research (Göksel Saraç, Aslan Türker, & Dogan, 2020). Conversely, low bulk density is a critical consideration, particularly for instant food powders, as it serves as a key indicator of susceptibility to agglomeration or caking in food products (Sharma, Jana, & Chavan, 2012). Upon conducting calculations for loose and tapped bulk density evaluation, the sample (C) consisting solely of einkorn flour exhibited the lowest results for both analyses. Tapped bulk density ranged between 0.65 g ml^{-1} and 0.74 g ml^{-1}, with statistically insignificant differences observed in composite flours containing lentil flour ($p>0.05$). However, the tapped bulk density value of sample C differed significantly from these samples ($p<0.05$), a trend consistent with observations

regarding loose bulk density, which also increased with the addition of lentil flours.

While soft wheat flour, being the least dense, may entail higher packaging costs due to increased space requirements per unit weight (Shittu, 2012), its lighter weight facilitates transportation. Furthermore, the low bulk density of wheat flour presents advantages in the preparation of complementary foods. The decreased bulk density of sample C, comprising only einkorn flour, can be attributed to its relatively low protein and moisture content (Owens, 2001). The structural strength of a powder plays a pivotal role, with powders possessing strong structural integrity resisting agglomeration and exhibiting low bulk density when dispersed in a conveying system. Conversely, structurally weak powders settle easily, resulting in higher bulk density. High friction between particles typically leads to lower bulk density, a phenomenon corroborated by previous studies (Abdullah & Geldart, 1999). Furthermore, the evaluation of flow behaviors through Hausner ratio analysis, derived from bulk and compressed density data, reveals distinct flow characteristics among the samples. Low Hausner ratio (<1.25) indicates better flow properties than higher ones; 1.25 to 1.5 indicates moderate flow characteristics and more than 1.5 indicates poor flow (Mahesh, 2018). Einkorn flour and composite flours with varying compositions demonstrate moderate flow behavior, as indicated by the Hausner ratio classification.

Water and Oil Absorption Capacity

Figure 1 illustrates the WAC values of composite flours formulated using einkorn flour alongside green, red, and yellow lentil flours. Notably, the S2 sample exhibited the highest WAC value (2.42 ± 0.41), whereas the S3 sample recorded the lowest value (1.43 ± 0.13). With the exception of red lentil flour, all other lentil flours significantly ($p<0.05$) augmented the WAC value of einkorn flour-based composite flours. This notable increase in water absorption capacity renders these composite flours viable for the production of bakery foods like bread and biscuits (Bello & Ekeh, 2014; Eriksson, Koch, Tortoe, Akonor, & Baidoo, 2014). In light of this, the composite flour containing green lentils and einkorn flour, boasting the highest WAC value, emerges as a promising alternative for the production of bakery goods such as biscuits and bread.

Absorption properties are primarily influenced by the chemical composition of the sample, which includes carbohydrates, proteins, and fiber that constitute the majority of the non-fat portion of food solids (Sciammaro et al., 2021). Powdered particles are composed of one or more biomolecules, such as lipids, proteins, carbohydrates and minerals. The impact of water on these particles depends on their composition and their capacity to adsorb water from the air (Yu, Chan, Gengenbach, & Denman, 2017). Moreover, the arrangement of biomolecules on the particle surface influences their interaction with water and other particles (Murrieta-Pazos et al., 2012). Proteins and lipids are mainly hydrophobic (Crowley, Gazi, Kelly, Huppertz, & O'Mahony, 2014). The chemical profiles of the powders are detailed in the Materials and Methods section. Composite flours showed varying lipid contents, with RLF having the highest at 1.5%. YLF was noted for its significantly lower carbohydrate content. Consequently, powders with higher protein and lipid content exhibit greater stability at high relative humidity and are less soluble in pure water (Chandrapala, Zisu, Palmer, Kentish, & Ashokkumar, 2014). In contrast, most minerals and carbohydrates, particularly saccharides, are hydrophilic and form soluble powders (Fournaise et al., 2020).

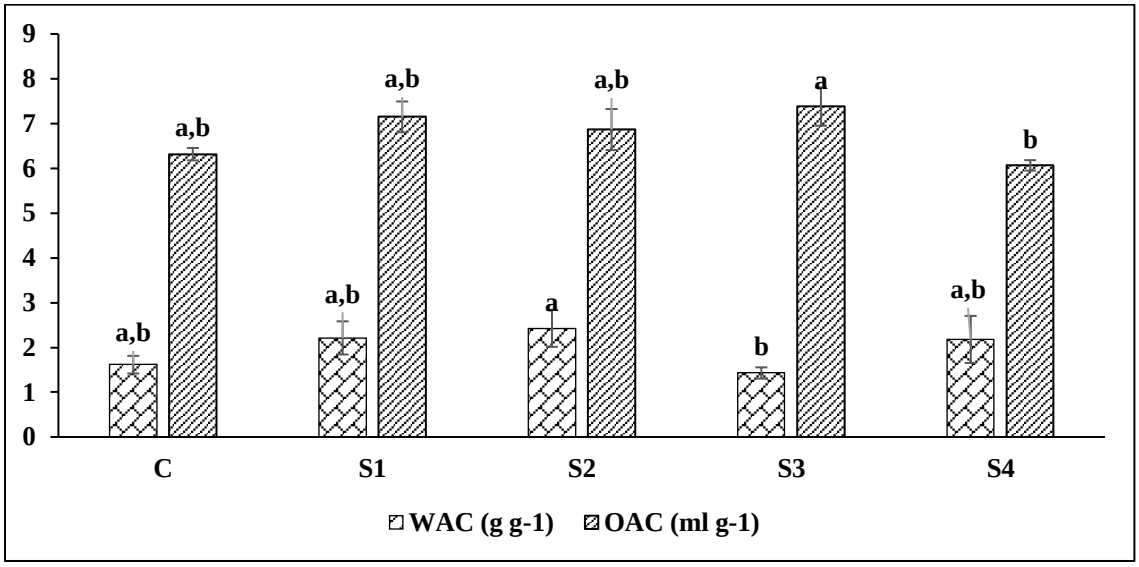

Figure 1. Water and oil absorption capacity of composite flours

The oil absorption capacity (OAC) of a foodstuff is pivotal in determining its ability to assimilate oil during biscuit development. With the inclusion of lentil flours in the formulation, the OAC content exhibited an increase, notably peaking in the S3 sample (Figure 1). This heightened OAC content within the composite flour may stem from the elevated protein content inherent in the investigated lentil flours. Such elevation could be attributed to the presence of proteins exposing more nonpolar amino acids to the oil, thereby amplifying hydrophobicity and consequentially enhancing oil absorption (Oluwamukomi, Oluwalana, & Akinbowale, 2011). Oil absorption capacity, calculated as the absorbed fat weight per protein or flour weight, hinges on the binding of fat by nonpolar amino acids residing in protein side chains. Elevated oil absorption capacity not only augments texture but also mitigates yield losses in processed foods like baked goods (Foschia, Horstmann, Arendt, & Zannini, 2017). Within this study, oil absorption capacities of composite flours were ranged from 6.07 ml g^{-1} to 7.37 ml g^{-1}. The water and oil absorption capacities of food proteins are depend on factors such as amino acid composition, protein structure, and surface polarity or hydrophobicity (Du, Jiang, Yu, & Jane, 2014a). Enhanced OAC not only refines texture but also contributes to favorable taste and mouthfeel in food products (Appiah, Asibuo, & Kumah, 2011). Consequently, a composite flour blend comprising einkorn flour and red lentil flour, boasting superior oil absorption capacity, may outperform other flours as a flavor enhancer. Moreover, the S3 sample's high oil absorption capacity suggests potential utility in products necessitating substantial oil absorption. However, Kinsella and Melachouris (1976) suggest that lipids bind more effectively to proteins exhibiting greater hydrophobicity, hinting at the affinity between nonpolar amino acid side chains and fat paraffin chains. Thus, it can be inferred that red lentil flour, with its heightened oil absorption capacity, probably has a higher amount of non-polar side chains in its protein molecules.

Functional properties of composite flours

Table 3 exhibits the variability in swelling power among composite flours, ranging from 12.37% to 14.25%. Notably, the type of lentil flour incorporated into the formulation did not yield statistically significant differences in the swelling power of the composite flours, as evidenced in Table 3. The swelling power values observed in this study align with those reported for wheat flour (12.75%) by Enwere (1998). The augmentation in swelling power within composite flours might be due to the formation of protein-amylose complexes. Swelling power serves as an indicator of granule absorption post-heating, thereby delineating the water retention capacity of starch molecules with hydrogen bonding, a phenomenon linked to starch and amino acid concentration (Iwe, Onyeukwu, & Agiriga, 2016). Variations in

flour swelling power may hinge on the amylose/amylopectin ratio and their respective properties concerning conformation, length and degree of branching and molecular distribution (Hoover, 2001).

The foaming capacity of a food or flour is measured by the extent of interfacial area generated during its agitation. Primarily, proteins are accountable for foaming. Foaming capacity and stability typically depend on the interfacial film forged by proteins, which facilitates the suspension of air bubbles and retards the coalescence rate (Awuchi, Igwe, & Echeta, 2019). Notably, the composite flour containing red lentil flour (S3) exhibited the highest foam capacity at 16.00%, followed by samples S4, S2, S1, and C, respectively (Table 3). Moreover, statistically significant differences in foam capacities among the samples was observed ($p<0.05$). This differences may stem from the variances among different lentil flours and the distinct physical properties of their principal proteins.

Table 3. Functional properties of composite flours

Samples	Swelling Power (%)	Foaming Capacity (%)	Dispersibility (%)
C	14.25±1.79^{a}	10.83±0.24^{c}	71.50±0.71^{a,b}
S1	14.15±1.14^{a}	10.83±0.24^{c}	72.50±0.71^{a}
S2	12.37±2.22^{a}	11.50±1.18^{b,c}	67.50±0.12^{c}
S3	14.22±2.69^{a}	16.00±1.41^{a}	73.50±0.71^{a}
S4	12.98±2.82^{a}	14.00±1.41^{a,b}	69.00±1.41^{b,c}

* Different letters within a column indicate statistically significant differences between the data ($p < 0.05$).

The protein dispersion in red lentil flour can diminish surface tension at the water–air interface, consistently resulting in the formation of a cohesive film enveloping the air bubbles in the foam (Kaushal, Kumar, & Sharma, 2012).

Legume/pulse flours are characterized by their elevated levels of protein and starch content, a factor that impacts the gelling capacity of these flours. This phenomenon arises from the competitive physical interplay between starch gelation and protein gelatinization for water absorption (Kaushal et al., 2012). The composite flour incorporating red lentils and einkorn flour demonstrated the highest foaming capacity, signifying that the protein content and the formation of protein-carbohydrate complexes in legumes might impact their foam capacity and stability. Red lentil flour exhibited commendable foam stability, likely attributable to the elevated surface activities of soluble proteins in continuous water phases (Kaur & Singh, 2005).

Table 3 presents the dispersibility results for both composite flour and einkorn flour. The findings indicate a notable ($p < 0.05$) alteration in flour dispersibility upon incorporating einkorn flour with red, yellow, and green lentil flour. Specifically, the composite flour formulated with red lentil flour exhibited the highest dispersibility rate, recorded at 73.50%.

Spread Ratio and Specific Volume of the Biscuits

The variations in composite flour compositions significantly influenced both the spread ratio and specific volume of the biscuits, as presented in Table 4. Throughout this study, the spread ratio of the biscuits ranged from 4.72 to 7.20, representing a tangible physical attribute. It's noteworthy that the control sample demonstrated a significantly ($p < 0.05$) lower spread ratio in contrast to the composite biscuits. This observation aligns with the findings of Durojaiye, Abubakar, Nwachukwu, Mohammed, and Ibrahim (2018) who stated that augmenting the addition of Bambara nut flour and black-eyed pea flour led to a higher spread ratio compared to whole wheat flour biscuits. Similarly, Chauhan, Saxena, and Singh (2016) observed an increase in spread ratio with higher substitution rates of wheat flour with amaranth flour. The lower spread ratio in control biscuits implies that the starches in the control (einkorn flour) exhibit

higher hydrophilicity compared to composite flours, thereby resulting in the reduced spread ratio of einkorn (control) biscuits. Differences in protein quality and water absorption properties can alter the water absorption capacity of flour and subsequently affect its spread ratio. Conversely, the robust water-binding property of fiber may also impact biscuit spread ratio (Ojha & Thapa, 2017). Spreading ratio is an important feature in predicting the quality and rising ability of flour used in the preparation of biscuits (Adeola & Ohizua, 2018). According to the Melese and Keyata (2022), the higher the value of the spread ratio, the more desirable the product and biscuits boasting a higher spread ratio are typically preferred. Hence, among the biscuits formulated with a mixture of all lentil flours (S4) and exhibiting the highest spread, is considered one of the most preferred.

Table 4. *Spread ratio and specific volume values of the biscuits*

Samples	Spread ratio	Specific volume (cm^3 g^{-1})
C	4.72±0.11^{c}	1.70±0.13^{a}
S1	6.87±0.29^{a}	1.24±0.11^{b}
S2	7.18±0.06^{a}	1.04±0.03^{c,d}
S3	5.77±0.24^{b}	1.16±0.02^{b,c}
S4	7.20±0.35^{a}	0.92±0.04^{d}

* Different letters within a column indicate statistically significant differences between the data ($p < 0.05$).

A biscuit of optimal quality is anticipated to exhibit a substantial specific volume, denoting the ratio of biscuit volume to biscuit weight (Ma & Baik, 2018). Specific volume measurements for control biscuits (1.70 cm^3 g^{-1}) were markedly higher compared to the lower values recorded for composite biscuits (0.92–1.24 cm^3 g^{-1}) (Table 4). The reduced volume observed in composite biscuit flour samples might stem from the reduced gluten content in the dough, impeding gas retention (Melese & Keyata, 2022). These findings consistent with those reported by Ostermann-Porcel, Quiroga-Panelo, Rinaldoni, and Campderrós (2017). In their study, researchers noted that, similar to our findings, elevating the addition of okara led to cookies with the lowest specific volume.

Microstructure of biscuits

Product attributes such as cell density, structural uniformity and size significantly influence the sensory characteristics of baked goods, including biscuits. SEM images illustrating the internal cross-sectional area of biscuits are provided in Fig. 2. The microstructural assessments conducted on biscuits incorporating einkorn and lentil flours revealed alterations in their structure, which varied depending on the type of lentil flour incorporated into the dough formulations.

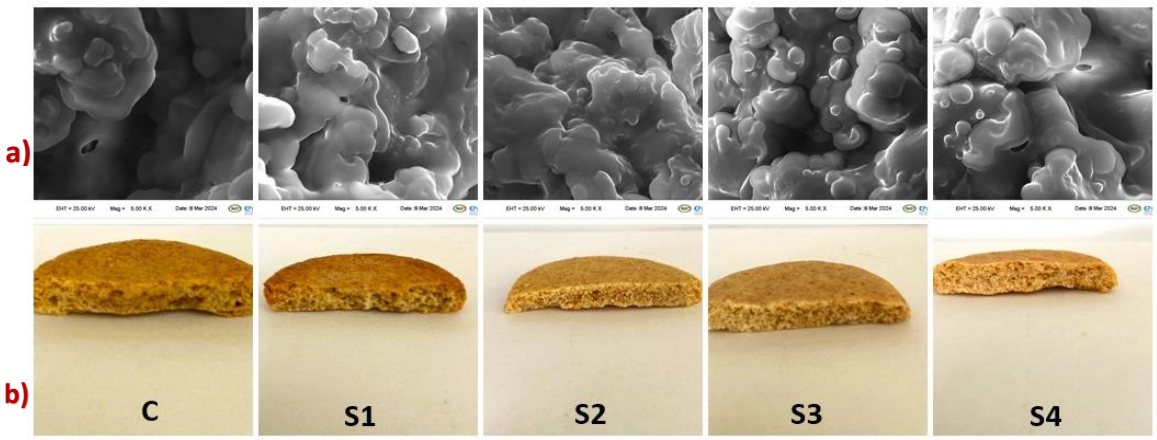

Figure 2. SEM images (a) and cross-sectional views (b) of biscuits

The control biscuit notably displayed a crumbly texture, contrasting with the other samples, which featured a more cohesive starchy-gluten composite network. Analysis of the biscuit micrographs (Figure 2a) revealed that the structure of C exhibited a smaller, more uniform, and smoother network compared to S1, S2, S3, and S4, indicating that the malting and fermentation processes induced changes contributing to the uniform shape in the respective samples. The observed pits in biscuit samples containing lentil flour may be attributed to the enzymatic hydrolysis of proteins (yielding amino acids) and starch (yielding sugars) (Claver, Zhang, Li, Zhu, & Zhou, 2010).

Sensory Evaluation of the Biscuits

The mean sensory assessments for both formulated biscuits and control samples, conducted by semi-trained panelists, are depicted in Figure 3a. Color serves as a crucial factor influencing the initial acceptability of baked goods. Notably, the color score of formulated biscuits exhibited an increment from 5.61 to 6.52. Furthermore, among the S1 coded biscuit samples containing einkorn and green lentil flour, the highest color score was observed. However, it's worth noting that the combination of red, yellow, and green lentil flours led to a decrease in consumer color preference.

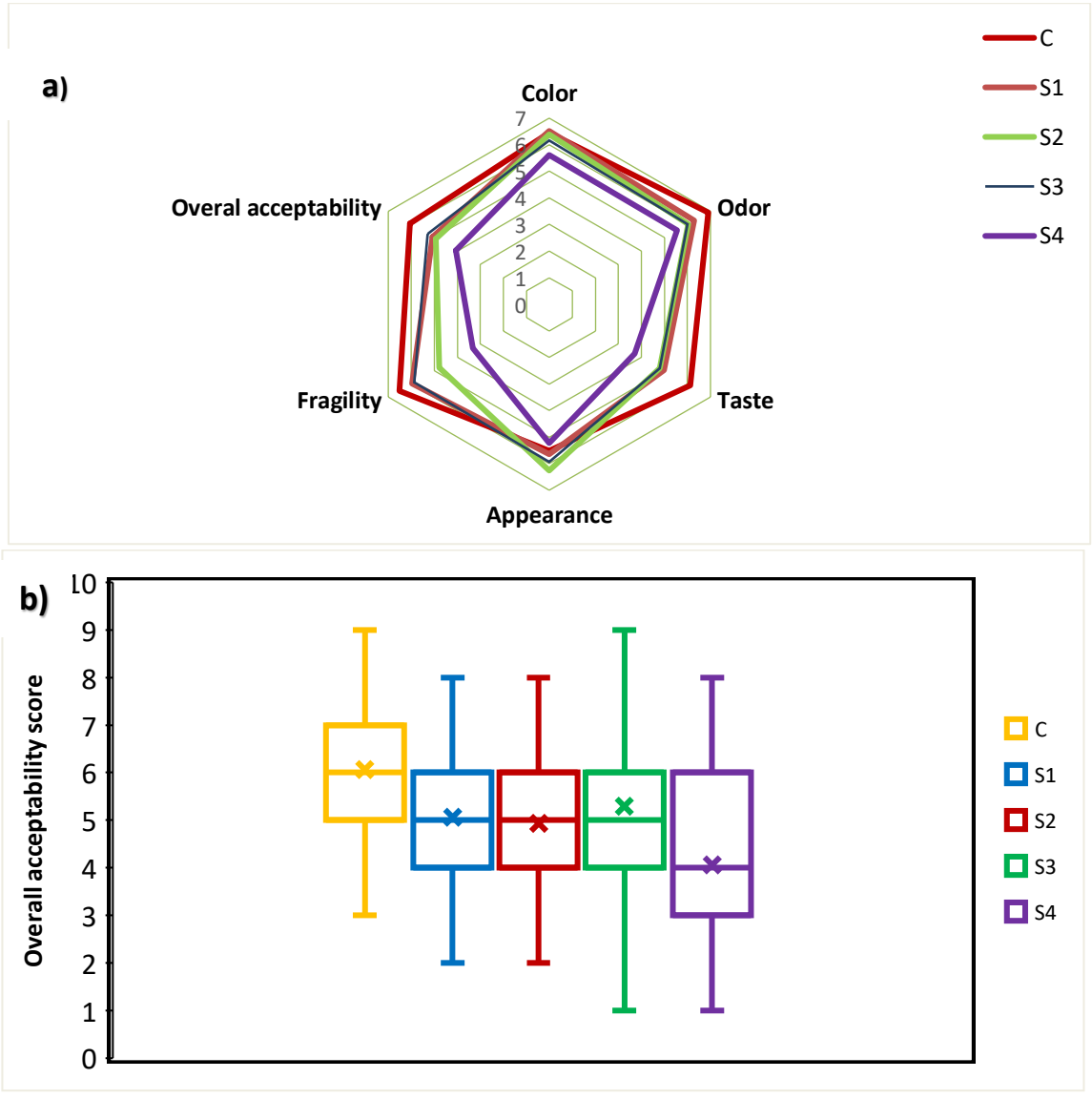

Figure 3. The sensory attributes (a) and overall acceptability (b) scores of the biscuits

Figure 3a illustrates the sensory odor scores ranging from 5.54 to 6.90. Notably, the variation in formulation led to a significant ($p < 0.05$) disparity in biscuit odor.

The taste scores for control samples and formulated biscuits ranged from 3.70 to 6.12, as

depicted in Figure 3a. Notably, the control flour biscuit achieved the highest taste score in comparison to the composite flour samples. As the lentil flours were incorporated, there was a decrease in taste score, with the lowest score recorded at S4. This could be attributed to the lentil flavors imparted by the utilized lentils.

Sensory panelist awarded the highest fragility score to the control biscuit comprising einkorn flour. Nonetheless, with the increased addition of composite flour, the score declined (Figure 3a).

Moreover, semi-trained panelist conducted evaluations on the biscuits regarding overall acceptability. The control biscuit scored 6.06 (±1.55), whereas the biscuit formulations with einkorn and green lentil flour scored 5.06 (±1.52), einkorn and yellow lentil flour scored 4.93 (±1.61), einkorn and red lentil flour scored 5.29 (±1.75), and biscuits with einkorn and all lentil flour scored 4.06 (±1.98) (Fig. 3b). Notably, adding red lentil flour to biscuits led to a product with comparatively consistent overall acceptability scores among the panelists. Additionally, its elevated mean score value indicates its sensory characteristics are similar to those of the control product. Such a bakery product holds promise for broad consumer preference (Figure 3a-b). Conversely, panelist showed a preference against biscuits incorporating all lentil flours together, as evidenced by their lower scores in color, odor, brittleness, and taste. Overall, the sensory evaluation clearly indicated a preference for biscuits containing red lentil flour, as they lacked apparent undesirable sensory attributes in the baked product with this flour addition.

In summary, this research consists of two key parts. The first part involved evaluating the physicochemical and powder flow properties of composite flours made with various lentils and einkorn flour. The second part focused on the physicochemical and sensory properties, as well as the morphological structures, of biscuits derived from these composite flours. The composite flour identified as S2, prepared with yellow lentil flour, exhibited the highest OAC, beneficial for enhancing texture and reducing yield losses in processed foods like baked goods. Similarly, the composite flour containing green lentils and einkorn flour showed a significant increase in WAC, making it suitable for bakery foods such as biscuits and bread. Additionally, bulk density considerations are crucial for economic and functional aspects of packaging and processing, while the Hausner ratio provides insights into the flow properties of the flours. These comprehensive evaluations indicate that composite flour blends of einkorn and lentil flours are promising candidates for use in various bakery products.

Conclusion

This study aims to formulate biscuits with improved physicochemical properties and sensory acceptability using composite flours incorporating einkorn flour along with green, red, and yellow lentil flours as base ingredients. The inclusion of lentil flours in the formulation led to an increase in OAC content, reaching its peak in sample S3, especially containing red lentil flour. This high OAC content in the composite flour may stem from the high protein content inherent in the investigated lentil flours' nature. The spread ratio of biscuits in this study is a true physical characteristic ranging from 4.72 to 7.20. The low spread ratio in control biscuits indicates that the starches in the control (einkorn flour) displayed higher hydrophilicity compared to the composite flours, thus resulting in the decrease in spread ratio of einkorn (control) biscuits. Differences in protein quality and water absorption properties can alter the flour's water absorption capacity and consequently affect the spread ratio. Biscuits prepared with einkorn flour, along with all lentil flours, which exhibited the highest spread ratio, can be considered the most preferred products. Overall, sensory evaluation clearly indicated a preference for biscuits containing red lentil flour, as they lacked obvious undesirable sensory properties in the baked product due to this flour addition. Consequently, biscuits incorporating composite flour were not as well received. It may be advantageous to experiment with a lower ratio of composite flour

in future formulations.

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Research Article/Araştırma Makalesi

Symbiotic ice-cream production using *Lactiplantibacillus plantarum* and oleaster (*Elaeagnus angustifolia* L.) flour

Lactiplantibacillus plantarum ve iğde (Elaeagnus angustifolia L.) unu kullanılarak simbiyotik dondurma üretimi

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ABSTRACT

The aim of this study was to produce a functional and low-calorie ice cream by incorporating oleaster flour (OF) into the ice cream mix formulation at different ratios as milk powder and/or sugar substitute. The ice cream was also probiotificated by supplementation of *Lactiplantibacillus plantarum* NRIC 1838, thus preparing symbiotic ice-creams. For this purpose, 8 different ice-cream formulations were designed and their physicochemical, bioactive, microbiological, thermal and sensory properties were analyzed. The results showed that dry matter content, pH values and total phenolic content of ice creams were in the range of 43.78-46.59 %, 5.65-6.38, and 0.25-0.94 mg GAE g^{-1}, respectively. Addition of OF made the samples darker, as indicated by lower *L** values. Additionally, the control samples had the highest brightness while the darkness of the ice-creams increased as the amount of OF in the formulations. Furthermore, the highest *a** and *b** values were determined in the ice cream sample supplemented with probiotic and OF while the lowest value was determined in the reference ice-creams enriched with probiotics. The cell counts of the ice cream mixes and samples were higher than 7 log CFU g^{-1}. The incorporation of OF, skimmed milk powder, and sugar in the different proportions and combinations led to a reduction in thermodynamic stability of ice-creams compared the control samples. The amount of OF was the most effective ingredient in the overall sensory acceptance of the produced samples. These results suggest that symbiotic ice-creams enriched with OF and *Lactiplantibacillus plantarum* NRIC 1838 exhibited good quality and sensory characteristics.

Key Words: Oleaster flour, *Lactiplantibacillus plantarum* NRIC 1838, symbiotic ice-creams, characterization.

ÖZ

Bu çalışmanın amacı, süt tozu ve/veya şeker yerine farklı oranlarda iğde ununun (İU) dondurma karışımına dahil edilerek fonksiyonel ve düşük kalorili bir dondurma üretimini sağlamaktır. Bu kapsamda üretilen dondurmalar *Lactiplantibacillus plantarum* NRIC 1838 kullanılarak probiyotik hale getirilmiş ve simbiyotik dondurmalar hazırlanmıştır. Bu

amaçla, 8 farklı dondurma formülasyonu oluşturulmuş ve bu dondurmaların fizikokimyasal, biyoaktif, mikrobiyolojik, termal ve duyusal özellikleri analiz edilmiştir. Sonuçlar, dondurmaların kuru madde içeriği, pH değerleri ve toplam fenolik içeriklerinin sırasıyla % 43.78-46.59, 5.65-6.38 ve 0.25-0.94 mg GAE g^{-1} olduğunu göstermiştir. Ayrıca, kontrol örneklerinin en yüksek parlaklık değerlerine sahip olduğu ve formülasyonlardaki İU miktarı arttıkça dondurma rengindeki koyuluk miktarının da arttığı tespit edilmiştir. Yanı sıra, probiyotik ve İU ile takviye edilmiş dondurma örneklerinde en yüksek *a** ve *b** değerleri belirlenirken, en düşük değer probiyotikle zenginleştirilmiş kontrol dondurmalarında belirlenmiştir. Dondurma karışımlarının ve örneklerinin hücre sayıları 7 log CFU/g'dan yüksektir. Farklı oranlarda ve kombinasyonlarda İU, yağsız süt tozu ve şekerin dahil edilmesi, kontrol örnekleriyle karşılaştırıldığında dondurmalardaki termodinamik stabiliteyi azaltmıştır. İU, üretilen örneklerin genel duyusal kabulünde en etkili bileşen olmuştur. Bu sonuçlar, İU ve *L. plantarum* NRIC 1838 ile zenginleştirilmiş simbiyotik dondurmaların iyi kalite ve duyusal özelliklere sahip olduğunu göstermektedir.

Anahtar Kelimeler: İğde unu, *Lactiplantibacillus plantarum* NRIC 1838, simbiyotik dondurma, karakterizasyon

Introduction

In recent years, probiotic dairy products have become a significant part of the functional food market in accordance with consumer demands. The term probiotic is defined by FAO/WHO (2002) as "a living microorganism, most of which belong to the genera of *Lactobacillus* and *Bifidobacterium*, that when taken in sufficient quantities, provides a positive effect on the health of the host organism" (Acu et al., 2021; Akman et al., 2023; Bagdat et al., 2024a,b; Di Criscio et al., 2010; El-Sayed et al., 2014). Furthermore, prebiotics are described as indigestible food components that benefit the host by promoting the growth and/or activity of probiotics in the colon. One approach to the management of intestinal microflora is the use of symbiotics, a combination of probiotics and prebiotics. The simultaneous presence of probiotics and prebiotics in the product allows better colonization ability in the colon compared to the use of prebiotics or probiotics alone (Acu et al., 2021; El-Sayed et al., 2014; Elkot et al., 2020).

Elaeagnus angustifolia L. (oleaster, Russian olive), a member of the family "*Elaeagnaceae*", comprises a group of flowering shrubs and has a wide geographical distribution in Asia, Central Asia, the Caucasus, and Europe. Fresh or dried reddish-brownish fruits ripen in September and are used different medicinal purposes such as anti-diarrheal, anti-inflammatory, anti-pyretic, diuretic, and tonic (Yavuz et al., 2022). The oleaster fruit consists of 50% oleaster powder, 15% crust, and 35% seed (Yavuz, 2019). It is rich in minerals, vitamins and dietary fibers, as well as exhibiting high bioactive properties. Oleaster flour (OF) is obtained by grinding of the dried oleaster fruits (Yavuz et al., 2022). The protein content of oleaster powder was determined to be 3.88%, dietary fiber content 20.10%, moisture content 21.96%, and ash content 1.85%. The TPC was measured at 3957.06±20.81 mg GAE kg^{-1}, while the DPPH inhibition percentage was found to be 6.48% (Yavuz, 2019). Due to its unique properties (*e.g.* floury structure, specific taste and chemical composition), it can be included as a functional ingredient in various food formulations such as cakes, chocolate, bakery products, ice-cream, infant food and yogurt (Sahan et al., 2013; Çakmakcı et al., 2014; Yavuz et al., 2021; Yavuz et al., 2022).

Functional ice cream can be defined as dairy products that contain basic ingredients such as air, emulsifiers, flavoring agents, milk fat, milk solids-not-fat, stabilizers and water as well as functional ingredients such as probiotics, prebiotics, bioactive peptides, antioxidants, various essential fatty acids and dietary fibers (Genovese et al., 2022). The relative high fat and sugar content of ice cream led to the high consumption of these nutrients, which raises the risk of obesity, especially pediatric obesity and health-related issues (Akalın et al., 2008; Drewnowski, & Greenwood, 1983). For this reason, there has been an increase demand in food industry related to the ice-cream including high-fiber content with low-calorie (Arslaner, & Salık, 2020). Different functional symbiotic ice-cream formulations were developed using different combinations ((Litesse ultra, Tagatose and polydextrose/ *Bifidobacterium bifidum, Bifidobacterium longum, and Lacticaseibacillus paracasei* (formerly *Lactobacillus paracasei*)) (Acu

et al., 2021); inulin/*Lacticaseibacillus casei* (formerly *Lactobacillus casei*) and *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus*) (Criscio et al., 2010); inulin, lactulose and Fructooligosaccharides/ *Bifidobaterium bifidum*, *L. casei, L. plantarum* (El-Sayed et al., 2014); black rice powder/ *Lactobacillus acidophilus* LA-5 (Elkot et al., 2022*);* inulin*/ Lactobacillus brevis* PML1 (Falah et al., 2021); inulin and *L. acidophilus* (Pandiyan et al., 2012); fructooligosaccharides/ *L. casei, L. plantarum* (Sabet-Sarvestani et al., 2021); banana flour/ *L. casei* TISTR 1463, *L. acidophilus* TISTR 1338 (Phuapaiboon, 2016). Moreover, Çakmakçı et al (2014) compared the quality, color, and sensory characteristics of ice cream using OF and oleaster crust at ratios of 1-3 (w:w) and investigated the contribution of oleaster to the nutritional and functional properties of ice cream. Although OF, as a dietary fiber source, has been used incorporated into several baked products such as white and gluten-free bread, cookie, gluten-free cake, biscuit, sponge cake and breakfast cereals (Yavuz et al., 2022; Şahin, 2023; Sahan et al., 2013; Lavini et al., 2021; Zangeneh et al., 2021; Tatari et al., 2022), to the best of our knowledge, no scientific data have been published on the use of OF as both a dietary fiber source and sugar substitute in ice cream production with the supplementation of probiotics. Therefore, the aim of the present work was to develop a new symbiotic combination using OF as prebiotics and *L. plantarum* NRIC 1838 as probiotics to produce functional ice-creams, and to investigate their physicochemical, microbiological, bioactive, rheological, and sensorial properties.

Materials and Methods

Materials

Pasteurized cow's milk (3.1 % milkfat; Mis, Ak Gida Company, Türkiye), pasteurized cream (35% milkfat; Mis, Ak Gida Company, Türkiye), powdered soybean lecithin (E322, Tito, İzmir, Türkiye), and sugar (Ismen Food Company, İstanbul, Türkiye) were purchased from supermarkets in Istanbul, Türkiye. Skimmed milk powder and pure salep were obtained from Aktar Diyari (Istanbul, Türkiye) while mature oleaster fruit (*Elaeagnus angustifolia* L., 20.1 % dietary fiber content) was kindly supplied from Ziya Organik Tarim (Istanbul, Türkiye). De Man Rogosa Sharp (MRS) agar (Merck, Germany) and MRS broth (Merck, Germany) were supplied.

Preparation of oleaster flour (OF)

Firstly, the oleaster fruits were meticulously cleaned with distilled water and scrubbing to eliminate any possible contaminants. Subsequently, the inner parts of the fruits were separated from the skins, dried in a hot air oven (Memmert UF-110, Germany) at 45-50 °C for 24 h, and then powdered using a grinder (Tefal 8100.31 coffee grinder, France) (Çakmakçı et al., 2015)

Preparation of probiotic inoculum

For this aim, *Lactiplantibacillus plantarum* strain NRIC 1838 was inoculated on MRS agar and left for incubation at 35 °C for 48 h. Then, grown cultures were collected with a sterile inoculation loop and transferred to sterile MRS broth and incubated at 37 °C for 24 h. Following a subsequent incubation at the same conditions, bacterial cells were collected by centrifugation at 9000 rpm, at 4 °C for 10 min (Centrifuge Multifuge X3 FR, Thermo Scientific). Finally, sterile peptone water was added into the collected cells and probiotic inoculum solution was prepared.

Ice cream production

In this study, 8 different ice cream formulations were prepared, as listed in Table 1. The ice cream mix samples were prepared following the flow chart given in Figure 1 (Sabet-Sarvestani, 2020).

Table 1. The recipes for different ice-cream mixes

Sample codes	Milk (%)	Cream (%)	Milk powder (%)	Sugar (%)	OF (%)	Lecithin (%)	Sahlep (%)	*L. plantarum*
C	65	7	7	20	0	0.5	0.5	-
CP	65	7	7	20	0	0.5	0.5	+
OF	65	7	7	0	20	0.5	0.5	-
OFP	65	7	7	0	20	0.5	0.5	+
OFS	65	7	7	10	10	0.5	0.5	-
OFSP	65	7	7	10	10	0.5	0.5	+
SPOF	65	7	17	0	10	0.5	0.5	-
SPOFP	65	7	17	0	10	0.5	0.5	+

C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder.

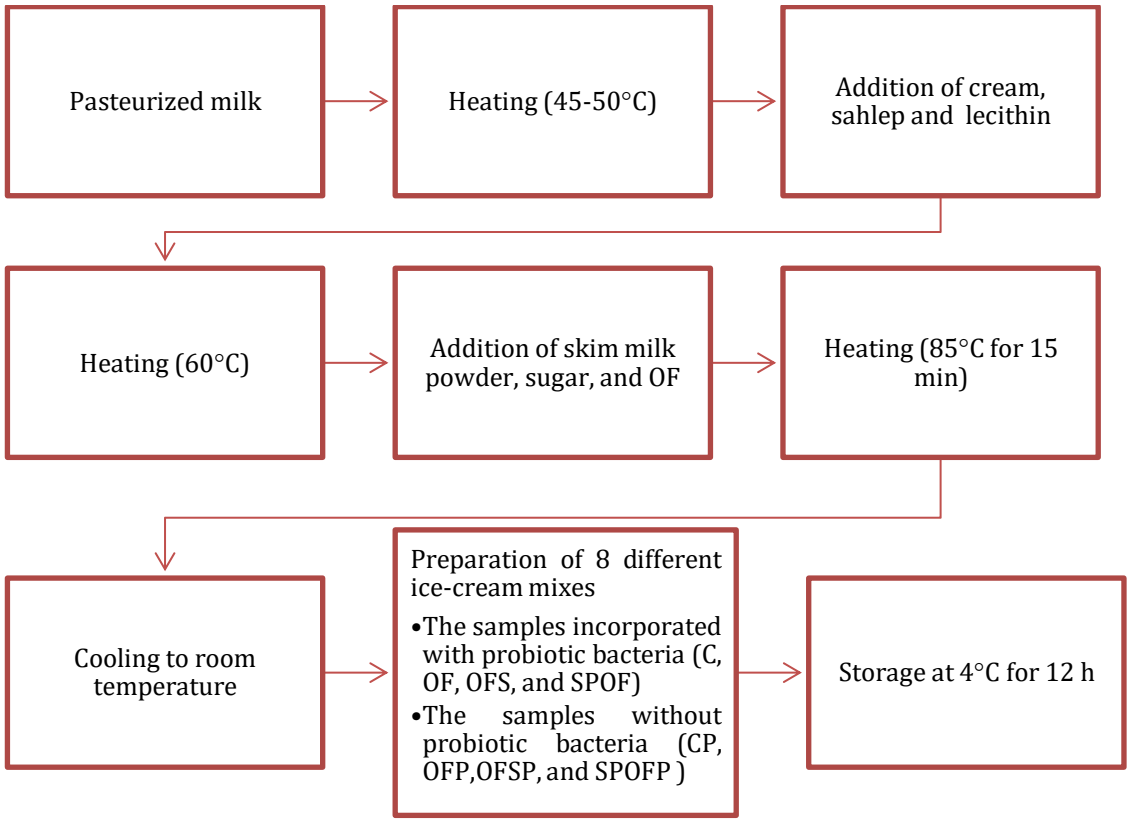

Figure 1. Process flowchart for the preparation of ice-cream mix.
C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder.

To begin with, milk was heated 45-50 °C and sahlep and lecithin were incorporated into it during medium heating (50ºC) and mixing. Lecithin is used in ice cream to act as an emulsifier, improve texture and creaminess, control crystallization, and facilitate homogenization. After addition of skim

milk powder, sugar and/or OF at 60 °C, the mixture was pasteurized at 85°C for 15 min. Finally, the ice cream mixes were rapidly cooled to room temperature and probiotic inoculums were added at a targeted initial level of ~10^7 kob mL^{-1}, which was determined by serial dilution and plating methods to ensure accurate microbial counts. Following the aging of ice cream mixes at 4°C for 12 h, ice cream was prepared using the ice cream machine (Delonghi, II Gelataio, ICK5000, China) at a constant rotation speed for 15 min. Then, the samples were placed in polypropylene food containers and stored at – 18 °C for 24 h. The samples were analyzed after the storage period (Sagdic et al., 2012). To facilitate a clearer understanding of the prepared ice cream formulations, they were schematized, and the analyses performed were presented in a graphical abstract format in Figure 2.

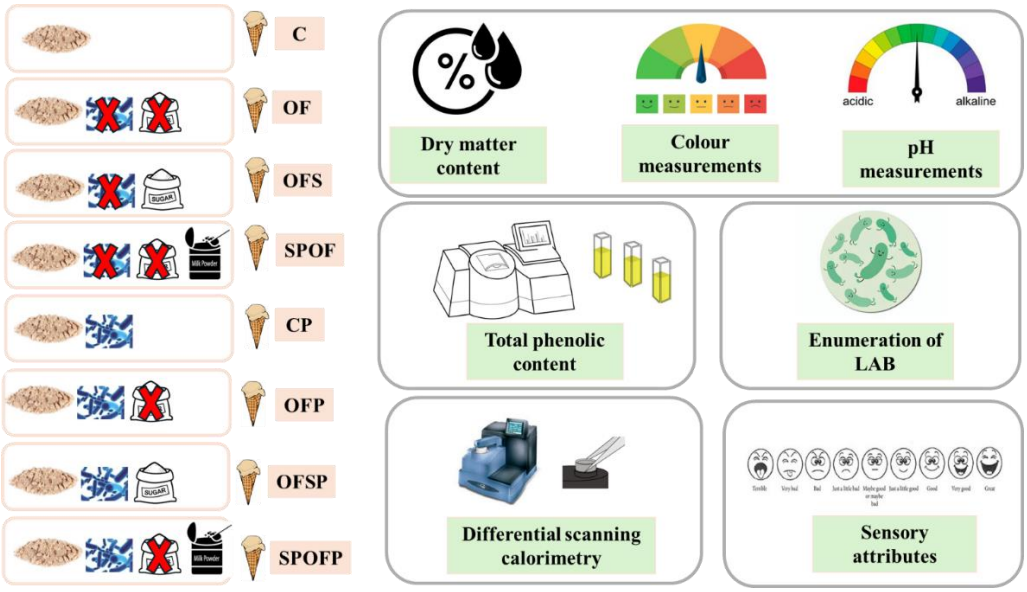

Figure 2. An overview of ice-cream formulations and analysis.
C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder.

Physico-chemical analysis

The official procedure was followed for the determination of dry matter content of the ice-cream samples (AOAC International, 2000). pH values of the samples were measured employing a Mettler-Toledo pHmeter (Model: S220 SevenCompact™ pH/Ion meter) (Karaman et al., 2014). Furthermore, the color measurements were performed using a portable colorimeter (CR-400, Minolta Camera Co., Osaka, Japan) after melting the samples to ensure a uniform surface, minimize reflection and refraction effects, and provide consistent sample states for accurate and comparable color readings.The *a**, *b** and *L** values measured in the colorimeter represented red-greenness, blue-yellowness and lightness-darkness, respectively (Yavuz et al., 2022; Kutlu et al., 2024). Following formulas were used to calculate the Δ*a**, Δ*b**, Δ*L** and Δ*E** (total color difference) values.

$$\Delta E^* = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2} \quad (1)$$

$$\Delta a^* = a_1^* - a_0^* \quad (2)$$

$$\Delta b^* = b_1^* - b_0^* \quad (3)$$

$$\Delta L^* = L_1^* - L_0^* \quad (4)$$

Total phenolic content (TPC)

In order to determine the TPC content of the ice cream samples, the methodology proposed by Karaman et al. (2014) was followed with minor

modifications. Firstly, ice-cream samples (10 g) were well-mixed with 5 mL of hexane and 50 mL of 80% methanol. This mixture was shaken and kept in dark at room temperature for 24 h. Then, the samples were transferred into centrifuge tubes and centrifuged at 9000 rpm for 10 min at 4 °C. Afterwards, the oil layers on the samples were removed with a syringe and centrifugation was performed again. Up till the oil was totally separated, the mixture was filtered through filter paper. After mixing the 0.5 mL sample and 2.5 mL Folin-Ciocalteau reagent for 3 min, 2 mL of sodium bicarbonate (7.5%) was added. These mixtures were kept in the dark at room temperature for 30 min and absorbance values were read at 760 nm wavelength. The results were given as mg gallic acid (GAE) equivalents per g of ice-cream sample (mg GAE g^{-1} sample) (Erol et al., 2023; Kutlu, 2024).

Enumeration of LABs

Enumaration of LABs in both ice-cream mixes and ice-creams was performed based on the protocol described by Sabet-Sarvestani et al. (2020). Briefly, 10 grams of sample was mixed with 90 mL of sterile peptone water using a stomacher. Afterwards, six decimal serial dilutions (10^1–10^6 CFU g^{-1}) were prepared and 0.1 mL of appropriate serial dilutions (10^4, 10^5 and 10^6 CFU g^{-1}) were spread plated onto MRS agar. Next, the plates were incubated at 37 °C for 48 h under aerobic conditions to facilitate the growth of LAB because *Lactiplantibacillus plantarum* is aerotolerant. Colonies were counted and the findings were given as log CFU g^{-1}.

Differential scanning calorimetry

Thermal properties of ice-cream samples were determined using a differential scanning calorimeter (DSC, Q100, TA Instruments Inc., New Castle, DE, USA) according to the applied method by Ertugay et al. (2020) with minor modifications. For this aim, 10 mg of sample was heated under nitrogen gas flow at a rate of 20 mL min^{-1}, with a heating rate of 5°C min^{-1}, between -20 °C and 20 °C, after being placed in hermetically sealed aluminum pans before loading into the instrument. Onset, midpoint, offset temperatures and enthalpy values of eight different ice cream samples were determined from the DSC thermograms.

Sensory attributes

A total of 20 panelists consisting of faculty members and undergraduate students from Yildiz Technical University Department of Food Engineering were selected as panelists in sensory evaluation. In the sensory analysis ice cream quality parameters (color, consistency, taste and aroma, odor, and overall acceptability) was evaluated by the panelists using a hedonic scale from 0 to 9 (0: very bad; 9: very good) (Sagdic et al., 2012).

Statistical evaluation

SAS software package, version 8.2 (SAS Institute Inc., Cary, NC) was employed for statistical evaluation of the obtained data using one-way analysis of variance (ANOVA). The statistical differences were evaluated using the Duncan's multiple range test at 95% of significance level.

Results and Discussion

Dry matter content

The type of milk used to prepare the mix has a significant impact on the characteristics of the product, and the physical properties of the ice cream mix fabricated by various processing techniques can change both the texture and appearance of the finished product (Elkot et al., 2022). In this study, the dry matter of ice-cream samples was OFSP (46.59 %) > OFP (45.57 %) > SPOF (45.44 %) > OFS (45.05 %) > C (44.82 %) > SPOFP (44.54 %) > CP (44.10 %) > OF (43.78 %) (Table 2). The results showed that the supplementation of probiotics into the ice-creams had no significant effect on the dry matter content ($p>0.05$). When sugar was substituted with OF in the formulation, a slight increase in the dry matter content was determined, which can be due to the higher water content of oleaster flour compared to the sugar. This finding suggested that while the type of ingredient affects the dry matter, the presence of probiotics alone does not significantly alter this parameter. Similar findings were reported

for ice-creams incorporated with tahini (Bayrakçı, 2018). Contrary, lower dry matter content values were reported for ice-creams incorporated with Kavılca fibre (Ertugay et al., 2020), ice-creams including OF (Çakmakçı et al., 2014), and the ice-cream samples enriched with black rice powder and *Lactobacillus acidophilus* LA-5 (Elkot et al., 2022). The variations between the dry matter content of the ice creams in the literature were due to the differences in the mix recipes of the ice creams. This study highlighted that ingredient substitutions and additions, such as OF and probiotics, could affect the dry matter content but did not impact all formulations in the same way.

Table 2. Some physico-chemical (dry matter, pH, and color values) properties of ice-cream samples.

Sample codes	Dry matter (%)	pH	*L**	*a**	*b**	*ΔE**
C	44.82 ± 0.62^{ba}	6.37 ± 0.01^{a}	86.33 ± 0.31^{a}	-1.53 ± 0.17^{b}	6.08 ± 0.43^{bc}	0.00
OF	43.78 ± 0.37^{b}	5.65 ± 0.01^{e}	67.45 ± 2.53^{c}	7.40 ± 0.57^{a}	21.68 ± 3.21^{bac}	26.07
OFS	45.05 ± 0.10^{ba}	5.93 ± 0.00^{b}	72.38 ± 2.80^{bc}	5.63 ± 1.11^{a}	18.83 ± 1.40^{ba}	20.21
SPOF	45.44 ± 0.47^{ba}	5.96 ± 0.01^{b}	68.32 ± 0.53^{c}	6.43 ± 0.12^{a}	15.30 ± 0.41^{bac}	21.74
CP	44.10 ± 0.44^{b}	6.38 ± 0.01^{a}	78.93 ± 1.93^{ba}	-2.47 ± 0.21^{b}	4.50 ± 1.24^{bac}	0.00
OFP	45.57 ± 0.67^{ba}	5.70 ± 0.01^{d}	66.19 ± 7.19^{c}	8.15 ± 3.75^{a}	2.33 ± 2.42^{a}	16.73
OFSP	46.59 ± 0.28^{a}	5.89 ± 0.01^{c}	70.03 ± 0.68^{cb}	4.80 ± 1.18^{a}	16.77 ± 1.19^{bac}	16.81
SPOFP	44.54 ± 0.10^{b}	5.95 ± 0.01^{b}	71.71 ± 5.91^{bc}	4.99 ± 1.79^{a}	21.65 ± 1.29^{a}	20.05

C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder.
^{a,b,c,d}: Means with different letters in the same column are significantly different ($p<0.05$).

pH

The pH values of the ice creams affect both the viability of probiotic bacteria and the sensory properties of the product. In this study, the following order in the pH values was determined among the ice cream samples, CP (6.38) > C (6.37) > SPOF (5.96) > SPOFP (5.95) > OFS (5.93) > OFSP (5.89) > OFP (5.70) > OF (5.65) (Table 2). Similar results were reported for ice-creams incorporated with OF (Çakmakçı et al., 2014), ice-creams enriched with free/encapsulated bacteria (*L. plantarum, L. casei* and *B. bifidum*) (El-Sayed et al., 2014), ice-creams enriched with cornelian cherry and *Bifidobacterium lactis* (Haghani et al., 2021). However, higher pH values were reported for ice-creams incorporated with Kavılca fibre (Ertugay et al., 2020) and ice-cream mixes incorporated with *Lactobacillus johnsonii* La1 (Alamprese et al., 2002). It is also noteworthy that OFSP, and OFP had higher pH values in comparison to SPOFP. This phenomenon may be due to decreased LAB activity as the sugar content in the formulation increased, because higher sugar concentrations can sometimes inhibit LAB metabolism.as previously reported by Falah et al. (2021). Moreover, OFP had the lowest pH value among the probiotic incorporated ice-cream samples, indicating the combination of *L. plantarum* and OF in ice-cream samples supplied a synergistic influence to enhance metabolism and activity of probiotic cells (Kemsawasd and Chaikham, 2020). This result indicated that the incorporation of *L. plantarum* with OF into the ice cream formulation was effective in maintaining a more acidic environment, which might favor the survival and growth of probiotics. Furthermore, the pH value of non-probiotic ice-creams were lower than their reference samples. This might be ascribed to the fact that the incorporation of phenolic compounds (*e.g.* hydroxycinnamic acid derivatives,

hydroxylbenzoic acid derivatives) (Karkar and Şahin, 2022) found in OF and milk proteins led to the pH reduction in comporison with their reference sample, impacting the overall acidity of the ice cream (Shazly et al., 2022).

Color properties

One of the most important quality factors influencing consumers' food choices is color, and visual quality includes the appearance of a product (Yavuz et al., 2022). *L** is regarded as a convenient unit of measurement for brightness, the feature that allows each color to be equated to a member of the grey scale between white and black. Among the tested samples, C was found to have the highest *L** value (86.33) (Table 2), followed by CP (78.93), OFS (72.38), SPOFP (71.71), OFSP (70.03), SPOF (68.32), OF (67.45), and OFP (66.19), respectively. From the experimental data, it was possible to observe that the *L** values of the ice cream samples decreased with the increase in the proportion of OF, indicating that the addition of OF affects the lightness of the ice cream. This was in agreement with previous reports informing that the *L** values of probiotic ice-creams decreased with a rise in level of cornelian cherry peel (Haghani et al., 2021). Likewise, Çakmakçı et al. (2014) reported that the brightness values decreased from 90.02 to 77.18 with the addition of 3% OF; this decrease may be due to the presence of brown pigments in OF. Similar *L** values also reported for ice cream mix prepared with fructooligosaccharides/ *L. plantarum* and *L. casei* (73.33-83.00) (Sabet-Sarvestani et al., 2021). Besides, the *a** values (+*a*-redness, − *a*-green) exhibited the following trend: OFP (8.15) > OF (7.40) > SPOF (6.43) > OFS (5.63) > OF (7.40) > SPOFP (4.99) > OFSP (4.80) > C (-1.53) > CP (-2.47) (Table 2). While *a** values were negative in control samples (C and CP), they were positive in enriched ice creams. The concentrations, probiotic supplemantion and types of ingredients used in formulations affected the *a** values. Increase the incorporation ratio of OF in formulation led to the increase in *a** values. The increase in *a** values with higher proportions of OF suggests that OF contributes to a more pronounced red hue in the ice cream. Likewise, Haghani et al. (2021) reported an increase in the *a** values of ice-cream samples from 0.36 to 27.6 upon supplementation of 9% of cornelian cherry peel into the probiotic ice-cream samples. The *a** values reported for the ice-creams incorporated with OF (-2.77- 2.04) (Çakmakçı et al., 2014). Moreover, the *b** values of ice-cream samples were found in the following order: OF (21.68) > SPOFP (21.65) > OFS (18.83) > OFS (18.83) > OFSP (16.77) > SPOF (15.30) > SPOF (15.30) > C (6.08) > CP (4.50) > OFP (2.33) (Table 2). The *b** values reported for the ice-creams incorporated with OF (7.57- 10.24) (Çakmakçı et al., 2014) and ice cream mix prepared with fructooligosaccharides/ *L. plantarum* and *L. casei* (15.33-22.67) (Sabet-Sarvestani et al., 2021). The higher *b** values in ice creams with OF indicate increased yellowness. The differences in *b** values may be due to the caretonoid content of OF, the fermentation process and the use of OF as a source of prebiotics. Δ*E** values for non-probiotic ice-creams and probiotic ice-creams were in the range of 0-26.07 and 0-20.05 (Table 2), respectively, showing that the color differences can be easily recognizable by eyes due to the *Δ*E*>3 (Atlar et al., 2024; Yavuz et al., 2022). The above results allowed us to confirm that the addition of phenolic compounds could potentially alter the color properties (Sagdic et al., 2012). Overall, we can conclude that supplementation of OF and probiotics led to a reduction in the lightness and yellowness values, but increased redness, so that OF could significantly affect the colour in ice-creams.

TPC

As fruit peels, cereal grains, vegetable seeds, and their pulp had high level dietary fiber content, they are great source of phenolic compounds (Akca and Akpınar, 2021). The data on TPC values presented in Table 3 indicated that TPC values of ice-cream samples ranged from 0.25 (C) to 0.94 (OFP) mg GAE g^{-1} depending on the ice-cream formulation. Both increase in the OF ratio and probiotic supplementation resulted in higher TPC

content. In a related work, an increase in the cornelian cherry content led to an increase in TPC content of probiotic ice-cream samples (Haghani et al., 2021). Similar TPC values were reported for the ice-creams supplemented with *L. casei,* dark blue and white *Myrtus communis* pulps (Öztürk et al., 2018); however, higher TPC values were noted for the ice-cream samples enriched with black rice powder and *L. acidophilus* LA-5 (Elkot et al., 2022). Among the ice-cream samples, the lowest TPC was determined in C and CP, which was in accordance with earlier findings (Öztürk et al., 2018). This indicated that the milk used in the ice cream formulation contained trace amounts of TPC or that the Maillard reaction occurred during pasteurization and this was the main reason for measuring TPC values for C and CP (Sagdic et al., 2012). Additionally, it was revealed that TPC results were close to each other in samples including equal amount of OF. A similar report showed that when *B. longum* + *B. bifidum* + *L. paracasei* subsp. *paracasei* mutual culture, raspberry, blackberry ready fruit sauces and raspberry sauce were used for the production of the ice-creams, TPC values were measured between 3.14 and 6.98 mg GAE g^{-1} (Acu, 2014). Overall, these findings highlighted that the incorporation of both OF and probiotics significantly enhanced the phenolic content of the ice cream, which could potentially improve its nutritional and functional properties.

Table 3. Total phenolic content (TPC) and LAB counts of ice-cream samples.

Sample codes	TPC (mg g^{-1} GAE)	Cell numbers (log CFU g^{-1})		Yield (%)
		Mix (N_0)	Ice-cream (N_1)	($logN_1 \times 100 \times N_0^{-1}$)
C	0.25 ± 0.01^{e}	-	-	-
OF	0.86 ± 0.03^{b}	-	-	-
OFS	0.77 ± 0.02^{c}	-	-	-
SPOF	0.82 ± 0.01^{cb}	-	-	-
CP	0.39 ± 0.01^{d}	7.32 ± 0.63^{a}	7.23 ± 0.14^{a}	98.77
OFP	0.94 ± 0.02^{a}	7.30± 0.17^{a}	7.38 ± 0.09^{a}	101.10
OFSP	0.83 ± 0.02^{cb}	7.37 ± 0.13^{a}	7.38 ± 0.13^{a}	100.14
SPOFP	0.93 ± 0.02^{a}	7.46 ± 0.21^{a}	7.43 ± 0.18^{a}	99.60

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Survival of probiotics

It is very crucial both to maintain the survival of probiotics during the extreme conditions in food processing such as freezing (dos Santos Leandro et al., 2013). In this study, probiotics were enumerated at both ice cream mix and ice cream samples in order to reveal the effect of ice cream formulation and processing in probiotic survival, as the results were presented in Table 3. The descending order of cell numbers of ice-cream mixes including probiotic bacteria were SPOFP (7.46 log CFU g^{-1}) > OFSP (7.37 log CFU g^{-1}) > CP (7.32 log CFU g^{-1}) > OF-P (7.30 log CFU g^{-1}). Moreover, cell numbers of ice-cream samples including probiotic bacteria were determined as CP (7.23 log CFU g^{-1}) > OFP = OFSP (7.38 log CFU g^{-}

^{1}) > SPOFP (7.43 log CFU g^{-1}) in increasing order (Table 3). Obviously, there was no significant differences on cell numbers of probiotics depending on the different formulations both in ice-cream mixes and samples, showing that aeration, freezing, and formulation had no effect on their viability. Similarly, dos Santos Leandro et al. (2013) reported that there was no significant difference on the probiotic viability (*L. delbrueckii* UFV H2b20) depending on the different formulations (low fat, fat free and high fat) after production of ice-cream. Also, Alamprese et al. (2002) and Alamprese et al. (2005) also noted that various sugar and fat concentrations had no significant effect on the viability of *L. johnsonii* La1 and *L. rhamnosus* GG. Besides, the populations of *L. plantarum* in SPOFP and CP samples were slightly negatively affected from ice-cream processing, which may be due to the toxic effect of oxygen during the aeration, resulting in thermal shock and injury (Haghani et al., 2021; Homayouni et al., 2008). However, the percent survival of probiotics for SPOF and CP samples was still >98.77%, indicating that probiotics were able to adapt to lower temperatures, as previously reported by Kemsawasd and Chaikham, (2020). Similarly, Sauvageot et al. (2008) also reported that insignificant decreases in the number of viable cells after cold storage treatment can be explained by the ability of *Lactobacillus* spp. bacteria to adapt to low temperature and cold shock conditions. Moreover, the percent yield for the ice-cream samples coded as OFP and OFSP exceeded 100%, showing that these formulations protected the viability of probiotics against adverse environmental conditions. Also, these findings showed the postive role of OF on the growth of probiotics. OF contains numerous bioactive compounds (Yavuz et al., 2022) that can protect probiotic cells from oxidative stress and toxicity, thus increase cell viability. In the ice-cream formulations of OFP and OFSP, the symbiotic effect between *L. plantarum* and OF was clearly observed. It is well known that the minimum allowable concentration of probiotic cells that can be added to food products for health benefits is 6 log CFU g^{-1} (Lee and Salminen, 1995). The cell counts of the ice cream mixes and samples were all higher than 7 log CFU g^{-1}, but storage and *in vitro* digestion studies are needed to observe the viability of probiotics in our future studies before they can be expressed as fully probiotic ice cream.

Thermal properties

DSC thermograms (Figure 3) of the ice-cream samples exhibited an endothermic peak, which was linked to the melting of ice. Similar characteristic DSC curves were also reported by Hwang et al. (2008). Onset, midpoint, offset temperatures and enthalpy results were given in Table 4. Among the samples whose thermographs were determined, the enthalpy values of all ice cream samples (except OFS) were found to be lower compared to the control samples. Hwang et al. (2008) reported that the decrease in the final moisture content and the amount of frozen water in the sample were two potential reasons for the decrease in enthalpy values. However, in our study, we couldn't find a relationship between moisture content and entalphy. In a related study (Hwang et al. 2008), different proportions of grape wine lees were incorporated into ice creams and this phenomenon led to a decrease in enthalpy values. Results revealed that midpoint of melting temperature were slightly decreased in OFS, SPOF, OFSP, and SPOFP compared to their control sample, showing that the incorporation of OF, skimmed milk powder and sugar and their combinations led to a reduction in thermodynamic stability of ice-creams. However, contrary results were also reported. For example, Soukoulis et al. (2009) found that the supplementation of four dietary fiber sources (apple, inulin, oat and wheat) in ice creams led to an increase in melting temperatures due to the restriction of the mobility of water molecules and increased thermodynamic stability of the formulations. However, as the molecular weights of the OF supplemented ice-creams were higher compared to the reference sample formulations, the increase in the proportion of OF in the formulation led to shift lower midpoint of melting temperature. For this

reason, the thermographs of OF and OFP were not visible in the Figure 3. This was probably due to the high-water retention capacity of OF and thus the decrease in the amount of freezable water. In summary, the DSC results revealed that the incorporation of OF affected the thermal properties and stability of the ice cream, with lower enthalpy values and shifted melting temperatures observed in the formulations with OF.

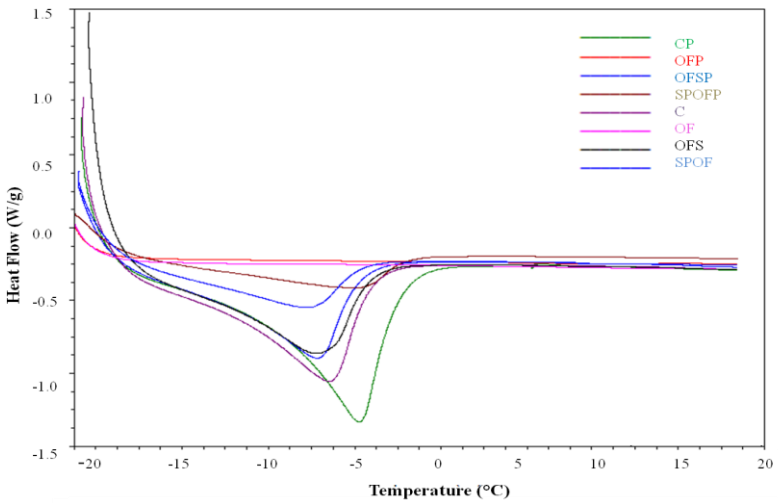

Figure 3. DSC thermographs of ice-cream samples.
C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder.

Table 4. Thermal properties of ice-cream samples.

Sample codes	Melting temperature			*ΔHm*
	Onset (°C)	Midpoint (°C)	Offset (°C)	(J g^{-1})
C	-11.28	-5.06	-0.77	42.57
OF	-	-	-	-
OFS	-13.41	-5.83	-0.98	47.59
SPOF	-12.99	-6.19	-1.41	17.53
CP	-8.61	-3.43	1.22	56.47
OFP	-	-	-	-
OFSP	-11.67	-5.74	-1.05	34.56
SPOFP	-12.76	-3.43	0.83	15.13

C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder.

Sensory evaluation

Overall acceptability scores of the ice cream samples were given in Figure 4. According to the sensory analysis, the acceptability of ice cream samples received higher scores in formulations in which OF was used as a sugar or milk powder substitute. However, they had lower sensory acceptability in formulations in which it was used

as a substitute for both sugar and skimmed milk powder. This phenomenan can be due to decrease in sweetness as a result of supplementing OF instead of sugar in the formulation. Furthermore, the presence of probiotic bacteria in the formulation did not affect the overall acceptability of the ice cream, indicating that this could likely mask the unpleasant flavor of the "probiotic" (Alamprese et al., 2005). These results were in line with the findings of Shazly et al. (2022). The findings revealed that the amount of OF was the most effective parameter in the sensory acceptance of the produced ice creams. Çakmakçı et al. (2014) reported that incorporation of OF in the ratio of 2 and 3% resulted in higher general acceptability in comparison to the reference sample. Overall, symbiotic ice cream with high bioactivity, rich in dietary fiber and sensory acceptable quality were produced with the incorporation of OF and probiotic bacteria in this study.

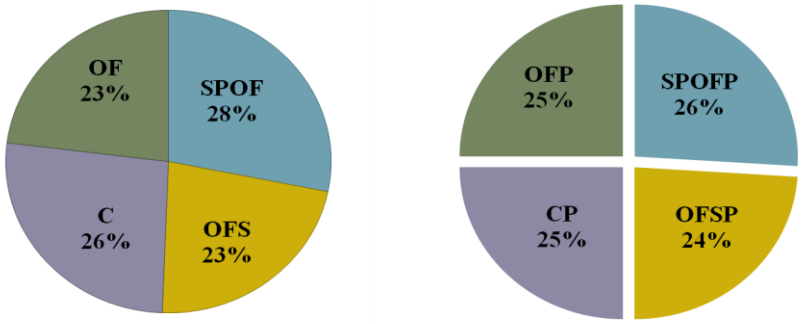

Figure 4. Overall acceptability results of ice-cream samples.
C: Control ice-cream without probiotic bacteria; CP: Control ice-cream including probiotic bacteria; OF: The non-probiotic, sugar-free ice-cream incorporated with oleaster flour; OFP: probiotic sugar-free ice-cream including oleaster flour; OFS: non-probiotic ice-cream including oleaster flour and sugar; OFSP: Ice cream including probiotic bacteria, oleaster flour and sugar; SPOF: non-probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder; SPOFP: probiotic, sugar-free ice cream including oleaster flour and high amount of skimmed milk powder.

Conclusions

The physicochemical, bioactive, microbiological, thermal, and sensory properties of prebiotic, probiotic, and symbiotic ice-cream samples produced with the supplementation of *L. plantarum* NRIC 1838 and/or OF were evaluated in the current study. The results revealed that the incorporation of probiotics into the ice-creams had no significant effect on the dry matter content while a slight increase in the dry matter content was observed when sugar was used instead of OF in the formulation. Incorporated ice-creams had lower pH value compared to their reference samples, due to the synergistic activity of OF and *L. plantarum* and presence of phenolic compounds in OF. Furthermore, supplementation of OF and/or probiotics led to a reduction in the lightness and yellowness values and provided an increase in TPC. Microbiological analysis revealed that aeration, freezing and formulation had no significant effect on viability, although a slight decrease in probiotic counts was detected after production of the ice creams compared to the mix forms. Enrichment with OF and/or probiotics decreased thermodynamic stability of the formulations. Sensory analysis revealed that the amount of oleaster powder was the most effective parameter in the sensory acceptance of the produced ice creams. Overall, the use of OF in the production of probiotic ice cream enabled the production of low-calorie ice cream, reducing production costs and adding economic value to the oleaster fruit for industrial purposes. As a recommendation, further study can be done for the examination of the changes in cell numbers during *in vitro* digestion

and storage as well as *in vitro* antidiabetic activities.

Acknowledgments

Prebiotic and probiotic (symbiotic) ice cream with oleaster flour and production method has been registered by the Turkish Patent and Trademark Agency in Türkiye. Patent Number: TR 2019/23160.

Conflict of Interest Statement

The authors declare no conflict of interest.

Data Availability Statement

The datasets produced throughout the present study can be obtained upon reasonable request from the corresponding author.

Author contributions

Beyza Nur SUREN: Methodology, Writing original draft; Sedanur SALMAN: Methodology, Writing original draft; Emel KAYA: Methodology, Writing original draft; Yagmur BUYUKKAL: Methodology, Writing original draft; Gozde KUTLU: Investigation; Writing original draft; Review & editing, Visualisation; Fatih TORNUK: Review & editing, Supervision, Resources.

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Research Article/Araştırma Makalesi

Evaluation of antioxidant activity and some physicochemical characteristics of pickled vine (*Vitis vinifera* L.) leaves

Salamura asma (Vitis vinifera L.*) yapraklarının antioksidan aktivitelerinin ve bazı fizikokimyasal özelliklerin değerlendirilmesi*

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ABSTRACT

Vine (*Vitis vinifera* L.) leaves have been used for centuries both to prepare various foods and for medicinal purposes. Vine leaves are processed to pickles for preserved for a long time. In this study, some physicochemical properties, total phenolic compounds and antioxidant activity of the pickled vine leaves produced by industrial or traditional methods from Narince variety grown in Tokat were determined. pH value (3.31-3.95), total acidity (0.3-1.72%), salt content (10.92-23.67%) were determined in pickled vine leaves produced by industrial or traditional methods. Total phenolic compounds for distilled water, 80% ethanol and 70% methanol extracts were determined in the range of 0.55-7.81 mg GAE g^{-1}, 3.98-15.66 mg GAE g^{-1}, 3.49-16.85 mg GAE g^{-1}, respectively. The cation radical scavenging activity (ABTS) for three solvents were determined in the range of 1.87-15.42 mg TE g^{-1}, 2.75-24.85 mg TE g^{-1}, 3.57-25.05 mg TE g^{-1}, while free radical scavenging activity (DPPH) for three solvents were determined in the range of 2.89-8.89 mg TE g^{-1}, 5.46-10.57 mg TE g^{-1}, 6.33-12.13 mg TE g^{-1}, respectively. As a result of the research, it was determined that the pickled vine leaves are rich source in terms of phenolic compounds and antioxidant activity but amount of salt in some samples was very high for consumption. The total phenolic compounds and antioxidant activity results of extracts obtained from pickled vine leaves using ethanol and methanol are close to each other but are higher than the results obtained distilled water.

Key Words: Antioxidant activity, Narince, Vine leaves, Phenolic compound, Physicochemical character

ÖZ

Asma (*Vitis vinifera* L.) yaprakları yüzyıllardır hem çeşitli yemeklerin hazırlanmasında hem de tıbbi amaçlarla kullanılmaktadır. Asma yaprakları uzun süre muhafaza edilmek amacıyla salamuraya işlenmektedir. Bu çalışmada, Tokat yöresinde yetiştirilen Narince çeşidinden endüstriyel ve geleneksel yöntemlerle üretilen salamura asma yapraklarının bazı fizikokimyasal özellikleri, toplam fenolik madde miktarları ve antioksidan aktiveleri belirlenmiştir. Endüstriyel ve geleneksel yöntemlerle üretilen salamura asma yapraklarının pH değerleri 3.31-3.95, toplam asitlik miktarı %0.3-1.72, tuz miktarı %10.92-23.67 aralığında tespit edilmiştir. Toplam fenolik madde miktarları saf su, %80 etil alkol ve %70 metil alkol ekstraktları için sırasıyla, 0.55-7.81 mg GAE g^{-1}, 5.00-15.66 mg GAE g^{-1}, 3.49-16.85 mg GAE g^{-1} aralığında belirlenmiştir. Katyon radikali giderme aktivitesi (ABTS) üç farklı çözücü için sırasıyla 1.87-15.42 mg TE g^{-1}, 2.75-24.85 mg TE g^{-1}, 3.57-25.05 mg TE g^{-1} aralığında, serbest radikali giderme aktivitesi (DPPH) ise sırasıyla 2.89-8.89 mg TE g^{-1},

5.46-10.57 mg TE g^{-1}, 6.33-12.13 mg TE g^{-1} aralığında tespit edilmiştir. Araştırma sonucunda, salamura asma yapraklarının fenolik bileşikler ve antioksidan aktivite açısından zengin bir kaynak olduğu fakat tuz değerlerinin tüketim için çok yüksek seviyede olduğu belirlenmiştir. Salamura asma yapraklardan etanol ve metanol kullanılarak elde edilen ekstraktların toplam fenolik madde ve antioksidan aktivite sonuçları birbirine yakın olmakla birlikte saf su kullanılarak elde edilen sonuçlardan daha yüksektir.

Anahtar Kelimeler: Antioksidan aktivite, Narince, Asma yaprağı, Fenolik bileşik, Fizikokimyasal özellik

Introduction

The using of functional food products has been increasing in the healthy eating model (Akin-Başçam, 2021). Functional foods are defined as foods containing or enriched with nutritional components such as vitamins, minerals and various bioactive compounds which are protecting from diseases, and increase the quality of life (Granato et al., 2020). Grape is considered a functional food with its bioactive content. Grape by-products attract attention due to their nutritional and functional compounds (Akin-Başçam, 2021). The nutritional and functional value of grapes and their derivatives are found, along with their potential for financial gain. Grapes have been employed in medicine to treat or prevent diseases such as gastroenteritis, diarrhea, nausea and skin disorders since centuries ago (Lacerda et al., 2016). Apart from the grape, studies have shown that grape by products such as seed, skin and leaf also present therapeutic effects (Silva et al., 2021). In pharmacological studies, vine leaf has been found many biological activities such as antioxidant (Amarowicz et al., 2008), antimicrobial (Ceyhan et al., 2012), antihypercholesterolemic (Devi and Singh, 2017) and neuroprotective (Dani et al., 2010). In traditional medicine, grape leaves can be used in the treatment of various conditions such as bleeding, hypertension, diarrhea, eye infections, diabetes and circulatory system disorders (Akin-Başçam, 2021). Numerous effects are believed to be brought on by phenolic chemicals contained in vine leaves (Khan et al., 2021). Vine leaves contain several types of phenolic compounds such as flavonoids, tannins, anthocyanins and procyanidins (Dani et al., 2010). It is known that the beneficial effects of the antioxidant activity of phenolic compounds are due to their ability to remove oxygen and delay lipid oxidation (Zhang et al., 2021). In addition to its biological activities, the vine leaf has even outgrown the grape commercially in some regions. An important part of the pickled leaves, which are exported from Türkiye and consumed in the domestic market, are produced in Tokat and Manisa regions (Cangi and Yağcı, 2012). Tokat takes the first place in Türkiye in the production of brine leaves. The structural and sensory character of the pickled vine leaf product from Tokat has made this product important in the domestic and foreign markets (Bal et al., 2019).

Production of pickled vine leaves by fermentation method is a storage method that has been used for many years. Pickled vine leaves are fermented products that are obtained as a result of the use of organic substances, primarily carbohydrates, by microorganisms. In the brine method, the fresh leaves are brined in salt water and subjected to fermentation. Then it is packaged and presented for consumption (Sat et al., 2002; Gülcü and Torçuk, 2016). Many delicious products of Tokat cuisine, which is rich in gastronomy and has a wide variety of options, are produced using pickled vine leaves (Yaylacı and Mertol, 2021).

In the literature, there is no comprehensive study available on the evaluation of physicochemical properties, total phenolic compounds and antioxidant activity on the pickled vine leaves. In this research, it was aimed to determine some physicochemical properties, total phenolic compounds and antioxidant activity of pickled vine leaves, which are traditionally and commercially produced from the leaves of the Narince grape variety in Tokat province in the Central Black Sea Region of Türkiye.

Material and Methods

Plant material

In the research, pickled vine leaves produced by fermenting the leaves of the Narince grape variety grown in the Tokat region were used. Traditional and industrially produced pickled vine leaves samples (15 samples) were obtained. While industrially produced (12 samples) vine leaves belonging to different brands were obtained from the Merkez district (Tokat province), traditionally produced (3 samples) vine leaves were obtained from Erbaa and Niksar districts (Tokat province). All samples were collected from the market in June 2020. Because the vine leaves that are harvested and fermented for the first time in the year are release to the market in this month. For each brand, two samples of one kilogram each with the same production dates and batch numbers were obtained. Selection criteria for pickled vine leaves were based on the Narince variety grown in the Tokat region in 2020. Because the pickled vine leaves of the Narince variety, which are grown and processed in Tokat, have a significant market share in Türkiye. After the samples were obtained, they were stored at 4-8 °C under refrigerator conditions in the laboratory of the Department of Food Engineering of Tokat Gaziosmanpaşa University. The brine leaves samples produced industrially are expressed with the "E" code, and the traditionally produced samples with the "G" code.

Chemical materials

Gallic acid, trolox, ethanol, methanol, ABTS and DPPH were supplied from Sigma-Aldrich (Germany). Silver nitrate ($AgNO_3$), Folin-Ciocalteu reagent, potassium peroxydisulfate ($K_2S_2O_8$), sodium carbonate (Na_2CO_3), potassium chromate (K_2CrO_4), phenolphthalein, sodium hydroxide (NaOH) were supplied from Merck (Darmstadt, Germany).

Physicochemical analysis

The pH, titration acidity, salt content and color analysis (L*, a*, b*) of samples were carried out according to the method applied by Cemeroğlu (2013).

Total phenolic compounds

Phenolic compound extraction from pickled vine leaves was carried out using different solvents (distilled water, ethanol (80%) and methanol (70%). Solvent concentrations were determined by preliminary experiments. Pickled vine leaves were ground. Then, 1 g of ground leaves was extracted with 50 ml of solvent at room temperature for 24 hours. The extract was then filtered and used for analysis. 100 µL of the prepared extracts were taken and 200 µL of Folin-Ciocalteu reagent and 2 mL of distilled water were added to it and left for 3 minutes at room temperature. At the end of the period, 1 mL of sodium carbonate (Na_2CO_3) solution (20%) was added to the mixture and mixed with vortex (Velp Scientifica, Italy). A spectrophotometer (PG Instrument, T80+, England) was used to measure the mixture at 765 nm after it had been incubated for one hour at room temperature. A calibration curve was created with different concentrations of gallic acid used as standard (0, 50, 100, 150, 250, 350, 500 mg L^{-1}). Total phenolic compounds values were represented as mg gallic acid equivalent (GAE) g^{-1} (Topuz and Bayram, 2022).

Antioxidant activity

Cation radical scavenging activity ($ABTS^{\bullet+}$)

7 mM ABTS and 2.45 mM $K_2S_2O_8$ solution were prepared and mixed 1:1 for ABTS stock solution. The prepared solution was kept at room temperature in the dark for 16 hours. 40 µL of the extracts (distilled water, 80% ethanol and 70% methanol) were taken and 4 mL ABTS was added to it and left in the dark at room temperature for 6 minutes. Mixture absorbance was measured in a spectrophotometer at 734 nm. ABTS values were represented as mg trolox equivalent (TE) g^{-1} (Re et al., 1999).

Free radical scavenging activity ($DPPH^{\bullet}$)

100 µL of the prepared extracts (distilled water, 80% ethanol and 70% methanol) was taken and 3.9 mL of the prepared DPPH solution (0.06 mM) was added. It was then mixed by vortex and kept in the dark for 30 minutes. Mixture absorbance was measured in a spectrophotometer at 517 nm. DPPH values were e represented as mg trolox

equivalent (TE) g^{-1} (Blasi et al., 2016).

Statistical analysis

Statistical analyses were done by using the Duncan test through the instrument of SPSS 22.0 (IBM, USA) statistical package program. Moreover, correlation coefficients were identified using the same program.

Results and Discussions

pH Values, Total Acidity and Salt Contents

The pH values of pickled vine leaves were detected in the range of 3.31-3.95 and the average pH value was 3.60 (Table 1).

Table 1. pH values, total acidity and salt contents of pickled vine leaves

Sample*	pH	Total acidity (%)*	Salt (%)
E1	3.83±0.01^{b}	0.56±0.05^{h}	15.83±0.07^{h}
E2	3.47±0.01^{g}	1.28±0.05^{c}	16.19±0.18^{g}
E3	3.95±0.01^{a}	0.48±0.05^{h}	10.92±0.07^{m}
E4	3.83±0.01^{b}	0.30±0.05^{i}	13.81±0.23^{j}
E5	3.71±0.01^{c}	0.56±0.05^{h}	15.44±0.12^{i}
E6	3.71±0.01^{c}	0.74±0.10^{g}	18.33±0.07^{d}
E7	3.56±0.01^{f}	0.68±0.05^{g}	12.79±0.14^{k}
E8	3.65±0.01^{d}	0.86±0.05^{f}	17.00±0.07^{f}
E9	3.48±0.01^{g}	1.16±0.09^{d}	17.36±0.18^{e}
E10	3.63±0.01^{e}	0.30±0.05^{i}	16.77±0.14^{f}
E11	3.31±0.01^{i}	0.98±0.09^{e}	23.67±0.07^{a}
E12	3.43±0.01^{h}	1.01±0.05^{e}	13.77±0.18^{j}
G1	3.54±0.01^{f}	1.72±0.05^{a}	16.89±0.18^{f}
G2	3.46±0.01^{g}	1.57±0.05^{b}	22.97±0.18^{b}
G3	3.42±0.01^{h}	1.49±0.10^{b}	22.43±0.18^{c}
Mean	3.60	0.92	16.94

Results are given as mean ± standard deviation.

* In terms of lactic acid

**Samples coded "E" and "G" represent industrial and traditional production, respectively.

The total acidity values of the pickled vine leaves were found between 0.30-1.72%. The average total acidity value is 0.92%. It was determined that the total acidity value of the brine leaves produced by the traditional method (G1, G2, G3) is higher. The salt content of pickled vine leaves was found in a wide range of 10.92-23.67%. The average value of salt content is 16.94%. Lactic acid bacteria break down carbohydrates in the media and convert sugar into lactic acid or lactic acid, CO_2, acetic acid and ethanol according to their homofermentative and heterofermentative properties (Bintsis, 2018). The main antimicrobial effect of lactic acid bacteria is the production of lactic acid and the resulting decrease in the pH value of the media (Alakomi et al., 2000). As a result of fermentation in vine leaves, with the increase in lactic acid in the media, total acidity increased, and pH value decreased.

In the study, pH values of pickled vine leaves were close to each other. The total acidity amounts of the samples were found to be higher in the traditionally produced pickled vine leaves. Generally, a decrease in pH values is observed in parallel with the titratable acidity values. But this case is not the same in all analyzed samples. There is no direct or predictable relationship between pH and titration acidity. The pH values of foods with the same titratable acidity value may differ from each other. The pH value does not depend on the concentration of acids present. However, it is affected by their dissociate abilities (AWRI, 2024).

Salt amounts of pickled vine leaves do not differ according to industrial or traditional methods. Although the salt values in the pickled vine leaves were in a wide range, a high level of salt was detected in the leaves. It is thought that this case may pose a risk in terms of health. In a study in the literature, vine leaves of different varieties were processed into canning using 1.5%, 3.5% and 5% salt (Göktürk et al., 1997), while Sultani variety vine leaves were processed into brine using 5% salt in another study (İç and Denli, 1997). It was determined that the pH, titratable acidity and salt values of the products obtained from the market are different from each other. This difference is not only between industrial and traditional production. Industrial and traditional production results also differ within themselves. Because the production process of pickled vine leaves may differ between brands. Therefore, standard production conditions should be established for the production of brine vine leaves.

Color Value

The L* value in a colorimeter symbolizes the transition from black to white between 0 and 100. The a* value determined in the colorimeter represents (+) redness and (-) greenness. The b* value specified in the colorimeter symbolizes (+) yellowness and (-) blueness (Cemeroğlu, 2013). As a result of color measurement, L* value (35.66)-(45.60), a* value (-4.38)-(-1.68), b* value (32.61)-(54.82) were determined. The color changes from green to yellow as a result of the fermentation of the vine leaves. The E4 sample had the highest value in terms of L* (brightness) and b* (yellowness) characteristics, while sample E2 was the highest value in terms of a* (greenness) characteristics (Table 2).

Although there are many derivatives of chlorophyll, the most important ones are chlorophyll a and chlorophyll b. Their most important duty is to provide the conversion of solar energy into chemical energy during photosynthesis in the plant. Solar rays are absorbed by chlorophyll a and chlorophyll b at different wavelengths, and the two chlorophylls complement each other. In the spectral area between 500-600 nm, very little light is absorbed and most of it is reflected, so plants appear green (Ergün, 2003). Chlorophyll pigments are broken down during processing (heat, light, acid, etc.) applied to foods and color changes are observed in the plant with the effect of fermentation, the lactic acid in the media increases. Thus, chlorophyll, which gives the leaf its color, is destroyed by the effect of acid. As a result, chlorophyll is converted to pheophytin and pheophorbides. As the leaves lose their green color, a color change is observed from mat green to olive yellow instead of bright green (Kazancı, 2008).

Table 2. Color values of pickled vine leaves

Sample***	L*	a*	b*
E1	43.09±0.80^{e**}	-3.07±0.25^{cd}	41.19±1.10^{cd}
E2	43.20±0.86^{e}	-1.68±0.40^{a}	42.20±0.40^{c}
E3	40.02±0.70^{g}	-2.25±0.10^{b}	32.61±1.39^{g}
E4	50.67±0.54^{a}	-2.82±0.06^{c}	54.82±1.28^{a}
E5	42.61±0.31^{e}	-2.88±0.11^{c}	39.95±0.43^{de}
E6	40.27±0.59^{g}	-2.30±0.11^{b}	35.54±1.27^{f}
E7	41.30±0.26^{f}	-3.34±0.05^{d}	45.90±1.03^{b}
E8	35.66±0.72^{i}	-2.80±0.27^{c}	35.62±1.04^{f}
E9	44.24±0.31^{d}	-4.53±0.17^{h}	40.81±0.62^{cde}
E10	37.29±0.30^{h}	-2.23±0.05^{b}	37.46±0.41^{f}
E11	45.60±0.11^{b}	-4.02±0.03^{f}	39.24±0.71^{e}
E12	44.48±0.28^{cd}	-4.47±0.01^{gh}	40.10±0.08^{de}
G1	50.22±0.46^{a}	-4.20±0.09^{fg}	45.63±1.05^{b}
G2	43.22±0.16^{e}	-3.73±0.13^{e}	36.03±0.70^{f}
G3	45.19±0.15^{bc}	-4.38±0.03^{gh}	42.25±0.42^{c}

Results are given as mean ± standard deviation.

** Small letters in the same column indicate statistical difference between leaf varieties. (P<0.05)

*** Samples coded "E" and "G" represent industrial and traditional production, respectively.

It is observed that there is a difference in the color values of pickled vine leaves obtained from the market. Although the pickled vine leaf samples belong to the same variety (Narince) and the same season (June 2020), it is possible that there are differences between the color values of samples. Because the processing parameters (salt, temperature, packaging, etc.) to brine also vary from brand to brand, in addition to the growing and harvesting conditions of the leaves. Therefore, it is possible to detect differences between the L*, a* and b* color values of the samples.

Total Phenolic Compounds

Grapes contain phenolic compounds such as flavanols and anthocyanins. Anthocyanins and flavanols are found in grape skins and leaves, whereas proanthocyanidins and other non-flavonoid substances are primarily found in the pulp and seeds (Di Lorenzo et al., 2019). According to a study, the highest levels of total phenolic compounds were discovered in seeds followed by leaves, while the lowest levels were discovered in skin and pulp (Šuković et al., 2020). According to studies, grape leaves are an important source of phenolic compounds (Labanca et al., 2020).

The gallic acid standard calibration curve (y=0.0028x+0.0836, R^2=0.99) is created to calculate total phenolic. The total phenolic compounds amount of the distilled water extract was determined to be lower than ethanol and methanol extract. The total phenolic compounds in the extracts obtained using distilled water were determined in the range of 0.55-7.81 mg GAE g^{-1}. The total phenolic compounds amount of the samples using ethanol or methanol was determined close to each other. The highest amount of phenolic compounds for both solvents was in the G2 pickled vine leaves. The amount of total phenolic compound was determined as 3.98-15.66 mg GAE g^{-1} in ethanol extracts and 3.49-16.85 mg GAE g^{-1} in methanol extracts (Table 3).

When the total phenolic compounds data were evaluated, it was determined that the amounts of phenolic compounds changed according to the type of solvent. The type of solvent to be used in the extraction of phenolic compounds is very important (Çoklar and Akbulut, 2016). The solvent system used in the extraction stage affects the profile of the phenolic compounds as well as the amounts of total phenolic compounds extracted from the plant material (Türkyılmaz et al., 2017).

While the high amount of total phenolic compounds was detected in the 80% ethanol extracts in some samples, the high amount of total phenolic compounds was detected in the 70% methanol extracts in the other samples. Because the solubility of phenolics may vary depending on the type of solvent used, the degree of polymerization of phenolics and the formation of insoluble complexes with other components (Yolci et al., 2022).

Table 3. The total phenolic compound of pickled vine leaves (mg GAE g^{-1})

Sample**	Water Extract	80% ethanol Extract	70% methanol Extract
E1	$1.26\pm0.13^{Bj*}$	5.00 ± 0.57^{Afgh}	5.28 ± 0.16^{Ai}
E2	2.85 ± 0.25^{Bf}	6.23 ± 0.39^{Ae}	6.15 ± 0.40^{Agh}
E3	0.55 ± 0.06^{Ck}	3.98 ± 0.16^{Ah}	3.49 ± 0.03^{Bj}
E4	1.55 ± 0.16^{Cij}	4.90 ± 0.05^{Bgh}	5.78 ± 0.43^{Ahi}
E5	1.51 ± 0.13^{Bij}	5.28 ± 0.40^{Aefg}	5.16 ± 0.04^{Ai}
E6	2.12 ± 0.09^{Cgh}	6.17 ± 0.03^{Bef}	6.86 ± 0.27^{Ag}
E7	2.24 ± 0.03^{Bg}	5.99 ± 0.05^{Aefg}	6.54 ± 0.45^{Agh}
E8	3.25 ± 0.09^{Ce}	9.00 ± 0.24^{Ac}	7.87 ± 0.11^{Bf}
E9	7.03 ± 0.14^{Bb}	12.38 ± 1.04^{Ab}	12.69 ± 0.56^{Ac}
E10	1.76 ± 0.08^{Bhi}	5.69 ± 0.11^{Aefg}	5.26 ± 0.40^{Ai}
E11	3.82 ± 0.32^{Bd}	7.69 ± 0.59^{Ad}	8.88 ± 0.48^{Ae}
E12	5.97 ± 0.05^{Bc}	12.19 ± 0.68^{Ab}	11.11 ± 0.53^{Ad}
G1	7.60 ± 0.18^{Ba}	14.54 ± 0.40^{Aa}	15.33 ± 0.35^{Ab}
G2	5.71 ± 0.37^{Bc}	15.66 ± 0.59^{Aa}	16.85 ± 0.45^{Aa}
G3	7.81 ± 0.30^{Ca}	15.15 ± 0.91^{Aa}	12.15 ± 0.25^{Bc}

Results are given as mean ± standard deviation.

* Small letters in the same column indicate the difference between samples, and capital letters in the same line indicate the difference between solvent extracts ($P<0.05$).

**Samples coded "E" and "G" represent industrial and traditional production, respectively.

In a study, total phenolic compound of pickled vine leaves of Narince Bağ, Narince Yerli, Narince Aşılı, Sultani Çekirdeksiz Aşılı were determined as 8.00, 11.56, 9.96, 10.34 mg GAE g^{-1}, respectively (Semerci, 2019). In another study, the total phenolic compound of fresh vine leaves grown under optimum conditions and drought stress were determined as 19.37 and 15.94 mg GAE g^{-1}, respectively (Król et al., 2014). The amounts of total phenolic compound obtained as a result of this study are very close to the values determined in fresh and pickled vine leaves in the literature. Kosar et al. (2007) stated that the total phenolic compound levels in leaves are not affected by the brining method. According to the literature, the total phenolic content of leaves gathered from various grape cultivars was found to vary by cultivar, and grape leaves had high phenolic compounds similar to berries. This showed that grape leaves are a rich source of phenolic and antioxidant compounds (Babalık and Baydar, 2019). The total phenolic compound of vine leaves from Sultani Çekirdeksiz, Sultan 1, Sultan 7, Saruhanbey, and Narince grape cultivars were found between 9.72 and 14.22 mg GAE g^{-1} (Güler and Candemir, 2014). Banjanin et al. (2021) researched the effect of grape varieties on total phenolic compounds amounts of vine leaves. Total phenolic compounds of vine leaves were determined between 12.98 mg GAE g^{-1} and 17.48 mg GAE g^{-1}.

Antioxidant activity of pickled vine leaves

The cation radical scavenging activity of the distilled water extract was determined to be lower than ethanol and methanol extract (Table 4). The trolox standard calibration curve ($y=-0.001x+0.6344$, $R^2=0.99$) is created to calculate

cation radical scavenging activity. When antioxidant activity is calculated using the calibration curve equation, cation radical scavenging activity in the extracts obtained using distilled water was found between 1.87-15.42 mg TE g^{-1}. The cation radical scavenging activity of the samples using ethanol or methanol was determined close to each other. Cation radical scavenging activity was determined in the range of 2.75-24.85 mg TE g^{-1} in ethanol extracts, and in the range of 3.57-25.05 mg TE g^{-1} in methanol extracts. The lowest cation radical scavenging activity was determined in water extracts. Although 80% ethanol and 70% methanol extracts are close to each other, it is not possible to determine which is best for the two solvents. The reason for obtaining different values for both solvents in the samples may be the composition and amount of compounds with antioxidant activity, such as phenolic compounds.

In a study, ABTS values of 8 vine leaf (immature and mature) varieties harvested in June and September varied between 311.59-715.85 µmol TE g^{-1} (Gülcü et al., 2020). ABTS values of the vine leaves of the Gohér variety harvested from the sun and shaded areas ranged from 0.65 to 1.88 µM TE g^{-1} (Bodó et al., 2017).

Table 4. Antioxidant activity of pickled vine leaves

Sample**	Cation radical scavenging activity (mg TE g^{-1})			Free radical scavenging activity (mg TE g^{-1})		
	Distilled water	**80% ethanol**	**70% methanol**	**Distilled water**	**80% ethanol**	**70% methanol**
E1	$2.82\pm0.42^{Aef*}$	4.80 ± 0.32^{Agh}	5.67 ± 1.77^{Agh}	$3.60\pm0.35^{Bg*}$	5.69 ± 0.06^{Ag}	6.81 ± 0.49^{Agh}
E2	3.02 ± 0.78^{Cdef}	12.02 ± 0.64^{Ae}	8.15 ± 0.95^{Bef}	4.07 ± 0.55^{Bfg}	6.56 ± 0.02^{Aef}	7.32 ± 0.27^{Ag}
E3	2.25 ± 0.18^{Bg}	2.75 ± 0.04^{Ah}	3.57 ± 0.42^{Ah}	2.89 ± 0.18^{Bh}	6.96 ± 0.51^{Ae}	6.33 ± 0.45^{Ah}
E4	1.95 ± 0.39^{Cfg}	5.27 ± 0.28^{Bg}	8.00 ± 0.60^{Aef}	4.39 ± 0.06^{Cef}	8.26 ± 0.20^{Bd}	9.15 ± 0.04^{Ade}
E5	2.17 ± 0.21^{Bf}	4.95 ± 0.95^{Bg}	8.30 ± 1.52^{Aef}	4.58 ± 0.06^{Cef}	6.04 ± 0.16^{Bfg}	7.01 ± 0.39^{Ag}
E6	4.25 ± 0.74^{Bde}	7.6 ± 0.67^{ABf}	10.90 ± 1.66^{Ad}	4.60 ± 0.59^{Cef}	7.26 ± 0.04^{Be}	8.57 ± 0.20^{Aef}
E7	3.72 ± 1.06^{Adef}	7.8 ± 2.65^{Af}	8.42 ± 0.07^{Ae}	4.33 ± 0.06^{Bef}	6.56 ± 0.33^{Aef}	7.24 ± 0.27^{Ag}
E8	4.65 ± 0.88^{Bde}	9.42 ± 1.13^{Af}	11.37 ± 0.28^{Ad}	4.97 ± 0.29^{Cde}	7.06 ± 0.41^{Be}	$8.19\pm0,14^{Af}$
E9	12.42 ± 0.35^{Bb}	16.95 ± 1.03^{Ad}	18.15 ± 0.25^{Ac}	3.93 ± 0.08^{Bfg}	9.65 ± 0.24^{Abc}	10.46 ± 0.39^{Ab}
E10	1.87 ± 0.14^{Bfg}	7.75 ± 0.39^{Af}	5.95 ± 1.10^{Afg}	7.85 ± 0.51^{Bb}	5.46 ± 0.31^{Ag}	7.18 ± 0.27^{Ag}
E11	4.82 ± 0.14^{Bd}	12.10 ± 0.67^{Ae}	11.40 ± 0.53^{Ad}	5.36 ± 0.02^{Cd}	7.14 ± 0.10^{Be}	8.57 ± 0.47^{Aef}
E12	9.55 ± 0.39^{Cc}	20.52 ± 1.20^{Bc}	25.05 ± 1.38^{Aa}	7.39 ± 0.06^{Cb}	10.19 ± 0.14^{Aab}	9.76 ± 0.04^{Bcd}
G1	12.02 ± 1.34^{Bb}	22.45 ± 0.25^{Abc}	22.07 ± 1.06^{Ab}	8.89 ± 0.06^{Ca}	10.57 ± 0.20^{Ba}	12.13 ± 0.04^{Aa}
G2	15.42 ± 1.56^{Ba}	24.85 ± 0.53^{Aa}	22.17 ± 0.85^{Ab}	8.60 ± 0.47^{Ba}	9.43 ± 0.31^{Bc}	11.71 ± 0.08^{Aa}
G3	9.22 ± 1.48^{Cc}	24.32 ± 0.71^{Aab}	17.35 ± 0.95^{Bc}	6.42 ± 0.33^{Bc}	9.60 ± 0.67^{Abc}	10.17 ± 0.22^{Abc}

Results are given as mean ± standard deviation.
* Small letters in the same column indicate the difference between examples, and capital letters in the same line indicate the difference between solvent extracts (P<0.05).
** Samples coded "E" and "G" represent industrial and traditional production, respectively.

The free radical scavenging activity of pickled vine leaves is given in Table 4. The trolox standard calibration curve (y=-0.0018x+0.5265, R^2=0.99) is created to calculate free radical scavenging activity. When antioxidant activity is calculated using the calibration curve equation, free radical scavenging activity in the extracts obtained using distilled water was found between 2.89-8.89 mg TE g^{-1}. Free radical scavenging activity was determined in the range of 5.46-10.57 mg TE g^{-1} in 80% ethanol extracts and 6.33-12.13 mg TE g^{-1} in 70% methanol extracts. The highest free radical scavenging activity in all three solvents was determined in the G1 sample.

Free radical scavenging analysis was carried out in the extracts obtained using distilled water, 80%

ethanol and 70% methanol. The free radical scavenging activity of the distilled water extract (except for E10 sample) was determined to be lower than ethanol and methanol extract. This may be due to the individual phenolic compound content of the pickled vine leaf of sample E10 or other antioxidant compounds in its content.

In a study, it was determined that the free radical scavenging activity of 8 vine leaf (immature and mature) varieties harvested in June and September varied between 14.54-30.24 µmol TE g^{-1} (Gülcü et al., 2020). In another study, it was determined that the DPPH activity of the extracts obtained from vine leaves using 8 different solvents varied between 714.71-6496.99 mg TE g^{-1} extract (Matloub, 2018). In a study, fresh vine leaf showed higher antioxidant activity as well as total phenolic compounds than frozen and canned vine leaf. This result was associated with the preservation method. These effects may induce the breakdown of antioxidant compounds or the destruction of the active metabolites (Jaradat et al., 2017).

Correlation coefficients between total phenolic compound and cation radical scavenging activity (ABTS), total phenolic compound and free radical scavenging activity (DPPH), cation radical scavenging activity (ABTS) and free radical scavenging activity (DPPH) are given in Table 5. It was determined that there was an inverse relationship between total phenolic compounds and ABTS, and between ABTS and DPPH in the E10 sample. A positive correlation was found between total phenolic compound and ABTS, total phenolic compounds and DPPH, and ABTS and DPPH in all samples except E10. In general, there is a relationship between phenolic compounds and antioxidant activity. Because phenolic compounds were considered to have antioxidant activities due to their behavior such as singlet oxygen quenchers, reducing agents, and hydrogen donor (Özer et al., 2018). There is a negative correlation between total phenolic compounds and DPPH in the E10 sample. This may be associated with the low free radical scavenging activity of the phenolic compound profile in the E10 sample.

Table 5. Correlation coefficients

	Correlation coefficients		
Sample*	**Total phenolic compound and ABTS**	**Total phenolic compound and DPPH**	**ABTS and DPPH**
E1	0.824	0.931	0.770
E2	0.877	0.939	0.741
E3	0.922	0.991	0.894
E4	0.959	0.853	0.753
E5	0.796	0.885	0.884
E6	0.903	0.969	0.936
E7	0.872	0.972	0.913
E8	0.865	0.939	0.971
E9	0.978	0.969	0.974
E10	0.971	-0.735	-0.838
E11	0.919	0.966	0.827
E12	0.902	0.987	0.900
G1	0.985	0.917	0.848
G2	0.907	0.769	0.444
G3	0.993	0.840	0.789

* Samples coded "E" and "G" represent industrial and traditional production, respectively.

Conclusion

In this present study, the pH values of the pickled vine leaves were close to each other. The total acidity amounts of the samples were found to be higher in the traditionally produced pickled vine leaves. Salt amounts of pickled vine leaves do not differ according to industrial or traditional methods. Although the salt values in the pickled vine leaves were in a wide range, a high level of salt was detected in the leaves. It is thought that this situation may pose a risk in terms of health. For total phenolic compounds and antioxidant activities (distilled water, 80% ethanol, 70%

methanol), it was found that the results obtained using 80% ethanol and 70% methanol were close to each other and total phenolic compounds and antioxidant activities of these extracts are higher than water extracts.

When the obtained data is evaluated, due to the lack of certain standards in the production of pickled vine leaves, serious differences were observed in quality parameters such as salt content. Determination of standard production parameters to produce of pickled vine leaves is important in terms of establishing a reliable market. The results obtained are important in terms of being a comprehensive study of the quality characteristics of Narince pickled vine leaves and being a source for future research and studies.

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Conflict of interest

The authors claim no conflict of interest.

Author contributions

Tuba Zorlu Ünlü: Sample collection, Analysis, Writing original manuscript. **Semra Topuz:** Methodology, Evaluation, Writing original manuscript, Reviewing & editing. **Mustafa Bayram:** Supervision, Evaluation, Writing-review & editing. **Cemal Kaya:** Writing-review & editing.

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Research Article/Araştırma Makalesi

Effect of early harvest on the aroma compounds and bioactive properties of natural olive oils

Erken hasadın naturel zeytinyağlarının aroma bileşiklerine ve biyoaktif özelliklerine etkisi

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ABSTRACT

In this study, the effect of harvest time on various quality characteristics of natural oils obtained from Kilis yağlık variety olives was determined. Free fatty acid (FFA), acid number, peroxide value, iodine number, refractive index, color, total phenol (Folin Ciocalteu) and antioxidant capacity (DPPH) values were determined in olive oils. Volatile compounds in olive oils were obtained by solid phase microextraction (SPME) and detected by gas chromatography-mass spectrometry (GC-MS). It was determined that %FFA and peroxide values increased as the harvest time progressed. Similar to the total phenol content determined as 516.61 mg GAE L^{-1} in early harvest and 77.70 mg GAE L^{-1} in normal harvest, antioxidant capacities also decreased with ripening. A total of 37 different volatiles were determined in olive oils, and a decrease in aldehyde ratio and an increase in alcohol and acid ratios were detected with ripening. While the rate of pleasant compounds (hexanal, α-farnesene, etc.) was high in early harvest olive oil, an increase in the rate of off-flavor compounds (acetic acid, nonanal, etc.) was observed in normal harvest olive oil. It has been determined that harvest time is an important factor in olive oil quality.

Key Words: Olive oil, early harvest, bioactive properties, aroma composition, quality

ÖZ

Bu çalışmada, Kilis yağlık çeşidi zeytinlerden elde edilen doğal yağların çeşitli kalite özellikleri üzerine hasat zamanının etkisi belirlenmiştir. Zeytinyağlarında serbest yağ asitliği (FFA), asit sayısı, peroksit sayısı, iyot sayısı, kırılma indisi, renk, toplam fenol miktarı (Folin Ciocalteu) ve antioksidan kapasitesi (DPPH) belirlenmiştir. Zeytinyağlarında bulunan aroma maddeleri katı faz mikro ekstraksiyon (SPME) ile elde edilmiş ve gaz kromatografisi-kütle spektrometrisi (GC-MS) aracılığıyla tespit edilmiştir. Hasat zamanı ilerledikçe %FFA ve peroksit sayısı değerlerinin artış gösterdiği tespit edilmiştir. Toplam fenol madde miktarı erken hasatta 516.61 mg GAE L^{-1}, normal hasatta 77.70 mg GAE L^{-1} olarak belirlenirken; antioksidan kapasiteleri de olgunlaşmayla düşüş götermiştir. Zeytinyağlarında toplam 37 farklı uçucu bileşik belirlenmiş olup, olgunlaşma ile aldehit oranında düşme, alkol ve asit oranlarında artış tespit edilmiştir. Erken hasat zeytinyağında hoş kokulu bileşiklerin (hekzanal, α-farnesen vb.) oranı daha fazla iken normal hasat zeytinyağında istenmeyen bileşiklerde (asetik asit, nonanal vb.) artış görülmüştür. Zeytinyağı kalitesinde hasat zamanının önemli bir faktör olduğu belirlenmiştir.

Anahtar Kelimeler: Zeytinyağı, erken hasat, biyoaktif özellik, aroma kompozisyonu, kalite

Introduction

Olive (*Olea europea L.*) is one of the plants frequently grown in Mediterranean weather. 90% of the olives produced in the world are processed for oil (Gündeşli and Küden, 2020). Olive oil is the oil obtained from olive tree fruits by physical methods. Natural olive oils are oils that can be consumed without chemical processing (unrefined) (García-Vico et al., 2017; Perestrelo et al., 2017). The term early harvest olive oil is used for olive oils obtained by using olives of various ripeness, from green to pink, at the beginning of the olive harvest season (September-October) (Dıraman and Dibeklioğlu, 2009). The mono and polyunsaturated fatty acid ratios of early harvest olive oils are optimal, and the amounts of important compounds such as phenol, tocopherol and chlorophyll are high (Dag et al., 2011).

Considering its nutritional and health benefits, olive oil has an important place among animal and vegetable oils (Kılıç, 2020). Olive oil, one of the products of the Mediterranean diet, is frequently preferred by consumers due to its bioactive properties and unique aroma (Armutçu et al., 2013; Kiralan et al., 2021; Zarrouk et al., 2008). Olive oil gains its bioactive properties from substances such as phenolic compounds it contains and the antioxidant effects of these substances (Armutçu et al., 2013; Çakmak Arslan, 2022; Zarrouk et al., 2008). Phenolic compounds are also responsible for the sensory properties of olive oil, such as bitterness, astringency, and pungency (Büyükgök and Saygın Gümüşkesen, 2017). The main phenolic compounds found in olive oil are tyrosol, hydroxytyrosol, oleuropein, shikimic acid, coumaric acid, caffeic acid, and p-ferulic acid (Boskou, 2006). The fact that olive oil is rich in unsaturated fatty acids such as oleic acid also contributes to its bioactive properties (Perestrelo et al., 2017).

Aroma is one of the important parameters affecting the sensory properties of olive oil. The aroma of olive oil is composed of approximately 200 compounds, including groups such as aldehydes, esters, terpenes, ketones, alcohols, and hydrocarbons (Kesen et al., 2013; Kiritsakis, 1998). 50-75% of the compounds in olive oil aroma are from the aldehydes group (Kiritsakis, 1998). C-5 and C-6 compounds in olive oil are formed through the lipoxygenase chain reaction, and these compounds are responsible for the characteristic aroma of olive oils (Zarrouk et al., 2008). The main aroma substances found in olive oils include hexanal, (E)-2-hexenal, (Z)-3-hexen-1-ol, and 1-hexanol (Kiritsakis, 1998). The compounds responsible for the aroma of olive oil are affected by factors such as harvest, storage, variety, maturity, growing conditions, and processing technique (Kılıç, 2020).

The olive varieties with high production potential in Kilis province are Kilis yağlık and Gemlik. No study has been found in the literature regarding the aroma of early harvest Kilis yağlık olive oil. The aim of this study is to determine some properties of early and normally harvested Kilis yağlık natural olive oils. It is aimed to determine the physical, chemical, and bioactive compounds and properties of natural olive oils as well as aroma composition. Within the scope of the study, the changes caused by the harvest time in olive oils were examined.

Materials and Methods

Materials

Early harvest (October) and normal harvest (November) olive oils obtained from the 2022 crop were supplied from an olive oil factory in Kocabeyli village of Kilis province. The samples were kept in a dark, cool place and packaged until analysis. Analyzes were performed in triplicate and the results are given as mean±standard deviation.

Quality analysis

The peroxide value of the oils was determined titrimetrically according to the AOCS (Cd 8-53), iodine number AOCS (Cd 1-25), FFA, and acid number AOCS (Cd 3d-63) methods. The refractive index was read according to the AOCS (Cc 7-25) method with an Abbe refractometer (Soif WYA-

2S, China). L*, a*, and b* values were determined using the Konica Minolta Chroma Meter (CR-400, Japan) color measurement device with the AOCS (Cc 13e-92) method (AOCS, 1997). Hue (Hue˚) and chroma (ΔC*) values were calculated using the formulas below (Artes et al., 2002).

Hue˚= arctg(b*/a*) (1)

ΔC*= (a*2 +b*2)1/2 (2)

Total phenol and antioxidant capacity

For the analysis of total phenol content and antioxidant capacity, the extraction process was performed according to Sousa et al. (2014). After adding 2.5 mL hexane and 2.5 mL methanol-water (80:20 v/v) to the olive oils (4 mL), the mixture was centrifuged for 10 min. The extraction process was repeated twice by removing the upper phase. The total phenol content of the oil extracts obtained was determined spectrophotometrically at 760 nm (Shimadzu UV-1700, Japan) using the Folin Ciocalteu colorimetric method, and the results are given as mg GAE L^{-1} (Singleton et al., 1999). The antioxidant capacity of the oils was determined spectrophotometrically according to the DPPH*(2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity method. The extract (150 µL) and DPPH solution (2850 µL) were kept in the dark for 24 h, and absorbance was measured at 515 nm. Results were calculated as trolox equivalent (µmolTE mL^{-1}) (Thaipong et al., 2006).

Determination of aroma composition

The extraction of aroma compounds in natural olive oils was determined using the SPME technique with modifications to the method of Szkudlarz et al. (2003). Oil samples were placed in 20 mL vials, and adsorption of volatile compounds was achieved at 45°C for 50 min using divinylbenzene carboxen polydimethylsiloxane fiber (50/30 µm, 2 cm, DVB/CAR/PDMS, Supelco Inc., USA). Separation of aroma substances was carried out using a DB-HeavyWax column (60 m x 0.25 mm x 0.25 µm) on a Shimadzu GC-MS-QP2020 (Kyoto-Japan) mass spectrophotometer connected to a Shimadzu GC-2010 Plus (Kyoto-Japan) gas chromatograph. Injection temperature is 250°C. Oven temperature: It was brought from 40°C to 80°C by increasing 3°C per minute and held for 1 minute, and then it was brought to 240°C by increasing 5°C per minute and held for 6 minutes. The flow rate of helium used as carrier gas is 1.05 mL min^{-1}. Peaks; were identified by comparing with mass spectra in Wiley 7.0, NIST-98, and Flavor 2L libraries, and aroma compounds of olive oil samples were given as % peak area. Analyses were performed in triplicate.

Results and Discussions

Physical and chemical properties of natural olive oils

The physical and chemical properties of olive oils are among the factors that determine the quality of olive oil and are affected by the maturity level of the olive. The %FFA, acid, and peroxide values of early harvest natural olive oil were found to be significantly lower than natural olive oil (Table 1). This is an expected situation, as it is known that the oil content of early harvested olives is low, but the oil is of superior quality and is not subject to spoilage reactions. Free fatty acidity is an important factor in the classification of olive oils. According to the Turkish Food Codex Communique on Olive Oil and Pomace Oil, early harvest olive oil meets the 0.8% free fatty acidity criterion of natural extra virgin olive oil, while normal harvest olive oil exceeds this limit and is classified as natural first olive oil (Anonymous, 2017). According to the communique, the upper limit value of the peroxide number, which provides information about the degree of oxidation, is 20 meq O^2 kg^{-1}. This value was achieved in olive oils from both harvest periods. The number of free fatty acids and peroxides affected by enzymatic activities increases depending on harvest time and fruit maturity (Mele et al., 2018). FFA values of Kilis yağlık olive oils obtained from different years and locations varied between 0.33-0.86% and peroxide values between 2.33-6.85 meq O^2 kg^{-1} oil (Arslan and Özcan, 2014). Kıralan et al. (2009) reported the

FFA value of Kilis yağlık olive oil as 0.41% and the peroxide number as 6.24 meq O^2 kg^{-1} oil. Piscopo et al. (2018) found that the FFA value and peroxide number of olive oils obtained from the Grossa di Gerace variety were 0.39% and 5.77 meq O^2 kg^{-1} in the early harvest period; they reported it as 1.01% and 7.81 meq O^2 kg^{-1} during the normal harvest period. %FFA and peroxide values in the literature were found to be compatible with the study. Erdoğan (2020) supported the conclusion that ripening causes an increase in acidity by stating the FFA values of Kilis yağlık olive oil in 6 different maturity periods as 0.40, 0.55, 0.66, 0.58, 0.93, and 0.98%. FFA is produced by the oxidation of aldehydes contained in aroma substances. While these aldehydes decrease with ripening, free fatty acidity increases (Sadeghi et al., 2019). The results of the current study support this situation.

The iodine amount in olive oils was determined to be higher in early harvest. The iodine number in oils provides information about the saturation and unsaturation values of the oil. The decrease in iodine number is attributed to the destruction of double bonds as a result of oxidation and polymerization (Alireza et al., 2010). It is possible to say that early harvest olive oil has not been oxidized and thus has a higher iodine binding capacity. The refractive index of normal harvest olive oil was determined to be higher. The refractive index of oils is affected by the degree of saturation and the presence of conjugated double-bonded fatty acids (Arya et al., 1969).

Table 1. Physical and chemical properties of natural olive oils

	Early harvest natural olive oil	**Normal harvest natural olive oil**
FFA (oleic acid%)	0.26±0.00	1.54±0.00
Acid number	0.51±0.00	3.06±0.00
Peroxide value (meq O_2 kg^{-1})	5.43±0.31	11.29±1.01
Iodine number	85.80±4.02	79.74±4.54
Refractive index	1.4683±0.00	1.4696±0.00
L*	14.64±0.27	19.08±0.42
a*	1.96±0.10	2.42±0.15
b*	1.29±0.16	1.73±0.11
ΔC*	2.35±0.16	2.98±0.12
Hue°	33.34±2.54	35.65±2.73

L*, a*, b* values and ΔC* and Hue° results calculated from these values were determined to be higher in normal harvest natural olive oil. The increase in L* value indicates that normal harvest natural olive oil is lighter in color. A low a* value in early harvest olive oil indicates a greener oil color. A high b* value indicates that normal harvest natural olive oil is more yellow. These changes are attributed to the decrease in carotenoid and chlorophyll content with ripening in olives (Arslan and Özcan, 2014). Both color tone and color saturation were determined to be higher in normal harvest natural olive oil.

Piscopo et al. (2018), L*, a*, and b* values in olive oils were 7.67, 0.63, and 2.67, respectively, in the early harvest Carolea variety; 7.65, 0.52, and 2.45 in the normal harvest Carolea variety; 7.36, 0.83, and 3.19 in the early harvest Grossa di Gerace variety. They stated that the values were 7.81, 0.78, and 2.90 in the normal harvest Grossa di Gerace variety.

Bioactive Properties of Natural Olive Oils

Phenolic compounds, which eliminate the negative effects of free radicals, contribute to oxidative stability. Phenolic compounds, which are an important quality criterion in this respect, also affect color, flavor, and sensory properties. The total phenol content of early harvest natural olive oil was determined to be approximately six times higher than that of normal harvest natural olive oil (Table 2). Similar to the total phenol, the DPPH antioxidant capacity of early harvest natural olive oil was found to be much higher. It has been

stated in the literature that there is a parallelism between the total amount of phenolic substance and antioxidant capacity (Piscopo et al., 2018). It has been determined that early harvest has a high effect on the bioactive properties of natural olive oil. This is an expected situation, as there are differences in the composition of olives with early harvest.

Table 2. Bioactive properties of natural olive oils

	Early harvest natural olive oil	**Normal harvest natural olive oil**
Total phenol (mg GAE L^{-1})	516.61±3.74	77.70±2.27
DPPH (µmol TE mL^{-1})	787.80±4.93	280.27±1.92

Rotondi et al. (2004) reported that the phenol content of olive oils decreased with ripening, decreasing from 441 mg GAE kg-1 to 209 mg GAE kg-1. Skevin et al. (2003) determined the total phenol amount as 193-387 in early harvest olive oils and 91-173 mg caffeic acid kg-1 oil in normal harvest. Piscopo et al. (2018) reported that the amount of phenolic substance and antioxidant capacity in oils obtained from two different types of olives changed depending on the harvest time. In early harvest olive oils, the total phenol amount is 337 and 453 mg GAE kg-1 and DPPH capacity is 24.47 and 33.66%; in normal harvest olive oils, the total phenol amount is 269 and 353 mg GAE kg-1 and DPPH capacity is 16.68 and 23.13%. It is also supported by these studies in the literature that the total phenol amount decreases with fruit ripening. Total phenol amount of olive oilsvaries depending on the variety, harvest year, harvest time, processing, and growing conditions (Büyükgök and Saygın Gümüşkesen, 2017). It is thought that the differences in the amount of phenol substances in the literature are due to these factors.

Aroma compounds of natural olive oils

Volatile and semi-volatile compounds determine sensory characteristics, and aroma is a very important quality criterion for olive oil. A total of 29 different aroma compounds, including 9 aldehydes, 7 terpenes, 5 alcohols, 4 acids, 2 esters, 1 hydrocarbon, and 1 ketone were detected in early harvest natural olive oil, and 35 different aroma compounds, including 9 aldehydes, 8 terpenes, 6 alcohols, 5 acids, 4 esters, 2 ketones, and 1 hydrocarbon, were detected in normal harvest natural olive oil (Table 3).

Adehydes constitute the main aroma component group of olive oils (Kesen et al., 2013). The main aldehyde compounds in all oil samples were (E)-2-hexenal, hexanal, (E)-2-heptanal, and nonanal. A decrease is observed in many of the aldehydes produced with ripening in olives (da Silva et al., 2012). This decrease in aldehydes was also determined within the scope of the study. Since aldehydes are found in higher amounts in early harvest olive oils, green, grass, fruit, and raw odour are felt to be more dominant in these oils. Karagoz et al. (2017) support the study by stating that hexanal content decreases from 39.8% to 16.6% with ripening in olive oil. The ratio of hexanal/nonanal aldehydes gives information about the oxidation state. If this ratio falls below 2, it indicates that the oil is oxidized (Kesen et al., 2013). Both early harvest and normal harvest olive oils remained above the value of 2, showing that they were of good quality.

Volatile alcohols produced by the action of the alcohol dehydrogenase enzyme contribute significantly to the aroma of olive oil (Kesen et al., 2013). Alcohols found in high amounts in olive oils are (Z)-3-hexen-1-ol, 1-hexanol, and 1-penten-3-ol. The amount of volatile alcohol in olive oils increased with ripening. In a study conducted in Spain, the study was supported by reporting that the amount of 1-hexanol increased as the harvest time progressed (Gomez-Rico et al., 2009). Since 1-hexanol is formed by the transformation of hexanal, it is considered normal that aldehydes decrease and alcohols increase. Arapoglou (2010) reported that an increase in alcohol was observed

with ripening in oils obtained from Throumbolia and Koroneiki olive varieties.

Table 3. Aroma compounds of natural olive oils (%)

RT*	Aroma group	Aroma description	Aroma compounds	Early harvest natural olive oil	Normal harvest natural olive oil
11.441	Aldehyde	Grass, fruit	Hexanal	16.25±2.20	9.97±0.54
17.430	Alcohol	Green, vegetable	1-Penten-3-ol	1.81±0.15	2.25±0.03
18.886	Aldehyde	Green, leaf	(*E*)-2-Hexenal	19.30±4.26	16.82±1.10
20.453	Hydrocarbon	Fruit, grass	(*E*)-5-Octadecene	n.d.	1.82±0.45
20.868	Ketone	Plant, fruit, mushroom	3-Octanone	n.d.	1.98±0.26
21.933	Ester	Fruit, dessert, pear	Hexyl acetate	0.55±0.03	0.91±0.15
22.603	Aldehyde	Waxy, orange, herbaceous	Octanal	1.87±0.22	0.68±0.05
24.171	Aldehyde	Green, oily	(*E*)-2-Heptenal	9.80±2.10	6.69±0.50
24.807	Ketone	Grass, fresh, plant	Methyl heptenone	n.d.	1.77±0.11
25.390	Hydrocarbon	-	(*Z*)-1-Ethyl-2-methylcyclopentane	2.36±0.20	n.d.
26.350	Alcohol	Fruit, alcohol	1-Hexanol	5.03±0.75	5.39±0.06
26.572	Ester	Oily, green	1-Octen-3-ol acetate	n.d.	0.78±0.25
27.437	Alcohol	Green, fruit	(*Z*)-2-Hexen-1-ol	1.20±0.27	1.80±0.11
27.620	Alcohol	Grass, banana	(*Z*)-3-Hexen-1-ol	8.90±1.05	9.40±1.12
28.135	Aldehyde	Waxy, orange peel	Nonanal	1.97±0.96	2.88±0.51
27.970	Ester	Fruit, waxy	Hexyl butanoate	2.85±0.10	1.71±0.26
28.430	Aldehyde	Fruit, soap, oily	(*E*)-2-Octenal	0.30±0.05	0.15±0.10
29.026	Alcohol	Earthy, mushroom	1-Octen-3-ol	n.d.	0.73±0.41
29.678	Acid	Sour, vinegar	Acetic acid	3.47±0.56	4.98±0.54
30.645	Aldehyde	Fatty, vegetable	(*E,E*)-2,4-Heptadienal	2.40±0.10	0.40±0.08
31.665	Terpene	Woody, spice, honey	α-Copaene	3.23±0.58	3.05±0.13
32.037	Aldehyde	Oily, grass	(*E*)-2-Nonenal	0.10±0.00	0.09±0.08
32.209	Terpene	Citrus, flower	Linalool	2.03±0.73	1.77±3.56
32.674	Ester	Sweet, bergamot	Linalyl acetate	n.d.	0.94±5.47
33.275	Ketone	-	4-Methyl-3-octanone	2.72±0.06	n.d.
34.130	Terpene	Flower	Lavandulol	2.51±0.64	2.22±2.25
35.240	Aldehyde	Oily, earthy	(*E*)-2-Decenal	1.63±0.26	1.04±0.20
35.712	Terpene	Citrus, sweet	(*Z*)-β-Farnesene	0.30±0.17	0.29±0.10
35.775	Acid	Butter, sharp	Butanoic acid	1.94±0.40	2.50±0.72
36.588	Terpene	Flower	α-Terpineol	0.10±0.02	0.90±0.50
37.895	Terpene	Orange, lavender, green	α-Farnesene	1.10±0.11	0.67±0.20
38.178	Terpene	Flower, citrus	Citronellol	n.d.	1.74±0.45
40.185	Terpene	Flower, sweet	Geraniol	0.21±0.00	1.57±0.25
41.355	Acid	Sweet, sharp	Hexanoic acid	n.d.	1.98±0.14
41.955	Alcohol	Flower, rose, orchid	Phenylethyl alcohol	0.50±0.04	1.82±0.02
46.180	Acid	Oily, rancid	Octanoic acid	1.82±0.86	2.94±0.93
48.430	Acid	Cheese	Nonanoic acid	3.78±1.10	5.38±0.03

*RT: Retention time, n.d.: Not detected. Aroma descriptions taken from https://www.thegoodscentscompany.com/.

With maturation, the aroma composition of olive oil changes and undesirable volatile compounds increase. Butanoic, hexanoic, and acetic acid rates increase with autooxaidation and fermentation of olive oils (Gonzalez and Aporico, 2013). An increase in the rates of acids determined in the study was also observed. Volatile composition of olive oils is affected by

many factors such as variety, region, maturity level, harvesting and processing method, storage time and conditions, and aroma extraction method (da Silva et al., 2012). The differences between literatures arise from these factors.

Conclusions

This study was conducted to determine the physical, chemical, bioactive properties, and volatile compounds of olive oils obtained from the Kilis yağlık variety at different harvest times. Free fatty acidity and peroxide value were determined to be lower, and physical properties were superior in early harvest olive oil. It was observed that the total phenol and therefore the antioxidant capacity decreased with ripening. Aroma composition is an important quality criterion because it affects the sensory properties of olive oil. It has been determined that the rate of pleasant smelling compounds such as green, grass, fruits, and vegetables is higher in early harvest olive oil. It has been determined that undesirable aroma compounds occur or increase in olive oil with ripening. Olives need to be processed at the optimum harvest time, where they provide both high oil yield and high quality (high bioactive properties, desired aroma) oil that can be obtained from olives. It is thought that the harvest time of the study will be a reference to evaluate the quality criteria of olive oil.

Conflict of interest:

The authors declare that they have no competing interests.

Author contributions:

The authors have an equal contribution. All authors have read and agreed to the published version of the manuscript.

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Araştırma Makalesi/Research Article

Sütün kaynatılmasının süt miR-191 düzeyine etkisinin araştırılması

Investigation of the effect of boiling on the level of milk miR-191

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ÖZ

MikroRNA'lar (miRNA'lar), gen anlatımının düzenlenmesinde etkin rol oynayan ~22 bp uzunluğunda küçük, kodlanmayan RNA dizileridir. Son yıllarda yapılan araştırmalarda inek sütünde bol miktarda miRNA bulunduğu tespit edilmiş ve inek sütü miRNA'larının gıda kalitesinde biyobelirteç olarak kullanım potansiyellerine yönelik bulgular elde edilmiştir. Ayrıca, güncel araştırmalar beslenme yoluyla inek sütü miRNA'larının insana transfer olarak önemli bir biyoaktif besin komponenti olabileceğini göstermektedir. Süt ve süt ürünlerinde üretim aşamalarında bozunmadan kalan inek sütü miRNA'larının insanların dolaşım sistemine geçerek farklı insan hastalıkları ile ilişkili önemli yolaklara etki edebileceği düşünülmektedir. Bu sebepten süt ve süt ürünlerinin miRNA içeriklerinin belirlenmesi önemlidir ve bu konuda güncel literatürde önemli bir boşluk olduğu gözlenmektedir. Bu çalışmada, literatürden insan homolog sekansına sahip ve inek sütünde bol miktarda bulunan miR-191'in kaynatma aşaması sonrası içme sütündeki miktarındaki değişiklik araştırılmıştır. Bu kapsamda süt örnekleri (çiğ süt ve pastörize süt) 100°C'de kaynatılarak örneklerden total RNA izolasyonu gerçekleştirilmiş ve elde edilen RNA'lardaki miR-191 miktarı RT-qPCR yöntemi ile analiz edilmiştir. Literatürde içme sütünün üretiminde kullanılan homojenizasyon ve pastörizasyon işlemlerinin miRNA spesifik farklı etkilere sebep olduğu gözlenmiştir. Gerçekleştirilen işlemler sonucunda literatüre uyumlu biçimde miR-191 miktarında kaynatılmış çiğ sütte %95.8 oranında (p<0.0001) ve kaynatılmış pastörize sütte %66.4 oranında (p=0.001) azalma gözlemlenmiştir. Bunun yanında çiğ süt ve pastörize sütte analiz edilen miR-191 için elde edilen CT değerleri arasında istatistiksel olarak anlamlı bir farklılık olduğu gözlenmiştir (p<0.0001). Çalışmamızın sonucu, sütün kaynatılmasının süt miRNA içeriği üzerindeki etkilerine ilişkin ön veriler ortaya koyarak işleme adımlarının süt miRNA bileşimi üzerine miRNA spesifik etkisinin olduğunu önemle vurgulamaktadır.

Anahtar Kelimeler: miRNA, çiğ süt, pastörize süt, kaynamış süt, RT-qPCR, miR-191

ABSTRACT

MicroRNAs (miRNAs) are small non-coding RNA sequences ~22 bp in length that play an active role in cellular processes. Recent studies have identified miRNA abundance in cow's milk, highlighting their nutritional impact and their potential utilization as biomarkers of food quality. However, current research suggests that dietary intake of cow's milk miRNAs may transfer to humans and have nutritional relevance for human health by entering human circulation and affecting important pathways associated with human diseases. Therefore, it is crucial to determine the miRNA content in milk and dairy products. The miR-191 has a similar sequence in cows and humans, and it has been previously shown to abundantly exist in cow milk. Here, we aimed to investigate the effects of boiling to the miR-191 levels in milk. Total RNA was isolated from raw and milk

boiled at 100°C, and miR-191 levels in raw and boiled milk were analyzed by RT-qPCR method. Previous research reported that homogenization and pasteurization processes used in milk production stages have miRNA-specific distinct effects. After heat treatments, the amount of miR-191 was reduced by 95.8% (p<0.0001) in boiled raw milk and 66.4% (p=0.001) in boiled pasteurized milk compared to pasteurized milk. Meanwhile, we observed a statistically significant difference (p<0.0001) in the CT values obtained by quantification of miR-191 in raw and pasteurized milk. The results of our study present preliminary data for the effects of boiling milk on the milk miRNA content and point out the significance of miRNA-specific effects of milk processing steps on milk miRNA composition.

Key Words: miRNA, raw milk, boiled milk, pasteurized milk, RT-qPCR, miR-191

Giriş

MikroRNA'lar (miRNA), gen ekspresyonunu düzenleyerek çeşitli biyolojik süreçlerde yer alan, yaklaşık 18-22 nükleotid uzunluğunda kodlama yapmayan RNA türleridir. Son yıllarda yapılan araştırmalarda inek sütünden elde edilen eksozomal miRNA'ların süt ve süt ürünleri endüstrisinde biyobelirteç olarak kullanılabilmesinin yanında önemli bir biyoaktif besin içeriği olma ve insan hastalıklarına etki etme potansiyelleri önemli bir araştırma konusu olmuştur (Baier ve ark., 2014; Melnik ve ark., 2017; Sadri ve ark., 2020; Rani ve ark., 2017; Abou el qassim ve ark., 2022; Abou el qassim ve ark., 2023). Süt miRNA'larından immün sistemdeki genleri hedefleyenlerinin, inek sütü tüketimine bağlı olarak bağışıklık sisteminde rol oynayabilecekleri ortaya atılmıştır (Baier ve ark., 2014). Ek olarak, çalışmalar süt eksozomlarının çok farklı türde (kolon kanseri hücrelerine, bağırsak hücrelerine, böbrek hücrelerine, makrofajlara ve insan periferal kan mononükleer) hücrelere transfer olabildiğini bildirmiştir (Benmoussa ve ark., 2020; Rani ve ark., 2017; Izumi ve ark., 2015; Izumi ve ark., 2012). İnek sütünde bol miktarda miRNA bulunur ve süt işleme aşamalarının süt ve süt ürünlerindeki miRNA içeriğine etkisi olduğu bilinmektedir (Rani ve ark., 2017; Abou el qassim ve ark., 2023; Howard ve ark., 2015). Fakat, bu miRNA'ların fizyolojik stabilitelerini ve biyoaktif potansiyellerini ortaya çıkarmak için daha fazla araştırmaya ihtiyaç vardır (Rani ve ark., 2017). Toplumumuzda yapılan anket araştırmalarında çiğ sütün daha lezzetli olması, katkı maddesi içermediği düşüncesi, besin içeriğinin diğer sütlere kıyasla daha yüksek olduğunun düşünülmesi gibi sebeplerden dolayı toplumun farklı kesimlerinden insanların farklı kaynaklardan çiğ süt aldığı ve çiğ sütü ev ortamında kaynatıp yoğurt, tatlı ve süt olarak tükettikleri gözlemlenmiştir (Arslan ve ark., 2020; Sevim ve ark., 2021). Normal şartlarda sütün ev ortamında kaynatılması durumunda süt kaynama noktası olan 100°C'ye kadar çıkmakta ve yaklaşık bu sıcaklıklarda değişken sürelerde tutulmaktadır. Marketten satın alınan pastörize süt ise pastörizasyon aşamasında en az 72°C'de 15 saniye veya 63°C de 30 dakika işlem görmektedir (Türk Gıda Kodeksi İçme Sütleri Tebliğ No: 2019/12, https://www.resmigazete.gov.tr/eskiler/2019/02/20190227-5.htm). Literatürde bu sıcaklık ve süre aralıklarında işlenmiş sütte işlevsel ve sağlam miRNA'ların eksozomlar ve benzeri mikroveziküller sayesinde korundukları raporlanmıştır (Kirchner ve ark., 2016). Bu korunan mikroveziküllerin sindirim boşluğunda zarar görmeden kana karıştığı ve yapıları sayesinde içlerinde bulunan molekülleri etkili bir biçimde hücrelere ilettikleri gözlenmiştir (Melnik ve ark., 2017). Sıcaklığın miRNA'ların degredasyonları üstündeki etkileri konusunda literatürde Melnik ve ark., (2014) tarafından yapılan çalışmada, miRNA miktarının sıcaklıkla azaldığı raporlanmıştır. miRNA benzeri kompleks ikincil ve üçüncül yapıya sahip ve sütte bulunan proteinlerin, kaynatma işlemi sonrasında önemli ölçüde azaldığı bilinmektedir (Tremonte ve ark., 2014). Oh ve arkadaşlarının (2014) çalışmasında immün sistemle ilişkili miRNA'ların ısıya dirençleri araştırılmış ve miRNA'ların kısa süreli yüksek sıcaklık uygulamasına (75°C/15 sn) uzun süreli düşük sıcaklık uygulamasına (63°C/30 dk) göre daha fazla direnç gösterdiği gözlenmiştir. Isı işlemlerinin süt miRNA'larına etkisinin araştırıldığı güncel bir araştırmada da, işlenmemiş süte pastörizasyon (85°C/15 sn) ve ultra yüksek sıcaklık işlemi (135°C/15 sn) uygulanmış, süt miRNA'larının bu işlemlere miRNA spesifik olarak farklı cevaplar verdiği gözlenmiştir (Zhang ve ark., 2022). Yakın tarihte Li (2022) tarafından yapılan çalışmada sütte yüksek miktarda bta-miR-191'e rastlanmıştır. Yaptığımız biyoinformatik analizlerde bta-miR-191'in insan miRNA'sı olan hsa-miR-191-5p ile yüksek homoloji gösterdiği saptanmıştır (Çizelge 1).

Çizelge 1. miR-191'in insan ve inekteki sekansları.
Table 1. Sequences of miR-191 in human and cow.

miRNA *miRNA*	**miRbase ID** *miRbase ID*	**Sekans** *Sequence*
hsa-mir-191-5p	MIMAT0000440	CAACGGAAUCCCAAAAGCAGCUG
bta-mir-191	MIMAT0003819	CAACGGAAUCCCAAAAGCAGCUG

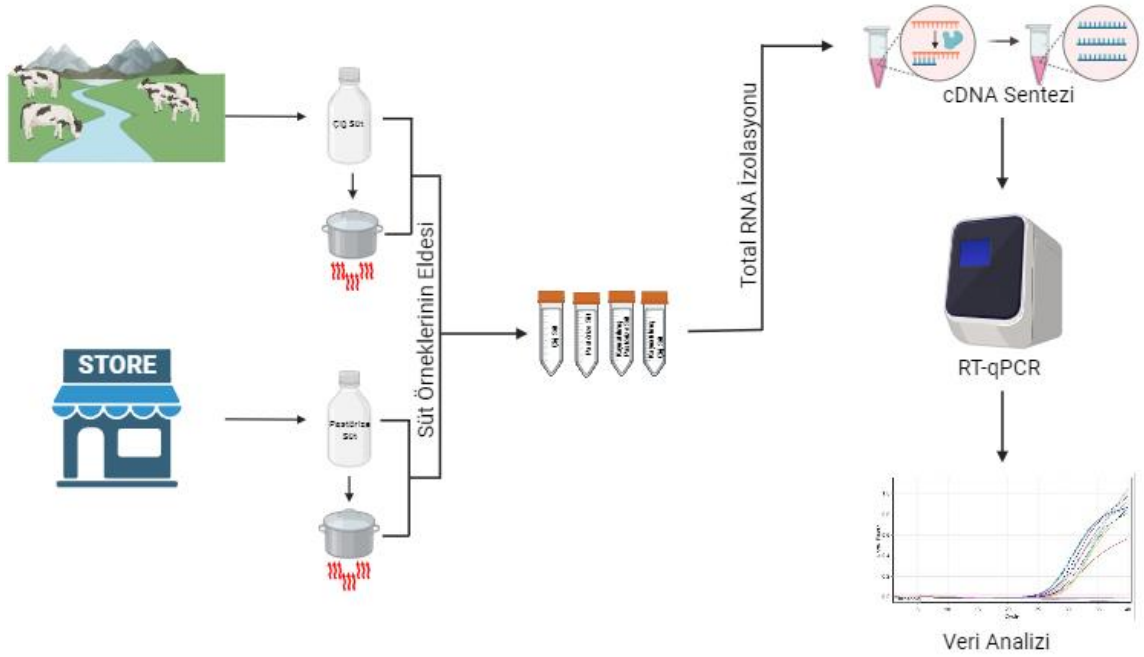
Hsa-miR-191-5p'nin farklı ekspresyon durumları literatürde insanlarda birçok kanser türü ile ilişkilendirildiği görülmüştür (Ashirbekov ve ark., 2020; Zhang ve ark., 2014; Tian ve ark., 2019; Polioudakis ve ark., 2015). Literatürde miR-191-5p sadece kanserle ilişkilendirilmemiş, farelerin nöronlarında *BDNF*'i hedefleyerek nörotoksisiteye karşı koruduğu, nöral hücre ölümünü tetiklediği ve farklı kardiyovasküler durumlarla ilişkilendiği de raporlanmıştır (Li ve ark.,2021; Wang ve ark., 2022; Yu ve ark., 2022; Licholai ve ark., 2021).

Bu sebeplerden dolayı süt ve süt ürünlerinin miRNA içeriklerinin öğrenilmesi ve bu miRNA'ların işlenme basamakları sonrasındaki miktarlarının tahmin edilmesi önemlidir. Bu çalışmada, toplum tarafından sık tercih edilen kaynaklardan elde edilmiş (çiftlik, seyyar satıcı, bakkal, market vb.) çiğ ve pastörize sütteki ve bu sütlerin en sık tüketilme şekli olan kaynatma sonrası sütte bol miktarda olduğu bilinen ve insan ile homolog olan miR-191'in miktarındaki değişim kantitatif olarak analiz edilmiştir. Literatür taramamızda sütün kaynatılması sonrası miR-191 seviyesinin incelendiği benzeri başka bir çalışma bulunamamıştır.

Materyal ve Metot

Süt örneklerinin eldesi

Çiftlikten ve marketten elde edilmiş çiğ süt ve pastörize sütün bir kısmı (≦ 50 ml) izolasyon işlemi için ayrılmıştır. Tremonte ve ark., (2014) tarafından kullanılan ev ortamında süt işlenmesi protokolüne göre süt ürünlerinin bir kısmı temizlenmiş behere aktarılarak düşük hızda manyetik karıştırıcılı ısıtıcı da köpük oluşumu gözlemlenen kadar (~30 dakika, 100°C) ısıtılmıştır. Isıtılmış sütün tekrardan oda sıcaklığına düşmesi beklenmiştir ve örnekler steril tüplere (≦ 50 ml) aktarılarak örneklerden Total RNA izolasyonu gerçekleştirilmiştir (Şekil 1).



Şekil 1. Çalışma iş akışı.
Figure 1. Workflow of the study.

Total RNA izolasyonu

Süt örneklerinden RNA izolasyonu için miRNeasy Serum/Plasma Advanced Kit (QIAGEN GmbH, Hilden, Almanya) kullanılmıştır. Başlangıç materyali olan 200 µl tam süt kullanılmış ve üretici firmanın protokolü takip edilmiştir. İzolasyon sonrası RNA saflığı ve miktar değerlendirmesi için Nanodrop 2000

Spektrofotometre (Thermo Fisher Scientific, Massachusetts, ABD), Qubit 4.0 (Thermo Fisher Scientific, Massachusetts, ABD) cihazları kullanılmıştır.

qRT-PCR yöntemi ile miRNA anlatım analizi

cDNA sentezi Sensiscript RT kit (QIAGEN GmbH, Hilden, Almanya) kullanılarak Thermal Cycler cihazında üretici firmanın protokolüne uyularak gerçekleştirilmiştir (Çizelge 2a). cDNA sentezi sonrası qRT-PCR yöntemi için LNA (Locked Nucleic Acid) ile geliştirilmiş, SYBR Green içeren miRCURY LNA miRNA kit ve miRCURY LNA miRNA PCR primerleri (QIAGEN GmbH, Hilden, Almanya) kullanılarak qPCR ve melting curve analizi gerçekleştirilmiştir. Tüm işlemler üretici firmanın protokolleri takip edilerek gerçekleştirilmiştir (Çizelge 2b).

Çizelge 2. cDNA sentezi (a) ve RT-qPCR (b) PCR şartları.
Table 2. PCR conditions for cDNA synthesis (a) and RT qPCT (b).

a)	**Sıcaklık** ***Temperature***	**Süre** ***Time***
Ters Transkripsyon ***Reverse Transcription***	42 °C	60 dk *min*
İnaktivasyon ***Inactivation***	95 °C	5 dk *min*
Tut ***Hold***	4 °C	∞

b)	**Sıcaklık** ***Temperature***	**Süre** ***Time***	
Başlangıç Aktivasyonu ***Initial Activation***	95 °C	2 dk *min*	
Denatürasyon ***Denaturation***	95 °C	10 sn *sec*	40 Siklus *Cycle*
Bağlanma/Uzama **Annealing/Extension**	56 °C	60 sn *sec*	
Erime Eğrisi ***Melt Curve***	60-95 °C		

Veri analizi ve yorumlanması

qRT-PCR analizi sonucunda elde edilen miRNA ekspresyon değerleri $2^{-\Delta\Delta CT}$ yöntemi ile normalize edilerek mir-191'e ait kat değişimi (Fold Change, FC) hesaplamaları yapılmıştır. CT değerlerinin normalizasyonu için sentetik cel-miR-39-3p CT değerleri referans alınmıştır. Analiz edilen miR-191 CT verileri paired ve unpaired t-test ile değerlendirilerek *p*-değerleri hesaplanmıştır. İstatistiksel analizler için SPSS programı (version 20.0) kullanılmıştır. *P*-değeri <0.05 istatistiksel olarak anlamlı kabul edilmiştir.

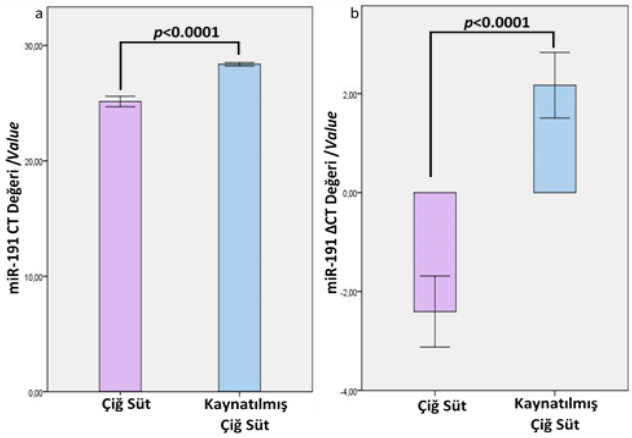
Araştırma Bulguları ve Tartışma

Kaynatma işleminin RNA miktarında yarattığı değişim

Farklı süt örneklerinin izolasyonu sonrası total RNA örnekleri Qubit HS (High Sensitivity) RNA assayi kullanılarak ölçülmüştür. Elde edilen total RNA miktarları, çiğ süt için 1.01 ng μl^{-1}, kaynatılmış çiğ süt için 0.758 ng μl^{-1}, pastörize süt için 0.42 ng μl^{-1} ve kaynatılmış pastörize süt için 0.582 ng μl^{-1} olarak gözlemlenmiştir. Daha önceki çalışmalarla kıyaslandığında kaynatılmış pastörize süt harici diğer örnekler literatürle uyumlu bulunmuştur.

Kaynatma işleminin miR-191 üstündeki etkisi

SPSS programında gerçekleştirilen analizler ile çiğ sütün kaynatılması sonrasında CT değerinde anlamlı bir artış (p<0.0001) gözlemlenmiştir (Şekil 2a). Çiğ süt ve kaynatılmış çiğ süt için cel-miR-39'un CT değerleri ile gerçekleştirilen normalizasyon sonrasında elde edilen ΔCT değerleri iki örnek arasında CT değerleriyle benzer anlamlı bir farklılık (p<0.0001) gözlemlenmiştir (Şekil 2b).



Şekil 2. miR-191 için çiğ süt ve kaynatılmış çiğ sütte tespit edilen Ct değerleri (a) ve ΔCt değerlerinin (b) karşılaştırılması.

Figure 2. Comparison of Ct values and ΔCt values detected in raw milk and boiled raw milk for miR-191.

$2^{-\Delta\Delta CT}$ yöntemi ile hesaplanmış kat değişimi sonucu kaynatılmış çiğ sütte, çiğ süte kıyasla miR-191 miktarında %95.8 oranında (p<0.0001) anlamlı bir azalma olduğu hesaplanmıştır (Çizelge 3).

Çizelge 3. Süt örnekleri için ortalama CT değeri ve miR-191 için gözlemlenen yüzdelik azalma.

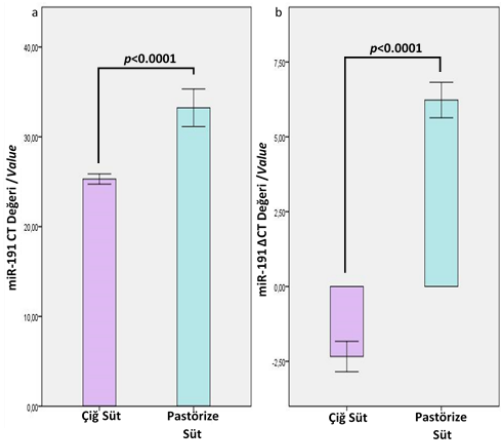
Table 3. Average CT values for mik samples and decrease percentages observed for miR-191.

	Ort. CT *Avr. CT*	**Kat Değişimi** *Fold Change*	**% Azalma** *Decrease %*	***p*-değeri** *p-value*
Çiğ Süt	25	1	-	-
Kaynatılmış Süt*	28	0.042	%95.8	p<0.0001
Pastörize Süt*	33	0.0025	%99.75	p<0.0001
Kaynatılmış Pastörize Süt**	31	0.336	%66.4	*P=0.001*

*Çiğ süt örneği ile kıyaslanmıştır.

** Pastörize süt örneği ile kıyaslanmıştır.

Çiğ süt ve pastörize süt kıyaslandığında CT ve ΔCT değerlerinde anlamlı bir fark olduğu gözlemlenmiştir (Şekil 3a/b). Kat değişim analizi ile çiğ ve pastörize süt örneklerinin miR-191 içeriğinde %99.75 miktarında anlamlı (p<0.0001) bir azalma bulunmuştur (Çizelge 3).

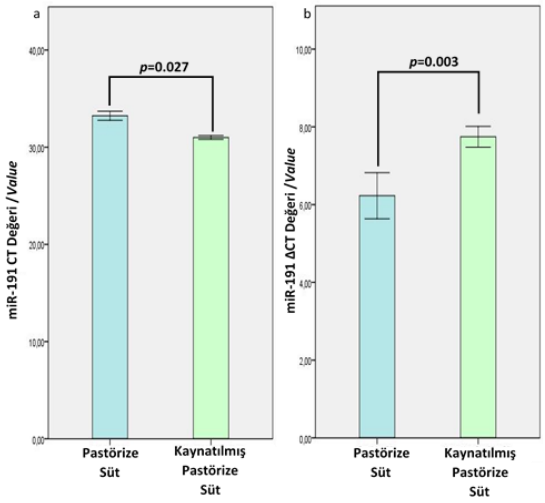


Şekil 3. miR-191 için çiğ süt ve pastörize sütte tespit edilen Ct değerleri (a) ve ΔCt (b) değerlerinin karşılaştırılması.

Figure 3. Comparison of Ct values and ΔCt values detected in raw milk and pasteurized milk for miR-191.

Normalizasyon öncesi pastörize süt ve kaynatılmış pastörize sütün CT değerlerinde beklenenin

dışında kaynatılmış pastörize sütün CT değerinde bir azalış gözlemlenmiştir (p=0.027) (Şekil 4a). Bu durum cel-miR-39 CT değerleri kullanılarak gerçekleştirilen normalizasyon sonrası elde edilen ΔCT değerlerinin analiz edilmesi sonrası literatürle uyumlu bir biçimde kaynatılmış pastörize sütte miR-191 içeriğinin düştüğünü göstermektedir (Şekil 4b).



Şekil 4. miR-191 için pastörize süt ve kaynatılmış pastörize sütte tespit edilen Ct değerleri (a) ve ΔCt (b) değerlerinin karşılaştırılması.

Figure 4. Comparison of Ct values and ΔCt values detected in pasteurized and boiled pasteurized milk for miR-191.

Pastörize sütü referans alarak gerçekleştirilen kat değişim analizleri sonucunda pastörize süt ile kaynatılmış pastörize süt arasında kaynatma işleminin sebep olduğu %66.4 oranından anlamlı (p=0.001) bir azalma gözlemlenmiştir (Çizelge 3).

Bu çalışma ve literatürdeki çalışmalar sonucunda miRNA'ların süt işleme süreçleri sonrası stabil kaldıkları görülmüştür. Çalışmamızda gerçekleşen işlemler (Kaynatma, pastörizasyon vb.) ile bağlantılı olarak miRNA miktarında beklenen bir azalma gözlenmiştir. Bununla birlikte işlemlerin sonunda kaynatılmış pastörize süt örneğinin dört tekrarının ikisi CT eşiğinin her ne kadar üstünde (>35) olsa dahi diğer iki örneğin RT-qPCR tekniği ile tespit edilebilen miRNA miktarına sahip olduğu görülmüştür.

Süt, süt ürünleri ve diğer gıdalarda bulunan miRNA'ların farklı mekanizmalar yardımıyla insan vücudunda bulunan farklı hücre tiplerine geçiş sağlayabileceği ve farklı patolojik durumlar oluşturabileceği daha önce gerçekleştirilen çalışmalar sonucunda gözlemlenmiştir (Baier ve ark., 2014, Benmoussa ve ark., 2020; Rani ve ark., 2017; Izumi ve ark., 2015; Izumi ve ark., 2012;). Yapılan çalışmalar sonunda süt ve süt ürünlerinin içeriklerinde miRNA'ların gıdalar işlenirken korunması eksozom benzeri partiküller ve Argonaute, Nucleophosmin 1 gibi farklı proteinlerin sayesinde olduğunu göstermektedir. Özellikle Argonaute protein 2'nin (Ago2) bu süreçte etkisinin olduğu ve hücre kültürü çalışmaları ile Ago2'nin bulunmadığı hücrelerde miRNA degradasyonun hızlandığı, Ago2'nin yüksek miktarda bulunduğu hücrelerde ise miRNA stabilitesinin artığı literatürde raporlanmıştır (Cieślik ve ark., 2023; Winter ve Diederichs, 2011). Domuz sütü ile yapılan bir çalışmada, miRNA'lar Ago2 proteinine bağlı olarak bulunduğu immünopresipitasyon assayleri ile gösterilmiştir (Zeng ve ark., 2021). Ago2, benzeri proteinler ve eksozomlar sayesinde gıda ürünlerinde bulunan miRNA'ların, ürünlerin geçtiği süreçlerin (pastörizasyon, homojenizasyon, kaynatma vb.) sonucunda tamamen degrade olmamalarının sebebi olabilir.

Yapılan çalışmalar sonucunda miR-191-5p ekspresyonun meme kanserinde prognozu etkilediği ve *C/EBPβ*'I hedefleyerek hücre döngüsünü bozup tümörogenezi tetiklediği, kolon kanserine sebep olabileceği, miR-191-5p'min *RXRA* ile etkileşerek prostat kanseri hücrelerine radyasyona karşı direnç verdiği ve hastaların tedavi sürecinde negatif etki yaratabileceği raporlanmıştır (Ashirbekov ve ark., 2020; Pan ve ark., 2023; Sharma ve ark., 2017; Zhang ve ark., 2014; Ray ve ark., 2015). miR-191-5p, 16 farklı kanser türünde [meme (kadın), kolon, akciğer, karaciğer, prostat, pankreas, mide, yumurtalık kanseri, hipofiz adenomu, özofagus skuamöz karsinomu, oral skuamöz karsinom, osteosarkom,

B-ALL, mesane, anaplastik büyük hücreli lenfoma ve akut myeloid lösemi (AML)] up regüle ve 6 farklı kanser türünde de (Şiddetli medulloblastom, retinoblastom, tiroid foliküler tümörü, erkek meme kanseri, CALL ve melanom) down regüle olduğu raporlanmıştır (Nagpal ve Kulshreshtha, 2014). Kanser haricinde İnsan Bağışıklık Yetmezliği Virüsü (HIV) ile enfekte kişilerde miR-191-5p'nin *NUP50* geninin ekspresyonu inhibe ederek HIV enfeksiyonun ilerleyişini yavaşlatabileceği gösterilmiştir (Zheng ve ark. 2021). Sütün içinde bol miktarda bulunan miR-191-5p'nin sebep olabileceği veya pozitif/negatif etkileyeceği durumlar söz konusudur. Literatürde gözlemlendiği üzere farklı miRNA'ların aynı işlemlere verdikleri cevaplar farklıdır. Gerçekleştirilmiş olan çalışmada tek bir miRNA'nın miktar analizleri incelenmiş olsa da literatürdeki farklı miRNA'lar ile kıyaslandığında gerçekleştirilen işlemler sonucunda hala ölçülebilecek miktarda miR-191'nin var olduğu gözlemlenmiştir. Analizlerin sonucu miRNA miktarındaki değişikliklerin gerçekleşen işlemler kaynaklı olabileceğini ortaya koymakla beraber analizler için ana örnekten ayrılan süt örneklerinin miRNA içeriklerinin homojen olmama ihtimali ve dış faktörlerin (sütün sağılma zamanı, tutulduğu sıcaklık, taşındığı materyallerin nükleaz içeriği vb.) de sonuçlara etki etme potansiyeli mümkündür.

SONUÇLAR

Sonuç olarak çiğ süt tüketiminin Hastalık Kontrol ve Korunma Merkezleri'ne [Centers for Disease Control and Prevention (CDC)] (https://www.cdc.gov/) göre Campylobacter, Cryptosporidium, E. coli, Listeria, Brucella ve Salmonella gibi farklı patojenik mikroorganizmaları bulundurmakla birlikte insana geçmesi durumunda farklı patolojiler ile ilişkili olduğu bilinen miR-191 ve benzeri miRNA'ları bol miktarda da içermektedir. Kaynatma ve benzeri süt işleme yöntemleri ile sütte bulunan miRNA'ların miktarlarının azaldığı hem gerçekleştirmiş olduğumuz çalışmada hem de literatürde farklı miRNA'lar için gözlemlenmiştir. Sonuçlarımız literatürdeki çiğ sütün ev ortamında kaynatılması ve bu durumun farklı miRNA dizilerine etkisi ile ilişkisine yönelik daha çok araştırma yapılması gerektiğini ortaya koymuştur. Elde edilebilecek bulgular ile farklı besinsel miRNA'ların gıdalardaki ve bu gıdaların işlenmesi sonrasındaki miktarlarının aydınlatılması üstünde oluşturulacak literatür kişiselleştirilmiş tedavi ve tamamlayıcı tıp alanlarında önemli olabileceği öngörülmektedir.

EKLER

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Çıkar Çatışması: Yazarlar arasında herhangi bir çıkar çatışması bulunmadığını beyan ederiz.

Yazar Katkısı: Proje yönetimi, danışmanlık Dilek Pirim; verilerin oluşturulması, işlenmesi, makale yazımı ve düzenlenmesi Fatih Atilla Bağcı ve Dilek Pirim tarafından gerçekleştirilmiştir.

Etik Kurulu Kararı: Bu çalışmada etik kurul onayına ihtiyaç yoktur.

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Araştırma Makalesi/Research Article

Zeytin yaprağı özütünün tereyağı, ayçiçek yağı ve patates cipsinde lipit oksidasyonu üzerindeki etkileri

Effects of olive leaf extract on lipid oxidation in butter, sunflower oil and potato chips

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ÖZ

Lipit oksidasyonu, yemeklik yağlarda duyusal kalite kusurlarına neden olmakla birlikte tüketici sağlığı açısından da risk oluşturan bir bozulma tipidir. Bu çalışmada, zeytin yaprağından elde edilen bir özüt (ZYÖ) ile sentetik bir antioksidan olan bütillenmiş hidroksitoluenin (BHT) tereyağı, ayçiçek yağı ve patates cipsindeki antioksidan özellikleri karşılaştırılmıştır. Bu amaçla, her iki antioksidan farklı konsantrasyonlarda (50, 100, 200 ppm) tereyağına ve ayçiçek yağına ilave edilmiş, bunlardan ayçiçek yağı kızartma yöntemiyle (170±5°C) patetes cipsi üretiminde kullanılırken tereyağı ise herhangi bir ısıl işleme tabi tutulmamıştır. Hızlandırılmış raf ömrü testinden sonra oksidasyon parametreleri olarak örneklerin tiyobarbitürik asit (TBA) içerikleri ve peroksit sayıları (PS) belirlenmiştir. Ayrıca, 2,2-difenil-1-pikrilhidrazil (DPPH) radikali giderme aktivitesine dayalı olarak ZYÖ ile BHT'nin toplam antioksidan kapasitleri (TAK) de karşılaştırılmıştır. ZYÖ ilave edilmiş tereyağı, ısıl işlem görmüş ayçiçek yağı ve patates cipsinin oksidasyon göstergeleri herhangi bir antioksidan bileşen eklenmemiş örneklerinkine (kontrol) nazaran önemli oranda düşük bulunmuştur ($P<0.05$). 50, 100 ve 200 ppm kosantrasyonlarında BHT eklenmiş tereyağlarında, aynı konsantrasyonlarda ZYÖ ilave edilmiş örneklere göre sırasıyla %48,92, %47,76 ve %50,97 oranında daha düşük miktarlarda TBA oluşmuştur. Benzer oranlar, tereğının PS değerleri için de saptanmıştır. Ayçiçek yağı örneklerinde ise ZYÖ eklenmiş örneklerin TBA değerleri BHT eklenmiş örneklerin ve kontrol örneğinin TBA değerlerine göre daha düşük bulunmuştur ($P<0.05$). Ancak, 100 ve 200 ppm'lik konsantrasyonlarda BHT ve ZYÖ kullanımı bu yağın PS düzeyi için anlamlı bir fark oluşturmamıştır ($P>0.05$). ZYÖ eklenmiş patates cipslerinin TBA içerikleri BHT'li örneklerinkine göre önemli düzeyde daha düşük bulunmuştur ($P<0.05$). TAK bakımından ise ZYÖ (79,12 mg TE (troloks eşdeğeri) g^{-1}) BHT'ye (67,39 mg TE g^{-1}) nazaran daha yüksek değer sergilemiştir ($P<0.05$). Çalışmanın sonuçları, doğal bir antioksidan olarak ZYÖ'nun lipit oksidasyonlarının önlenmesinde BHT'ye alternatif olabileceğini göstermiştir.

Anahtar Kelimeler: Zeytin yaprağı özütü, oksidasyon, antioksidan, patates cipsi, tereyağı

ABSTRACT

Lipid oxidation causes sensory quality defects in edible oils and also poses a health risk. In this study, the antioxidant properties of an extract obtained from olive leaves (OLE) and butylated hydroxytoluene (BHT), a synthetic antioxidant, were compared in butter, sunflower oil, and potato chips. For this purpose, both antioxidants were added to butter and sunflower oil at different concentrations (50, 100, 200 ppm). Sunflower oil was used in the production of potato chips at 170±5 °C, while butter was not subjected to any heat treatment. After the accelerated shelf life test, the oxidation parameters of the samples were determined by measuring thiobarbituric acid (TBA) and peroxide values (PV).

Additionally, the total antioxidant capacities (TAC) of OLE and BHT were compared based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The oxidation parameters of OLE added butter, heat-treated sunflower oil, and potato chips were found to be significantly lower than those of the samples without any antioxidant component added (control) ($P<0.05$). In butters with BHT added at 50, 100, and 200 ppm concentrations, the formation of TBA was reduced by 48,92%, 47,76%, and 50,97%, respectively, compared to the samples with OLE added at the same concentrations. Similar rates were also determined for PV of butter. In sunflower oil samples, the TBA values of samples with OLE added were found to be lower ($P<0.05$). However, the use of BHT and OLE at concentrations of 100 and 200 ppm did not result in a significant difference in the PV of this oil ($P>0.05$). The TBA contents of potato chips with OLE added were found to be significantly lower than those of samples with BHT ($P<0.05$). In terms of TAC, OLE (79,12 mg TE (trolox equivalent) g^{-1}) exhibited a higher value than BHT (67,39 mg TE g^{-1}) ($P < 0.05$). The results of the study showed that OLE, as a natural antioxidant, could be an alternative to BHT in preventing lipid oxidation.

Key Words: Olive leaf extract, oxidation, antioxidant, potato chips, butter

Giriş

Gıdaların üretimi ve depolanma süreçlerinde en yaygın bozulma tiplerinden biri de yağların oksidasyonuyla sonuçlanan reaksiyonlardır. Özellikle, kızartma gibi yüksek sıcaklıklarda gerçekleşen ısıl işlemler yağlarda oksidasyon, hidroliz ve polimerizasyon gibi bozulma reaksiyonlarına neden olmaktadır (Jiménez ve ark., 2017). Bu reaksiyonlarla oluşan bileşikler, yağlarda duyusal kusurlara neden olmakla birlikte onların raf ömürlerini de kısaltmaktadır. Diğer taraftan, termal oksidasyon bileşikleri diyabet, nörodejeneratif hastalıklar, aterogenez, inflamasyon ve kanser gibi hastalıklar açısından risk oluşturabilmektedir (Romojaro ve ark., 2013; Wu ve ark., 2019).

Gıda sektöründe, ısıl uygulamalardan kaynaklı olarak yağlarda meydana gelen oksidasyonunun önlenmesinde çoğunlukla sentetik antioksidanlar kullanılmaktadır. Bu amaçla en yaygın kullanılan antioksidanlar bütillenmiş hidroksitoluen (BHT), bütillenmiş hidroksianisol (BHA), tert-bütilhidrokinon (TBHQ) ve propil gallattır (PG). Bu antioksidanların, düşük sıcaklıklarda etkili olabildikleri ancak, kızartma gibi daha yüksek sıcaklık (~180 °C) gerektiren uygulamalarda kararsız yapılarından ötürü etkinliklerini yitirebildikleri bildirilmektedir (Esposto ve ark. 2015; Wu ve ark., 2019). Ayrıca, sentetik antioksidanların insan sağlığı üzerindeki olası zararları da gıda sektöründeki kullanımlarının sınırlandırılmasına ilişkin bir gerekçe olarak kabul görülmeye başlanmıştır (Farag ve ark., 2007). Bu nedenle, derin kızartma olarak bilinen yüksek sıcaklıklardaki ısıl işlemlere karşı termal dayanımı yüksek ve sağlık için tehlike yerine, fayda sağlayabilecek doğal antioksidanların kullanımı daha etkin bir yaklaşım olarak önem arz etmektedir. Bu amaçla, üzerinde en çok durulan ürünlerden biri de zeytin yaprağı olup son yıllarda bu yaprağın antioksidan özellikleriyle ilgili yapılan bilimsel çalışmalar yoğunluk kazanmıştır. Zeytin yaprağı, yüksek polifenol içeriğinden ötürü hem gıdaları oksidasyona karşı koruma hem de çeşitli hastalıklar açısından tüketici sağlığını koruma potansiyeline sahiptir (Difonzo ve ark., 2021; Wu ve ark., 2019).

Literatürde, zeytin yaprağının antioksidan rolleri dahil olmak üzere bazı gıdalar üzerindeki etkilerini araştıran çeşitli çalışmalar bulunmaktadır. Fakat, bu çalışmalarda yaprağın kullanılan formu (özüt veya tüm) ve dozu, çalışılan zeytinin çeşidi ve uygulanan gıda veya yağ türü gibi farklılıklara bağlı olarak elde edilen sonuçlar geniş bir farklılık sergilemektedir. Jiménez ve ark. (2017), zeytin ve avokado yapraklarının patates kızartmasında kullandıkları ayçiçekyağı ve kanola yağları üzerindeki antioksidan etkilerini karşılaştırmışlar. Çelik ve ark. (2021), zeytin yaprağı ilavesinin zeytinyağının kalite özelliklerinin pozitif etkilendiğini aktarmaktadır. Benzer bir çalışmada, yabani zeytin (delice) yaprağının zeytinyağının fonksiyonel özelliklerine etkileri konu edinmiştir (Baccouri ve ark., 2022). Diğer bir çalışmada, pirina özütü ilave edilmiş palm yağı, zeytinyağı ve ayçiçek yağlarının kızartma sonrasında özütten kaynaklı olarak önemli oranda polifenol içerdiği belirlenmiştir (Orozco-Solano ve ark., 2011). Macit ve Kizil (2022), pişirilmiş somon balığında zeytinyaprağı özütü kullanımının heterosiklik aromatik amin oluşumunu azaltabildiğini

belirtmişlerdir.

Belirli yağlarda zeytin yaprağı özütünün kullanımı ve antioksidan özelliklerinin belirlenmesinde, uygun doz ve sürdürülebilirlik açısından daha fazla çalışmaya ihtiyaç duyulmaktadır.. Bu çalışmada, ZYÖ ve BHT'nin tereyağı ve ayçiçek yağlarında oksidasyon önleyici etkilerinin karşılaştırılması amaçlanmıştır. Çalışmada, ayrıca, ZYÖ ve BHT eklenmiş ayçiçek yağıyla kızartılan patates cipslerindeki oksidasyon düzeyinin belirlenmesi de hedeflenmiştir.

Materyal ve Metot

Zeytin yaprağı özütü

Zeytin yaprağından özüt üretiminde, Korkmaz, (2023) tarafından uygulanan metot biraz değiştirilerek kullanılmıştır. Taze olarak toplanmış (200 g) zeytin yaprakları, oda koşullarında (20-25 °C) bir tepsi üzerinde serilerek kurutulmuş ve bir baharat öğütücü (Premier PRG 259; İstanbul, Türkiye) yardımıyla toz haline getirilmiştir. Bu tozdan 10 g tartılarak bir erlene alınmış ve üzerine 90 mL metanol-su karışımı (80:20 v/v) eklenerek bir homojenizatörde (Ultra-Turrax T25 Basic, IKA, Staufen, Almanya) 7000 rpm hızda 60 s süreyle homojenize edilmiştir. Karışım, 50 mL'lik falkon tüplere alınarak soğutmalı bir santrifüjde (Universal 320 Hettich, Westphalia, Almanya) 5000 rpm hızda 5 dk süreyle 10 C°'de santrifüj edilmiştir. Üst faz alınarak dipte kalan çökeltiyle ektraksiyon iki kez daha tekrarlanmıştır. Toplanan üst fazlar bir evaporatörde (Heidolph, Schwabach, Almanya) 900 mmHg vakum altında 33 °C tutularak metanol uçurulmuştur. Elde edilen ZYÖ, kapalı bir tüp içerisine alınarak çalışmadaki yağlarda kullanılıncaya kadar -18 °C' de muhafaza edilmiştir.

Patates cipsi

Cips üretiminde kullanılan patatesler ve ayçiçek yağı yerel bir manavdan temin edilmiştir. Patatesler (~1 kg) yıkanıp kabukları soyulduktan sonra 2-3 mm kalınlık ve 3-5 cm çapındaki ince dilimler halinde kesilmiş ve örnekleme amacıyla bu dilimler 7 eşit partiye bölünmüştür. Bu arada, yeteri kadar ayçiçek yağı (200 mL) bir tavaya konulmuş ve üzerine gerekli konsantrasyonda ZYÖ (veya BHT) ilave edilerek manyetik karşıtırıcıda 1 saat boyunca oda sıcaklığında karıştırılmıştır. Sonra, yağ bir ocakta 170±5 °C'ye kadar ısıtılıp patates dilimleri yağın içine bırakılmış ve cips kıvamına gelinceye kadar (2 dk) kızartılmıştır. Her bir patates partisinin kızartılmasında tavadaki yağ yenisiyle değiştirilmiştir. Ayrıca, antioksidan madde olarak ZYÖ veya BHT her seferinde farklı konsantrasyonda (50, 100 ve 200 ppm) kullanılmıştır. Kontrol amacıyla bir parti cipsin üretiminde herhangi bir antioksidan bileşen kullanılmamıştır.

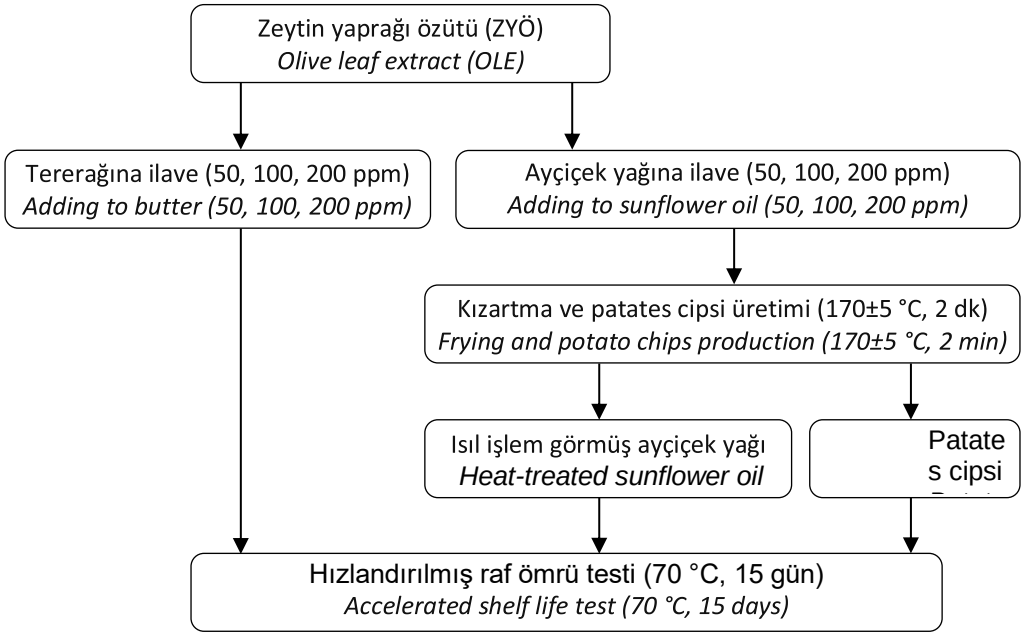
Tereyağı

Yerel bir marketten temin edilen tereyağı (2 kg) 40 °C'de eritilerek 7 eşit kısma bölünmüş ve cips üretiminde uygulandığı gibi, her bir kısma üçer farklı konsantrasyonlarda olacak şekilde ZYÖ ve BHT ilave edilerek manyetik karıştırıcı (38 °C) ile 1 saat karıştırılmıştır. Kontrol örneğinde antioksidan maddeler ilave edilmemiştir.

Hızlandırılmış raf ömrü testi (oksidatif stabilite)

Tereyağı, elde edilen patates cipsleri ve patates cipslerinin üretiminde kullanılan kızartılmış ayçiçek yağı örnekleri hızlandırılmış raf ömrü testine tabi tutulmuştur. Bu test, Zhang ve ark. (2016) tarafından kullanılan metot biraz değiştirilerek uygulanmıştır.

Ayçiçek yağı ve tereyağı örneklerinden 100'er g, cips örneklerinden ise 50'şer g tartılıp alüminyum kaselere alınmıştır. Daha sonra, kaselerdeki örnekler 70 °C'ye ayarlanmış ve oksijen gazıyla beslenen bir etüvde (Memmert UN55, Schwabach, Almanya) 15 gün boyunca bekletilmiştir. Bu süre sonunda çalışma örnekleri kapalı tüplere alınarak analizlerde kullanılıncaya kadar -18°C'de muhafaza edilmiştir. Üretimler iki tekerrürlü olarak gerçekleştirilmiştir. Çalışmanın deneysel tasarımına ilişkin akış şeması Şekil 1'de gösterilmiştir.



Şekil 1. Çalışmanın deneysel tasarımına ait akış şeması (ZYÖ: Zeytin yaprağı özütü)
Figure 1. Flow chart of the experimental design of the study (OLE: Olive leaf exract)

Tiyobarbitürik asit (TBA) analizi

Örneklerin TBA analizi, Kamacı (2021) tarafından kullanılan metoda göre yapılmıştır. 10 g numune tartılarak 50 mL'lik bir balona aktarılmıştır. Üzerine 47,5 mL saf su ilave edilerek yaklaşık 2 dk süresince karıştırılmıştır. Ardından, bu karışıma 4 M'lık HCl'den 2,5 mL ilave edilerek pH değeri 1,5'e ayarlanmıştır. Kaynama sırasında köpük oluşumunu engellemek amacıyla balon içine birkaç tane sünger taşı konularak balon damıtma düzeneğine yerleştirilmiştir. Damıtma hızı, 10 dk içerisinde 50 mL distilat elde edilecek şekilde ayarlanmıştır. Oluşan distilatlardan 5 mL alınarak ayrı ayrı tüplere aktarılmış, her birinin üzerine %90'lık (v/v) glasiyel asit içerisinde hazırlanmış TBA çözeltisinden (0,02 M) 5 mL eklenerek iyice karıştırılmış ve ağızları sıkıca kapatılmıştır. Kör (şahit) çözelti için distilat yerine 5 mL saf su kullanılmıştır. Tüpler kaynayan bir su banyosunda 35 dk sürecince tutulmuş ve akabinde 10 dk soğuk suda bekletilerek soğuması sağlanmıştır. Daha sonra, bir UV-spektrofotometresi (Biochrom Libra S70 Dual) kullanılarak çözeltinin absorbansı kör örneğe karşı 538 nm'de okunmuştur. TBA miktarı eşitlik 1'e göre hesaplanıp mg malonaldehit (MDA) kg^{-1} olarak ifade edilmiştir.

$$TBA\ (mg\ MDA\ kg^{-1}) = 7{,}8\ x\ A \qquad (1)$$

Burada; A, 538 nm'deki absorbans değeri; 7,8 ise absorbans değerini kg örnek başına mg MDA'ya dönüştürmek için kullanılan sabit sayıdır.

Peroksit sayısı (PS) analizi

PS, Uluslararası Zeytin Konseyi'nin (IOC, 2017) zeytinyağı için belirlenen resmi yöntemine göre belirlenmiştir. 3 g örnek tartılıp bir erlene alınmış ve üzerine 10 mL kloroform ilave edilerek hızlıca karıştırılmıştır. Sonra, üzerine 15 mL asetik asit ve 1 mL potasyum iyodür ilave edilerek erlenin ağzı sıkıca kapatılmış ve iyice karıştırılmıştır. 1 dk bekleme süresinden sonra, erlenin kapağı açılıp üzerine 75 mL saf su ilave edilmiş ve karıştırılıp indikatör olarak %1'lik nişasta çözeltisinden 2-3 damla ilave edilmiştir. Daha sonra, karışımın rengi açılıncaya kadar 0,01 N'lik sodyum tiyosülfat ile titrasyon yapılarak PS eşitlik 2'ye göre hesaplanmıştır.

$$PS\ (meqO_2\ kg^{-1}) = (V\ x\ T\ x\ 1000)/m \qquad (2)$$

Burada: V, harcanan sodyum tiyosülfat çözeltisinin hacmi (mL); T, sodyum tiyosülfat çözeltisinin molaritesi (mol L^{-1}); m, tartılan örnek miktarını (g) ifade eder.

Toplam antioksidan kapasitesi (TAK)

ZYÖ ve BHT'nin TAK değerleri, DPPH radikali

giderme kapasitelerinin ölçülmesiyle belirlenmiştir. TAK analizi, Korkmaz (2023) tarafından kullanılan metot biraz modifiye edilerek yapılmıştır. Stok çözeltiler için BHT ve ZYÖ'nün herbirinden 10 mg tartılarak ayrı deney tüpleri içerisine alınmış ve her birinin üzerine 10 mL metanol eklenerek çözülmeleri sağlanmıştır. Öncelikle ZYÖ ve BHT için IC_{50} değerleri hesaplanmıştır. IC_{50}, başlangıçtaki DPPH radikallerinin %50'sini gidermek için gereken antioksidan maddenin konsantrasyonudur. Bu amaçla, stok çözeltilerden seyreltme yoluyla BHT ve ZYÖ örneklerinin herbirinden dört farklı (0.25, 0.125, 0.0625 ve 0,03125 mg mL^{-1}) konsantrasyonlarda çözeltiler elde edildi. Daha sonra, 3 mL antioksidan çözelti üzerine 1 mL 60 µM DPPH solüsyonu ilave edilerek iyice karıştırılmış ve 30 dk karanlıkta bırakılmıştır. Sonra, bir UV-spektrofotometresinde 517 nm'de absorbans ölçümü yapılarak DPPH giderme oranı eşitlik 3'e göre hesaplanmıştır. Kontrol örneği olarak sadece DPPH çözeltisi kullanılmıştır. Sonuçlar mg TE kg^{-1} olarak ifade edilmiştir.

$$DPPH\ (\%) = [\frac{(Kontrol_{abs} - Örnek_{abs})}{Kontrol_{abs}}]x\ 100 \qquad (3)$$

Burada: $Kontrol_{abs}$ ve $Örnek_{abs}$, sırasıyla kontrol ve örneğin absorbans değerini ifade etmektedir. Troloksa ait IC_{50} değerinin hesaplanmasında bu bileşiğin farklı konsantrasyonlardaki (1, 0.5, 0,25, 0.125, 0.0625 mg mL^{-1}) çözeltileri kullanılmıştır.

İstatistiksel analizler

TBA ve PS analizlerinden elde edilen veriler arasındaki farkların önemi tek yönlü varyans analizi (ANOVA) ile belirlendi. Ortalamalar arasındaki farklar için çoklu karşılaştırma testi olarak Duncan testi uygulanmıştır. Ayrıca, TAK verileri arasındaki farkların önemi için ise bağımsız t testi yapılmıştır. İstastistiksel analizler SPSS paket programı (IBM SPSS Statistics 24 for Windows, IBM, New york, ABD) kullanılarak yapılmıştır.

Araştırma Bulguları ve Tartışma

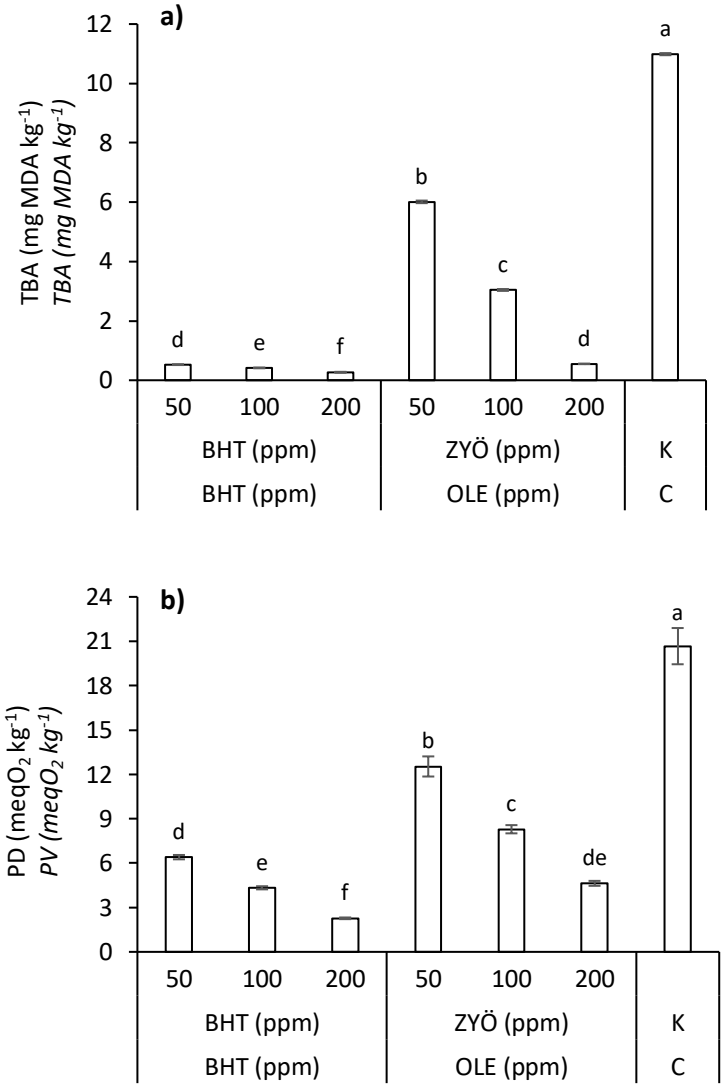
Tereyağının TBA ve PS sonuçları

TBA reaktif bileşikleri, lipit peroksidasyonunun yan ürünleri olarak oluşmakta ve gıdalardaki lipit oksidasyonu için bir belirteç olarak kullanılmaktadır (Zhang et al., 2016). Raf ömrü testi sonunda tereyağında belirlenen TBA ve PS ortalamaları Şekil 2'de gösterilmiştir. Kontrol örneğine göre, ZYÖ ve BHT'nin ilave edildiği tereyağı örneklerinde TBA ve PS'nin belirgin olarak daha düşük olduğu tespit edilmiştir ($P<0.05$). Ancak, 50, 100 ve 200 ppm konsantrasyonları için ZYÖ eklenmiş tereyağlarının TBA düzeylerinin BHT eklenmiş örneklere göre sırasıyla 11,33, 7,23 ve 2,03 kat daha yüksek olduğu belirlenmiştir ($P<0.05$).

Öte yandan, tereyağına ilave edilen antioksidan maddelerdeki konsantrasyon artışı ile TBA değerlerindeki değişim incelendiğinde, ZYÖ konsantrasyonundaki artışın TBA değerinde kademeli olarak azalmaya neden olduğu görülmektedir (Şekil 2a). Nitekim, ZYÖ'nün tereyağındaki konsantrasyonunun 50'den 200 ppm seviyesine artırılması TBA değerini yaklaşık 11 kat düşürürken, BHT'nin aynı orandaki artışı için TBA değerini 1.96 kat azaltmıştır. Demirkaya (2013), piyasadan temin ettiği 50 farklı tereyağı örneğinin TBA düzeylerinin 0,078-0,236 mg MDA kg^{-1} arasında olduğunu bulmuştur.

Tereyağının BHT ve ZYÖ içeriklerindeki artışa bağlı olarak PS değerinde meydana gelen azalma oranları her iki antioksidan için benzerlik göstermiştir (Şekil 2b). Ancak, aynı konsantrasyonlar için BHT, ZYÖ'ye göre tereyağının PS düzeyini daha fazla oranda düşürmüştür. Her üç farklı konsantrasyona ait ortalama PS, BHT ve ZYÖ için sırasıyla 4,33 ve 8,45 $meqO_2\ kg^{-1}$ olarak belirlenirken, kontrol örneğinde bu değer 20,67 $meqO_2\ kg^{-1}$ olarak tespit edilmiştir ($P<0.05$). Önceki çalışmalarda da tereyağına çeşitli bitkisel ürünler olarak çay özütü (Gramza-Michalowska ve ark. 2007), kurutulmuş limon (Maqbool ve ark., 2023) ve hurma çekirdeği tozu (Mansour ve Sindi, 2024) ilavesinin antioksidan etki gösterdiği rapor edilmektedir. ZYÖ veya diğer bitkilerin yağlarda TBA ve PS değerini düşürmeye dayalı antioksidan rolleri içeriklerindeki doğal antioksidanlardan

kaynaklanmaktadır. Zeytin yaprağında güçlü antioksidan etkiye sahip fenolik maddeler olarak oleuropein, ligstrosit ve bunlardan türevlenen hidroksitorosol, tirosol, oleokantal ve oleasein gibi bileşikler bulunmaktadır (Nikou ve ark., 2022).



Şekil 2. Tereyağının TBA (a) ve PS değerleri (BHT: Bütillenmiş hidroksitoluen, ZYÖ: Zeytin yaprağı özütü, K: Kontrol, TBA: Tiyobarbitürik asit, PS: Peroksit sayısı, MDA: Malondialdehit. $^{a-f}$ Aynı sütunda farklı küçük harflerle gösterilen ortalamalar arasındaki farklar istatistiksel olarak anlamlıdır (P<0.05))

Figure 2. TBA values (a) and PV (b) of butter (BHT: Butylated hydroxytoluene, OLE: Olive leaf extract, C: Control, TBA: Thiobarbituric acid, PV: Peroxide values, MDA: Malondialdehyde. a-fDifferences between means shown with different letters in the same column were statistically significant (P<0.05))

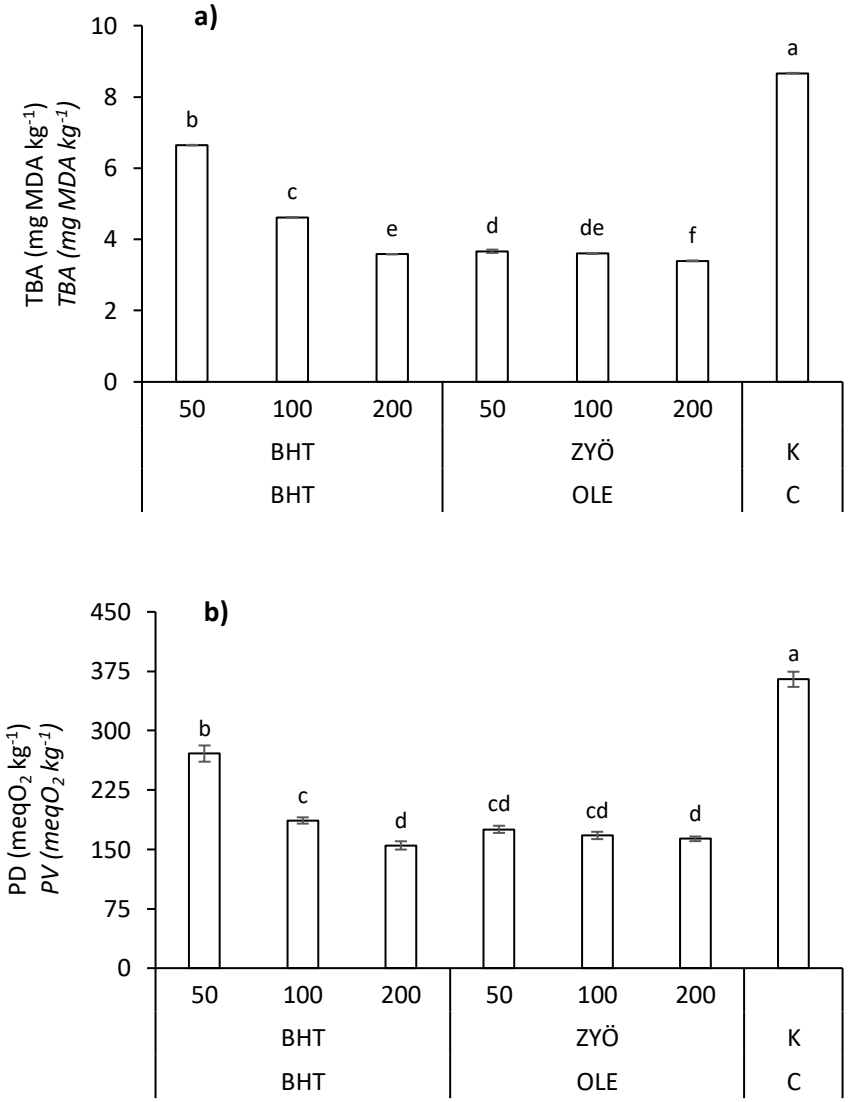
Ayçiçek yağının TBA ve PS sonuçları

Ayçiçek yağının TBA ve PS verileri Şekil 3'te verilmiştir. TBA değerleri, kontrol örneğine nazaran BHT ve ZYÖ eklenmiş ayçiçek yağlarında daha düşük bulunmuştur (P<0.05). 50 ppm BHT içeren ayçiçek yağındaki TBA miktarı 6,65 mg MDA kg^{-1} iken, aynı konsantrasyonda ZYÖ içeren örneğin TBA düzeyinin bu değerin yaklaşık yarısı (3,67 mg MDA kg^{-1}) kadar olduğu tespit edilmiştir

(P<0.05). Ancak, dikkate değer bir bulgu olarak, BHT konsantrasyonundaki artışın ZYÖ'deki artışa oranla ayçiçek yağının TBA içeriğini daha yüksek oranda düşürdüğü belirlenmiştir. Örneğin, BHT konsantrasyonun 50'den 200 ppm'e artırılması, ayçiçek yağındaki TBA değerini %46,2 oranında azaltırken (P<0.05), ZYÖ konsantrasyonundaki aynı artış, bu yağın TBA değerinde %7,4 oranında düşürmüştür (P<0.05) (Şekil 3a). Bu çalışmanın bulguları, Farag ve ark. (2007) tarafından bildirilen verilerle benzerlik göstermektedir. Bahsedilen araştırmacılar, 5 farklı konsantrasyonlarda (400-2400 ppm) polifenol içerecek şekilde zeytin yaprağı

suyu ilave edilmiş ve 25 saat kızartma işlemi (180±5 °C) gören ayçiçek yağındaki TBA içeriklerinin artan polifenol konsantrasyonuyla orantılı olarak azaldığını rapor etmektedirler.

Herhangi bir antioksidan ilave edilmeyen ayçiçek yağı örneğinin PS değerinin 50, 100 ve 200 ppm BHT eklenmiş örneklere göre sırasıyla 1,35, 1,95 ve 2,35 kat; aynı ZYÖ konsantrasyonları için ise sırasıyla 2,08, 2,18 ve 2,23 kat daha yüksek olmuştur. 50 ppm'lik konsantrasyonda BHT içeren ayçiçek yağının hızlandırılmış raf ömrü testinin sonundaki PS düzeyi aynı konsantrasyonda ZYÖ içeren örneğe göre 1,55 kat daha yüksek bulunmuştur (P<0.05). Bununla beraber, BHT eklenmiş ayçiçek yağlarının PS değerinin artan konsantrasyona paralel olarak kademeli bir şekilde azalırken, ZYÖ'nun artan konsantrasyonları ayçiçek yağının PS değerinde daha düşük oranlarda azalmaya neden olmuştur (Şekil 3b).



Şekil 3. Ayçiçek yağının TBA (a) ve PS (b) değeleri (BHT: Bütillenmiş hidroksitoluen, ZYÖ: Zeytin yaprağı özütü, K: Kontrol, TBA: Tiyobarbitürik asit, PS: Peroksit sayısı, MDA: Malondialdehit. [a-f] Aynı sütunda farklı küçük harflerle gösterilen ortalamalar arasındaki farklar istatistiksel olarak anlamlıdır (P<0.05))

Figure 3. TBA values (a) and PV (b) of sunflower oil (BHT: Butylated hydroxytoluene, OLE: Olive leaf extract, C: Control, TBA: Thiobarbituric acid, PV: Peroxide values, MDA: Malondialdehyde. [a-f]Differences between means shown with different letters in the same column were statistically significant (P<0.05))

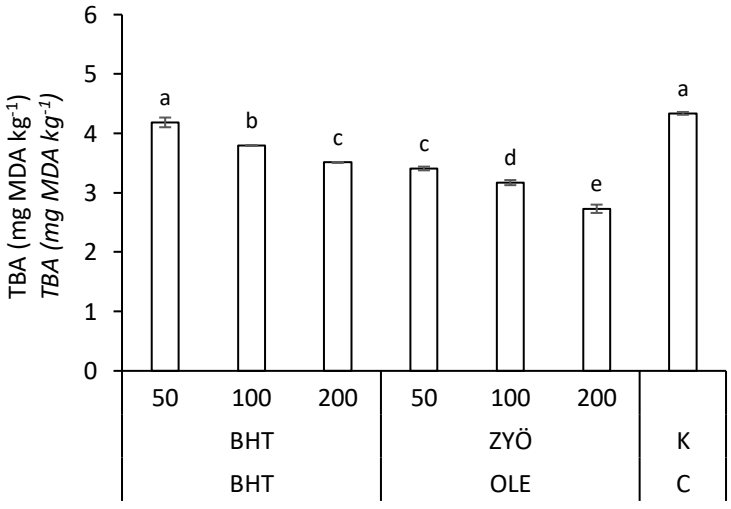
Birçok araştırmacı, bitki ekstraktlarının yağlarda antioksidan etki olarak PS düzeyini düşürebildiğini rapor etmektedir. Kontogianni ve Gerothanassis (2012), 200 ppm konsantrasyonlarda ZYÖ ilave edilmiş ve 40 °C'de kapalı şişe içerisinde 200 saat süreyle karanlıkta oksidasyona maruz bırakılan ayçiçek yağı örneklerindeki PS değerlerini bu çalışmada elde edilen değerlerden daha düşük

olarak 30-105 meqO2 kg^{-1} aralığında bulmuştur. Bu farkın, ZYÖ üretiminde kullanılan çözücüler dahil olmak, uygulanan ektraksiyon metodunun yanı sıra oksidasyon sırasındaki sıcaklık, süre ve ışık gibi koşulların farklılığından ileri geldiği tahmin edilmektedir. Guo ve ark. (2016), biberiye ektresinin hem kızartma (180 °C) hem de hızlandırılmış depolama koşullarında (65 °C, 24 saat) palm yağında antioksidan etki gösterdiğini ve yapay antioksidanlar olarak BHA ve TBHQ'ye alternatif olabilecek etkiler gösterebildiğini tespit etmişlerdir. Diğer bir çalışmada, bu bitki özütünün soya yağı ve ayçiçek yağı karışımındaki antioksidan etkileriyle ilgili rolleri bildirilmektedir (Chammem ve ark., 2015). Bitkilerdeki fenolik bileşikler oksidasyonunun ilk aşamasında oluşan lipit radikaline bir hidrojen atomu vererek hidroksiperoksit oluşumunu engelleyebilmektedir (Farag ve ark., 2007).

Patates cipsinin TBA sonuçları

50 ppm konsantrasyonunda BHT içeren ayçiçek yağında kızartılmış cipslerin TBA değerinde (4,18 mg MDA kg^{-1}) kontrol örneğine (4,34 mg MDA kg^{-1}) göre anlamlı bir azalma meydana gelmemiştir (P>0.05). Buna karşın, aynı konsantrasyonda ZYÖ ilave edilmiş ayçiçek yağında kızartılmış cips örneğinde (3,41 mg MDA kg^{-1}), kontrol örneğine göre % 21,4 oranında daha az miktarda TBA oluşmuştur (P<0.05). 100 ve 200 ppm konsantrasyonları için de ZYÖ içeren patates cipslerinde TBA daha düşük oranlarda belirlenmiştir (P<0.05) (Şekil 4). Bu durum, ZYÖ'deki fenolik bileşikler ve/veya diğer antioksidan etkili bileşenlerin kızartma sırasında patates cipslerine geçişiyle ve buna bağlı olarak raf ömrü süresince de antioksidan etki gösterebilmesiyle ilişkilendirilmektedir (Orozco-Solano ve ark., 2011).

Jiménez ve ark. (2017), zeytin yaprağından etanol-su karışımını (1:1 v/v) çözücü olarak kullanarak elde ettiği özütün ayçiçek yağında kızartılan patateslerde antioksidan etki gösterdiğini ve buna bağlı olarak termal oksidasyonun diğer bir göstergesi olarak polar bileşiklerin oluşumunu engellediğini bulmuşlardır. Aynı çalışmada, karşılaştırma amacıyla gallik asit cinsinden zeytin yaprağı özütüyle aynı konsantrasyonda toplam fenolik madde içeren avokado yapraklarından elde edilen özütün ise örneklerde prooksidan etki gösterdiği bildirilmiştir'.



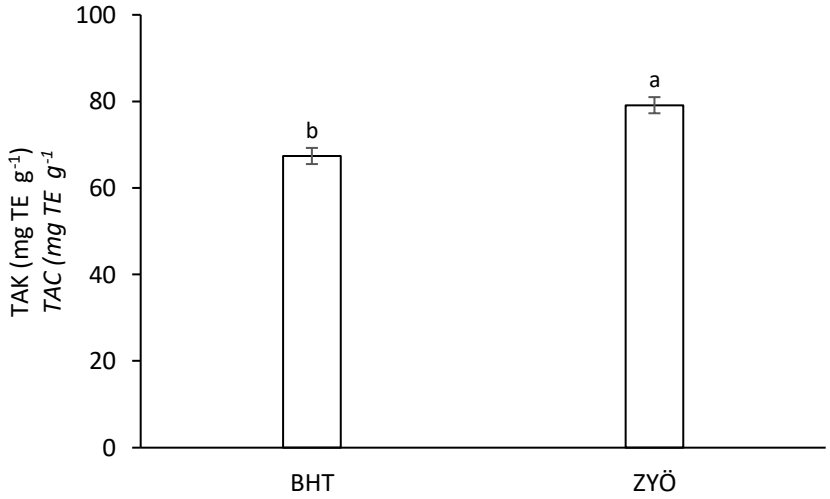
Şekil 4. Patates cipsinin TBA değeleri (BHT: Bütillenmiş hidroksitoluen, ZYÖ: Zeytin yaprağı özütü, K: Kontrol, TBA: Tiyobarbitürik asit, MDA: Malondialdehit. $^{a-d}$ Aynı sütunda farklı küçük harflerle gösterilen ortalamalar arasındaki farklar istatistiksel olarak anlamlıdır (P<0.05))

Figure 4. TBA values of potato chips (BHT: Butylated hydroxytoluene, OLE: Olive leaf extract, C: Control, TBA: Thiobarbituric acid, MDA: Malondialdehyde. $^{a-d}$Differences between means shown with different letters in the same column were statistically significant (P<0.05))

ZYÖ ve BHT'nin toplam antoksidan kapasiteleri

ZYÖ ve BHT'nin DPPH radikali temizleme

kapasitesine göre tespit edilen TAK değerleri Şekil 5'te gösterilmiştir. BHT ve ZYÖ'nun TAK değerleri sırasıyla 67,39 ve 79,12 mg TE g^{-1} olarak belirlenmiştir (P<0.05). Bu bulgu, aynı içeriklerde kullanım durumunda zeytin yaprağındaki fenolik bileşiklerin BHT'ye göre daha yüksek antioksidan aktivite sergileyebileceğini göstermektedir. Başka bir çalışmada da farklı zeytin çeşitlerinin yapraklarından elde edilen özütlerin BHT'ye göre daha fazla oranda DPPH temizleme kapasitesileri sergilediği belirlenmiştir (Ben Salah ve Abdelmelek, 2012). Bitkilerin antioksidan özellikleri, çoğunlukla polifenolik bileşiklerle beraber çeşitli pigmentler ve tokoferoller gibi doğal antioksidan maddelerden ileri gelmektedir. ZYÖ'nün göreli olarak yüksek TAK değerinin daha çok oleuropein ve bundan türevleri olan sekoiridoitlerden ileri geldiği düşünülmektedir (Esposto ve ark., 2015).



Şekil 5. BHT ve ZYÖ'nün TAK değerleri (BHT: Bütillenmiş hidroksitoluen, ZYÖ: Zeytin yaprağı özütü, TAK: Toplam antioksidan kapasite, TE: Troloks eşedeğeri. [a-b]Aynı sütunda farklı küçük harflerle gösterilen ortalamalar arasındaki farklar istatistiksel olarak anlamlıdır (P<0.05))

Figure 5. TAC of BHT and OLE (BHT: Butylated hydroxytoluene, OLE: Olive leaf extract, TAC: Total antioxidant capacity, TE: Trolox equivalent. [a-b]Differences between means shown with different letters in the same column were statistically significant (P<0.05))

Sonuçlar

Bu çalışmada, ZYÖ'nün tereyağı, ayçiçek yağı ve patates cipsindeki oksidasyona karşı etkileri araştırılmıştır. Çalışmada, karşılaştırma amacıyla bu gıdalarda bir sentetik antioksidan olarak BHT de kullanılmıştır. ZYÖ'nün, lipit oksidasyon göstergeleri olarak depolama sırasında tereyağındaki TBA ve PS düzeylerini düşürebildiği, artan kullanım dozuyla orantılı bir şekilde bu etkinin de arttığı ve tereyağında 200 ppm'lik bir içerikte kullanımı durumunda BHT ile yakın oranlarda antioksidan özellik sergileyebildiği saptanmıştır. Benzer şekilde, ayçiçek yağının kızartmaya dayalı ısıl işlemi sırasında veya hızlandırılmış raf ömrü testi sonrasında ZYÖ'nün TBA ve PS'yi düşürücü etki gösterdiği ve ilave edilen dozlar bakımından hem ayçiçek yağında hem de patates cipslerinde BHT'ye göre daha fazla oranda TBA değeri düşürebildiği belirlenmiştir. Ayrıca, DPPH temizleme yeteneğine dayalı TAK değeri açısından ZYÖ'nün BHT'ye nazaran daha yüksek antioksidan aktiviteye sahip olduğu tespit edilmiştir. Bu çalışma, ZYÖ'nün yemeklik yağlarda oksidasyona dayalı bozulma reaksiyonlarının önlenmesinde veya yavaşlatılmasında BHT yerine kullanılabileceğini ortaya koymuştur.

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Araştırma Makalesi/Research Article

Bazı Yabancı Otların Yaprak Ekstraklarının Kök-Ur Nematodu *Meloidogyne incognita* Üzerinde Nematisidal Etkilerinin Değerlendirilmesi

Evaluation of The Nematicidal Effects of Some Weed Leaf Extracts on The Root-Knot Nematode Meloidogyne incognita

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ÖZ

Bu çalışmanın amacı, köygöçüren (*Cirsium arvense* (L.) Scop.), deve dikeni (*Silybum marianum* (L.) Gaertn.), sinir otu (*Plantago major* L.) ve şeytan elması (*Datura stramonium*)'nın metanol ekstraktının *Meloidogyne incognita*'ya karşı toksikolojik potansiyelini *in vitro* ve kontrollü koşullar altında domates üzerinde değerlendirmektir. Çalışma her bitki için dört konsantrasyon (125, 250, 500 ve 1000 ppm) ile yürütülmüştür. Çalışma tesadüf parselleri deneme deseninde 5 tekerrürlü olarak kurulmuştur. *İn vitroda* dokuz ml ekstrakt içeren petrilere 20 adet ikinci dönem larva (L2) ml^{-1} içeren 1 ml süspansiyon eklenmiştir ve 48 saat sonra ölü bireyler sayılarak ölüm oranı kaydedilmiştir. Kontrollü koşullar altında yürütülen denemede nematoda hassas Gülizar F1 domates fideleri saksılara şaşırtıldıktan beş gün sonra her saksıya 500 L2 ile nematod inokulasyonu gerçekleştirilmiştir. Nematod inokulasyonundan 24 saat sonra bitkilerin metanol ekstraktı 30 ml/saksı konsantrasyonda olacak şekilde toprağa uygulanmıştır. Elli gün sonra köklerdeki gal ve yumurta paketleri sayılmıştır. *İn vitroda* en yüksek L2 mortalitesi tüm bitkilerde 1000 ppm konsantrasyonunda tespit edilmiştir. Bu konsantrasyonda köygöçüren (%81.6), deve dikeni (%85.4) ve şeytan elması (%84.2) bitkilerinin L2 üzerindeki öldürücü etkisi sinir otundan (%67.8) yüksek bulunmuştur. Kontrollü koşullar altında domates köklerinde gal ve yumurta paketi sayısı ortalamasında 1000 ve 500 ppm konsantrasyonları arasında önemli bir fark saptanmamıştır. En düşük gal ve yumurta paketi sayısı ise deve dikeni ve şeytan elması bitkilerinin uygulamalarında belirlenmiş ve %80'in üzerinde baskılayıcı etki bulunmuştur. Çalışmada deve dikeni ve şeytan elması ekstraktının kök-ur nematodunun kontrolünde etkili bir şekilde kullanılabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Metanol ekstrakt, Nematisidal etki, Devedikeni, Şeytan elması

ABSTRACT

The aim of this study was to determine the toxicological potential of the methanol extract of creeping thistle (*Cirsium arvense* (L.) Scop.), milk thistle (*Silybum marianum* (L.) Gaertn.), greater plantain (*Plantago major* L.) and jimsonweed (*Datura stramonium*) plant against *Meloidogyne incognita in vitro* and under controlled conditions on tomato. The study was conducted with four concentrations (125, 250, 500 and 1000 ppm) for each weed plant. The study was set up in a randomized trial design with 5 replications. *In vitro*, 1 ml of suspension containing 20 second stage juvenile (J2) ml^{-1} was added to petri dishes containing nine ml of extract, and after 48 hours, dead individuals were counted and the mortality rate was recorded. Five days after the nematode-sensitive Gülizar F1 tomato seedlings were transplanted into the pots under controlled conditions, nematode

inoculation was carried out with 500 J2 in each pot. Twenty four hours after nematode inoculation, methanol extracts of the plants was applied to the soil at 30 ml/pot concentration. Fifty days later, galls and egg masses in the roots were counted. *In vitro*, the highest J2 mortality was found in all plants at 1000 ppm concentration. At this concentration, the mortality effect of creeping thistle (81.6%), milk thistle (85.4%) and jimsonweed (84.2%) plants on J2 was found to be higher than broadleaf plantain (67.8%). Under controlled conditions, no significant difference was detected in the average number of galls and egg masses on tomato roots between 1000 and 500 ppm concentrations. The lowest number of galls and egg masses was determined in milk thistle and jimsonweed applications, and over 80% suppressive effect was found. Consequently, the extract of milk thistle and jimsonweed can be used effectively in the control of root knot nematode.

Key Words: Methanol extract, Nematicidal effect, Milk thistle, Jimsonweed

Giriş

Dünya tarım alanlarında birçok bitkide önemli verim ve ekonomik kayıplarına neden olan kök-ur nematodları (*Meloidogyne* spp.) obligat ve sabit endoparazit beslenme özelliğindedir (Khan ve ark., 2011). Kök-ur nematodları hemen hemen tüm bitki türlerini enfekte edebilmekte ve bitkinin besin maddesini tüketerek gıda üretiminin miktarında ve kalitesinde önemli bir azalmaya neden olabilmektedir (Kiewnik ve Sikora 2006; Adekunle ve Akinlua, 2007). Aynı zamanda kök-ur nematodu ile enfekteli ürünler bakteriyel ve fungal hastalıklara daha yatkın hale gelmektedir (Ashraf ve Khan, 2010). Kök-ur nematodlarının 98'den fazla türü olmasına rağmen (Jones ve ark., 2013), *Meloidogyne incognita, M. javanica, M. arenaria* ve *M. hapla*, tüm kök ur nematodu türlerinin popülasyonlarının %95'ini temsil etmektedir (Dong ve ark., 2012). Kök-ur nematodlarının popülasyonunu azaltmak için, doğal düşmanların kullanılması, kültürel uygulamaların geliştirilmesi, dayanıklı çeşitlerin yetiştirilmesi ve nematisitlerin uygulanması gibi çeşitli yaklaşımlar kullanılmaktadır (Williamson ve Kumar, 2006; Khan ve Kim, 2007; Salim ve ark., 2016). Nematisitlerin kök-ur nematodu kontrolünde etkili bir yöntem olduğu bilinmekle beraber topraktaki yararlı mikroorganizmaların dağılımında azalma, kimyasallara dirençli zararlılarda artış, kalıntı toksisitesi nedeniyle çevre kirliliği ve insan vücudu için kanserojenik etkiler bildirilmiştir (Nico ve ark., 2004; Kiewnick ve Sikora, 2006). Bu nedenlerden dolayı tarımsal faaliyetleri, çiftçileri, tüketicileri veya çevreyi etkilemeyen etkin bir kök-ur nematodu mücadelesi için yeni alternatiflerin geliştirilmesine yönelik ilgi artmaktadır. Yabancı otlar, antagonizma yolu ile nematodların baskılanmasını sağlayabilir. Fenolik asitler, terpenler, terpenoidler, glikozitler, alkaloidler ve flavonoidler gibi allelokimyasalların üretimi yoluyla kültür bitkileri ve toprak organizmaları ile rekabet ederler (Tran ve ark., 2016). Son zamanlarda nematod kontrolü araştırmalarının önemli bir konusu, nematisidal aktiviteye sahip, allelokimyasallar açısından zengin, hedef dışı organizmalara olumsuz etkisi olmayan ve biyolojik olarak kolayca parçalanabilen bitkisel preparatların değerlendirilmesidir. Kök-ur nematodlarına karşı bitkisel ekstraktların kullanımı ile ilgili pek çok çalışma bulunmaktadır (Taba ve ark., 2008; Erdoğuş, 2022). Brassiceae, Lamiaceae, Asteraceae, Apiacae, Rutaceae ve Lauraceae familyalarındaki çoğu bitki nematisidal aktivite göstermektedir (Andres ve ark., 2012; Göze Özdemir ve ark., 2022). Kadife çiçeği (*Tagetes* spp.), çıngırak kutusu (*Crotalaria spectabilis*), krizantem (*Chrysanthemum* spp.), sarımsak (*Allium sativum*), tarçın (*Cinnamomum verum*) ve neem (*Azadiracta indica*) bitki paraziti nematodlara karşı nematisidal özelliği en iyi bilinen bitki örnekleridir (Satti ve Naser, 2006; Kong ve ark., 2007).

Bu çalışmanın amacı köygöçüren (*Cirsium arvense* (L.) Scop.: Asteraceae), deve dikeni (*Silybum marianum* (L.) Gaertn.: Asteraceae), sinir otu (*Plantago major* L.: Plantaginaceae) ve şeytan elması (*Datura stramonium*: Solanaceae)'nın yaprak metanol ekstraktının *M. incognita*'ya karşı toksikolojik potansiyelini *in vitro* ve kontrollü koşullar altında domates üzerinde değerlendirmektir.

Materyal ve Yöntem

Yaprak ekstraktının hazırlanması

Her yabancı otun yaprağı ayrı bir küvette homojen bir şekilde karıştırılmış ve makas ile kesilerek küçük parçalara ayrılmıştır. Daha sonra bu küvetlerden her yabancı ot için 5 g yaprak tartılmış ve konik bir şişeye alınmıştır. Üzerine 80 ml metanol eklenerek 40°C'de 130 rpm'de bir gece çalkalayıcıda bekletilmiştir. Daha sonra üzerine tekrar 80 ml metanol eklenerek aynı işlem dört kez daha tekrarlanmıştır (Vinodhini ve ark., 2019). Beş gün sonunda oluşan yaprak ekstraktı süzülerek süspansiyon konsantresi ayrılmış ve denemeler kuruluncaya kadar +4°C'de buzdolabında bekletilmiştir.

İkinci Dönem Larvaların Elde Edilmesi

Denemede iklim odası koşullarında (24±1 °C, %60±5 nem) kitle üretimi Tueza F1 domates çeşidinde devam ettirilen *M. incognita* ISP izolatı kullanılmıştır. Kitle üretimi yapılan urlu domates köklerinden binoküler mikroskop altında yumurta paketleri çıkarılarak distile su içeren 9 cm petri içerisinde elekler içerisine alınarak 28°C'de üç gün inkübe edilmiştir. Bu şekilde yumurta paketlerinden ikinci dönem larvaların (L2) çıkışları sağlanmıştır. Işık mikroskobu altında L2 sayımları yapılarak eppendorf tüpler içerisine alınmış ve denemede kullanılmak üzere +4 °C'de saklanmıştır (Göze Özdemir ve ark., 2022).

Yabancı ot yapraklarının metanol ekstraktlarının in vitro koşullarda Meloidogyne incognita'nın ikinci dönem larvaları üzerindeki nematisidal etkisinin belirlenmesi

Denemede köygöçüren, deve dikeni, sinir otu ve şeytan elması'nın yapraklarından elde edilen metanol ekstraktının 4 konsantrasyonu (125, 250, 500 ve 1000 ppm) kullanılmıştır. Deneme tesadüf parselleri deneme deseninde her konsantrasyon için 5 tekerrür olacak şekilde 6 cm çaplı petri kaplarında yürütülmüştür. *Meloidogyne incognita* L2 lm üzerindeki etkisini belirlemek için, her bir ekstraktın konsantrasyonundan 9 ml petri kaplarına dökülmüş ve içine yeni yumurtadan çıkmış 20 L2 ml^{-1} içeren 1 ml süspansiyon eklenmiştir. Dokuz ml steril damıtılmış su içeren petriye bir ml L2 süspansiyonu eklenerek 10 ml'ye tamamlanmış ve kontrol olarak kullanılmıştır (Vinodhini ve ark., 2019). Kırksekiz saat sonra ışık mikroskobunda sayım yapılmış, nematodlar ince bir iğne ile dokunulduklarında hareket etmezlerse ölü olarak kabul edilmişlerdir. Deneme 2 kez tekrarlanmış ve yüzde ölüm değerleri (m) Abbott formülü ile hesaplanmıştır (Finney, 1978). Daha sonra ortalamaları alınarak istatistiki analize tabi tutulmuştur.

m = 100 (1- (nt/nc)) (m = ölüm yüzdesi, nt = uygulamadan sonra canlı nematodların sayısı ve nc = su kontrolündeki yaşayabilirlerin sayısı)

Kontrollü koşullar altında domateste Meloidogyne incognita'ya karşı yabancı ot yaprak ekstraktlarının etkisinin araştırılması

Çalışma iklim odası koşullarında (24±1 °C, %60±5 nem) saksılarda, nematoda hassas olduğu bilinen 35 günlük Özkan F1 domates fideleri ile yürütülmüştür. Olympos Fide (Kumluca, Antalya, Türkiye)'den temin edilen domates fideleri yaklaşık 600 g (%68 kum, %21 Silt ve %11 kil) steril toprak karışımı içeren plastik saksılara (13X14 cm) her tekerrüre 1 domates fidesi gelecek şekilde şaşırtılmıştır. Denemede her yabancı ot yaprak ekstraksiyonunun 4 (125, 250, 500 ve 1000 ppm) konsantrasyonu ile çalışılmıştır. Deneme tesadüf parselleri deneme deseninde her konsantrasyon için 5 tekerrürlü olacak şekilde kurulmuştur. Şaşırtmadan 5 gün sonra her saksıya 500 L2 gelecek şekilde nematod inokulasyonu gerçekleştirilmiştir (Vinodhini ve ark., 2019). Nematod inokulasyonundan 24 saat sonra ekstraktlar her saksıya 30 ml konsantrasyonda toprağa uygulanmış ve iyice karışması sağlanmıştır (Kabil ve Adam, 2020). Negatif kontrol olarak nematod inokulasyonu yapılmış ve ekstrakt yerine 30 ml distile su uygulanmış bitkiler kullanılmıştır. Nematod inokulasyondan 50 gün sonra deneme sonlandırılmıştır. Daha sonra bitkiler sökülüp, bitkinin köklerindeki topraklardan arındırılması için temiz su ile yıkanmış kökler asit fuksinle boyandıktan sonra gal ve yumurta paketi sayımı yapılmıştır (Moltmann, 1988).

İstatistiksel analiz

İn vitro ve kontrollü koşullar altında yürütülen deneme sonucu elde edilen verilerin istatistiksel analizi için SPSS (versiyon 20.0) programı kullanılmış ve ortalamalar arasındaki farkları test etmek için varyans analizi (ANOVA) yapılmıştır. Ortalamalar, P≤ 0.05'te Tukey HSD testi ile karşılaştırılmıştır.

Araştırma Bulguları ve Tartışma

Kontrol ile karşılaştırıldığında tüm yabancı ot uygulamalarının konsantrasyonlarının etkili olduğu belirlenmiştir. *İn vitroda* en yüksek yüzde ölüm tüm yabancı otların 1000 ppm konsantrasyonunda tespit edilmiştir. Ektraktların uygulama konsantrasyonu seyreltildikçe yüzde ölüm oranlarının azaldığı görülmektedir. Köygöçüren, deve dikeni ve şeytan elmasının 1000 ppm konsantrasyonunda L2 üzerindeki öldürücü etkisi sinir otu uygulamasından yüksek bulunmuştur. En düşük (125 ppm) konsantrasyonda deve dikeni ve şeytan elması ekstraktlarının uygulamalarının ölüm değerlerinin %50'nin üzerinde olduğu saptanırken, köygöçüren ve sinir otu uygulamalarında sırası ile %35.8 ve 16.5 tespit edilmiştir (Çizelge 1).

Çizelge 1. Yabancı ot yapraklarının metanol ekstraktlarının *in vitro* koşullarda *Meloidogyne incognita*'nın ikinci dönem larvaları üzerindeki nematisidal etkisi

Table 1. Nematicidal effect of methanol extracts of weeds on second stage juvenile of Meloidogyne incognita in vitro

Konsantrasyon *Concentration*	Yüzde ölüm±Standart Hata *Percent mortality±Standart Error*			
	Sinir otu Broadleaf plantain	Köygöçüren *Creeping thistle*	Deve dikeni *Milk thistle*	Şeytan elması *Jimson weed*
1000 ppm	67,8±1,9 a B*	81,6±2,1 a A	85,4±1,6 a A	84,2±1,1 a A
500 ppm	56,6±2,4 b B	63,4±0,9 b AB	70,6±0,8 b A	74,8±1,5 b A
250 ppm	23,8±2,0 c C	47,6±2,3 c B	56,0±1,3 c A	59,2±0,7 c A
125 ppm	16,5±1,5 c C	35,8±2,2 d B	52,0±2,0 c A	51,8±2,5 d A
Kontrol *(Control)*	1,2±1,9 d	1,2±1,9 e	1,2±1,9 d	1,2±1,9 e

*Aynı sütundaki küçük harfler konsantrasyonlar arasındaki farkı, aynı satırdaki büyük harfler ise yabancı otlar arasındaki istatistiki farkı göstermektedir (p≤0.05).

* *Lowercase letters in the same column indicate the difference between concentrations, and uppercase letters in the same row indicate the statistical difference between weeds (p≤0.05).*

Kontrollü koşullar altında en yüksek gal (130,4/kök) ve yumurta paketi (120,0/kök) sayısı ortalaması kontrolde tespit edilmiştir. Ekstraktların uygulama konsantrasyonları seyreltildikçe gal ve yumurta paketi sayısı artmıştır. En yüksek konsantrasyon 1000 ppm de gal sayısı ortalaması 6.6-19.6 arasında değişim gösterirken, 125 ppm de 18.2-41.8 arasında değişmiştir. Benzer şekilde yumurta paketi sayısı ortalaması 1000 ppm de 6.4-16.8 arasında değişim gösterirken, 125 ppm de 16.6-27.0 arasında değiştiği bulunmuştur. Ancak aynı ekstraktın 250 ve 125 ppm konsantrasyon uygulamalarında köklerdeki gal ve yumurta paketi sayısı değerlendirildiğinde aralarındaki fark istatistiki olarak önemli bulunmamıştır (p≤0.05). En az gal ve yumurta paketi sayısı deve dikeni ve şeytan elması uygulamalarında 1000 ve 500 ppm konsantrasyonlarında tespit edilmiştir. Köygöçüren uygulamalarının gal ve yumurta paketi sayısı ortalaması1000, 500 ve 250 ppm de sinirotu uygulaması ile aynı istatistiki grupta yer almasına rağmen, 125 ppm de düşük bulunmuş ve aralarındaki farkın önemli olduğu saptanmıştır (p≤0.05). Kontrolle karşılaştırıldığında 1000 ppm konsantrasyonunda yabancı otların ekstrakt uygulamalarının köklerdeki gal ve yumurta paketi üzerindeki baskılayıcı etkisinin %80'in üzerinde olduğu belirlenmiştir (Çizelge 2).

Çizelge 2. Kontrollü koşullar altında yabancı ot yaprak ekstraktlarının uygulandığı domates köklerinde *Meloidogyne incognita* gal ve yumurta paketi sayısı

Table 2. Number of Meloidogyne incognita galls and egg masses on tomato roots in weed leaf extracts application under controlled conditions

Konsantrasyon Concentration	Gal sayısı ortalaması± Standart hata Number of galls±Standar Error				Yumurta paketi sayısı ortalaması± Standart hata Number of egg masses±Standart Error			
	Sinir otu *Broadleaf plantain*	Köygöçüren *Creeping thistle*	Deve dikeni *Milk thistle*	Şeytan elması *Jimson weed*	Sinir otu *Broadleaf plantain*	Köygöçüren *Creeping thistle*	Deve dikeni *Milk thistle*	Şeytan elması *Jimson weed*
1000 ppm	19,6± 1,5 a B*	10,4± 0,9 a AB	7,8± 0,8 a A	6,6± 0,5 a A	16,8± 1,8 a B	9,6± 0,8 a AB	7,8± 0,8 a A	6,4± 0,7 a A
500 ppm	29,0± 1,4 a C	17,8± 1,8 a BC	11,4± 1,0 ab AB	7,6± 1,3 a A	25,8± 1,7 ab C	14,8± 2,0 a BC	11,0± 0,7 a AB	7,0± 0,7 a A
250 ppm	41,6± 2,8 b C	41,4± 3,3 b C	17,8± 1,4 bc B	8,8± 0,8 ab A	35,4± 2,0 bc C	34,0± 3,7 b C	15,2± 1,5 ab B	6,0± 0,3 a A
125 ppm	41,8± 2,6 b C	33,0± 2,5 b B	23,8± 1,3 c AB	18,2± 1,0 b A	38,0± 2,9 c C	27,0± 1,9 b B	21,0± 1,5 b AB	16,6± 0,6 b A
Kontrol (Control)	130,4± 4,6 c	130,4± 4,6 c	130,4± 4,6 d	130,4± 4,6 c	120,0± 4,1 d	120,0± 4,1 c	120,0± 4,1 c	120,0± 4,1 c

*Aynı sütundaki küçük harfler yabancı otların konsantrasyonları arasındaki farkı, aynı satırdaki büyük harfler ise yabancı otlar arasındaki istatistiki farkı göstermektedir (p≤0.05).

* *Lowercase letters in the same column indicate the difference between the concentrations of weeds, and uppercase letters in the same row indicate the statistical difference between weeds (p≤0.05).*

İn vitro ve kontrollü koşullarda yürütülen çalışmalarda kontrolle karşılaştırıldığında tüm yabancı ot yaprak methanol ekstraktlarının kök-ur nematodu üzerinde baskılayıcı etkisi belirlenmiştir. En düşük etki sinir otu uygulamalarında bulunurken, en yüksek etki deve dikeni ve şeytan elması uygulamalarında saptanmıştır. Çalışmada *invitro da* şeytan elmasının L2 üzerindeki yüzde ölüm oranı %84.2 belirlenmiştir. Chaudhary ve ark. (2013) 25-100 mg ml^{-1} arasında test edilen şeytan elması tohumlarının sıcak su ve etanol ekstraktlarının, *M. incognita* L2'si üzerinde %75-100 ölüm oranına neden olduğunu bildirmişlerdir. Benzer şekilde, L2'ye karşı 500 mg L^{-1}'de test edilen yaprak ve gövde ekstraktları, 72 saatlik maruziyetten sonra %68 ve %70 gibi nispeten yüksek ölüm oranlarıyla sonuçlanmıştır (Elbadri ve ark., 2008). Adekunle ve ark. (2007) *in vitro*da deve dikeni yaprak methanol ekstraktının yumurtadan çıkış üzerindeki inhibasyon etkisini %39 olarak bildirirken, L2 üzerindeki etkisini 11. günde %100 olarak belirtmektedir. Bu çalışmada ise deve dikeni ekstrakt uygulamasından 48 saat sonra %80'nin üzerinde ölüm bulunmuştur. *Plantoago lanceolata* ekstraktı yumurtadan çıkmayı %75 oranında azaltırken, *M. incognita* L2'sı üzerinde 5 gün içinde ölüm meydana gelmiştir (Adekunle ve ark., 2006). Çalışmada ise 48saat sonra *P. major* ekstrakt uygulamasında L2 üzerinde %55'den fazla ölüm tespit edilmiştir.

Kontrollü koşullarda gal ve yumurta paketi üzerindeki baskılayıcı etkide deve dikeni ve şeytan elmasının 1000 ve 500 ppm konsantrasyonları arasında istatistiki olarak önemli bir fark bulunmamıştır. Deve dikeni ve şeytan elması ekstraktının 1000 ppm konsantrasyonunda köklerdeki gallenme üzerindeki baskılayıcı etkisi sırasıyla %93.5 ve %94.5 olarak belirlenmiştir. Köygöçüren uygulamasının genel anlamda baskılayıcı etkisi sinirotu uygulamasından daha yüksek tespit edilmiştir. Şeytan elması yaprak ekstraktlarının %0,5-%1 oranında ekim öncesi uygulamaları gal sayılarını önemli ölçüde azaltmıştır (Mateeva ve Ivanova, 2000). Asteraceae familya içerisindeki bazı bitkilerinin nematisidal etkisi ile ilgili çalışmalar bulunmaktadır (Tsay ve ark., 2004). Deve dikeni Asteraceae üyesi bir bitkidir. D'Addabbo ve ark. (2013) Asteraceae familyasında seskiterpenlerin

nematisidal metabolit olduğunu bildirmektedirler. Seskiterpenoidlerin çoğunun mikrobiyal etmenlere tepki olarak üretilen antibiyotik bileşikler işlevinde olduğu bilinmektedir (Tiring ve ark., 2021). *In vitro* ve in vivo sonuçları, *Chrysanthemum coronarium*'un esansiyel yağının ve Asteraceae türlerinden elde edilen organik bileşiklerin nematisit olarak kullanılabileceğini göstermektedir (Perez ve ark., 2003). Akdeniz ülkelerinde popüler bir Asteraceae türü olan *Inula viscosa*'nın sürgünlerinde nematisit aktiviteye sahip bileşikler olduğu bulunmuş ve yapraklarından nematisit seskiterpenik asitler (kostik asit ve izokostik asit) izole edilmiştir (Oka ve ark. 2001). Daha sonra tarla koşullarında bu bileşikler denendiğinde marul bitkilerinde *M. javanica*'nın oluşturduğu gallerin %40'lık bir oranda azalma meydana getirdiği gözlemlenmiştir (Oka ve ark. 2006). Aydınlı ve Mennan (2014), 12 bitki ekstraktının *M. arenaria* üzerindeki etkisini araştırmışlar ve yumurta paketi üzerindeki baskılayıcı etkide *P. lanceolata* 12 bitki ekstraktı içerisinde 3. sırada yer almıştır.

Deve dikeni ve şeytan elmasının yaprak ekstraklarının nematisidal etkisi benzer bulunmuştur. Ancak şeytan elması belladona alkaloid ailesinin bir üyesi olan hallüsinojenik bir bitkidir. Hiyosiyamin, skopolamin ve atropin gibi alkoloidlerin özellikle tohum ve çiçeklerde olduğu belirtilmektedir (Taştan ve ark., 2024). Şeytan elmasının antimikrobiyal aktiviteye sahip olduğu bildirilmektedir (Kaushik ve Goyal, 2008; Taştan ve ark., 2024). Yapılan literatür araştırmalarında şeytan elmasından kaynaklı zehirlenmelerin insan sağlığına olumsuz etkisi ile ilgili çok sayıda makaleye rastlanmıştır (Türkseven ve ark., 2021; Yöntem ve ark., 2021). Deve dikeni bitkisi ise farmakolojik özellik yönünden oldukça zengin görülmüş ve karaciğer-kalp rahatsızlıklarında kullanımına dair araştırmalara rastlanmıştır (Kocaman ve Dabak, 2015; Gürsili ve Yeşilkaya, 2020). Deve dikeni'nin insan sağlığı üzerinde olumsuz bir etkisinin olmaması avantaj sağlamaktadır.

Sonuçlar

Kök-ur nematodlarına alternatif yöntemlerin oluşturulmasında deve dikeni ve şeytan elmasının ekstraktlarının kullanılabileceği belirlenmesine rağmen, insan sağlığı açısından değerlendirildiğinde deve dikeni ektraktının daha ön planda olması gerektiği düşünülmektedir. Bu nedenle arazi çalışmaları da yürütülerek etkinliklerin belirlenmesi gerekmektedir. Ayrıca içeriğindeki etken bileşiklerin belirlenmesiyle yeni nematisitlerin geliştirilmesine katkı sağlanabilecektir.

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Araştırma Makalesi/Research Article

Bazı Yerel Biber Tiplerinden Elde Edilen Melezlerin Morfolojik Özelliklerinin ve Domates Lekeli Solgunluk Virüsüne (TSWV) Dayanıklılığının Belirlenmesi

Assessment of Morphological Traits and Resistance to Tomato Spotted Wilt Virus (TSWV) in Hybrids Derived from Various Indigenous Pepper Varieties

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ÖZ

Tokat Biberi (*Capsicum annuum* L.), Tokat'ta uzun yıllardır yetiştirilen ve tüketilen popüler bir biber çeşididir. Bu çeşidin üretimi çeşitli biyotik stres faktörleri tarafından engellenmektedir. Bunlardan biri de biber tarımında önemli ekonomik kayıplara neden olan domates lekeli solgunluk virüsüdür (TSWV). Bu araştırmada, yerel biber hatları kullanılarak TSWV'ye dayanıklı hibrit bitkiler yetiştirilmiş ve bu hibritlerin verim, meyve özellikleri ve dayanıklılık seviyeleri araştırılmıştır. S5 generasyonuna kadar kendilenmiş üç farklı 'Tokat Biberi' hattı, TSWV'ye dayanıklı ve TSW geni taşıyan 2 donör bitki ile melezlenmiştir. Melez bitkiler topraksız tarım koşullarında yetiştirilmiş ve TSWV bitkilere hem mekanik yollarla hem de çiçek tripsleri (*Frankliniella occidentalis*) kullanılarak bulaştırılmıştır. Ebeveyn bitkilerin pazarlanabilir verimleri bitki başına 2.260 kg ile 2.727 kg arasında değişirken, melez genotiplerin verimleri bitki başına 1.917 kg ile 2.527 kg arasında değişmiştir. Melez genotiplerin pazarlanabilir verimleri ebeveynlerden düşük olmuştur. Melez bitkilerde TSWV'ye karşı heterozigot dayanıklılık belirlenmiştir. Tül serada hava sıcaklığı 32°C'nin üzerine çıktığında melez bitkilerde dayanıklılığın kırıldığı görülmüştür. Çalışmada melez bitkilerin meyve şekli 3 ve 4 loblu, meyve rengi açık yeşil, yeşil ve koyu yeşil olarak gözlenmiştir. Sonuç olarak, yerel biber hatlarından geliştirilen melez bitkilere TSWV dayanıklılık geni TSW başarıyla aktarılmıştır. Melez bitkilerin veriminde düşüş meydana gelirken meyve görünümlerinde bir değişim olmamıştır. Yüksek sıcaklıklarda dayanıklılık kırılmıştır. Literatürde de TSW geninin bazı durumlarda kırıldığı sıklıkla belirtilmektedir. Çalışma sonunda 'Tokat Biberi' popülasyonlarından TSWV'ye dayanıklı hibrit çeşitlerin geliştirilmesi amaçlanmaktadır.

Anahtar Kelimeler: TSWV, Tokat Biberi, Dayanıklılık, Kalite, Verim

ABSTRACT

Tokat Pepper (*Capsicum annuum* L.) is a popular variety of pepper that has been cultivated and consumed in Tokat for many years. The production of this cultivar is hindered by various biotic stress factors, one of which is the tomato spotted wilt virus (TSWV) that causes significant economic losses in pepper farming. In this research, TSWV-resistant hybrid plants were cultivated using local pepper lines, and the yield, fruit characteristics, and resistance levels of these hybrids were analysed. Three

different strains of 'Tokat Pepper', which had undergone self-fertilisation to reach the fifth generation, were interbred with two donor plants that were resistant to TSWV and carried the TSW gene. The hybrid plants were cultivated in soilless farming conditions and TSWV was transmitted by both mechanical means and the use of floral thrips (*Frankliniella occidentalis*). While the marketable yields of the parent plants ranged from 2.260 kg per plant to 2.727 kg per plant, the yields of the hybrid genotypes varied from 1.917 kg per plant to 2.527 kg per plant. Marketable yields of the hybrid genotypes were lower than those of the parents. The hybrid plants exhibited heterozygous resistance to TSWV. However, it was observed that resistance in hybrid plants was compromised when the air temperature surpassed 32°C in the screenhouse. The study revealed that fruit shape of the hybrid plants was either 3 or 4 lobed, and fruit colour ranged from light green to dark green. In conclusion, the TSWV resistance gene TSW has been effectively transferred to hybrid plants that were bred from native pepper varieties. Although hybrid plants showed lower yield, there was no visible effect on the fruit's appearance. Resistance was observed to break down at high temperatures, as it has been frequently reported in literature that the TSW gene may exhibit such problems. The study discovered that hybrid varieties of 'Tokat Pepper' can be developed to resist TSWV.

Key Words: TSWV, Tokat Biberi, Resistance, Quality, Yield

Giriş

Güney Amerika orijinli olan biber (*Capsicum annuum*), dünya genelinde birçok alanda yaygın olarak yetiştirilmektedir. Biber üretimi ve tüketimi 20. yüzyıl boyunca düzenli olarak artmıştır. *Solanaceae* familyasının diğer türlerinden olan domates ve patates gibi biber de popüler türler arasında yer almaktadır. Açık alanda yetiştiriciliğinin yanında Hollanda ve Kanada gibi kuzey ülkeleri ile İspanya, Türkiye, İtalya gibi Akdeniz ülkelerinde örtü altında popüler ürünlerden biri olmuştur (Pardossi ve ark., 2004). Acı ve tatlı biberler geniş bir tüketim şekline sahiptirler. Taze olarak değişik şekillerde tüketilmesinin yanında salça, sos, turşu, kurutulmuş ürün, pul biber, toz biber gibi çok değişik şekillerde de tüketilmektedir. Ayrıca içerdiği fitokimyasallar sayesinde insan sağlığı açısından da önemli bir sebzedir (Bosland ve Votava, 2003; Emmanuel-Ikpeme ve ark., 2014).

Dünya genelinde bu kadar popüler olan biberin yetiştiriciliğinde ve tüketimindeki pazar talepleri ve bunun oluşturduğu rekabet üreticileri daha yüksek verimli ve kaliteli çeşitler kullanmaya yöneltmiştir. Bu durum biber ıslahının gelişmesinde önemli bir pozitif etki oluşturmuştur. Biber ıslahında diğer önemli türlerde olduğu gibi meyve kalitesi, verim, hastalıklara dayanıklılık, erkencilik, stres toleransı ve bitki morfolojisi en önemli özelliklerdir. Biber ıslahında ıslahçıların ve üreticilerin en çok üzerinde durduğu özelliklerden biri üniformitedir. Dünyada 1980'li yıllarda bitki ıslahında yaşanan gelişmeler etkisini biber ıslahında da göstermiş ve 1980'den sonra her geçen gün ıslah çalışmaları artarak devam etmiştir. Biber ıslahındaki bu gelişme biber üretiminde ve veriminde de artışlar sağlamıştır. Dünya biber üretimi toplamda 1980 yılında 2.48 milyon hektar alanda 9.25 milyon ton iken, 2000 yılında 3.33 milyon hektar alanda 23.34 milyon ton, 2021 yılında ise 2,05 milyon ha alanda 36.29 milyon ton üretim yapılmıştır (Fao, 2021). Türkiye biber üretimi ise 377.905 ha alanda 1.445.275 ton kapya biber, 123.388 ha alanda 420.918 dolma biber 277.868 ha alanda 1.064.633 ton sivri biber ve 23.228 ha alanda 160.469 ton çarliston biber üretimi yapılmıştır (Tüik, 2022).

Üretim miktarındaki ve birim alandan elde edilen üründeki bu artışta biber ıslahının önemli rolü bulunmaktadır. Birden çok biyotik ve abiyotik stres faktörüne dayanıklı ve/veya tolerant ve aynı zamanda verim ve kalitesi yüksek hibrit çeşitler söz konusu olduğunda dışa bağımlılık artmaktadır. Oysa biberde domates lekeli solgunluk virüsü ve L4 virüsü hastalıklarına karşı dayanıklı hibrit çeşitler geliştirmek mümkündür. Günümüzde TSWV'ye karşı dayanıklı çeşit geliştirmek en popüler ıslah çalışmaları arasında yer almaktadır. TSWV'ye dayanıklılık *Capsicum annuum* türünde bulunmamaktadır, ancak *Capsicum chinense* (Boiteux ve ark., 1993), *Capsicum frutescens, Capsicum baccatum* (Boiteux ve ark., 1993) ve *Capsicum pubescens* (Nuez ve ark., 1994) türlerinde dayanıklılık bulunmaktadır. *Capsicum chinense* türü içinde PI-152225 ve PI-159236

hatları dayanıklılık taşımakta ve bu hatlar *Capsicum annum* ile melezlenebilmektedir (Cheng ve ark., 1989; Roggero ve ark., 2002). PI-152225 hattı ile *Capsicum annuum* arasında yapılan melezleme çalışmalarında dayanıklı hibritler elde edilebilmektedir ve TSWV'ye dayanıklılık TSW dominant tek gen tarafından kontrol edilmektedir (Boiteux, 1995; Jahn ve ark., 2000; Çetin, 2023).

Bu çalışmada domates lekeli solgunluk virüsüne dayanıklı F6 kademesinde saf hatlar ile dolmalık ve üç burun biber tipleri TSWV'ye dayanıklı hatlarla melezlenerek TSWV'ye dayanıklı hibritler elde edilip, yerel biber tiplerinde TSWV hastalığına dayanıklı çeşit adayları geliştirilecektir.

Materyal ve Metot

Bu çalışma 2019-2020 yılları arasında Tokat Gaziosmanpaşa Üniversitesi Tarımsal Araştırma ve Uygulama Merkezi arazisinde yer alan tül sera ve tam otomasyonlu serada yürütülmüştür.

Denemenin melezleme ve melez tohum üretimlerinin yapıldığı sera oluk altı yüksekliği 5 metre, taban alanı 2000 m^2, ısı ve gölge perdesi bulunan, üstleri kelebek havalandırmalı, yanları uzay havalandırmalı, havalandırma boşlukları böcek ağı ile kapalı, sulama ve gübreleme sistemi otomasyona bağlı, içinde hem fide yetiştirme bölümü hem de topraksız yetiştiriciliğe uygun altyapıya sahip bölümler mevcuttur.

Melez genotiplerin verim ve bitki gelişim durumlarının belirlenmesi için yapılan çalışmalar tül serada yürütülmüştür. Bu sera 10 m eninde, 40 m uzunluğunda (400 m^2), yanları 40 metre insekt net ile, ve üst çatısı %50 gölge tülü ile kaplı gotik çatılıdır. Denemenin yürütüldüğü Tokat ili Karadeniz ve İç Anadolu Bölgeleri arasında geçit iklimine sahip olup, denizden yüksekliği 612 m'dir.

Araştırmada kullanılan biber çeşitleri ve hatları

Araştırmada kullanılan Tokat Biberi'nin özellikleri İnce kabuk, meyve eti ince, kızartmaya uygun, üç burun yapısında ve meyvenin baş kısmı uç kısmına göre daha kalın olan bölgeye özgü bir çeşittir. Daha önce yürütülen ve F6 kademesine kadar kendilenmiş hatlar ve anter kültürü çalışmaları sonucunda geliştirilmiş dayanıklı hatlardan oluşan gen havuzundan verim denemeleri ve morfolojik gözlemler sonucunda seçilen 3 hat denemede ana ebeveyn olarak kullanılmıştır. Bu hatlar üç burun, koyu yeşil dolmalık ve açık yeşil dolmalık meyve tipine sahip genotiplerdir. Denemede donör bitki olarak TSWV dayanımı olan bir dolmalık (D1) ve bir üçburun (Ü1) hat kullanılmıştır. Donör bitkiler bir ıslah firmasının gen havuzundan seçilerek alınmıştır. Denemede kullanılan donör bitkilerin özellikleri Çizelge 1'de, bitki yapısı ve temin edilen tohumların resimleri Şekil 1'de dolmalık ve üç burun hatların resimleri Şekil 2'de verilmiştir.

Çizelge 1.Çalışmada kullanılan TSWV ye karşı dayanıklı donör hatlar

Table 1. TSWV resistant donor lines used in the study

Donör Hatlar *Donor Lines*	**Dayanım Durumu** *Endurance Status*	**Homozigotluk Düzeyleri** *Homozygosity Levels*	**Orijini** *Origin*
Dolma (D1)	TSW, PC, L4	IL	SEM 334
Üç burun (Ü1)	TSW, L4	F5	Ticari F_2 popülasyonundan

Şekil 1. Donör bitkiler ve tohumları

Figure 1. Donor plants and seeds

Şekil 2. Denemede kullanılan yerel biber genotipleri

Figure 2. Local pepper genotypes used in the experiment

Ebeveyn hatların yetiştirilmesi ve melezlemelerin yapılması

Çalışmada melezleme çalışmaları 2019 yılında yapılmış ve melez bitkilerin tohumları elde edilmiştir. Melezlemelerin yapılışı ve melez tohumların elde edilmesi aşağıdaki gibi gerçekleştirilmiştir. Ebeveyn hatların tohum ekimleri 25 Nisan 2019 tarihinde yapılmıştır. Fide dikimleri 25 Mayıs 2019 tarihinde yapılmıştır. Her ebeveyn için 10 bitki yetiştirilmiştir. Ebeveyn bitkiler serada topraksız tarım koşullarında yetiştirilmiştir. Yetiştirme ortamı olarak 1:1 oranında torf-perlit karışımı kullanılmıştır. Bitkiler Hoagland besin solüsyonu ile gübrelenmiştir. Ana ebeveyn olarak kullanılan bitkilerde ilk çiçekler bitkiden uzaklaştırıldıktan sonra diğer çiçekler melezlemede kullanılmıştır. Tozlayıcı olarak kullanılan bitkilerin çiçekleri 1 gün önceden kapatılmış, ertesi gün sabah erken saatlerde çiçek tomurcukları alındıktan sonra polen taneleri bir pensin ucu ile anterlerden çıkarılarak ana bitkilerin stigmaları üzerine aktarılmıştır. Ana bitkilerde melezleme yapılan çiçekler taç yaprakları açılmamış ancak açılmak üzere olan çiçekler seçilmiş, bir pens ile dikkatlice taç yaprakları açılmış, anterler patlatılmadan iplikçiklerinden tutularak çiçekten uzaklaştırılmıştır. Melezlemesi yapılan çiçeklerde çanak yapraklar stigmayı kırmadan stigma etrafına doğru kapatılmış ve bir cam bant ile izolasyonu yapılmıştır. Melezlemesi yapılan çiçeklere ana ebeveyn x donör ebeveyn adı ve melezleme tarihi yazılarak etiketlenmiştir. Ayrıca ana ve donör bitkilerde her genotip için 10 adet çiçekte kendileme yapılarak ebeveyn hatlarda tohum üretimine devam edilmiştir. Melezleme ve kendilemesi yapılan çiçeklerin gelişmesi ile oluşan meyveler tam olgunluğa ulaştıklarında hasat edilmiş ve bir gün karanlıkta bekletildikten sonra tohumları çıkarılmıştır. Tohumlar hasat edildikten sonra %15 nem içerecek şekilde oda sıcaklığında ve karanlık ortamda kurutulmuş ve daha sonra thiram ile muamele edilerek paketlenmiştir.

Melez hatların yetiştirilmesi

Melez tohumların elde edilmesi yaklaşık 6 ay

sürmüştür. Melez bitkilerin verim, TSWV dayanıklılığı ve morfolojik özellikleri 2020 yılında tül serada yürütülen çalışmada incelenmiştir. Melez bitkiler 2019 yılında ebeveyn hatların yetiştiriciliğinde uygulanan yöntemin aynısı kullanılarak yetiştirilmiştir. Melez bitkilerde fideler 6 Haziran 2020 tarihinde dikilmiştir.

Patolojij çalışmalar

Denemede patolojik testlemeler için Tokat'ta biber tarımının yoğun olarak yapıldığı Kazova yöresinde biber tarlaları gezilmiş, TSWV belirtisi gösteren bitkilerden hastalıklı bitki parçaları alınmış ve naylon torbalara konmuştur. Ayrıca bu bitkilerde çiçekler sallanarak çıkan tripsler bir tüp içine alınmıştır. Enfekteli bitkilerden alınan doku parçaları testlenecek bitkilerin yan dallarının gövdelerinde yara yüzeyi oluşturulmuş ve bu yüzeylere sürtme yöntemi ile bulaştırma yapılmıştır. Ayrıca tüp içine toplanan tripslerden her bitkinin çiçeğine trips bulaştırması yapılmıştır (Şekil 3; Şekil 4).

Virüsler genellikle 15 günlük kuluçka evresi sonunda etkilerini göstermeye başlamaktadırlar. Bu özellik dikkate alınarak inokulasyondan yaklaşık 1 ay sonra bitki ve meyvelerde gözlemler yapılmış ve hastalık gelişme düzeyleri kaydedilmiştir. Patoloji çalışması 5 tekerrürlü olarak yürütülmüş ve her tekerrürde 6 bitki kullanılmıştır.

Şekil 3. Melezleme aşamalarından görünüm
Figure 3. View of the hybridisation stages

Şekil 4. Yerel genotiplerin dayanıklı hatlarla melezleme sonucu ortaya çıkan melez bitkiler
Figure 4. Hybrid plants resulting from crossbreeding local genotypes with resistant lines

Moleküler çalışmalar

Melez bitkilerde patolojik testlemenin yanında moleküler testleme de yapılmıştır. Biberde lekeli solgunluk virüsüne dayanıklılık TSW geni ile kontrol edilmektedir. Melez bitkilerde testlemelerde TSW geni markır yardımı ile test edilmiştir.

Dayanıklılık geninin varlığını tespit edebilmek için DNA ekstraksiyonu DNA Purification Kiti kullanılarak yapılmıştır. İzolasyon sonucunda elde edilen DNA'lar kalıp olarak kullanılmış ve Moury ve ark. (2000) tarafından geliştirilmiş (TSW genine

özelleşmiş) CAPS markırı (SCAC 568) kullanılarak PCR (Thermo Fisher scientific, ABD) işlemi gerçekleştirilmiştir.

Çalışmada PCR işleminde, genomik DNA, 10×PCR buffer, dNTP karışımı, 25mm MgCl2, forward (5'GTGCCAGAGGAGGATTTAT3') ve reverse (5'GCGAGGTGGACACTGATAC3') primerlerin (10 pmol µl-1) her birinden 1 µl ve 5 U µl Taq polymerase enzimi içeren karışım kullanılmış ve 3 dakika başlangıç denatürasyonu, 35 döngü 95 °C'de 50 saniye denatürasyon, 57 °C'de 45 saniye bağlanma, 72 °C'de 50 saniye uzama, bunu takiben 72 °C'de 10 dakika son uzama koşullarında PCR yapılmıştır. PCR ürünlerinin kontrolü için PCR ürünleri %1.2 oranında hazırlanan, içerisinde 10 mg/ml ethidium bromid bulunan agaroz jelde 100 V'da 1 saat elektroforez işlemine tabi tutulmuştur. Elektroforez işlemi sonunda görüntüleme cihazında görüntüleme işlemi yapılmıştır ve ayrıca araştırmada promega enzimi kullanılmıştır (Tablo 2).

Çizelge 2. Tsw ve lokusların PCR amplifikasyonu için kullanılan primer dizileri
Table 2. Primer sequences used for PCR amplification of Tsw and loci

Primer name ***Primer adı***	**Primer sequences (5–3)** ***Primer dizilişi(5-3)***	**PCR product (bp)** ***PCR ürünü (bp)***	**Locus** ***Lokus***	**Restriction enzyme** ***Kısıtlama enzimi***	**References** ***Kaynaklar***
SCAC 568	GTGCCAGAGGAGGATTTAT GCGAGGTGGACACTGATACT	568	TSW	Xbal	(Moury ve ark. 2000)

Bulgular ve tartışma

Erkenci verim

Denemede erkenci verim değerleri bitki başına meyve sayısı ve meyve ağırlığı olarak belirlenmiştir. Denemede kullanılan ana ebeveyn genotipler ve bunlardan elde edilen melezlerin erkenci meyve sayıları 20.06 adet/bitki (Koyu dolmalık x D1 melezi) ile 33.00 adet/bitki (Koyu dolmalık x Ü1 melezi) arasında, erkenci verimleri 0.393 kg/bitki (Koyu dolmalık x D1 melezi) ile 0.847 kg/bitki (Koyu dolmalık ana ebeveyn) arasında değişmiştir. Erkenci meyve sayısı bakımından melez bitkiler ana ebeveynlere göre daha üstün olurken, erkenci verim bakımından melez bitkiler ana ebeveynlerden daha düşük verim vermiştir. Gerek erkenci meyve sayısı ve gerekse erkenci verim bakımından genotipler arasındaki farklar önemli çıkmamıştır. Erkenci meyve sayısı ve erkenci meyve verimine göre varyans analiz sonuçları Çizelge 3'te, ana ebeveyn ve melez genotiplerin erkenci meyve sayıları ve erkenci meyve verimleri Çizelge 4'de verilmiştir.

Çizelge 3. Erkenci meyve sayısı ve erkenci meyve verimine ait varyans analizi
Table 3. Variance analysis of early fruit number and early fruit yield

Erkenci meyve sayısı - *Number of early fruits*					
Varyans Kaynağı	**Kareler Toplamı**	**Düzeltme Faktörü**	**Kareler Ortalaması**	**F**	**p**
Genotip	550.501	8	68.813	1.871	0.128
Hata	662.011	18	36.778		
Toplam	20640.384	27			
Erkenci meyve verimi - *Early fruit yield*					
Varyans Kaynağı	**Kareler Toplamı**	**Düzeltme Faktörü**	**Kareler Ortalaması**	**F**	**P**
Genotip	0.520	8	0.065	2.477	0.052
Hata	0.472	18	0.026		
Toplam	12.491	27			

Çizelge 4. Genotiplerin erkenci verimleri
Table 4. Early yields of genotypes

Genotip *Genotype*	Erkenci verim *Early yields* Meyve sayısı (adet/bitki) *Number of fruits (pcs/plant)*	Verim (kg/bitki) *Yield (kg/plant)*
Üç Burun	24.05	0.770
Açık Dolmalık	22.16	0.703
Koyu Dolmalık	29.28	0.847
Üç Burun x D1	22.33	0.493
Koyu Dolmalık x D1	20.06	0.393
Açık Dolmalık x D1	27.59	0.550
Açık Dolmalık x Ü1	31.22	0.650
Üç Burun x Ü1	31.72	0.773
Koyu Dolmalık x Ü1	33.00	0.693
	ö.d.	ö.d.

ö.d.: Uygulamalar arasındaki farkların istatistiksel olarak önemli olmadığını ifade eder

Pazarlanabilir verim

Bitki başına düşen pazarlanabilir meyve sayısı 76.95 adet/bitki (Üç burun ana ebeveyn) ile 102.81 adet/bitki (Açık dolmalık x Ü1 melezi) arasında değişirken, pazarlanabilir meyve verimi 1.917 kg/bitki (Açık dolmalık x D1) ile 2.727 kg/bitki (Açık dolmalık ana ebeveyn) arasında değişmiştir. Pazarlanabilir meyve sayısı bakımından melez genotipler ana ebeveynlere göre daha üstün performans göstermiş ve genotipler arasındaki farklılıklar P≤0,01 düzeyinde önemli bulunmuştur. Pazarlanabilir meyve sayısında melez bitkilerde ana ebeveynlere göre artış sağlanırken, üç burun x Ü1 melezi ana ebeveyne göre daha üstün verim vermiş, diğer melezlerin verimleri ana ebeveynlerin altında kalmıştır. Denemede pazarlanabilir meyve sayısı bakımından genotipler arasındaki farklılıklar P≤0,01 düzeyinde, pazarlanabilir verim bakımından genotipler arasındaki farklılıklar P≤0,001 düzeyinde önemli çıkmıştır. Pazarlanabilir meyve sayısı ve pazarlanabilir meyve verimi Çizelge 5'te, ana ebeveyn ve melez genotiplerin pazarlanabilir meyve sayıları ve meyve verimleri Çizelge 6'da verilmiştir.

Çizelge 5. Pazarlanabilir meyve sayısı ve pazarlanabilir meyve verimine ait varyans analizi
Table 5. Analysis of variance for the number of marketable fruits

Pazarlanabilir meyve sayısı - *Number of marketable fruits*					
Varyans Kaynağı	**Kareler Toplamı**	**Düzeltme Faktörü**	**Kareler Ortalaması**	**F**	**p**
Genotip	1591.800	8	198.975	3.510	0.013
Hata	1020.301	18	56.683		
Toplam	238688.382	27			
Pazarlanabilir meyve verimi - *Marketable fruit yield*					
Varyans Kaynağı	**Kareler Toplamı**	**Düzeltme Faktörü**	**Kareler Ortalaması**	**F**	**P**
Genotip	1.899	8	0.237	6.653	0.000
Hata	0.642	18	0.036		
Toplam	141.398	27			

Çizelge 6. Genotiplerin pazarlanabilir verimleri
Table 6. Marketable yields of genotypes

Genotip *Genotype*	Pazarlanabilir verim / *Marketable yields*	
	Meyve sayısı (adet/bitki) *Number of fruits(pcs/plant)*	Verim (kg/bitki) *Yield (kg/plant)*
Üç Burun	76.95 b	2.260 bc
Açık Dolmalık	88.13 ab	2.727 a
Koyu Dolmalık	90.69 a	2.593 ab
Üç Burun x D1	101.22 a	2.107 c
Koyu Dolmalık x D1	102.00 a	2.027 c
Açık Dolmalık x D1	93.44 a	1.917 c
Açık Dolmalık x Ü1	102.81 a	2.160 c
Üç Burun x Ü1	93.88 a	2.527 ab
Koyu Dolmalık x Ü1	92.44 a	2.093 c
	**	***

** Uygulamalar arasındaki farkların P≤0,01 düzeyinde önemli olduğunu ifade eder
*** Uygulamalar arasındaki farkların P≤0,001 düzeyinde önemli olduğunu ifade eder

Iskarta verim

Pazarlanabilir kalitede olmayan bozuk şekilli meyvelerin ıskarta olarak değerlendirilmiş ve genotiplerin ıskarta meyve sayıları 1.22 adet/bitki (Açık dolmalık x D1 melezi) ile 3.78 adet/bitki (Koyu dolmalık x D1) arasında, ıskarta meyve verimi 0.01 kg/bitki (Açık dolmalık x D1 melezi) ile 0.06 kg/bitki (Üç burun ana ebeveyn) arasında değişmiştir.

Iskarta verim bakımından ana ebeveynler ile melezler arasında anlamlı bir ilişki bulunamamıştır. Sayı ve ağırlık olarak ıskarta verim bakımından genotipler arasındaki farklar önemli çıkmamıştır. Iskarta meyve sayısı ve ıskarta meyve verimine göre varyans analiz sonuçları Çizelge 7'de, ana ebeveyn ve melez genotiplerin ıskarta meyve sayıları ve ıskarta meyve verimleri Çizelge 8'de verilmiştir.

Çizelge 7. Iskarta meyve sayısına ait varyans analizi
Table 7. Analysis of variance for the number of discarded fruits

Iskarta meyve sayısı - *Number of discarded fruits*					
Varyans Kaynağı	**Kareler Toplamı**	**Düzeltme Faktörü**	**Kareler Ortalaması**	**F**	**P**
Genotip	24.927	8	3.116	0.765	0.637
Hata	73.350	18	4.075		
Toplam	270.294	27			
Iskarta meyve verimi - *Discarded fruit yield*					
Varyans Kaynağı	**Kareler Toplamı**	**Düzeltme Faktörü**	**Kareler Ortalaması**	**F**	**P**
Genotip	0.006	8	0.001	0.809	0.603
Hata	0.016	18	0.001		
Toplam	0.062	27			

Çizelge 8. Genotiplerin Iskarta verimleri
Table 8. Discard yields of genotypes

Genotip *Genotype*	Erkenci verim / *Early yield*	
	Meyve sayısı (adet/bitki) *Number of fruits (pcs/plant)*	Verim (kg/bitki) *Yield (kg/plant)*
Üç Burun	3.61	0.06
Açık Dolmalık	2.55	0.05
Koyu Dolmalık	1.45	0.03
Üç Burun x D1	2.17	0.03
Koyu Dolmalık x D1	3.78	0.05
Açık Dolmalık x D1	1.22	0.01
Açık Dolmalık x Ü1	3.00	0.04
Üç Burun x Ü1	1.39	0.02
Koyu Dolmalık x Ü1	3.55	0.05
	ö.d.	ö.d.

ö.d. ** Uygulamalar arasındaki farkların önemli olmadığını ifade eder.

Toplam verim

Denemede bitki başına düşen toplam meyve sayısı 80.56 adet/bitki (Üç burun ana ebeveyn) ile 105.81 adet/bitki (Açık dolmalık x Ü1 melezi) arasında değişirken, toplam meyve verimi 1.93 kg/bitki (Açık dolmalık x D1 melezi) ile 2.78 kg/bitki (Açık dolmalık ana ebeveyn) arasında değişmiştir. Bitki başına düşen toplam meyve sayısı bakımından melez genotipler ana ebeveynlere göre daha üstün performans göstermiş ve genotipler arasındaki farklılıklar P≤0,05 düzeyinde önemli bulunmuştur. Toplam verimde üç burun x Ü1 melezi ana ebeveyne göre daha üstün verim vermiş, diğer melezlerin verimleri ana ebeveynlerin altında kalmıştır. Denemede toplam meyve verimi bakımından genotipler arasındaki farklılıklar P≤0,01 düzeyinde önemli çıkmıştır. Bitki başına toplam meyve sayısı ve toplam meyve verimi varyans analiz sonuçları Çizelge 9'da, ana ebeveyn ve melez genotiplerin toplam meyve sayıları ve meyve verimleri Çizelge 10'da verilmiştir.

Çizelge 9. Toplam meyve sayısına ait varyans analizi
Table 9. Analysis of variance of total number of fruits

Toplam meyve sayısı - *Total number of fruits*					
Varyans Kaynağı	**Kareler Toplamı**	**Düzeltme Faktörü**	**Kareler Ortalaması**	**F**	**P**
Genotip	1591.325	8	198.916	3.125	0.021
Hata	1145.840	18	63.658		
Toplam	251730.471	27			
Toplam meyve verimi - *Total fruit yield*					
Varyans Kaynağı	**Kareler Toplamı**	**Düzeltme Faktörü**	**Kareler Ortalaması**	**F**	**P**
Genotip	1.931	8	0.241	6.397	0.001
Hata	0.679	18	0.038		
Toplam	146.117	27			

Çizelge 10. Genotiplerin Toplam verimleri
Table 10. Total yield of genotypes

Genotip *Genotype*	Toplam Verim *Total Yield*	
	Meyve sayısı (adet/bitki) *Number offruits(pcs/plant)*	**Verim (kg/bitki)** *Yield (kg/plant)*
Üç Burun	80.56 b	2.32 bc
Açık Dolmalık	90.68 ab	2.78 a
Koyu Dolmalık	92.14 ab	2.62 ab
Üç Burun x D1	103.39 a	2.13 cd
Koyu Dolmalık x D1	105.78 a	2.07 cd
Açık Dolmalık x D1	94.67 ab	1.93 d
Açık Dolmalık x Ü1	105.81 a	2.20 cd
Üç Burun x Ü1	95.26 ab	2.55 ab
Koyu Dolmalık x Ü1	96.00 a	2.15 cd
	*	**

* Uygulamalar arasındaki farkların P≤0,05 düzeyinde önemli olduğunu ifade eder.
** Uygulamalar arasındaki farkların P≤0,01 düzeyinde önemli olduğunu ifade eder.

Ortalama meyve ağırlığı

Denemede kullanılan ana ebeveyn genotiplerin ve bunlardan elde edilen melezlerin ortalama meyve ağırlığı 19.87 adet/bitki (koyu dolmalık x D1 melezi) ile 31.14 adet/bitki (açık dolmalık ana ebeveyn) arasında değişmiştir. Melez genotiplerin ortalama meyve ağırlığı ebeveynlere göre daha düşük çıkmıştır. Ortalama meyve ağırlığı bakımından genotipler arasındaki fark P≤0,001 düzeyinde önemli çıkmıştır. Ortalama meyve ağırlığına göre varyans analiz sonuçları Çizelge 11'de, ana ebeveynler ve melez genotiplerin ortalama meyve ağırlıkları Çizelge 12'de verilmiştir.

Çizelge 11. Ortalama meyve ağırlığına ait varyans analizi
Table 11. Analysis of variance of average fruit weight

Varyans Kaynağı	Kareler Toplamı	Düzeltme Faktörü	Kareler Ortalaması	F	P
Genotip	472.669	8	59.084	13.600	0.000
Hata	78.197	18	4.344		
Toplam	16842.496	27			

Çizelge 12. Genotiplerin ortalama meyve ağırlıkları
Table 12. Mean fruit weights of genotypes

Genotip *Genotype*	**Ortalama Meyve Ağırlığı (g)** *Mean Fruit Weight (g)*
Üç Burun	29.39 ab
Açık Dolmalık	31.14 a
Koyu Dolmalık	28.74 ab
Üç Burun x D1	20.74 c
Koyu Dolmalık x D1	19.87 c
Açık Dolmalık x D1	20.54 c
Açık Dolmalık x Ü1	21.10 c
Üç Burun x Ü1	26.86 b
Koyu Dolmalık x Ü1	22.69 c

*** Uygulamalar arasındaki farkların P≤0,001 düzeyinde önemli olduğunu ifade eder.

Meyve özellikleri

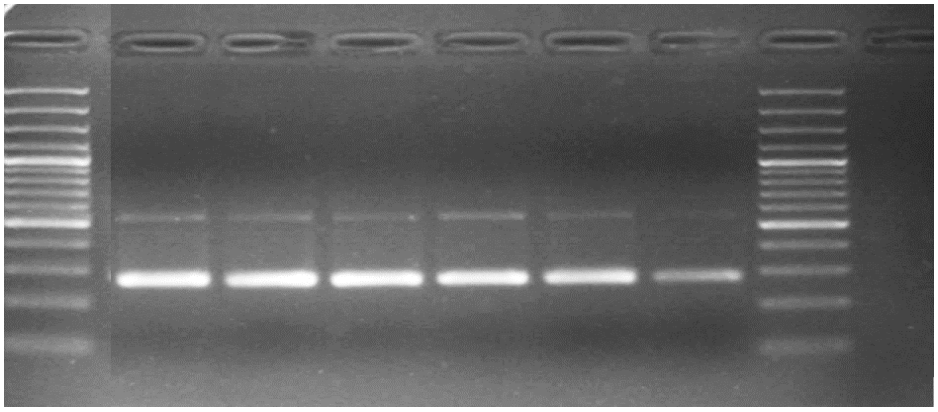
Denemede kullanılan melez bitkilerin meyve renkleri ana ebeveynlerin rengine benzer çıkmış ve koyu yeşil, açık yeşil ve yeşil olarak gözlenmiştir. Melez bitkilerin meyve şekli ana ebeveynlerin meyve şekline benzer çıkmış, uzun yuvarlak ve oval yuvarlak şeklinde gözlenmiştir. Meyvede lob sayıları da ana ebeveynin lob sayısına benzer çıkmış ve 3 ve 4 loblu olarak gözlenmiştir. Çalışmada kullanılan ana ve melez bitkilerin meyve rengi, meyve şekli ve lob sayıları çizelge 13'te verilmiştir.

Çizelge 13. Genotiplerin meyve rengi, meyve şekli ve lob sayıları
Table 13. Fruit colour, fruit shape and number of lobes of genotypes

Genotip *Genotypes*	**Meyve Rengi** *Fruit colour*	**Meyve Şekli** *Fruit Shape*	**Lob Sayısı** *Number of lobes*
Koyu Dolmalık	Koyu Yeşil	Yuvarlak-Uzun	4
Açık Dolmalık	Açık Yeşil	Yuvarlak-Oval	3
Üç Burun	Yeşil	Yuvarlak-Oval	3
Koyu Dolmalık X Ü1	Koyu Yeşil	Yuvarlak-Uzun	4
Koyu Dolmalık X D1	Koyu Yeşil	Yuvarlak-Uzun	4
Açık Dolmalık X Ü1	Açık Yeşil	Yuvarlak-Oval	3
Açık Dolmalık X D1	Açık Yeşil	Yuvarlak-Oval	3
Üç Burun X Ü1	Yeşil	Yuvarlak-Oval	3
Üç Burun X D1	Yeşil	Yuvarlak-Oval	3

TSWV dayanıklılık düzeylerinin moleküler ve patolojik bulguları

Denemede kullanılan melez bitkilerde TSWV dayanıklılığının belirlenmesinde Moury ve ark. (2000) tarafından geliştirilen ve TSW genine özelleşmiş CAPS markırı (SCAC 568) kullanılmıştır. Deneme sonucunda melez bitkilerde TSW geni SCAC 568 primeri ile heterozigot profil göstermiştir. Melez bitkilerin TSWV'ye dayanımları heterozigot dayanıklılık şeklinde değerlendirilmiştir. Taq polymerase enzimi kesim sonuçları Şekil 5'te verilmiştir.



Şekil 5. Melez genotiplerin TSW genine ait görünüm
Figure 5. View of TSW gene of hybrid genotypes

Tokat biberi olarak bilinen yerel dolmalık ve üç burun biber hatlarının TSWV dayanımlı donör bitkilerle melezlenmeleri ile elde edilen melez bitkilerde dayanıklılık düzeyi hem patolojik testleme hem de markır tekniği kullanılarak test edilmiştir. Başlangıçta herhangi bir simptom görülmemiş, ancak özellikle tül serada sıcaklığın 35^{o}C'nin üzerine çıkması ile beraber hem ana ebeveyn bitkilerde hem de melez bitkilerde yaprak ve meyvelerde TSWV simptomları görülmüştür. Simptomlar bitkiler yaşlandığında daha da belirgin hale gelmiştir.

Ayrıca aynı melezin farklı bitkilerinde TSWV'ye dayanıklılık konusunda farklı sonuçlar ortaya çıkmış, bitkilerin yaklaşık % 50'si TSWV zararı daha şiddetli görülmüştür. Domates lekeli solgunluk virüsü domates ve biber başta olmak üzere birçok bitkide zarar oluşturmaktadır ve *Frankliniella occidentalis* Pergande bu virüsün taşınmasında ve yayılmasında oldukça etkili bir vektördür (German ve ark., 1992).

Biberde *Capsicum chinense* Jacq türünün farklı aksesyonlarında dayanıklılık tespit edilmiş olup, majör bir gen olan TSW geni TSWV'ye dayanıklılık sağlamaktadır (Black ve ark., 1991; Boiteux, 1995; Moury ve ark., 1997). Ancak yapılan çalışmalar TSW geninin zaman zaman etkisiz kaldığı ve kırıldığını ortaya koymuştur. Özellikle genç bitkilerin 30 ^{o}C'nin üzerindeki sıcaklılara maruz kalması, bitkinin yaşı, iklim koşulları, patojenite ve bitkinin maruz kaldığı inokulum yoğunluğu bitkideki dayanıklılığın kırılmasına neden olmaktadır (Black ve ark., 1991; Nuez ve ark., 1994; Gil-Ortega ve Luis, 1994; Salomon ve ark., 2016).

Roggero (2002)'da yüksek sıcaklıklar ve enfeksiyonun bitkiye bulaştığı döneme bağlı olarak biberde TSWV'ye dayanıklılığın kırıldığını belirtmektedir. Denemede TSWV dayanımının yüksek sıcaklıklarda kırılmasına yönelik elde edilen bulgular literatür sonuçları ile benzerlik göstermiştir.

Moury ve ark. (1998), biberde TSWV'ye dayanıklılığı TSW lokusundaki heterozigotluk veya homozigotluk ile ilişkilendirmektedir. Araştırıcılar hibrit bir biber bitkisi sürekli yüksek sıcaklıklarda yetiştirildiğinde TSW lokusu heterozigot ise homozigot olana göre daha az dayanıklı olmaktadır. Bu nedenle de TSW lokusu homozigot çeşitlerin geliştirilmesini önermektedirler.

Ayrıca, Çelik ve ark. (2018), TSWV'ye dayanıklı 3 hattı donör bitki olarak kullanarak Serademre 8 bitkisi ile melezlemişler ve Fı bitkilerinde dayanıklılığın %49,5 olduğunu, melez bitkileri F5 kademesine kadar kendilediklerinde ise dayanıklılığın %98'e kadar ulaştığını belirlemişlerdir. Denememizde de hibrit çeşitlerde dayanıklılığın bitkiler bazında farklı düzeylerde ortaya çıkması bu çalışma ile benzerlik göstermiştir.

Sonuçlar

Tokat biberi popülasyonlarından teksel seleksiyon yoluyla elde edilen dolmalık ve üç burun yerel biber hatlarından TSWV'ye dayanıklı melez bitkiler elde etmek için yürütülen çalışmada 2 dolmalık ve bir üç burun biber hattı ana ebeveyn olarak kullanılmış ve TSWV'ye dayanıklılık geni olan TSW genini taşıyan dolmalık ve üç burun donör biber hatları ile melezlenmiştir. Melezlemeler

sonucunda 6 melez genotip elde edilmiş, melez genotiplerde meyve özellikleri, verim ve TSWV dayanım düzeyleri araştırılmıştır. Çalışmada melezlemeler başarıyla tamamlanmış ve yeterli düzeyde Fı tohum elde edilmiştir.

Verim özellikleri bakımından bazı melez bitkiler ana ebeveynlere göre daha üstün performans gösterememişlerdir. Genotipler arasında önemli farklılıklar oluşmakla beraber, melez bitkilerin pazarlanabilir ve toplam verimleri ana ebeveyn hatlardan düşük çıkmıştır. Benzer şekilde melez bitkilerin ortalama meyve ağırlıkları da ana ebeveyn bitkilerin altında kalmıştır. Meyve şekli, meyve rengi ve meyvedeki lob sayısı %100 ana ebeveynin aynısı çıkmıştır. Patolojik testlemelerde vejetasyon döneminde bitkilerin gelişimi sağlıklı şekilde devam etmiş, ancak sera içi sıcaklıkların 30 °C'nin üzerine çıkması ile birlikte melez bitkilerde TSW geninin etkisiz kaldığı ve dayanıklılığın kırıldığı gözlenmiştir. Bu dönemde özellikle melez bitkilerde yaprak ve sürgünlerde TSWV belirtileri görülmüş ve ilerleyen dönemlerde belirtiler meyvelerde de görülmeye başlanmıştır. Melez bitkilerde TSW dayanıklılık geninin incelendiği çalışmada melez bitkilerin heterozigot dayanıklılığa sahip oldukları anlaşılmıştır.

Bu veriler ışığında ortaya çıkan en önemli sonuç eğer biberde TSWV'ye dayanıklı melez bitkiler geliştirilmek isteniyorsa dayanıklılığın kaynağı olarak kabul edilen TSW geninin ana ebeveynde olması tercih edilmelidir. Yerel popülasyonlardan TSWV'ye dayanıklı melez bireyler elde edilecekse öncelikli olarak TSW geni taşıyan donör bitkilerle melezlendikten sonra geriye melezlemeler yapılarak dayanıklı ve aynı zamanda kendine has özellikleri kaybolmamış hatların (yarıyol materyali) elde edilmesi gerekir. Bu durum zaman alsa da ıslah tekniği açısından uygulanması oldukça kolay tekniklerden biridir.

Denemede TSW geninin özellikle yüksek sıcaklık koşullarında kırıldığı gözlenmiştir. Literatürde de yüksek sıcaklık, patojen yoğunluğu, ırk farklılığı gibi faktörlere bağlı olarak TSW geninin kırıldığı genel olarak kabul görmektedir. Bununla beraber günümüzde biberde domates lekeli solgunluk virüsüne karşı dayanıklılık kaynağı olarak sadece TSW geni bilinmekte ve kırılması söz konusu olmasına rağmen koruyucu etkisi göz ardı edilememektedir. Dolayısıyla bir yandan TSW geninin dayanıklılıkta kullanılırken, diğer yandan yüksek sıcaklık, saldırgan patojen vb. durumlarda TSWV'ye karşı koruyuculuğunu devam ettirecek yeni dayanıklılık kaynaklarının bulunması ve *C. annuum* aktarılması gerekmektedir.

Yazarların Katkısı

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan ederler.

Beyanlar

Çıkar çatışması: Yazarlar herhangi bir çıkar çatışması olmadığını beyan etmektedir.

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Research Article/Araştırma Makalesi

The Effect of 24–Epibrassinolide on vegetative growth of Sweet Ann strawberry seedling under lime stress conditions

24–Epibrassinolide'in Kireç Stresi Koşullarında Sweet Ann Çilek Fidelerinin Vejetatif Büyümesi Üzerine Etkisi

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ABSTRACT

The research was conducted in 2022-2023 in Yozgat. The study was set up to determine the responses of the Sweet Ann strawberry variety to different lime levels and the effects of 24-Epibrassinolide (24-eBL) applications on vegetative growth. It was observed that plant growth and development were negatively affected by the increase in lime doses. It was found that 24-eBL applications (BR) increased leaf and root fresh weight, iron and zinc uptake in calcareous conditions. In terms of leaf fresh weight, it was found to be higher in 0% Lime x 0 mg l^{-1} BR, 0% Lime x 1 mg l^{-1} BR, 0% Lime x 2 mg l^{-1} BR and 5% Lime x 0 mg l^{-1} BR applications compared to other applications, and it was determined that they were statistically in the same group. Regarding leaf area, the highest leaf area in the Lime x BR interaction was determined as 32.13 cm^2 in the 0% lime x 0 mg l^{-1} BR combination and 33.60 cm^2 in the 0% lime x 1 mg l^{-1} BR combination. Leaf chlorophyll content (SPAD) was statistically highest in 0% lime x 1 mg l^{-1} BR, 5% lime x 0 mg l^{-1} BR and 10% lime x 0 mg l^{-1} BR combinations. The highest stoma conductivity values were observed from 0% lime x 0 mg l^{-1} BR and 0% lime x 1 mg l^{-1} BR applications. Considering the lipid peroxidation (MDA) gave statistically significant the highest values 10% lime x 2 mg l^{-1} BR, 5% lime x 1 mg l^{-1} BR and 5% lime x 0 mg l^{-1} BR applications. While the highest N (%) and P (%) contents in the leaves were measured in the 0% lime x 0 mg l^{-1} BR application, the highest K (%) content was detected in the 5% lime x 1 mg l^{-1} BR application.

Key Words: Strawberry, lime, Brassinosteroid, vegetative growth, nutrient elements

ÖZ

Araştırma 2022-2023 yıllarında Yozgat'ta yürütülmüştür. Çalışma, Sweet Ann çilek çeşidinin farklı kireç seviyelerine ve 24-Epibrassinolide (BR) uygulamalarının vejetatif büyüme üzerine etkilerini belirlemek amacıyla kurulmuştur. Bitki büyüme ve gelişmesinin kireç dozlarındaki artıştan olumsuz etkilendiği görülmüştür. Kireçli koşullarda BR uygulamalarının yaprak ve kök taze ağırlığını, demir ve çinko alımını artırdığı tespit edilmiştir. Yaprak yaş ağırlığı en yüksek %0 Kireç x 1 mg l^{-1} BR, %0 Kireç x 0 mg l^{-1} BR, %0 Kireç x 2 mg l^{-1} BR ve %5 Kireç x 0 mg l^{-1} BR uygulamalarında belirlenmiştir. Yaprak alanı bakımından, Kireç x BR etkileşiminde en yüksek yaprak alanı 32,13 cm^2 ile %0 kireç x 0 mg l^{-1} BR ve 33,60 cm^2 ile %0 kireç x 1 mg l^{-1} BR kombinasyonunda belirlenmiştir. Yaprak klorofil içeriği SPAD değeri açısından

incelendiğinde, Kireç x BR etkileşimi %0 kireç x 1 mg l^{-1} BR, %5 kireç x 0 mg l^{-1} BR ve %10 kireç x 0 mg l^{-1} BR kombinasyonlarında istatistiksel olarak en yüksek bulunmuştur. Stoma iletkenliği en yüksek değerleri %0 kireç x 0 mg l^{-1} BR ve %0 kireç x 1 mg l^{-1} BR uygulamalarından elde edilmiştir. Kireç x BR interaksiyonu bakımından lipid peroksidasyon (MDA) değerine bakıldığında, istatistiksel olarak en yüksek değerleri %10 kireç x 2 mg l^{-1} BR, %5 kireç x 1 mg l^{-1} BR ve %5 kireç x 0 mg l^{-1} BR uygulamalarında tespit edilmiştir. Yapraklarda N (%) ve P (%) içeriği en yüksek 0% kireç x 0 mg l^{-1} BR uygulamasında ölçülürken en yüksek K (%) içeriği 5% Lime x 1 mg l^{-1} BR uygulamasında tespit edilmiştir.

Anahtar Kelimeler: Çilek, kireç, Brassinosteroid, vejetatif büyüme, besin elementleri

Introduction

The cultivated strawberry (Fragaria x ananassa L.) is a member to the Rosaceae family of the Rosales order and is included in the genus Fragaria. It is a fruit consumed with pleasure by many people in the world and its cultivation is carried out in very different ecologies. Strawberries are cultivated in northern European countries, countries close to the equator and countries with temperate and subtropical climates between these two areas (Pio et al., 2019). The areas with the highest strawberry production in our country and in the world have a temperate and subtropical climate between 28° and 60° latitude (Ağaoğlu, 2013; Türemiş and Ağaoğlu, 2013).

Strawberries can be grown in areas with an annual rainfall of 250 mm, in cold areas with an altitude of up to 3500 m, in subtropical climates, even in the Arctic, where it is constantly bright during the summer months, provided that it is irrigated (Türemiş and Ağaoğlu, 2013). Although strawberry plants can be grown in a wide range of temperatures, cultivated species are damaged at temperatures below -5°C. When the temperature drops further, serious damage occurs (Warmund, 1993). Optimum temperatures for strawberries are between 20 and 26°C. Growth and development slow down at temperatures below 20°C and stop at temperatures higher than 35°C (Galletta and Bringhurst, 1990). The effect of climatic characteristics on plant growth and development is very important. Fall and winter climatic conditions have a great influence on their development in spring (Kronenberg et al., 1976). When strawberries are exposed to low temperatures in winter, deficiency in flower formation or fewer flowers are observed in the spring (Guttridge, 1958). Covered cultivation with or without heating is practiced to reduce cold damage. In this way, earliness is also ensured (Hancock and Simpson, 1995).

According to FAOSTAT 2022 data, world strawberry production is 9.569.865 tons and this production is carried out on approximately 3.976.030 decares of land. Strawberry production in our country increased from 669.195 tons in the 2021 production season to 728.112 tons in 2022. China ranked first in the world strawberry cultivation by producing 3.354.804 tons on an area of 1.267.770 da, while the USA ranked 2nd by producing 1.261.890 tons of strawberries on an area of 212.870 da. Türkiye ranked 3rd by producing 728.112 tons of strawberries on an area of 222.720 da. Egypt ranked fourth with 637.842 tons of production and Mexico ranked fifth with 568.272 tons of production (FAOSTAT, 2024).

Half of the cultivated agricultural areas in the world are located in arid and semi-arid regions and ¼ of these are composed of calcareous soils (Bates, 1982). It is known that the lime content of the soils of our country is high except for the Eastern Black Sea Region. High lime and pH cause damages by preventing the uptake of some nutrients such as phosphorus, iron and zinc by the plant. It is not possible to remove this stress caused by lime. As a solution, it is tried to reduce the pH of the soil by using sulfur preparations, but this requires a period of time and is not applicable in large areas. Therefore, there is a need for alternatives, simple-to-use, transferable to practice, and non-harmful to human health alternatives and approaches to provide stress resistance in plants (Koç, 2022; Çetin and Koç, 2023).

Until the first half of the 1900s, it was believed that plant hormones consisted of five classes including auxin, cytokinin, gibberellin, ethylene

and abscisic acid. However, in recent studies, some compounds such as brassinosteroids, jasmonates, nitric oxide and salicylic acid, which are synthesized by plants and are determined to play very important vital roles in the plant structure, have been added to the category of plant hormones (Koç, 2022; Çetin and Koç, 2023). It is recognized that plants develop some specific mechanisms to survive under stress conditions and that internal hormones play an extremely important role during this period by increasing or decreasing. Especially these substances, called 'new generation hormones', stand out. Some of these include plant steroids, which comprise more than 70 compounds with structural similarities to insect, animal and human steroid hormones. Brassinosteroids (BRs), a new class of plant hormones, are a specific group of plant steroids commonly found in plants. At very low concentrations, BRs have been found to regulate plant growth and development such as cell division, elongation and expansion, photomorphogenesis, reproductive organ development, leaf senescence, total biomass and yield increase, as well as adaptation to environmental stresses (Surgun et al. 2012).

Brassinosteroids are involved in root growth and development together with several auxin signaling genes. Brassinosteroids increase primary root outgrowth at small doses and supress it at higher doses (Mussig et al., 2003). They control lateral root growth by auxin (Nemhauser et al., 2004; Bao et al., 2004). External application of BRs at the root formation stage increased root elongation in wild plants (Mussig et al., 2003). High lime levels cause severe chlorosis in strawberries and significantly reduce plant growth and yield (Balci, 2021).

This experiment was conducted to determine the responses of Sweet Ann strawberry cultivar to different lime levels and the effects of 24-epibrassinolide (24-eBL) applications on vegetative growth.

Materials and Methods

Material

This research was conducted between 2022-2023 in the research application greenhouse of Yozgat Bozok University, Faculty of Agriculture, and Department of Horticulture. Frigo seedlings of Sweet Ann variety were used in the experiment. Sweet Ann strawberry variety is a neutral day plant and has round, conical shaped, large, hard, bright red and sweet fruits (Noğay, 2017). These seedlings were planted in 2 liter pots filled with peat:perlite mixed at a ratio of 1:1 and slacked lime (CaO) was added at different rates (0, 5, and 10%). When the seedlings had 4 leaves and 15 days after the first application, three different doses (0, 1 and 2 mg l^{-1}) of 24-eBL were applied.

Seedlings in pots were watered with ½ Hoagland solution. The content of Hoagland solution used in plant nutrition is given in Table 1 (Gül, 2019).

Table 1. Content of fertilizer used in plant nutrition.

Nutrient	mg l^{-1}
N	210
P	40
K	250
Ca	150
Mg	50
Fe	2
Mn	0.75
Cu	0.50
Zn	0.40
B	0.10
Mo	0.05

Method

A 1:1 mixture of peat:perlite was used as a growing medium. Lime was added to this medium at 0%, 5%, and 10% by weight (w.w^{-1}). Frigo seedlings of Sweet Ann variety were planted in 2 liter pots on 28.05.2022. Four lime doses and three 24-eBL concentrations were used in the study. Strawberry plants were irrigated three times a week for 10 weeks after planting and once a week with ½ Hoagland solution. In addition, the normal irrigation program was continued and cultural maintenance of the plants was maintained throughout the study.

Approximately 25 days after transplanting, when the first 4 leaves of the seedlings reached full size, three different concentrations (0, 1 and 2 mg l^{-1}) of 24-eBL were sprayed on the above-ground organs of the plants. The second application of 24-eBL was made 15 days after the first application by spraying the leaves at the same rate and in the same way.

This research was completed 10 weeks after planting in order to determine the effects of BR applications on vegetative growth of strawberry seedlings in calcareous environments. Leaf, stem and root fresh and dry weight (g), number of leaves per plant (pcs), leaf area (ADC BioScientific Area Meter AM300, cm^2), leaf color (L, a, b value Minolta CR 400), leaf chlorophyll value were determined in three replications with 3 leaves in each replicate (Konica Minolta SPAD-502 Plus, Chlorophyll Meter, SPAD), anthocyanin content of the leaves was measured with three replicates and 3 leaves in each replicate (Opti Science ACM-200 Plus, Anthocyanin Meter, ACI), stoma conductivity was measured with "Leaf Porometer" device (Decagon Leaf Porometer Model SC^{-1}, mmol m-2s-1).

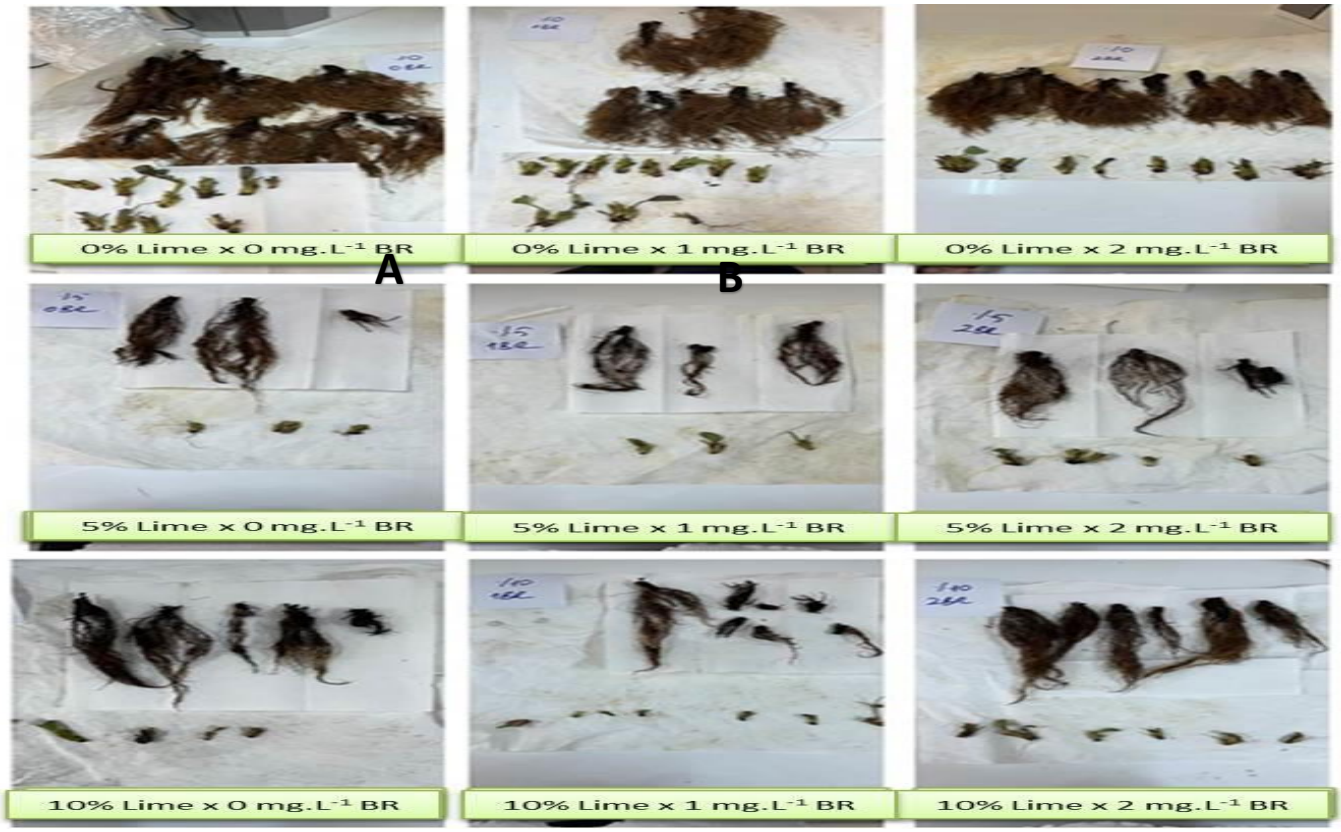

Figure 1. Effect of lime x BR combinations on root growth

Lipid peroxidation: 0.5 g of fresh leaves were taken from each replicate of each treatment and homogenized by adding 6 ml of 10% TCA and this mixture was centrifuged at 10,000 g for 15 min. After taking 2 ml of supernatant from the centrifuged samples, 2 ml of 0.6% thiobarbutric acid (TBA) containing 20% TCA was added and boiled at 100 °C for 30 min and then placed in an ice bath. Afterwards, absorbance readings were taken at 400, 532 and 600 nM in a spectrophotometer and MDA content was calculated according to the formula in Zhang et al. (2008).

MDA (µmol.g^{-1} TA)=6.45 x (A532 - A600) - 0.56 x A450

Nutrient elements in leaves: Leaf samples taken from the plants were first washed in tap water, then washed with 0.1N HCl and finally washed twice with pure water and the excess water was removed with filter paper. The leaves were dried at 70ºC for 48 hours. The dried leaf samples were ground and burned with a mixture

of 5ml HNO3 and 2ml H_2O_2 in a microwave system (CEM-MarsXpress) and total macro and micro elements were determined by ICPAES (Inductively Coupled Plasma-Atomic Emission Spectrometer) (Soltanpour et al., 1979).

Statistical analysis

The experiment was established with three lime doses (0, 5, and 10%), three different concentrations of 24-eBL (0, 1 and 2 mg l^{-1}), 3 replications and 3 plants in each replicate according to the factorial design method in randomized blocks. The results obtained were evaluated using SPSS 20.0 package program. As a result of statistical analysis, 'Duncan multiple comparison test' was applied to determine the difference between the averages.

Results and Discussions

Plant Fresh and Dry Weight (g): When the interaction table (Table 2) of three different BR treatments at different lime doses was examined, it was seen that there was a statistically significant difference in leaf fresh weight, stem and root wet and dry weights, but there was no statistically significant difference only in leaf dry weight.

The highest values in terms of leaf fresh weight were found in 0% Lime x 1 mg l^{-1} BR (3.21 g), 0% Lime x 0 mg l^{-1} BR (3.20 g), 0% Lime x 2 mg l^{-1} BR (3.14 g) and 5% Lime x 0 mg l^{-1} BR (3.14g) treatments. Leaf dry weights ranged from 1.07 (10% Lime x 2 mg l^{-1} BR) to 1.21 g (5% Lime x 0 mg l^{-1} BR).

In terms of stem fresh weight, the highest values were measured in 0% Lime x 0 mg l^{-1} BR (3.14 g), 0% Lime x 1 mg l^{-1} BR (3.01 g) and 0% Lime x 2 mg l^{-1} BR (3.18 g) treatments. In terms of stem dry weight, the highest values were determined in 0% Lime x 0 mg l^{-1} BR (0.86 g), 0% Lime x 1 mg l^{-1} BR (0.76 g) and 0% Lime x 2 mg l^{-1} BR (0.85 g) treatments.

The highest root fresh weight was determined in 10% Lime x 2 mg l^{-1} BR treatment (3.48 g), while the highest root dry weight was measured in 10% Lime x 2 mg l^{-1} BR (1.28 g) and 10% Lime x 0 mg l^{-1} BR (1.19 g) treatments.

Table 2. Effects of Lime x BR treatments on leaf, stem, root fresh and dry weight

Lime doses (%)	BR doses (mg l^{-1})	Leaf		Stem		Root	
		Fresh weight (g)*	Dry weight (g) NS	Fresh weight (g)*	Dry weight (g)*	Fresh weight (g)*	Dry weight (g)*
0	0	3.20 ab	1.09	3.14 a	0.86 a	3.24 b	0.80 d
	1	3.21 a	1.11	3.01 a	0.76 ab	3.26 b	0.98 cd
	2	3.14 ac	1.17	3.18 a	0.85 a	3.01 c	0.99 cd
5	0	3.14 ac	1.21	0.65 e	0.20 f	1.65 g	0.92 cd
	1	3.03 cd	1.18	0.67 e	0.23 ef	1.60 g	0.85 d
	2	2.91 d	1.15	1.18 c	0.60 bc	2.20 f	0.89 cd
10	0	2.90 d	1.16	1.22 c	0.40 de	2.73 d	1.19 ab
	1	3.04 bd	1.10	0.90 d	0.30 ef	2.46 e	1.05 bc
	2	3.01 cd	1.09	1.52 b	0.51 cd	3.48 a	1.28 a

* The differences between the means shown with different letters in the same column are statistically significant (p≤ 0.05)
NS Non-significant

Karlıdag (2011) determined that when strawberry were grown under salt stress conditions and BR was applied through leaves, their fresh and dry weights increased. Balcı (2018) reported that 24-eBL application at different doses had no statistically significant effect on leaf fresh and dry weights of strawberry under cadmium (Cd) stress conditions. In terms of root weights, foliar 24-eBL applications had no effect on dry weights under Cd stress conditions, while the effect on root fresh weights was statistically significant. It was reported that 24-eBL increased the fresh and dry weights of stems and roots when applied to short-day strawberry cultivars.

Number of Leaves per Plant, Leaf Area (cm^2) and Leaf Color (L, a*, b*):

In the lime x BR interaction, the number of leaves per plant and b value expressing yellow color were found to be insignificant, while leaf area, a* value expressing red color and L values were found to be statistically significant (Table 3).

Table 3. Effects of lime x BR interaction on leaf number, leaf area and leaf color

Lime doses (%)	BR doses (mg l^{-1})	Number of Leaves (pcs)^{NS}	Leaf Area (cm^2)*	Leaf Color		
				L*	a*	b^{NS}
0	0	5.44	32.13 ab	32.00 b	2.94 b	11.01
	1	5.78	33.60 a	32.55 b	9.75 a	11.60
	2	7.56	26.87 b	32.59 b	9.63 a	11.63
5	0	6.67	14.47 cd	40.88 a	9.71 a	12.51
	1	7.00	12.51 cd	36.55 ab	10.45 a	17.59
	2	6.50	11.93 d	33.73 ab	9.92 a	14.25
10	0	5.50	10.68 d	35.90 ab	10.88 a	19.79
	1	4.67	10.42 d	37.93 ab	12.31 a	16.23
	2	5.06	19.05 c	34.87 ab	10.22 a	16.86

* The differences between the means shown with different letters in the same column are statistically significant (p≤ 0.05)
NS Non-significant

Leaf area was statistically highest with 32.13 cm2 (0% lime x 0 mg l^{-1} BR) and 33.60 cm2 (0% lime x 1 mg l^{-1} BR). L value was statistically highest at 5% and 10% lime doses and 0 mg l^{-1}, 1 mg l^{-1} and 2 mg l^{-1} BR doses. The a* value of all treatments (9.63-12.31) except 0% lime x 0 mg l^{-1} BR dose (2.94) were statistically in the same group. The b* value varied between 11.01 (0% lime x 0 mg l-1 BR) and 19.79 (10% lime x 0 mg l^{-1} BR).

Chlorophyll (SPAD), Anthocyanin (ACI), Lipid Peroxidation (MDA) and Stoma Conductivity:

The differences between chlorophyll value, anthocyanin content, stoma conductance and MDA values of Lime x BR interaction were found statistically significant ($p<0.05$). The highest leaf chlorophyll content was obtained in the interaction of 0% lime x 1 mg l^{-1} BR, 5% lime x 0 mg l^{-1} BR, 10% lime x 0 mg l^{-1} BR (Table 4). High lime content in the growing medium causes a decrease in the amount of active Fe in the leaf and consequently a decrease in the amount of chlorophyll (Byrne and Rouse, 1995; Pestana et al., 2001). An increase in soil pH causes a decrease in the amount of Fe in the leaf, negatively affecting the synthesis and amount of chlorophyll. It has been reported in many studies that leaf chlorophyll content SPAD value is related to chlorophyll concentration (Schaper & Chocko, 1991; Daşgan, 1999; Eker, 2001). Karlıdağ et al. (2011) reported that foliar application of BR significantly increased leaf chlorophyll content in strawberries under salt stress. Çoban (2014), in his study on salt stress, determined that the effect of salt applications on chlorophyll content was negative. In the study, it was determined that 0.5 and 1.5 mg l^{-1} 24-eBL applied at 0 and 100 mM salt concentrations had a positive effect by increasing the amount of chlorophyll in plants. Altaş (2016) stated that maximum light yield and total chlorophyll content of two maize varieties decreased statistically significantly under salinity stress. Kara (2018) found a very close relationship between the chlorophyll concentrations and SPAD values of the varieties due to Fe deficiency and stated that the SPAD value decreased as the lime dose increased. Balcı (2018) reported a significant effect on chlorophyll content in Albion strawberry variety as a result of statistical analysis of the data obtained from 24-eBL applications against different doses of cadmium. It is known that BR applications improve the chlorophyll content of plants under Cd stress conditions (Hayat et al., 2010).

The highest leaf anthocyanin content (ACI) was obtained in the interaction of 5% lime x 0 mg l^{-1} BR (6.94) and 5% lime x 2 mg l^{-1} BR (8.36) in Table 4. There are research reports that BRs synthesized in plants increase the accumulation of secondary metabolites with antioxidant properties, which are extremely important for human health. As a matter of fact; Farooq et al. (2009) found that 24-eBL and 28-hBL application increased soluble phenolic compounds and anthocyanin content in plants under drought stress in paddy plants. Again, Farooq et al. (2010) determined that exogenous 24-eBL application increased anthocyanin amounts in their drought stress study in paddy plants. Balcı (2018) evaluated the effects of 24-eBL applications on Cd stress on Albion variety and determined that it had no effect on leaf anthocyanin and membrane permeability.

Considering the lime x BR interaction, 0% lime x 0 mg l^{-1} BR and 0% lime x 1 mg l^{-1} BR, applications gave the highest stoma conductance values. The combinations of 5% and 10% lime doses with BR were not effective in stoma conductance. Yu et al. (2004) reported that 24-eBL application promoted photosynthesis in cucurbits and was associated with V-ATPase, which is thought to have effects on hypocotyl elongation. Acharya and Assmann (2009) reported that BRs are effective in the management of stomatal apertures such as auxin, cytokinin and ethylene. Kara (2018) applied 0%, 5%, 10%, 15%, 15% and 20% lime doses to seven different strawberry cultivars and reported that the difference between the averages of stomatal permeability in terms of lime doses was significant, the highest was measured in Hilal77 cultivar and the lowest in Bolverim77 cultivar. In his study, he determined that while higher values were determined in the control, the values decreased due to the increase in lime doses.

When lipid peroxidation (MDA) value was considered, 10% lime x 2 mg l-1 BR, 5% lime x 1 mg l^{-1} BR and 5% lime x 0 mg l^{-1} BR treatments gave the highest values statistically (Table 4). Yan et al. (2013) reported that the amount of MDA increased with increasing amount of methyl jasmonate as a result of Cd toxicity in red peppers. It was reported that MDA concentration decreased and cell damage decreased with the application of MeJA to diseased plants (Sun et al., 2013). It was stated that the application of giberellic acid (GA3) in pepper increased CAT and SOD activity and decreased the amount of MDA; GA3 application had no statistical effect on cell damage (Uzal, 2017). Zhang et al. (2008) reported that the amount of MDA in strawberry varied between 3.12- 4.87 µmol.g^{-1}FW. Gündoğdu et al. (2019) reported that the amount of MDA varied in strawberry varieties as a result of the application of giberellic acid and methyl jasmonate, Honeoye variety was determined to have a higher amount of MDA, while Sweet Ann variety was determined to have a lower amount of MDA. The highest MDA value was measured from Honeoye variety with MDA content of 29.42 µmol g-1FW with 0.25 mM dose application of methyl jasmonate and 30.88 µmol g^{-1}FW with 100 ppm dose of giberellic acid.

Table 4. Effects of Lime x BR interaction on Chlorophyll, Anthocyanin, Stoma conductance and MDA

Lime doses (%)	BR doses (mg l^{-1})	Chlorophyll (SPAD)*	Anthocyanin (ACI)*	Stoma conductance (mmol $m^{-2}s^{-1}$)*	MDA (µmol g^{-1} FW)*
0	0	34.82 bc	5.44 bd	211.31 a	4.85 bc
	1	36.99 ab	5.64 bd	178.50 ab	3.14 de
	2	36.01 bc	5.61 bd	161.86 bc	3.09 de
5	0	39.14 a	6.94 ab	117.36 cd	6.08 ab
	1	33.56 c	4.79 cd	164.14 bc	6.31 a
	2	33.57 c	8.36 a	85.82 d	3.91 cd
10	0	40.02 a	6.34 bc	130.15 cd	2.19 e
	1	27.48 d	4.09 d	161.79 bc	4.25 cd
	2	33.90 bc	4.91 bd	88.11 d	6.49 a

* The differences between the means shown with different letters in the same column are statistically significant (p≤ 0.05)

Nutrient Elements in Leaves

Nitrogen (N%) and phosphorus (P%) contents in the leaves of Sweet Ann strawberry cultivar were found to be statistically significant ($p<0.05$) in terms of lime x BR interaction (Table 5). The highest amounts of N and P were determined as 1.63% and 0.22% in 0% lime x 0 mg l^{-1} BR interaction, respectively. As lime and BR doses increased, %N and %P in leaves decreased. In his study, Kara (2018) determined that the differences between the averages were not significant when leaf nitrogen (%N) content was examined in terms of varieties and lime doses. Yağmur et al. (2021) reported that the effect of powdered lime and slurry applications on the macro and micro element amounts of pepper plant leaves was statistically significant, these applications significantly increased the N and P contents of the leaves compared to the control application and powdered lime application provided the highest total nitrogen amount. In a study conducted to determine the effect of liming materials applied to acidic soil on yield and mineral matter content in maize plant, it was found that these applications to maize plant increased total N, P, K, Ca and Mg contents (Kant et al., 2006). Kaçar and Katkat (2006) stated that increasing the pH of the growing medium decreased phosphorus uptake. Kara (2018) found that different lime doses applied to the soil significantly affected the amount of leaf phosphorus in all varieties, the highest amount of phosphorus was determined in the control and 5% lime dose, while this amount decreased with increasing lime dose. Balcı (2022), in his study in which he examined the P contents in leaves, stems and roots of strawberry seedlings uprooted at three different periods by MEL applied in a calcareous environment, stated that P uptake in leaves decreased as the amount of lime in the growing medium increased.

Leaf potassium content (% K) was found to be the highest (1.07%) in 5% lime x 1 mg l^{-1} BR combination. As a result of the study conducted by Kara (2018), when the amount of leaf potassium was analyzed in terms of lime doses, the differences between lime doses were found to be statistically significant. The highest and lowest values differed according to lime dose and strawberry varieties. The effect of lime applications was found to be insignificant in Doruk77, Hilal77, Erenoğlu77, Bolverim77 varieties and significant in Dorukhan77 and Ata77 strawberry varieties.

The highest values of leaf magnesium content (% Mg) were found in 10% lime x 2 mg l^{-1} BR combination (0.62%) and 10% lime x 0 mg l^{-1} BR combination (0.61%). Kara (2018) reported that leaf magnesium content did not show significant differences in varieties and lime applications. Balcı (2022) determined that the effect of MEL applications on Mg contents in strawberries was significant in all three removals. The Mg content was found to be in the range of 0.84-0.60% in the first harvest one month after planting, 0.63-0.31% in the second harvest and 0.50-0.30% in the third harvest (fruiting period).

Leaf calcium (Ca) content was highest at 5% and 10% lime doses. In the lime x BR interaction, 1.90 ppm Ca was found to be the highest in the 5% lime x 2 mg l^{-1} BR combination. Kara (2018) reported that the amount of leaf calcium (Ca) was not statistically significant when analyzed in terms of lime doses in his study, but there was an increase in the amount of Ca in the varieties due to the increase in lime doses. Balcı (2022) determined the highest Ca content in the leaves of Albion strawberry cultivar in 1% lime/0% MEL application in all removals. In his study, the Ca content of the plants in the control group applied 5 µM MEL was higher than the plants in the same group, while MEL applications decreased the Ca content of the plants applied 1% lime.

Leaf iron content was statistically significant ($p<0.05$) in terms of lime x BR interaction (Table 5). In the lime x BR interaction, the highest iron value was measured in 10% lime x 2 mg l^{-1} BR combination (119.50 ppm). Unlike our results, Kara (2018) found the highest leaf iron value in the control (0%) dose, while the amount of leaf iron decreased with increasing lime doses. Balcı

(2018) reported that the highest Fe content in strawberry leaves was obtained from 0 lime/10 µM MEL and 1% lime/5 µM MEL applications at the first uprooting. The Fe content in the leaves was found to be between 24.40-19.37 ppm at the flowering stage and 29.38-20.36 ppm at the fruiting stage.

Leaf copper (Cu) content was found to be statistically significant ($p<0.05$) in terms of lime x BR interaction. The highest copper content in the leaves of Sweet Ann strawberry cultivar was determined in 0% lime x 0 mg l^{-1} BR interaction (11.56 ppm). Cu content in leaves decreased as lime and BR doses increased.

Leaf zinc (Zn) content of Sweet Ann strawberry cultivar was found to be statistically significant ($p<0.05$) in terms of lime x BR interaction. The highest Zn content was obtained from 2 mg l^{-1} BR application with 46.29 ppm and from 10% lime x 2 mg l-1 BR interaction with 59.26 ppm. As the lime and BR doses decreased, the % zinc content in the leaves also decreased.

Tablo 5. Effects of lime x BR applications on some nutrients in leaves

Lime doses (%)	BR doses (mg l^{-1})	N (%)*	P (%)*	K (%)*	Mg (%)*	Ca (ppm)*	Fe (ppm)*	Cu (ppm)*	Zn (ppm)*
0	0	1.63 a	0.22 a	0.77 f	0.37 g	1.52 h	81.78 g	11.56 a	41.86 f
	1	1.49 b	0.11 b	0.83 d	0.43 f	1.65 g	84.62 f	11.48 b	35.58 h
	2	1.42 c	0.09 c	0.80 e	0.43 f	1.66 g	80.30 i	10.14 f	31.74 i
5	0	1.37 d	0.07 d	0.80 e	0.46 e	1.75 d	97.66 e	9.36 h	43.36 d
	1	1.33 e	0.07 d	1.07 a	0.59 c	1.68 f	109.02 b	9.20 i	47.86 c
	2	1.34 e	0.08 cd	0.83 d	0.53 d	1.90 a	81.62 h	9.56 g	47.88 b
10	0	1.42 c	0.05 e	0.89 c	0.61 ab	1.82 b	106.50 c	10.30 e	38.78 g
	1	1.48 b	0.08 cd	0.95 b	0.60 bc	1.74 e	103.54 d	11.04 c	42.72 e
	2	1.28 f	0.04 e	0.53 g	0.62 a	1.76 c	119.50 a	10.50 d	59.26 a

* The differences between the means shown with different letters in the same column are statistically significant ($p \leq 0.05$)

Conclusions

Considering the rapid increase in population and the decrease in agricultural lands day by day, it is of great importance to ensure that the highest yield is obtained from agricultural lands. Water and nutrient needs of plants should be met at optimum level. There are many factors that limit the usefulness of nutrients and agricultural production. Plants try to survive by developing many responses to all biotic and abiotic factors that they perceive as stress factors. These responses vary according to the effectiveness of the stressor and the genetic characteristics of the plant. Thanks to these stress responses, plants are able to adapt to the stress factors they experience in order to survive.

Calcareous soils are directly linked to plant development, such as soil-water relations, fertility and nutrient availability. Excess CaCO3 in the soil affects soil pH, and high pH decreases the availability of plant nutrients and leads to nitrogen losses in the form of ammonia. There is a decrease in the solubility of phosphorus, and the usefulness of microelements such as Fe, Cu, Zn and Mn decreases with increasing pH levels (Grattan & Grieve, 1999).

In order to prevent all these unfavorable soil conditions from limiting plant growth and increasing productivity, various applications are made. Brassinosteroids, defined as the sixth group of hormones, can be listed as cell division and expansion, cellular differentiation, lateral root development, maintenance of apical dominance, flowering, senescence and increasing stress tolerance (Rao et al., 2002; Savaldi-Goldstein & Chory, 2006). BRs promote growth by accelerating cell elongation and division. The positive effects of BRs in plant response to stress have been confirmed by many studies (Divi et al., 2010). At the end of the study, it was concluded that foliar application of 24-eBL had a positive effect on vegetative growth and nutrient uptake

under calcareous soil conditions.

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Conflict of interest:

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. The authors declare no conflict of interest.

Author contributions:

This article was prepared from the A.K. statistical analysis, writing, editing, and submitting the manuscript; G.Z. data collection and analysis and writing.

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Research Article/Araştırma Makalesi

Determination of Genetic Variation of Ayhan Eggplant (*Solanum melongena* L.) Genotypes using Some Plant and Fruit Characteristics

Ayhan Patlıcanı (Solanum melongena L.) Genotiplerinin Bazı Bitki ve Meyve Özellikleri Kullanılarak Genetik Varyasyonunun Belirlenmesi

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ABSTRACT

The different climatic conditions of Turkey contribute to the diversity of genetic resources. It is very important to detect, describe, protect and evulate this genetic diversity. One of these genetic resources is the Ayhan eggplant, which is grown locally in and around Ayhan village of Avanos district of Nevşehir province. The most commonly used method for detecting and identifying genetic resources is morphological characterization using some plant and fruit features. For morphological characterization, observations and measurements of 41 fruit and vegetative characters of 29 genotypes of Ayhan eggplant were used and genetic similarity/differences were determined among eggplant genotypes. A dendogram was created using the 41 features examined for morphological characterization, and while the genetic similarity was determined as 0.91-1.00, two main groups were obtained in the dendogram. These two main groups are divided into two separate subgroups. While there are control genotypes in the first group, there are genotypes of Ayhan eggplant in the other group. The results of present study show that, Ayhan eggplant population has important genetic variation. These eggplant population can be used for eggplant breeding programs.

Key Words: Eggplant, genetics diversity, plant, fruit characteristics

ÖZ

Türkiye'nin farklı iklim koşulları genetik kaynakların çeşitliliğine katkıda bulunmaktadır. Bu genetik çeşitliliğin tespiti, tanımlanması, korunması ve değerlendirilmesi oldukça önemlidir. Bu genetik kaynaklardan biri de Nevşehir ili Avanos ilçesine bağlı Ayhan köyü ve çevresinde yerel olarak yetiştirilen Ayhan patlıcanıdır. Genetik kaynakların tespiti ve tanımlanmasında en yaygın kullanılan yöntemlerden birtanesi de, bazı bitki ve meyve özelliklerini kullanarak morfolojik karakterizasyondur. Morfolojik karakterizasyon için Ayhan patlıcanının 29 genotipine ait 41 meyve ve bitkisel karakterin gözlem ve ölçümleri kullanılmış ve patlıcan genotipleri arasında genetik benzerlik/farklılıklar belirlenmiştir. Morfolojik karakterizasyon için incelenen 41 özellik kullanılarak bir dendogram oluşturulmuş ve genetik benzerlik 0,91-1,00 olarak belirlenirken, dendogramda iki ana grup elde edilmiştir. Bu iki ana grup iki ayrı alt gruba ayrılmaktadır. Birinci alt grupta kontrol genotipleri bulunurken, ikinci grupta ise Ayhan patlıcanı genotipleri bulunmaktadır. Bu çalışmanın sonuçları Ayhan patlıcan populasyonunun önemli genetik varyasyona sahip olduğunu göstermektedir. Bu patlıcan popülasyonu patlıcan ıslah programları için kullanılabilir niteliktedir.

Anahtar Kelimeler: Patlıcan, Genetik çeşitlilik, Bitki, meyve özellikleri

Introduction

Turkey has many regions with different characteristics in terms of climate and soil. Since it is on one of the first regions where agriculture was practiced, it has become a center of diversity and a micro gene center of most cultivated plant species. In this way, an intense plant endemism emerged. Plant genetic resources are in danger of extinction due to various reasons. Preserving the diversity of plant genetic resources of cultivated species is very important for the sustainability of plant production (Tan and İnal, 2003). Plant diversity is decreasing due to reasons such as increased land work, the spread of improved varieties instead of local varieties, natural disasters, urbanization, differentiation of agricultural systems and control methods, and consumption by collecting from nature. For this reason, many countries have initiated studies on the detection, protection and preserve of plant resources (Tan, 1992).

Plant genetic resources carry genetic codes that enable and maintain the adaptation of the plant to the environmental and climate factors in the region where it is grown for a long time. Plant genetic resources will provide significant adapting to different conditions caused by environmental factors and climate changes, creating resistance to current diseases and pests, and meeting new needs in the future. There are different studies conducted in many countries regarding plant genetic researches, and these studies are continuing rapidly today (Zhukovsky, 1951). Genetic resources are defined as basic living resources consisting of wild and modern varieties for the purpose of increasing the performance of plants and developing new varieties (Şakiroğlu, 2010).

Since Turkey is in a geographically and climatically favorable location with various ecological regions, it is very rich in terms of animal and plant diversity. Ecological diversity has led to the formation of three plant geography regions. 75% of the 11,600 plant species seen in Europe are represented by over 9,500 taxa in Turkey. Turkey's flora includes important local cultivated plants as well as many wild relatives of these species (Harlan, 1951; Harlan, 1995).

Turkey is the micro gene center of many vegetables. Many types of vegetables originate from Anatolia. As a result of the adaptation process to eco-geographic regions and farmer choices, local vegetable varieties vary greatly between regions. Especially in the vegetable species grown in Turkey's micro areas or small producers, traditional agricultural methods used and natural hybridizations resulting from the combination of some species play a major role in the emergence of different forms (Tan, 2010). Local genetic resources form the basis of phenotypic variation, which is one of the most important factors in variety breeding studies (Bliss, 1981; Balkaya et al., 2010).

So far, genetic resources collection studies have been carried out mostly in pepper, melon, tomato, broad bean and watermelon species in Turkey. Seeds of vegetable genetic resources originating from Turkey are collected and preserved in many important seed gene banks abroad. There are a total of 14,348 vegetable genetic resources originating from Turkey in 18 vegetable species in the world seed gene banks (Balkaya et al., 2017). As of the 1990s, instead of local varieties; Hybrid varieties have become popular due to advantages such as yield, quality, standardization and resistance to stress conditions. Most of the vegetables grown under greenhouses are hybrid tomatoes, peppers, cucumbers, eggplants and zucchinis(Balkaya et al., 2015).

Eggplant (*Solanum melongena* L.) is a member of the Solanaceae family and is a tropical plant. While eggplant grows as an annual in hot climates, it is a perennial plant in tropical climates. The origine of eggplant is India and some wild species are found in Africa. In Turkey, eggplant is grown under cover and in open fields conditions. Eggplant species have high sugar, high anthocyanin, phenol, free reducing sugar, amide proteins, dry matter and glycoalkaloid content. High glycoalkaloid content creates a bitter taste (Ali et al., 2011). Although it is very important in terms of vitamin

and mineral content, its fruit is a powerful antioxidant. It is very rich in minerals, vitamins and some polyphenols (Sudheesh et al., 1999; Nisha et al., 2009). Therefore, it is a vegetable with economic value(Topçu et al., 2016).

As a result of variety breeding programs carried out using local genetic resources in the last fifty years, 75 open-pollinated vegetable varieties in 15 vegetable species have been registered. Eggplant varieties including Topan 374 (2), Kemer 27 (2), Balıkesir -76 (1), Pala-49 (1), Halep-18 (2), Aydın Siyahı -55 (2)(Balkaya et al., 2017).

One of the most important of these genetic resources is the eggplant genotype called "Ayhan eggplant", which is grown in Nevşehir and its surroundings. Ayhan eggplant has round-shaped fruits that are lilac-tinged, purple and white in color. Although it is used for roasting, it is consumed in different ways, such as dried, fresh and pickled, especially by the people in the region. However, since production is continued by producers obtaining their own seeds, productivity gradually decreases, while susceptibility to diseases and the introduction of new diseases and pests into production areas have started to restrict production. At the same time, non-uniform fruit size and color are frequently encountered in markets. If the productivity and quality of this variety are not increased, there is a possibility that the variety specific to the region will be completely removed from production over time. In this case, determining the genetic diversity in the region is very important. Based on this, this study aimed to develop a standard variety with the selection breeding method and to determine the variation within the Ayhan eggplant population by using morphological characterizations to be used in possible breeding studies.

Materials And Methods

Plant materials

In present study, 29 Ayhan eggplant genotypes and also, one Yamula eggplant, Adana Topak eggplant and Kemer eggplant genotype were used as the control group (Table 1). Ayhan eggplant materials, including 29 genotypes, were collected from the Ayhan Village region of Avanos district of Nevşehir province (Figure 1.) Genotypes were collected from 29 different producer eggplant fields, showing variation in morphological characteristics.

Figure 1. The region where the eggplant genotypes used in the study were collected.

Table 1. Eggplant genotypes used in the study

Number	Genotype Code	Variety
1	AYH-1	Ayhan Eggplant
2	AYH-2	Ayhan Eggplant
3	AYH-3	Ayhan Eggplant
4	AYH-4	Ayhan Eggplant
5	AYH-5	Ayhan Eggplant
6	AYH-6	Ayhan Eggplant
7	AYH-7	Ayhan Eggplant
8	AYH-8	Ayhan Eggplant
9	AYH-9	Ayhan Eggplant
10	AYH-10	Ayhan Eggplant
11	AYH-11	Ayhan Eggplant
12	AYH-12	Ayhan Eggplant
13	AYH-13	Ayhan Eggplant
14	AYH-14	Ayhan Eggplant
15	AYH-15	Ayhan Eggplant
16	AYH-16	Ayhan Eggplant
17	AYH-17	Ayhan Eggplant
18	AYH-18	Ayhan Eggplant
19	AYH-19	Ayhan Eggplant
20	AYH-20	Ayhan Eggplant
21	AYH-21	Ayhan Eggplant
22	AYH-22	Ayhan Eggplant
23	AYH-23	Ayhan Eggplant
24	AYH-24	Ayhan Eggplant
25	AYH-25	Ayhan Eggplant
26	AYH-26	Ayhan Eggplant
27	AYH-27	Ayhan Eggplant
28	AYH-28	Ayhan Eggplant
29	AYH-29	Ayhan Eggplant
30	KNT-1	Yamula Eggplant
31	KNT-2	Adana Topak Eggplant
32	KNT-3	Kemer Eggplant

The genotypes used in the study were inbred for 2 years and seeds were obtained by selection to represent the population. Seedlings of the genotypes were germinated in a 3:1 peat:perlite media and transfered to the Research and Training area of Erciyes University Department of Horticulture at the 3-4 true leaf stage. The experiment was set up according to the random plot design, with 3 replications of each genotype and 5 plants in each replication. The planting spacing was determined as 40 cm above the row and 70 cm between the rows. Soil analysis was done before planting and fertilization was done according to the prepared program. Other cultural treatments such as irrigation and disease and pest management were carried out. A total of 15 plants from each genotype were used for plant measurements. For fruit measurements, 3 fruits were taken from each plant. The average of the measurements taken was used in the characterization.

Morphological Characterization

For morphological characterization, 14 plant characteristics and 27 fruit characteristics determined by the rules of UPOV (International Union for the Protection of New Plant Varieties) (2002) were examined. The examined features are shown in Table 2.

Table 2. Vegetative and fruit characteristics used in morphological characterization

Plant characteristics		
Number	**Characteristics**	**Descriptions**
1	Plant : Attitude	Erect, semi-erect, recumbent (3,5,7)
2	Plant: Height	Short, middle, long (3,5,7)
3	Plant : Length of stem (from cotyledon to node of first flower	Short, middle, long (3,5,7)
4	Stem : Anthocyonin coloration	Absent, present (1,9)
5	Stem : Intensity of anthocyonin coloration	Slight, Middle, strong (3,5,7)
6	Stem : Hairness	Slight, Middle, much (3,5,7)
7	Branch :Length of internodes	Short, middle, long (3,5,7)
8	Leaf : Size	Small, middle, big (3,5,7)
9	Leaf : Margin	Whole, Serrated, Wavy (3,5,7)
10	Leaf : Degree of sinuation of margin	Slight, Middle, strong (3,5,7)
11	Leaf :Blistering	Absent, present (1,9)
12	Leaf :Spininess	Absent, Slight, Medium, Strong, Very Strong (1,3,5,7,9) 9
13	Leaf :Color	Green, Bluish Green, Violet Green(3,5,7)
14	Flower : Purple color	Light, Medium, Dark(3,5,7)
Fruit characteristics		
Number	**Characteristics**	**Descriptions**
1	Fruit : Length	Measured data
2	Fruit : Diameter	Measured data
3	Fruit : Ratio length /diameter	Measured data
4	Fruit : General shape	Pear, Solid, Sphere, Cylindrical (1,3,5,7,9)
5	Fruit : Size of pistil scar	Small, Medium, Large (3,5,7)
6	Fruit: Shape of apex	Notched, Round, Pointed(3,5,7)
7	Fruit : Depth of indentation	Superficial, Medium, Deep (3,5,7)
8	Fruit : Curvatıre (only for cylindirical types	Ansent, Slight, Medium, Much, Very Much (1,3,5,7,9)
9	Fruit : Color of skin at commercial harvesting	White, Yellow, Green, Lilac, Purple (1,3,5,7,9)
10	Fruit :Intensity of color of skin	Light, Medium, Dark (3,5,7)
11	Fruit : Glossiness at harvest maturity	Slight, Medium, High (3,5,7)
12	Fruit : Status of stripes	Absent, present (1,9)
13	Fruit : Intensity of stripes	Low, Medium, High (3,5,7)
14	Fruit : Ribs	Absent, present (1,9)
15	Fruit : Prominence of ribs	Slight, Medium, Large (3,5,7)
16	Fruit : Size of calyx	Small, Medium, Large (3,5,7)
17	Fruit : Anthocyanin coloration below calyx	Slight, Medium, High (3,5,7)
18	Fruit : Anthocyanin coloration of calyx	Very Little, Little, Medium, Much, Very Much (1,3,5,7,9)
19	Fruit : Anthocyanin coloration below calyx	Absent, present (1,9)
20	Fruit : Intensity ofa Anthocyanin coloration below calyx	Slight, Medium, High (3,5,7)
21	Fruit : Spinyness calyx	Very Little, Little, Medium, Much, Very Much (1,3,5,7,9)
22	Fruit : Color of flesh	Whitish, Greenish (1,9)
23	Fruit :Color of skin (at physiological ripeness)	Yellow, Soil Color, Brown (3,5,7)
24	Fruit : Time of beginning of flowering	Early, Medium, Late (3,5,7)
25	Fruit : Time of physiological ripeness	Early, Medium, Late (3,5,7)
26	Fruit : Weight	Measured data
27	Fruit : Firmness	Measured data

Data analysis The features examined during morphological

characterization were evaluated as described above and analyzed in the computer package program NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.11, Exeter Software, Setauket, N.Y., USA, Rohlf, 2000). Similarity indices were calculated according to the Dice (1945) method, and the dendrogram was created according to the UPGMA (Unweighted Pair-Group Method With Arithmetic Average) method.

Results And Discussıon

Vegetative characteristics

In the study, a total of 14 vegetative traits in 32 genotypes were examined (Table 3) According to plant characteristics findings; Looking at the plant posture, it was seen that 25 genotypes grew upright, 5 genotypes grew semi-erectly, and 2 genotypes grew horizontally. In terms of plant height, it was determined that 12 genotypes were tall, 12 genotypes were medium height, and 8 genotypes were short. In terms of plant stem length, it was determined that 1 genotype was long-stemmed, 1 genotype was medium-stemmed, and 30 genotypes were short-stemmed. The anthocyanin content of plants is an important factor. In terms of anthocyanin coloration in the stem, coloration was observed in 17 genotypes, while no anthocyanin coloration was observed in the stem in 15 genotypes. It was observed that anthocyanin coloration was slight in 12 of the 17 genotypes, moderate in 5 genotypes, and strong in 1 genotype. While the hairiness on the stem was slight in 29 genotypes, moderate hairiness was observed in 3 genotypes. It was determined that internode length was long in 2 genotypes, medium in 10 genotypes, and short in 20 genotypes. In plants, leaf size and leaf color are important for the photosynthesis efficiency of the plant. In terms of leaf size, 5 genotypes had large leaves, 10 genotypes have medium-sized leaves and 17 genotypes have small leaves. The leaf edge shape was toothed in 1 genotype, has the entire structure in 1 genotype, and was wavy in the remaining 30 genotypes. Among the genotypes with a wavy leaf edge structure, the wavy degree is middle in AYH-2, AYH-4, AYH-5 and AYH-31 genotypes, AYH-30 had a strong degree in one genotype and a slight degree in the remaining 27 genotypes. Leaf spininess was not observed in any genotype. Leaf blistering was only AYH-3. and AYH-4. was observed in genotypes. In terms of leaf color, only the AYH-30 genotype had a bluish green color, while all the remaining genotypes have a green color. In plants, flower color is a factor that attracts pollinator insects and ensures pollination. As for flower color, 4 genotypes were determined as dark purple, 6 genotypes as medium purple and 22 genotypes as light purple (Table 3).

Fruit characteristics

27 fruit characteristics were observed on the genotypes (Table 4 and Table 5). According to the fruit characteristics results; all fruit characteristics are directly proportional to the prominence of the variety and the demand of the fruits by the consumer. The fruit length of the genotypes used in the study was measured between 82 mm and 196 mm and the fruit diameter was measured between 36 mm and 62 mm. Fruit length/fruit diameter ratios were determined as the lowest 1.72 mm and the highest 3.92 mm. Such as fruit shape, curvature, skin color, fruit brightness, etc. fruit characteristics are phenotypic advantages that are very important. In terms of fruit shape, AYH-3, AYH-13, AYH-19 and AYH-32 genotypes were spherical, while all genotypes had a cylindrical structure. Female flower marks in eggplant fruits are the traces left behind from the structures that remain on the fruit after pollination and fruit set formation, but fall off from the tip of the fruit after the fruit matures. Fruit pistil scar size was large in 7 genotypes, medium in 10 genotypes and small in 15 genotypes. The fruit tip shape is round in 9 genotypes and all genotypes have a pointed structure. Fruit tip paddy depth was not found in any genotype. In terms of curvature in the fruit, AYH-2, AYH-8 and AYH-17 genotypes were slightly curved, AYH-4, AYH-24, AYH-27 and AYH-32 genotypes were moderately curved, and no curvature was found in the remaining genotypes.

There is no uniform fruit color in the genotypes used in the present study. It was observed that the commercial shell color of the genotypes was white in 5 phenotypes, purple in 15 genotypes and lilac in 12 genotypes. In terms of fruit skin color density, apart from the white genotypes, it was determined that it was slight in 5 genotypes, medium in color in 13 genotypes, and dark in 9 genotypes. In terms of stripes on the fruits of the genotypes, stripes were found in 22 of them, and no stripes were observed in the other 10 genotypes. It was determined that the stripes were dense in 9 of the striped genotypes, moderate in 5 genotypes, and sparse in 8 genotypes. It was observed that the brightness of the fruit at harvest was high in AYH-31 and AYH-32 genotypes, and moderate in the other 30 genotypes.

Fruit vascularity was seen in 16 genotypes but not in 16 genotypes. In genotypes with vascularity, the protrusion of the vessels was large in 1 genotype, medium in 4 genotypes, and mild in 11 genotypes. The size of the fruit flower envelope was determined as large in 9 genotypes, medium in 16 phenotypes and small in 7 genotypes. Anthocyanin, which has many benefits for human health, is abundant in eggplant fruits. Anthocyanin coloration below calyx on the fruit was found in 11 genotypes but not in 21 genotypes. The intensity of anthocyanin coloration under the flower calyx was high in 2 genotypes, moderate in 7 genotypes, and slight in 2 genotypes. Anthocyanin coloration of the fruit flower calyx was moderate in 1 genotype, slight in 13 genotypes, and was not observed in the remaining genotypes. In the genotypes in which anthocyanin coloration of the fruit flower envelope was observed, the coloration intensity was moderate in 1 genotype and low in 13 genotypes. The prickliness of the fruit flower envelope was seen at a medium level in 1 genotype, at a low level in 6 genotypes, and the remaining genotypes do not have thorniness. Two different colors were observed in the fruit in terms of flesh color. The AYH-32 genotype had greenish flesh color, while all other genotypes have whitish flesh color. At physiological maturity, the skin color of the fruit was yellow in AYH-30 and AYH-31 genotypes, while it was soil colored in other genotypes(Figure 2). Flowering onset time and fruit physiological maturity time are early in AYH-30 and AYH-32 genotypes, and intermediate in all remaining genotypes. Fruit weight of the genotypes varied between 47.7 g and 214.7 g, and fruit flesh hardness varied between 1.3 kg/cm2 and 4.1 kg/cm2 (Table 4). There is wide phenotypic variation among Ayhan eggplant fruits, as in vegetative characteristics.

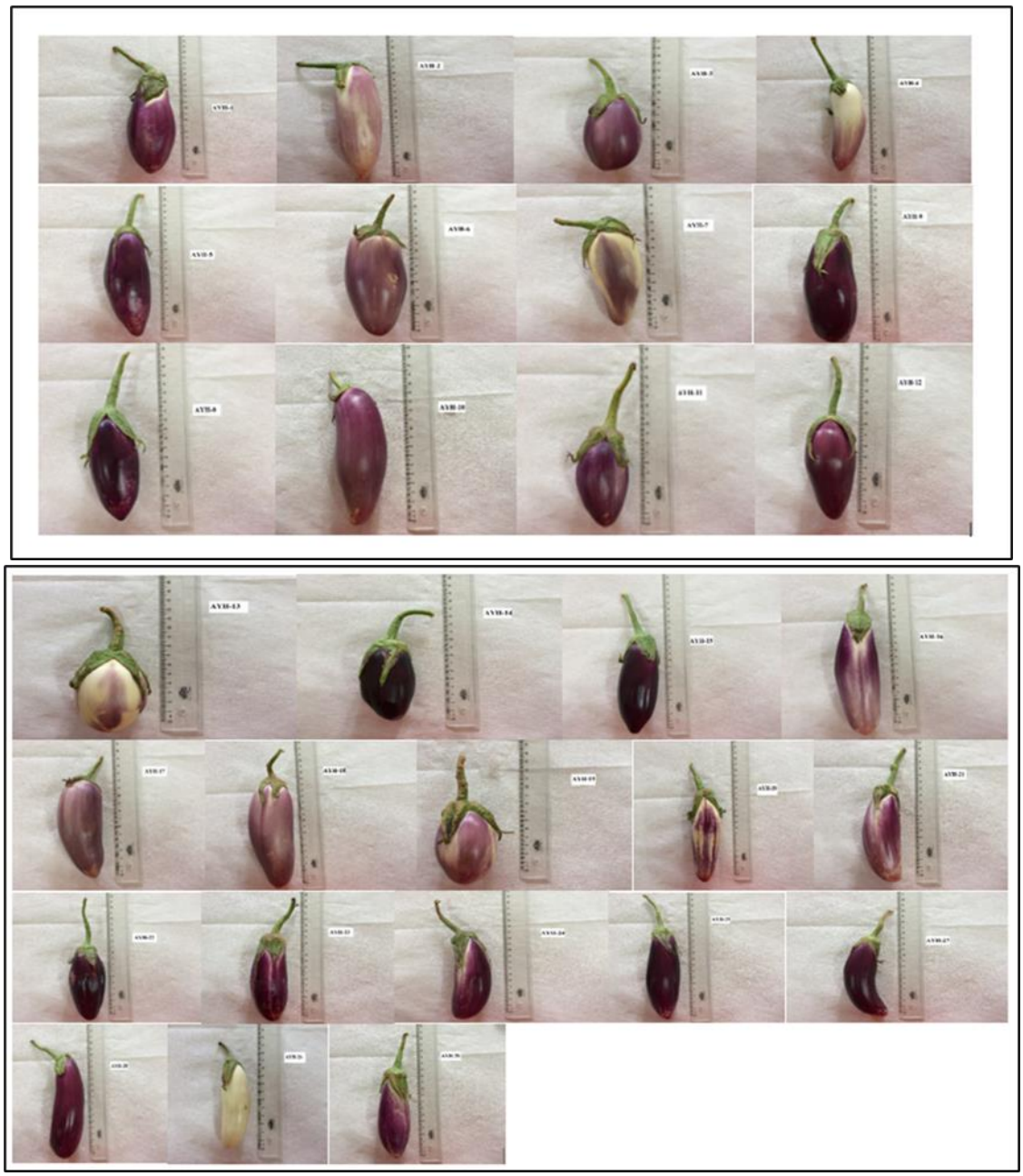

Figure 2. Fruit photos of Ayhan eggplant genotypes

A dendogram was created using the 41 features examined for morphological characterization, and while the genetic similarity was determined as 0.91-1.00, two main groups were obtained in the dendogram. These two main groups are divided into two separate subgroups. While there are control genotypes in the first subgroup, there are genotypes of Ayhan eggplant in the other subgroups (Figure 3).

Especially since the vegetable genotypes grown locally in Turkey are produced with seeds obtained from the producers' production area, there is genetic segregation, and the genotypes have population characteristics. Therefore, it is possible to obtain differences within the same population. As a matter of fact, in a study conducted by Uysal (2023), morphological characterization was made using 16 vegetative traits and 14 fruit traits in 28 Yamula eggplant genotypes, 1 Manisa eggplant genotype as a control group, and 3 Kemer eggplant genotypes. According to the results, the genetic similarity between eggplant genotypes was determined to be between 0.27-0.84. Two main groups were obtained in the dendrogram. These two groups are divided into two separate subgroups. The first group consisted of genotypes belonging to control groups, while the second group consisted of genotypes belonging to Yamula eggplant. In another study conducted by Topçu et al. (2016), 100 eggplant lines used in breeding programs were characterized morphologically and molecularly. For morphological characterization,

observations and measurements of 32 morphological features were made using the criteria specified by UPOV, and as a result, it was determined that the genotypes were divided into 17 groups.

On the other hand, Çakır (2018) aimed to characterize local eggplant populations originating from Turkey according to their morphological characteristics and to determine the level of genetic variation in the current population. At the beginning of the study, a gene pool consisting of eighty eggplant genotypes was created. The present collection has been reported to show significant phenotypic diversity in fruit characteristics. Principal Component Analysis explained 71.38% of the total variation based on the first four principal component axes. This result showed that there was a high degree of variation among eggplant genotypes.

In the dendrogram, the geotypes in the 1st main group had lilac fruit color and round fruit structure, while the other groups included genotypes with calyx thorniness and oval fruit structure. Fruit shape and color were effective in the genetic separation of eggplant genotypes (Figure 3).

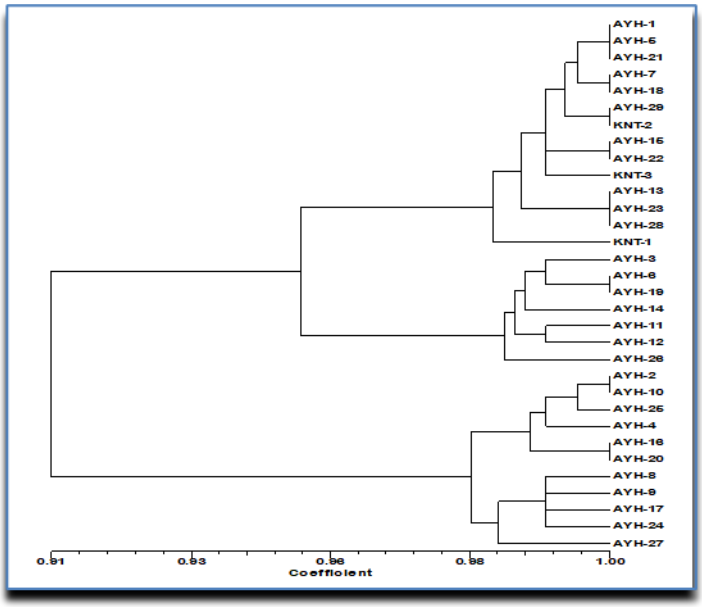

Figure 3. Dendrogram created as a result of morphological characterization data analysis

Conclusion

It is possible to determine similar variation in other eggplant varieties produced in Turkey. However, although this genetic difference is important in terms of breeding, it creates negativities in terms of production in terms of yield, resistance/sensitivity to diseases and pests, and some fruit quality criteria. Therefore, the solution to this problem is to determine the variation within the populations that constitute local genetic resources and to use the obtained variation in developing efficient and high-quality varieties, on the other hand, genotypes bearing all the characteristics of the local variety are determined through selection taking into account variation and are evaluated as standard varieties after yield trials. In this study, the genotypes of the eggplant variety called Ayhan eggplant, determined from the areas where eggplant is produced in Ayhan Village of Avanos district of Nevşehir province, were evaluated taking into account morphological features and variation was determined using eggplant genotypes commonly grown in Turkey. The variation obtained can be used in the development of new hybrid eggplant varieties, or it is possible to develop a new standard variety and bring it into production through selection using the genotypes in question. According to the results of present study, an important variation was observed among Ayhan eggplant genotypes, and Ayhan eggplant genotypes could be used as a good source for future breeding studies.

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Authors' Contribution: The authors declare that they contributed equally to the article.

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Table 3. Vegetative characteristics of some Ayhan eggplant and control genotypes.

Genotpe	Plant Attitude	Plant Height	Length of stem	Stem Anthocyonin coloration	Intensity of anthocyonin coloration	Stem Hairness	Length of internodes	Leaf Size	Leaf Margin	Degree of sinuation of margin	Leaf Blistering	Leaf Spininess	Leaf Color	Flower Purple color
AYH-1	Erect	Long	Short	Present	Medium	Slight	Short	Big	Whole	Slight	Absent	Absent	Green	Medium
AYH-2	Erect	Short	Short	Absent	Absent	Slight	Medium	Big	Wavy	Medium	Absent	Absent	Green	Light
AYH-3	Semi Erect	Short	Short	Absent	Absent	Slight	Short	Medium	Wavy	Slight	Absent	Present	Green	Medium
AYH-4	Horizantal	Short	Short	Absent	Absent	Slight	Medium	Big	Wavy	Medium	Absent	Present	Green	Dark
AYH-5	Semi Erect	Long	Short	Absent	Absent	Slight	Medium	Medium	Wavy	Medium	Absent	Absent	Green	Dark
AYH-6	Erect	Long	Short	Present	Medium	Slight	Short	Medium	Wavy	Slight	Absent	Absent	Green	Dark
AYH-7	Erect	Medium	Short	Present	Slight	Slight	Medium	Small	Wavy	Slight	Absent	Absent	Green	Medium
AYH-8	Erect	Short	Short	Present	Slight	Slight	Medium	Medium	Wavy	Slight	Absent	Absent	Green	Dark
AYH-9	Semi Erect	Medium	Short	Present	Slight	Slight	Short	Small	Wavy	Slight	Absent	Absent	Green	Light
AYH-10	Erect	Medium	Short	Absent	Absent	Slight	Short	Small	Wavy	Slight	Absent	Absent	Green	Light
AYH-11	Erect	Long	Short	Absent	Absent	Slight	Short	Small	Wavy	Slight	Absent	Absent	Green	Medium
AYH-12	Erect	Long	Short	Present	Slight	Slight	Medium	Medium	Wavy	Slight	Absent	Absent	Green	Medium
AYH-13	Erect	Medium	Short	Present	Medium	Slight	Medium	Small	Wavy	Slight	Absent	Absent	Green	Light
AYH-14	Erect	Short	Short	Present	Slight	Slight	Short	Small	Wavy	Slight	Absent	Absent	Green	Light
AYH-15	Erect	Short	Short	Present	Slight	Slight	Short	Big	Wavy	Slight	Absent	Absent	Green	Light
AYH-16	Erect	Medium	Short	Present	Slight	Slight	Short	Small	Wavy	Slight	Absent	Absent	Green	Light
AYH-17	Semi Erect	Medium	Short	Present	Slight	Slight	Short	Small	Wavy	Slight	Absent	Absent	Green	Light
AYH-18	Erect	Medium	Short	Absent	Absent	Slight	Medium	Small	Wavy	Slight	Absent	Absent	Green	Light
AYH-19	Erect	Long	Short	Present	Slight	Slight	Short	Small	Wavy	Slight	Absent	Absent	Green	Light
AYH-20	Erect	Long	Short	Present	Medium	Slight	Short	Medium	Wavy	Slight	Absent	Absent	Green	Light
AYH-21	Semi Erect	Long	Short	Absent	Absent	Slight	Medium	Medium	Wavy	Slight	Absent	Absent	Green	Light
AYH-22	Erect	Long	Short	Present	Slight	Slight	Short	Medium	Wavy	Slight	Absent	Absent	Green	Medium
AYH-23	Erect	Medium	Short	Absent	Absent	Slight	Short	Big	Wavy	Slight	Absent	Absent	Green	Light
AYH-24	Erect	Short	Short	Present	Slight	Slight	Short	Small	Wavy	Slight	Absent	Absent	Green	Light
AYH-25	Erect	Medium	Short	Absent	Absent	Slight	Short	Small	Wavy	Slight	Absent	Absent	Green	Light
AYH-26	Erect	Long	Short	Absent	Absent	Slight	Medium	Big	Wavy	Slight	Absent	Absent	Green	Light
AYH-27	Erect	Long	Short	Absent	Absent	Slight	Short	Small	Wavy	Slight	Absent	Absent	Green	Light
AYH-28	Erect	Medium	Short	Absent	Absent	Slight	Short	Small	Wavy	Slight	Absent	Absent	Green	Light

AYH-29	Erect	Medium	Short	Absent	Absent	Slight	Long	Small	Wavy	Slight	Absent	Absent	Green	Light
KNT-1	Horizantal	Short	Short	Present	Strong	Medium	Short	Small	Wavy	Strong	Absent	Absent	Bluish Green	Light
KNT-2	Erect	Medium	Medium	Absent	Absent	Medium	Short	Medium	Wavy	Medium	Absent	Absent	Green	Slight
KNT-3	Erect	Long	Long	Present	Slight	Medium	Long	Medium	Serrated	Medium	Absent	Absent	Green	Slight

Table 4. Fruit characteristics of some Ayhan eggplant and control genotypes.

Genotype	Status of stripes	Intensity of stripes	Size of calyx		Anthocyanin coloration below calyx	Anthocyanin coloration below calyx	Anthocyanin coloration of calyx	Spinyness calyx	Color of flesh	Fruit Color of skin (at physiological ripeness)	Time of beginning of flowering	Time of physiological ripeness	Fruit firmness(kg/cm2)	Fruit weight(g)
AYH-1	Present	Slight	Medium	Absent	Absent	Slight	Low	Absent	Whitish	Soil	Medium	Medium	2.9	182.3
AYH-2	Present	Slight	Small	Absent	Absent	Absent	Absent	Medium	Whitish	Soil	Medium	Medium	2.2	176.7
AYH-3	Absent	Absent	Big	Absent	Absent	Absent	Absent	Low	Whitish	Soil	Medium	Medium	1.9	89.7
AYH-4	Absent	Absent	Medium	Absent	Absent	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	2.6	93.2
AYH-5	Present	Medium	Medium	Absent	Absent	Slight	Low	Low	Whitish	Soil	Medium	Medium	2.1	148.2
AYH-6	Absent	Absent	Small	Absent	Absent	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	2.1	146.3
AYH-7	Absent	Absent	Small	Absent	Absent	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	2.1	84.6
AYH-8	Present	Slight	Big	Present	Medium	Absent	Absent	Low	Whitish	Soil	Medium	Medium	1.8	162.9
AYH-9	Present	Slight	Big	Present	Medium	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	2.3	82.5
AYH-10	Absent	Absent	Small	Present	Slight	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	2.2	124.9
AYH-11	Present	Slight	Medium	Absent	Absent	Slight	Low	Absent	Whitish	Soil	Medium	Medium	2.3	54.5
AYH-12	Absent	Absent	Medium	Present	High	Slight	Low	Absent	Whitish	Soil	Medium	Medium	2.1	61.3
AYH-13	Absent	Absent	Big	Absent	Absent	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	2.4	65.7
AYH-14	Present	Slight	Big	Present	High	Slight	Low	Low	Whitish	Soil	Medium	Medium	2.7	73.8
AYH-15	Present	Slight	Medium	Present	Medium	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	2.5	122.6
AYH-16	Present	Slight	Medium	Absent	Absent	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	2.4	143.6
AYH-17	Absent	Absent	Small	Present	Slight	Slight	Low	Absent	Whitish	Soil	Medium	Medium	2.9	107.7
AYH-18	Absent	Absent	Small	Absent	Absent	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	2.8	214.7
AYH-19	Absent	Absent	Big	Absent	Absent	Slight	Low	Absent	Whitish	Soil	Medium	Medium	1.8	100.4
AYH-20	Absent	Absent	Medium	Absent	Absent	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	2.3	121.4
AYH-21	Present	Slight	Medium	Absent	Absent	Slight	Low	Absent	Whitish	Soil	Medium	Medium	2.5	97.8
AYH-22	Present	Big	Medium	Present	Medium	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	2.2	73.8
AYH-23	Absent	Absent	Big	Absent	Absent	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	2.7	118
AYH-24	Present	Medium	Big	Absent	Absent	Medium	Medium	Absent	Whitish	Soil	Medium	Medium	2.6	80

AYH-25	Present	Medium	Medium	Present	Medium	Slight	Low	Absent	Whitish	Soil	Medium	Medium	2.5	103.6
AYH-26	Present	Medium	Medium	Absent	Absent	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	2.4	47.7
AYH-27	Present	Slight	Medium	Present	Medium	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	1.7	78
AYH-28	Present	Slight	Medium	Present	Medium	Absent	Absent	Absent	Whitish	Soil	Medium	Medium	1.3	152
AYH-29	Absent	Absent	Medium	Absent	Absent	Slight	Low	Low	Whitish	Soil	Medium	Medium	2.9	84
KNT-1	Absent	Absent	Big	Absent	Absent	Slight	Low	Low	Whitish	Yellow	Early	Early	4.1	128.2
KNT-2	Absent	Absent	Medium	Absent	Absent	Slight	Low	Medium	Whitish	Yellow	Medium	Medium	3.6	155.6
KNT-3	Absent	Absent	Small	Absent	Absent	Slight	Low	Absent	Greenish	Soil	Early	Early	2.9	164.8

Table 5. Fruit characteristics of some Ayhan eggplant and control genotypes.

Genotip	Fruit : Length	Fruit : Diameter (mm)	Fruit : Length / Diameter	Fruit General shape	Size of pistil scar	Shape of apex	Depth of indentation	Curvatıre	Color of skin at commercial harvesting	Intensity of color of skin	Status of stripes	Intensity of stripes	Glossiness at harvest maturity
AYH-1	164	62	2.64	Cylindrical	Medium	Pointed	Absent	Absent	Lilac	Dark	Present	Sparse	Medium
AYH-2	168	52	3.23	Cylindrical	Small	Pointed	Absent	Slight	White	Dark	Present	Intense	Medium
AYH-3	93	54	1.72	Sphere	Medium	Pointed	Absent	Absent	Lilac	Medium	Present	Medium	Medium
AYH-4	133	43	3.09	Cylindrical	Small	Pointed	Absent	Medium	White	Medium	Present	Intense	Medium
AYH-5	148	56	2.64	Cylindrical	Big	Pointed	Absent	Absent	Purple	Medium	Absent	Intense	Medium
AYH-6	115	61	1.88	Cylindrical	Small	Round	Absent	Absent	Lilac	Medium	Present	Medium	Medium
AYH-7	114	46	2.47	Cylindrical	Small	Pointed	Absent	Absent	White	Medium	Present	Intense	Medium
AYH-8	143	59	2.42	Cylindrical	Big	Pointed	Absent	Slight	Purple	Medium	Absent	Intense	Medium
AYH-9	103	47	2.19	Cylindrical	Small	Pointed	Absent	Absent	Purple	Medium	Present	Sparse	Medium
AYH-10	174	52	3.34	Cylindrical	Small	Round	Absent	Absent	Lilac	Medium	Present	Intense	Medium
AYH-11	90	47	1.91	Cylindrical	Medium	Pointed	Absent	Absent	Lilac	Dark	Present	Sparse	Medium
AYH-12	82	45	1.82	Cylindrical	Medium	Pointed	Absent	Absent	Lilac	Dark	Present	Sparse	Medium
AYH-13	139	49	2.83	Sphere	Medium	Round	Absent	Absent	White	Dark	Present	Intense	Medium
AYH-14	89	51	1.74	Cylindrical	Medium	Pointed	Absent	Absent	Purple	Dark	Absent	Intense	Medium
AYH-15	143	53	2.69	Cylindrical	Big	Pointed	Absent	Absent	Purple	Dark	Absent	Intense	Medium
AYH-16	159	52	3.05	Cylindrical	Medium	Round	Absent	Absent	Lilac	Dark	Present	Intense	Medium
AYH-17	115	51	2.25	Cylindrical	Medium	Pointed	Absent	Slight	Lilac	Slight	Present	Intense	Medium
AYH-18	162	59	2.74	Cylindrical	Small	Round	Absent	Absent	Lilac	Slight	Present	Intense	Medium
AYH-19	110	56	1.96	Sphere	Medium	Round	Absent	Absent	Lilac	Slight	Present	Sparse	Medium

AYH-20	143	47	3.04	Cylindrical	Small	Round	Round	Absent	Purple	Medium	Present	Intense	Medium
AYH-21	122	48	2.54	Cylindrical	Big	Pointed	Absent	Absent	Purple	Slight	Present	Sparse	Medium
AYH-22	113	44	2.56	Cylindrical	Big	Pointed	Absent	Absent	Purple	Dark	Absent	Sparse	Medium
AYH-23	133	47	2.82	Cylindrical	Small	Pointed	Absent	Absent	Purple	Medium	Present	Sparse	Medium
AYH-24	130	40	3.25	Cylindrical	Big	Pointed	Absent	Medium	Purple	Medium	Present	Medium	Medium
AYH-25	136	43	3.16	Cylindrical	Small	Pointed	Absent	Absent	Purple	Dark	Present	Sparse	Medium
AYH-26	102	36	2.83	Cylindrical	Small	Pointed	Absent	Absent	White	Dark	Absent	Medium	Medium
AYH-27	133	39	3.41	Cylindrical	Big	Pointed	Absent	Medium	Purple	Medium	Absent	Medium	Medium
AYH-28	156	53	2.95	Cylindrical	Medium	Round	Absent	Absent	Purple	Medium	Present	Medium	Medium
AYH-29	120	47	2.55	Cylindrical	Small	Pointed	Absent	Absent	Lilac	Medium	Present	Medium	Medium
KNT-1	116	48	2.42	Cylindrical	Small	Pointed	Absent	High	Lilac	Slight	Absent	Medium	Medium
KNT-2	137	54	2.53	Sphere	Small	Round	Absent	Absent	Purple	Medium	Absent	Medium	High
KNT-3	196	50	3.92	Cylindrical	Small	Pointed	Absent	Medium	Purple	Dark	Absent	Medium	High

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.

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Note: You can use the web pages related to **APA Referencing Style** on the internet.

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