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Optimization of Aquafaba Production by Response Surface Methodology and Application in Plant-Based Mayonnaise

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Abstract: Aquafaba is the liquid left over after the chickpeas are boiled and canned. Aquafaba, which is used as an egg replacer in vegan products, is a valuable by-product that has become popular in recent years. The components that are transferred from chickpea into water during the boiling process provide foaming and emulsion-forming capabilities. The composition and functionality of aquafaba are affected by parameters such as boiling time, pH, and solid-liquid ratio. The purpose of this study was to investigate the effects of pH, time and solid-liquid ratio on various characteristics of aquafaba and to optimize aquafaba production using Response Surface Methodology. The °Brix, pH, foaming capacity, stability and emulsion activity index values were measured in 15 aquafaba samples produced according to the experimental design. The °Brix values of the samples ranged from 1.12 to 2.22. The foaming properties of the samples at pH 4.0 yielded significantly higher foam value and stability compared to the other samples. While the foam volume varied between 58.5 and 74.5%, the foam stability varied between 4.6-83.8%. The emulsion activity index was affected by the solid-liquid ratio and varied between 96.8 and 99.1%. In conclusion, aquafaba characteristics changed depending on pH, time, and solid-liquid ratio. The optimization was performed according to the Box-Behnken design and the optimum parameters were identified as pH: 3.58, time: 80 min and solid-liquid ratio: 0.25. Characteristics of plant-based mayonnaise prepared with optimized aquafaba was compared with commercial regular and vegan mayonnaise samples. The quality characteristics of the product can be improved by making further formulation revisions. Within the scope of this study, aquafaba was successfully used in the formulation of plant-based mayonnaise.

Keywords: Aquafaba, response surface methodology, plant-based mayonnaise.

Aquafabanın Yanıt-Yüzey Metodolojisi ile Optimizasyonu ve Bitkisel Bazlı Mayonez Üretiminde Kullanımı

Özet: Aquafaba, nohutun haşlama ve konserveleme işleminden sonra kalan sıvı kısmı ifade etmektedir. Vegan ürünlerde yumurta ikamesi olarak kullanılan aquafaba son yıllarda popülerliği artan değerli bir yan üründür. Haşlama işlemi sırasında suya geçen bileşenler köpük ve emülsiyon oluşturma ve stabilize etme özelliği sağlamaktadır. Aquafabanın bileşimi ve fonksiyonel özellikleri haşlama süresi, pH, katı-sıvı oranı gibi değişkenlerden etkilenmektedir. Bu araştırmanın amacı pH, süre ve katı-sıvı oranının aquafabanın karakteristik özellikleri üzerindeki etkilerini araştırmak ve aquafaba üretimini Yanıt-Yüzey Metodolojisi kullanarak optimize etmektir. Deney tasarımına göre üretilen 15 aquafaba örneğinde °Brix, pH, köpük oluşturma kapasitesi, köpük stabilitesi ve emülsiyon aktivite indeksi değerleri ölçülmüştür. Elde edilen numunelerin °Brix değerleri 1,12 ila 2,22 arasında ölçülmüştür. pH 4,0'da numunelerde köpürme özellikleri diğer numunelere kıyasla önemli düzeyde yüksek ölçülmüş olup; köpük hacmi %58,5 ile %74,5 arasında değişirken, köpük stabilitesi %4,6-83,8 arasında değişmiştir. Emülsiyon aktivite indeksi katı-sıvı oranından önemli düzeyde etkilenmiş ve %96,8–99,1 arasında değişmiştir. Sonuç olarak aquafabanın karakteristik özellikleri pH, süre ve katı-sıvı oranına bağlı olarak değişmiştir. Box-Behnken tasarımına göre optimizasyon yapılmış ve optimum koşullar pH 3,58, süre 80 dk ve katı-sıvı oranı 0,25 olarak üretildiğinde sağlanmıştır. Optimum koşullarda üretilen aquafaba ile hazırlanan bitki bazlı mayonez piyasadan temin edilen standart mayonez ve vegan mayonez ile karşılaştırılmıştır. Mayonez örneklerinin pH, °Brix, asitlik ve kıvam analizleri yapılmıştır. Gerekli iyileştirmeler yapılarak istenilen özelliklerde ürün elde edilmesi mümkündür. Bu çalışma kapsamında aquafaba bitkisel bazlı mayonez formülasyonunda başarılı şekilde kullanılmıştır.

Anahtar kelimeler: Aquafaba, yanıt-yüzey metodolojisi, bitki bazlı mayonez.

Article

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1. Introduction

Chickpea is an important legume that is grown and consumed widely in Türkiye. Chickpea is a good source of protein, carbohydrates and essential micronutrients. It is commercially processed in the form of canned chickpeas, chickpea flour and roasted chickpeas. During all these processes, several by-products are formed. The most important by-product during the processing of chickpeas is aquafaba. Aquafaba is the liquid part that remains after the chickpeas are boiled and separated from the grains. Aquafaba has functional properties such as foaming, emulsifying and gelling. However, these functional properties vary according to the processing conditions of chickpeas as well as chickpea cultivar, grain-water ratio, pH, boiling time, temperature etc. In recent studies, aquafaba is frequently encountered as an egg alternative in various products, especially for the vegan consumers. In a study conducted by Buhl et al. (2019), aquafaba samples were collected from different brands of canned products and compared with egg white (Buhl et al., 2019). In another recent study, aquafaba was obtained from a company in powder form and was used as a gelling agent in plant-based yoghurt samples (Raikos et al., 2020).

Mayonnaise is a product that aquafaba can be applied easily. It is an emulsified viscous sauce. Egg yolk provides the emulsion in the structure. Due to the proteins in its composition, it can form and stabilize an emulsion. In a study by Raikos, Hayes, and coworkers (2020), aquafaba was collected from canned chickpeas and a vegan mayonnaise recipe was optimized using aquafaba. The stability of the obtained samples was investigated. The aquafaba/oil ratio had a minor impact on colour properties and no significant effect was observed on the physicochemical stability of the mayonnaise during cold storage (Raikos, Hayes, et al., 2020). In another recent study, the temperature of soaking water, the soaking time and the boiling time were optimized and the produced aquafaba was used in mayonnaise. Aquafaba prepared by soaking chickpeas in 4 °C water for 16 h and boiling for 30 min achieved the highest emulsion capacity and stability (He et al., 2021). Since it is not possible to obtain exactly same composition in every batch, various gums and starches can be used to provide consistency to support the structure.

In majority of the studies from the current literature, aquafaba is collected from the canned products. There are a few studies focusing on the optimization of aquafaba processing parameters. The main goal of this study was to investigate the effects of processing conditions on aquafaba characteristics using Response Surface Methodology and to optimize aquafaba production on a lab scale. The application of aquafaba in mayonnaise formulation can enable development of plant-based products that can be consumed by vegan individuals.

2. Materials and Methods

2.1 Materials

The chickpeas used in the production of aquafaba were supplied from a market and the same brand product was used throughout the experiments. The ingredients used in mayonnaise production were also obtained from the market.

2.2 Processing techniques

An experimental design was created using the Response Surface Methodology, Box-Behnken design to observe the effects of pH, solid-liquid ratio and boiling time on aquafaba characteristics.

Parameter levels were determined based on preliminary experiments. The experimental design is presented in Table 1.

Table 1. The experimental design created by the response-surface methodology.

Tablo 1. Yüzey-yanıt metodolojisi ile oluşturulan deney tasarımı.

Sample	pH	Time (min)	Solid-liquid ratio
1	2.0	80	0.225
2	4.0	70	0.225
3	4.0	60	0.250
4	4.0	60	0.200
5	2.0	70	0.200
6	4.0	80	0.200
7	2.0	70	0.250
8	2.0	60	0.225
9	6.0	80	0.225
10	4.0	80	0.250
11	4.0	70	0.225
12	6.0	70	0.200
13	6.0	60	0.225
14	4.0	70	0.225
15	6.0	70	0.250

2.3 Preparation of the samples

Chickpeas were soaked in tap water for 24 h at a chickpea: water ratio of 1:3 (w/v). With this process, chickpea grains were softened by absorbing water and increased in volume. At the end of 24 h, chickpeas were washed several times with water and cleaned, then weighed and placed in a pot. For each treatment, 400 g of chickpeas were used and water was added according to the determined solid- liquid ratio. After the solid-liquid ratio was adjusted, the pot was placed on the induction cooker and the boiling process was carried out according to the time determined in the experimental design at a temperature of 100 °C under constant heating. After the boiling process, chickpeas and aquafaba were separated from each other by a strainer. Aquafaba was transferred into a glass beaker and cooled in a water bath.

The pH value of the samples was adjusted with citric acid and trisodium citrate according to the experimental design at 20°C. Aquafaba samples were analyzed the same day after preparation.

2.4 pH analysis

pH values were measured when the temperature of the aquafaba samples was dropped to around 20°C. Three replicate measurements were made for each sample. The pH value was measured with a pH meter (Mettler Toledo, USA).

2.5 Analysis of Soluble Solid Matter (°Brix)

The total soluble solids content was measured with a digital refractometer (ATAGO 5000a, Japan). Results were expressed in °Brix at 20°C (Tüfekci & Fenercioğlu, 2010).

2.6 Foaming Capacity and Stability

Foam volume and foam stability were measured according to method of Makri et al. (2005), with slight modifications (Makri et al. 2005). Approximately 30 mL of aquafaba sample was transferred into a beaker. Afterwards, it was whipped for 2 min with a hand mixer (Bosch, Germany) at 1400 rpm. The foam was transferred into a measuring cylinder. The first reading on the scale was recorded as the foaming capacity. After waiting for 30 min, the amount of foam was read again and % foam stability and % foam volume were calculated using equation 1 and 2, respectively (Lafarga et al., 2019). Analysis was repeated three times for each sample.

$Foaming\ capacity\ (\%)$: $((VF - Vo)\ /Vf)\ * 100$ (Equation 1)
Vo: volume before homogenization
VF: volume after homogenization

$Foaming\ stability\ (\%)$: $((V30/Vf)\ * 100$ (Equation 2)
Vf: Initial foam
V30: Foam volume after 30 min

2.7 Emulsion Activity Index

A measuring cylinder was used to measure 40 mL of aquafaba and 60 mL of sunflower oil. The ingredients were transferred into a plastic beaker and homogenized (Silverson L5M-A, England) for 1 min. The obtained emulsion was transferred into a measuring cylinder and kept at room temperature. The emulsion volume was recorded at time 0 and 1 h. Emulsion activity index was calculated according to the equation 3 (Meurer et al., 2020). The analysis was performed in three replicates for each sample.

$EAI\ (\%)$: $(V60/Vi)\ * 100$ (Equation 3)
Vi: Initial emulsion volume
V60: emulsion volume after 60 min

2.8 Optimization and Validation

It is indicated that the foam and emulsion properties of aquafaba also change depending on the processing conditions. The results of the measurements made on the foam and emulsion properties were used to reach the optimum conditions. With the optimization, the foam and emulsion properties of aquafaba were maximized. Optimum levels of this study were determined as: pH 3.58, time 80 min, and the solid-liquid ratio 0.25. For validation, the analyzes were repeated under these conditions.

2.9 Production of Plant-Based Mayonnaise

Plant-based mayonnaise was produced with the optimized aquafaba. The mayonnaise formulation is presented in Table 2. Mayonnaise was produced in 500 g with using Thermomix (Thermomix Vorwerk, Germany) without applying heat treatment. First, salt, sugar and aquafaba were mixed at a speed of 2.5 and 350 rpm for 2 min. Starch, xanthan gum and 75 g of sunflower oil were mixed in a separate bowl and added to the aquafaba mixture in the Thermomix. After mixing, the speed was increased to 3.5 and 450 rpm. The remaining 175 g of sunflower oil was added slowly. The subsequently added oil was introduced slowly over approximately 4 min. After adding the oil, apple vinegar was added and mixed for 3 min. After the mayonnaise production was completed, it was stored at +4 °C.

Mayonnaise produced with aquafaba was compared with commercial products which is the regular mayonnaise and vegan mayonnaise. All analyses applied to mayonnaise were performed for these three products.

Table 2. Formulation of the plant-based mayonnaise.
Tablo 2. Bitkisel bazı mayonez formülasyonu.

Ingredient	Amount (g)
Salt	5.00
Sugar	10.00
Aquafaba	207.10
Potato starch	12.00
Xanthan gum	0.90
Sunflower oil	250.00
Apple vinegar	15.00

2.10 Characterization of plant-based mayonnaise

Analysis of Soluble Solid Matter (°Brix)

°Brix measurement of the mayonnaise sample was performed using a refractometer (Atago 5000a, Japan) at 20°C.

pH and Acidity Analyses

The pH values of the mayonnaise samples were measured using a pH meter (Mettler Toledo, USA), and the acidity values were measured using a titrator (Metrohm, Switzerland).

Consistency analysis

Bostwick consistometer was used to determine the consistency of mayonnaise samples at 20°C. Sample was placed in the reservoir of the consistometer and the cover was released. Bostwick values of the sample at the end of 30 and 60 secs were determined (Rüzgar & Yazıcı, 2022).

Heat Stability of Mayonnaise Samples

Heat stability of mayonnaise samples was determined according to the method used by Huang et al. (2016) with slight modifications (Huang et al., 2016). Approximately 10 g of mayonnaise was weighed and transferred into a 250 mL glass beaker. The water bath was pre-set to 80°C (Nüve BM 30, Türkiye). The beakers were placed in a hot water bath for 1 h. Afterwards, oil release from the samples was observed.

Sensory analyses

In total, 21 panelists between the ages of 23–36 participated in the sensory analyses. Panelists were requested to evaluate the products in terms of color, taste, mouthfeel, sourness, viscosity, spreadability, odor, taste and general acceptability. During the taste session, the panelists were requested to taste mayonnaise with fries and alone. The panelists were requested to answer the demographic questions and to give a score from 1 to 5 for each parameter. The significance level of the difference between samples was examined statistically by ANOVA test.

2.11 Statistical analysis

All analyses were performed in three replicates. Statistical analyses were performed using Minitab (Minitab Inc., USA). ANOVA test was applied and the difference between the results was determined at 95% confidence level using the Tukey test ($p < 0.05$).

3. Results and Discussion

3.1 pH and soluble solid content of the aquafaba

The pH values of the produced aquafaba samples varied between 5.96-6.14 and the °Brix values ranged between 1.12-2.22. Since the pH values specified in the experimental design were adjusted after the measurements, the possible reason for the variance observed was the changing solid-liquid ratio and time parameters. To examine the effect of solid-liquid ratio on initial pH and °Brix, comparisons were made between samples with the same duration. The change in the solid-liquid ratio in the samples with the same duration caused a statistically significant difference on the pH and °Brix value. As the solid-liquid ratio changed, the °Brix value of aquafaba changed. The dissolved solids content was measured higher in the samples with high this ratio. Accordingly, the pH value also changed due to the components that pass from chickpea to boiling water.

When the solid-liquid ratio was kept constant and the effect of the time on the initial pH and °Brix value was examined, no significant difference was observed. Presumably, the components that passed from chickpea to water did not change after a certain period of time and did not cause a significant difference between 60-70 and 70-80 min.

3.2 Foaming Capacity and Stability

When the parameters affecting the foaming capacity were examined, the interaction of pH2 and pH*time significantly affected the foaming capacity of the samples. The created regression model was meaningful. In the study, where the foam and emulsion properties of aquafaba were tried to be improved, the solid-liquid ratio and pH significantly affected the foam capacity (Lafarga et al., 2019).

The graphs of the foam capacity obtained from the, Response-Surface Method were shown in Fig. 1-3. The foam volume of aquafaba reached maximum levels when the pH was around 4, the solid/liquid ratio was around 0.25 and the time was between 70-75 min. Samples in which the pH were adjusted to 4 during the foam volume analysis were visibly higher than the other pH levels. While the proteins in aquafaba were best around pH 4, the foaming capacity reaches maximum levels.

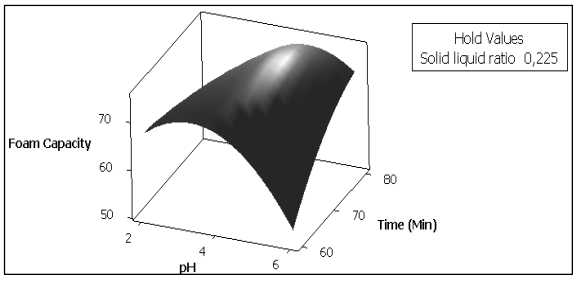

Figure 1. The effect of pH and time on foam capacity of aquafaba.

Şekil 1. pH ve sürenin aquafaba köpük kapasitesine etkisi.

The foam stability of aquafaba was significantly affected by pH2. As with the foam capacity, pH significantly affected the foam stability. The conditions between pH 4-5, solid/liquid ratio around 0.25 and duration longer than 70 min provide the best conditions for foam stability. Foam was produced at points where was around the pH 2 had the lowest stability. The higher the solid/liquid ratio was caused the higher protein and carbohydrate content in the water. Accordingly, the stability of the foam was at higher levels with a high solid/liquid ratio.

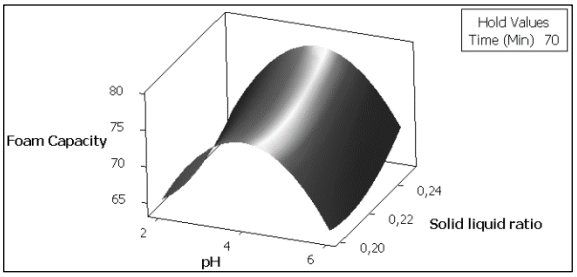

Figure 2. The effect of pH and solid/liquid ratio on foam capacity of aquafaba.

Şekil 2. pH ve katı/sıvı oranının aquafaba köpük kapasitesine etkisi.

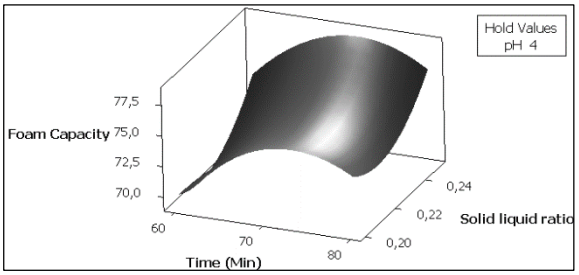

Figure 3. The effect of time and solid/liquid ratio on foam capacity of aquafaba.

Şekil 3. Süre ve katı/sıvı oranının aquafaba köpük kapasitesine etkisi.

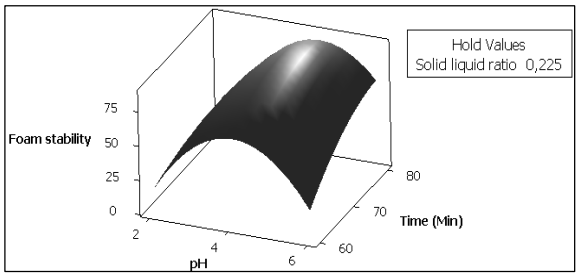

Figure 4. The effect of pH and time on foam stability of aquafaba sample.

Şekil 4. pH ve sürenin aquafaba köpük stabilitesine etkisi.

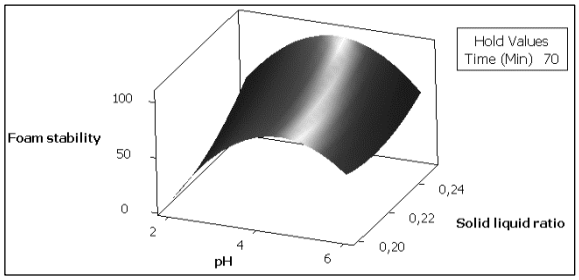

Figure 5. The effect of pH and solid/ liquid ratio on foam stability of aquafaba samples.

Şekil 5. pH ve katı/sıvı oranının aquafaba köpük stabilitesine etkisi.

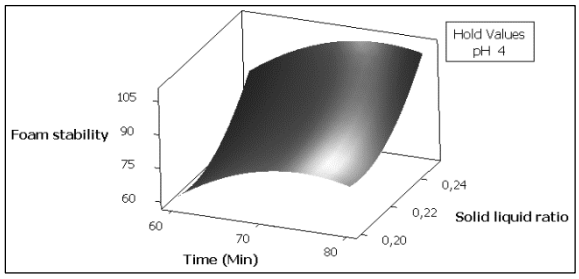

Figure 6. The effect of time and solid/ liquid ratio on foam stability of aquafaba samples.
Şekil 6. Süre ve katı/sıvı oranının aquafaba köpük stabilitesine etkisi.

3.3. Emulsion activity index

Solid/liquid ratio, pH2 and pH*time interaction significantly affected the emulsion activity index.
The effect of the parameters was presented in the Fig. 7-9 on the emulsion activity index. In contrast to the foam properties, the emulsion activity index was calculated higher when value was close to the pH 2. In cases where the time and solid/liquid ratio were high, the emulsion activity index was at higher levels.

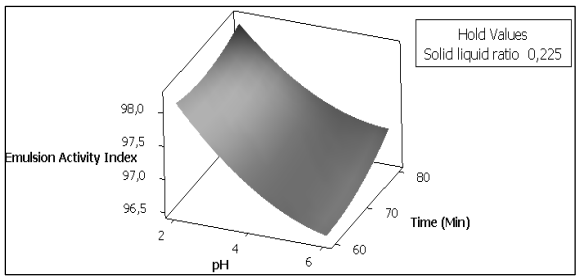

Figure 7. The effect of the pH and time on the emulsion activity index.
Şekil 7. pH ve sürenin emülsiyon aktivite indeksine etkisi.

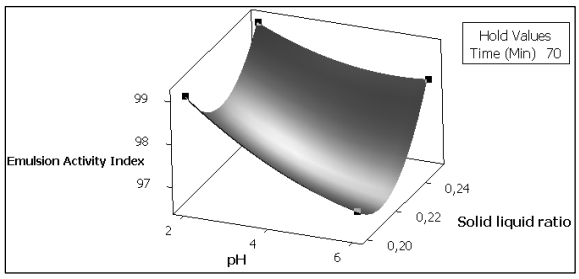

Figure 8. The effect of pH and solid/ liquid ratio on the emulsion activity index.
Şekil 8. pH ve katı/sıvı oranının emülsiyon aktivite indeksine etkisi.

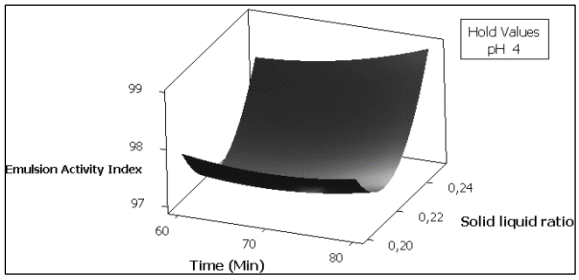

Figure 9. The effect of time and solid/ liquid ratio on the emulsion activity index.
Şekil 9. Süre ve katı/sıvı oranının emülsiyon aktivite indeksine etkisi.

3.4 Optimization and validation

The results of aquafaba produced with optimized conditions are given in Table 3. The sample produced under optimized conditions for foam volume and foam stability was measured at the highest levels. As a result, the foam properties reached maximum levels when the pH was around 3.5. Considering the emulsion activity index, the measured value was like the samples. In a study in which the solid/liquid ratio and the time were tried to be optimized, the optimum conditions were calculated as the situation where the solid/liquid ratio was 1.5/3.5 and the duration was 60 min. Optimum foam volume was measured as 88.3 ± 2.36 and foam stability as 55 ± 2.45 (Alsalman et al., 2020).

Table 3. Characteristics of the optimized sample.
Tablo 3. Optimize örneğin karakteristikleri.

Optimized sample	
pH	6.10 ± 0.03
°Brix	2.000 ± 0.006
% Foam capacity	76.0 ± 1.1
% Foam stability	85.6 ± 1.8
Emulsion activity index	97.9 ± 0.9

3.5 Characteristics of plant-based mayonnaise

pH and acidity

Characteristics of the mayonnaise samples are presented in Table 4. The lowest pH was measured in commercial vegan mayonnaise. While there was no significant difference in pH values of mayonnaise which is produced with aquafaba and regular mayonnaise from the market; the pH of commercial vegan mayonnaise is significantly different from the others. In a study by Karas and coworkers, commercially produced mayonnaise samples with different fat contents were taken and physicochemical analyzes were applied. The pH value of aquafaba was measured 3.74 and 4.66, in another study, the lowest pH value was measured as 3.27 (Karas et al., 2002). The values measured in this study were lower than the values in other studies. The reason for the difference between the pH values of the samples was the amount of acid added and formulation differences.

Table 4. Characteristics of mayonnaise samples.
Tablo 4. Mayonez numunelerinin özellikleri.

	pH	°Brix	Acidity (%)	Bostwick value (cm)
Mayonnaise with aquafaba	3.40±0.02^{A}	11.2±0.3^{C}	0.40±0.03^{A}	1.1-1.4^{A}
Regular commercial mayonnaise control	3.40±0.02^{A}	25.8±0.8^{A}	0.40±0.006^{A}	0^{B}
Commercial vegan mayonnaise	2.90±0.02^{B}	21.9±0.6^{B}	0.40±0.02^{A}	0^{B}

* There is a statistically significant difference between the values shown with capital letters within the same column (p<0.05).

No significant difference was observed in the acidity values of the samples (p>0.05). In the study conducted by Hakimian et al., the pH value of the control samples was measured as

0.61% (Hakimian et al., 2022). In another study, acidity was measured as 0.50% (Pradhananga et al, 2016). The acidity value of mayonnaise used in this study was observed to be relatively lower compared to the studies in the literature.

Soluble Solid Content

°Brix is an important parameter for sauce type products. It provides an idea about mouthfeel and consistency. When two mayonnaise samples with different °Brix values are compared, it will be observed that the sample which has higher °Brix value has more intense consistency and more mouth-satisfying taste. In the present study, significant differences were observed between the °Brix values of the mayonnaise samples. The °Brix value of the mayonnaise produced with aquafaba was approximately half of the °Brix value of other products. Significantly lower °Brix values observed in the newly developed mayonnaise formulation can result in negative effects on the consistency and stability of the products. Generally, increases in soluble solid content contribute to higher viscosity of the sauces (Gamonpilas et al., 2011). This can be accomplished via using various stabilizers such as hydrocolloids in the product formulation. Increasing the soluble solid content in the formulation can also have a significant effect on both the consistency and mouthfeel of the product. In a study aiming to reduce the amount of oil in mayonnaise formulation, various stabilizers including xanthan gum and sodium carboxymethyl cellulose were used. The °Brix value of mayonnaise produced using xanthan gum was measured as 23 (Than & Win, 2020).

Consistency

It was observed that the commercial products did not move in 60 sec, while the mayonnaise sample produced with aquafaba moved 1.1 cm in 30 sec and 1.37 cm in 60 sec. In terms of consistency and viscosity of the product produced with aquafaba was lower than commercial product. In a study focusing on fat reduction in mayonnaise, modified starch was added, and the control sample (80% fat content) moved 0.2 cm in 30 sec (Carcelli et al., 2020). The mayonnaise produced for this study moved more in 30 sec, and therefore its consistency was found to be lower.

Heat Stability

At the end of 60 min, while water started to accumulate on the surface of the vegan mayonnaise and aquafaba mayonnaise, it was clearly observed that the amount of separated oil was higher in the regular mayonnaise control. As a result, the thermal stability of regular mayonnaise was found to be lower than plant-based mayonnaise samples.

Figure 10. Heat stability of mayonnaise samples.
Şekil 10. Mayonez örneklerinin ısı stabilitesi.

Sensory Characteristics

Sensory scores of the mayonnaise sample are presented in Table 5. Mayonnaise produced with aquafaba was whitish in color, while vegan mayonnaise was brighter yellow than regular mayonnaise. According to the taste parameter, vegan mayonnaise received the lowest ratings from the panelists since it was indicated to leave an artificial and oily taste in the mouth.

In terms of sourness, the formulation of the product with aquafaba needs to be improved. When the odor was examined in all 3 products, no significant difference was detected in terms of odor. When considering the overall acceptability, vegan mayonnaise received the lowest score.

The general comments about the product with aquafaba were additionally provided by the panelists which included that it did not leave an aftertaste and that it was perceived to be significantly better than expected.

Table 5. Sensory properties of mayonnaise samples.
Tablo 5. Mayonez örneklerinin duyusal özellikleri.

	Color	**Taste**	**Mouthfeel**	**Sourness**	**Viscosity**	**Spreadability**	**Odor**	**Aftertaste**	**Overall Acceptability**
Mayonnaise with aquafaba	3.3±1.0^{B}	4.0±0.7^{A}	3.9±0.6^{AB}	3.9±1.1^{A}	4.6±0.5^{A}	4.3±0.9^{A}	4.2±0.9^{A}	3.9±1.1^{A}	4.1±0.5^{AB}
Regular commercial mayonnaise	4.7±0.6^{A}	4.0±1.1^{A}	4.3±0.7^{A}	4.0±0.9^{A}	4.7±0.6^{A}	4.7±0.5^{A}	4.5±0.7^{A}	3.9±1.2^{A}	4.4±0.7^{A}
Vegan mayonnaise	3.8±1.2^{B}	3.5±1.1^{A}	3.4±1.2^{B}	3.7±11^{A}	4.2±1.2^{A}	4.2±1.0^{A}	4.1±1.2^{A}	3.9±1.2^{A}	3.7±1.3^{B}

* There is a statistically significant difference between the values shown with capital letters within the same column (p<0.05).

4. Conclusion

The effects of processing parameters such as boiling time, pH and solid-liquid ratio on aquafaba properties were investigated. According to the results obtained from Response Surface Methodology, maximum values for foaming capacity, foam stability and emulsion activity index were obtained when the solid-liquid ratio was approximately 0.25 and time over 70 min. Maximum levels for foaming capacity and stability were obtained at pH 4, whereas pH 2 resulted in maximum emulsion activity index, and optimized samples were validated.

Plant-based mayonnaise was prepared with the optimized aquafaba and compared with commercial samples. The °Brix value and consistency of the sample produced with aquafaba were significantly lower than the commercial products. These differences can be eliminated with revisions on the formulation of mayonnaise produced with aquafaba.

By including thickeners such as starches and gums in the formulation, it is possible to match the consistency and °Brix values of commercial samples. On the other hand, the desired yellowish color can be obtained by the addition of natural colorants. While improving the taste of mayonnaise by adding lactic acid to the formulation; at the same time, the shelf life of the product can be extended.

5. Acknowledgements

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6. Conflicts of interest

The authors declare no conflict of interest.

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Effect of Processed Foods on Advanced Glycation End Products: Cancer Cases

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Abstract: Advanced glycation end products (AGEs) are heterogeneous compounds that occur endogenously and exogenously during metabolism. These compounds increase because of processed food consumption. Nowadays, fast-paced living conditions lead individuals to consume processed food. High numbers of processed foods consumed because of nutrition cause inflammation in metabolism. Inflammation causes atherosclerosis, diabetes, kidney diseases, cancer, liver, and some neurodegenerative diseases. The purpose of this review study was to detail the relationship between AGEs and some types of cancer depending on nutrition and dietary habits. For this purpose, cancer types such as breast cancer, colorectal cancer, and pancreatic cancer, which have been common in recent years, were discussed. AGEs bind to receptors on cells, affect certain transcription factors, prevent cancer cell apoptosis, and support proliferation. Studies have shown that the number of AGEs is affected by nutrition and dietary habits. In this context, it has been shown that phenolic compounds, vitamins, and limited AGE intake play an important role in minimizing the effects of these products. This review study revealed the effects of AGEs on cancer and examined in detail the conditions that affect the formation of these products. When the studies are evaluated, it is aimed to raise public awareness by emphasizing that the formation of advanced glycation end products is directly related to nutritional habits and food processing methods, that it causes different diseases, especially cancer, and how its formation can be limited.

Keywords: Glycation, advanced glycation end products, nutrition, cancer.

İşlenmiş Gıdaların İleri Glikasyon Son Ürünlerine Etkisi: Kanser Vakaları

Özet: İleri glikasyon son ürünleri (İGS) metabolizmada endojen ve eksojen olarak oluşan heterojenik bileşiklerdir. Bu bileşikler işlenmiş gıda tüketimine bağlı olarak artmaktadır. Günümüzde hızlı ve tempolu yaşam koşulları bireyleri işlenmiş gıda kullanımına yönlendirmektedir. Beslenme sonucunda alınan yüksek miktarda işlenmiş gıdalar metabolizmada inflamasyona neden olmaktadır. İnflamasyon ise metabolizmada ateroskleroz, diyabet, böbrek hastalıkları, kanser, karaciğer ve bazı nörodejeneratif hastalıkların oluşumuna neden olmaktadır. Bu derleme çalışmasının amacı ise İGS'ler ile bazı kanser türleri arasındaki ilişkiyi beslenme ve diyet alışkanlıklarına bağlı olarak detaylandırmaktır. Bu amaçla son yıllarda sık rastlanan meme kanseri, kolorektal kanser ve pankreas kanseri gibi kanser türleri ele alınmıştır. İGS'lerin hücrelerdeki reseptörlere bağlanarak belirli transkripsiyon faktörlerini etkileyip kanser hücrelerinin apoptozunu engellediği ve proliferasyonu desteklemektedir. Yapılan çalışmalar, beslenme ve diyet alışkanlıklarına göre İGS'lerin miktarlarını etkilediğini göstermektedir. Bu bağlamda fenolik bileşiklerin, vitaminlerin ve kısıtlı İGS alımının, bu ürünlerin etkilerini en aza indirmede önemli rol oynadığı gösterilmiştir. Bu derleme çalışması, İGS'lerin kanser üzerindeki etkilerini ortaya koyarak, bu ürünlerin oluşumunu etkileyen durumları detaylı bir şekilde incelemiştir. Çalışmalar değerlendirildiğinde, ileri glikasyon son ürünlerinin oluşumunun beslenme alışkanlıkları ve gıda işleme yöntemleriyle direkt olarak ilişkili olduğunu, kanser başta olmak üzere farklı hastalıklara neden olduğunu ve oluşumunun nasıl sınırlandırılabileceği vurgulanarak, kamu bilincinin oluşturulması hedeflenmiştir.

Anahtar Kelimeler: Glikasyon, ileri glikasyon son ürünleri, beslenme, kanser.

Review

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1.Introduction

Glycation (Glycosylation) is a biochemical process involving the interaction of carbohydrates with free amino groups in lipids and nucleic acids through "*maillard"* or "*browning reactions*". These reactions can result from high-temperature cooking or prolonged slow-cooking processes (Dariya and Nagaraju, 2020).

Glycation reactions lead to the formation of advanced glycation end products (AGEs). They can be classified based on their formation as endogenous (formed in the organism) or exogenous (formed through lifestyle factors such as nutritional habits, smoking, etc), their molecular weight as heavy or light, or their toxicity properties as toxic or non-toxic. One of the exogenous sources, dietary AGEs, can be associated with an individual's eating habits. Examples of glycation products acquired through diet include Nε-carboxymethyl-lysine (CML), Nε-1-carboxyethyl-lysine (CEL), methylglyoxal (MGO), pyrraline, and glycolaldehyde. Dietary AGEs (dAGEs) formed by the interaction of exogenous proteins and carbohydrates at high temperatures occur more rapidly than endogenously formed AGEs. Therefore, dietary habits are known to increase the rate of AGEs (Gill et al., 2019; Lugt et al., 2020; Rungratanawanich et al., 2021; Twarda-Clapa et al., 2022).

Accumulation of AGEs in the body leads to various health problems and metabolic effects such as insulin resistance, lipid resistance, arterial stiffness, inflammation, tissue damage, apoptosis, bone cell injuries, cellular stress, neurodegeneration, hepatic fibrosis, and cirrhosis. Moreover, these products trigger hyperlipidemia, oxidative/carbonyl stress, antioxidant deficiency in the body, and aging. Because of these reactions, the onset and progression of numerous diseases, including cancer, diabetes, kidney diseases, heart diseases, osteoporosis, aging, gout, and liver diseases, are observed. In addition, some factors such as diabetes, age, gender, smoking, obesity, and lifestyle are known to influence the increase in glycation (Ahmad and Farhan, 2016; Dariya and Nagaraju, 2020). Recent and ongoing studies continue to explore the impact of AGEs on various health conditions. The objective of this study was to investigate the formation of AGEs and their association with cancer types such as breast, pancreas, and colorectal cancers, shedding light on the factors influencing AGE formation (Uribarri et al., 2010; Rungratanawanich et al., 2021) (Figure 1).

2. What are AGEs and Theirs Effects on Metabolism?

AGEs, which are a natural mechanism in the body, are known to have a high potential for toxicity because of the quantity of production and types of binding. These products are formed through the glycation, oxidation, and carbonylation pathways. The Maillard reaction, representing the initial step of glycation, involves the nucleophilic reaction between a free amino group of a protein (such as lysine, arginine, or cysteine) and a carbonyl group of a sugar molecule (glucose, fructose, etc.). These two compounds undergo a chemical reaction to form a Schiff base, which is then re-arranged intra-molecularly into a more stable ketoamine or Amadori product (AP) within hours. These glucose-modified proteins undergo advanced reactions to form AGEs. They occur through irreversible reactions, leading to protease-resistant cross-linking of peptides and proteins, contributing to protein accumulation and amyloidosis (Ahmed, 2005; Vicil and Ulutaş, 2020).

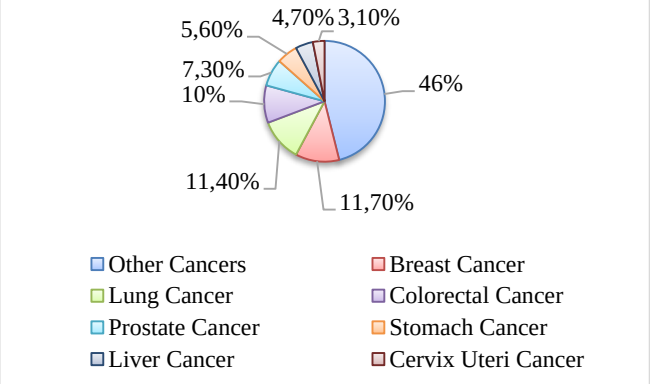

Figure 1. Estimated number of new cases in 2020 (WHO, 2023).
Şekil 1. 2020'de tahmini yeni vakalar (WHO, 2023).

Moreover, the cross-linking of long-lived proteins by AGEs is known to accelerate the normal aging process of cells and tissues. AGEs can be found in various forms of lipoproteins and lipid components, contributing to problems such as macroangiopathy, microangiopathy, and amyloidosis. Studies have suggested some diseases such as atherosclerosis, cataracts, diabetes, inflammation, cancer, kidney and liver diseases, and neurodegenerative problems by AGE formation. Although the pathological properties of AGEs were initially attributed primarily to chemical features such as protein cross-linking, recent studies indicate that AGEs also impact cellular processes and signaling. AGEs can directly bind to extracellular membranes or interact with cell surfaces through specific receptors (RAGEs) (Lyu et al., 2023).

When we evaluated recent studies, it was observed that AGEs can alter the structure, tissue, activity, and other characteristics of cell matrix proteins by cross-linking them. Glycation reactions have changed collagen and elastin, proteins in the extracellular matrix membranes. Through this process, collagen can change endothelial cell activity, leading to the formation of atherosclerotic plaques. In terms of biochemical insight, a recent study suggested that CML may increase vascular calcification through activation of pyruvate dehydrogenase kinase 4. These correlated atherosclerotic plaques and vascular calcification are interrelated problems that can lead to renal failure, coronary artery disease, and oxidative stress (Zhu et al., 2018).

When examining the mechanisms through which AGEs may cause diseases, it can be said that they play a significant role in diabetes complications. In these complications, AGEs bind to specific cell receptors, triggering various signaling pathways. The receptors most commonly bound by AGEs in the body are referred to as the receptor for advanced glycation end products (RAGE). RAGE, belonging to the immunoglobulin superfamily, is a transmembrane receptor and is the most commonly bound receptor by AGEs in humans (Jangde et al., 2020).

Many diseases occur because of AGE binding to RAGEs. The production of RAGE increases in various metabolic conditions such as diabetes and inflammation. RAGEs are found in many cells throughout the body, including macrophages, endothelial cells, neurons, and connective tissue cells. When bound to RAGE, AGEs activate extracellular and intracellular pathways. As a result of these reactions, a transcription factor called NF-κB (nuclear factor-kappa B) can become active (Asadipooya and Uy, 2019).

This activated transcription factor increases the expression of inflammatory cytokines, adhesion molecules, and other cellular mediators. These changes lead to increased oxidative stress, production of proinflammatory cytokines, increased expression of adhesion molecules, changes in procoagulant factors, and vasoconstriction (Asadipooya and Uy, 2019).

3. Nutrition and Cancer Risk: Biochemistry of AGEs/RAGEs

Accumulation of AGEs and their metabolites such as α-dicarbonyls such as methylgloxal, glyoxal, and diacetyl results in dysfunction of proteins, nucleotide bases, and DNA repair enzymes. Although tumor cells use the same metabolic network as normal cells, they have re-programmed metabolism to meet uncontrolled replicative demands. AGEs/RAGE increase the glycolysis pathway and trigger inflammatory cytokines with NF-κB. Depending on increased glycolysis and inflammatory cytokines, the amount of inflammation around the tumor also increases (Rojas et al., 2010). AGEs play a pivotal role 25% of all cancer types and increase tumorigenic signaling pathways; they are responsible for low oxygen levels (hypoxia) around tumor cells and inflammation (Xu et al., 2010). The AGEs–RAGE interaction activates NADPH oxidases and produces reactive oxygen species (ROS) under hypoxic conditions. These trigger interactions between hypoxia and AGEs. Hypoxia also causes the formation of new blood vessels around active cancer cells. Increased cancer cells facilitate the nutrition and proliferation of cancer cells. *In vitro* and *in vivo* studies have shown that blocking RAGE can suppress invasive capacity and angiogenesis (Liang et al., 2011; Nedić et al., 2013).

In metabolism there is mechanism that called "autophagy" to reduce wear and tear of tumor cells. It minimizes stress by breaking down damaged cellular components and ensuring cell survival and resistance to death. The key inhibitor of this process is the target of rapamycin (mTOR) and its regulator, AMP-activated protein kinase (AMPK). The AGEs–RAGE interaction maintains autophagy by reducing mTOR phosphorylation and ensures the survival of tumor cells. In addition, RAGE increases proliferation resistance by activating the AMPK/mTOR signaling pathway and regulating autophagy (Li et al., 2018). The effects of AGEs and RAGEs on the recent increase in cancer are diverse. For example, their role in various cancer types, such as pancreatic, colon, and breast cancer, has been emphasized.

Considering that cancer is currently an increasing public health problem, it is important to highlight the role of factors such as food safety, processed foods, and dietary habits in cancer formation. AGEs in foods play a crucial role in this age. In this review, we will explore in-depth the relationship between AGEs and cancer, providing examples of how this mechanism is effective in different cancer types (Figure 2).

3.1 Breast cancer

Breast cancer typically originates in breast tissue and often manifests in the mammary glands (milk-producing glands) or milk ducts (channels carrying milk to the mammilla). Cancer usually begins with uncontrolled and abnormal cell growth in the breast tissue. According to World Health Organization (WHO) data, in 2020, 2.3 million women worldwide were diagnosed with breast cancer, resulting in 685.000 deaths attributed to this disease. By the end of 2020, 7.8 million women who had been diagnosed with breast cancer in the last 5 years were alive, establishing it as the most prevalent cancer globally. This cancer type is more frequently observed in women over 40 years old compared with other age groups (Abdulrahman and Rahman, 2012). 5-10% of women, the

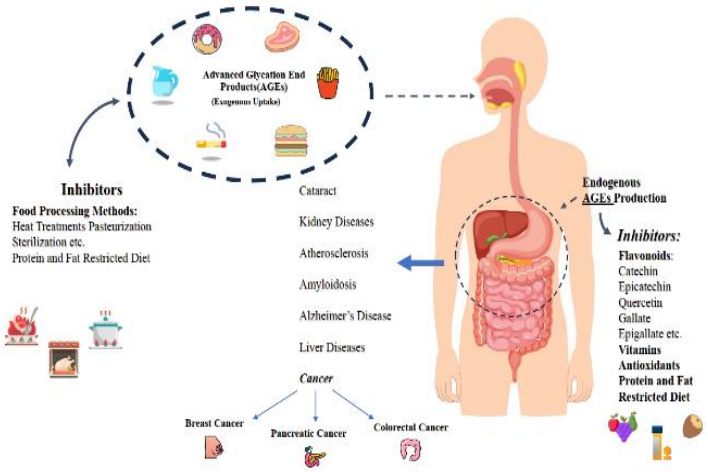

Figure 2. AGE sources and their relationship with diseases.
Şekil 2. İleri Glikasyon Son Ürünlerinin kaynakları ve ilişkili hastalıklar.

development of this cancer type is known to occur due to the inheritance of an autosomal dominant gene, often involving mutations in the breast cancer gene 1 and 2 (BRCA1 and BRCA2) genes. In addition, risk factors for breast cancer include female reproductive hormones (endogenous and exogenous), nutritional factors (especially dietary AGEs intake), benign breast disease, re-productive history, and environmental factors. Treatment involves radiotherapy, chemotherapy, andsurgical interventions. Breast cancer represents the most prevalent form of cancer and is the primary contributor to cancer-associated mortality among women globally (Maxwell and Nathanson, 2013; Alexander et al., 2010). Moreover, there is a significant association between diabetes and breast cancer. Several studies and experiments have demonstrated a connection between diabetes and breast cancer. The most prevalent in circulation in individuals with diabetes is glycated albumin. In a study conducted by Sharaf et al. using a breast cancer cell line, the effects of methylglyoxal-derived bovine serum albumin AGEs on cell proliferation, migration, and invasion were examined (Sharaf et al., 2023). The results indicated that MGO-BSA-AGEs increase proliferation, migration, and invasion of breast cancer cells, and these effects occur through the AGEs receptor (RAGE). Blocking this receptor has been suggested to eliminate the observed changes (Sharaf et al., 2023). MGO-BSA-AGEs, through RAGE, enhance certain unwanted activities within the cell and regulate cancer cell invasion and migration by contributing to other signaling proteins (Sharaf et al., 2023). In another study conducted in 2023, focus was on carboxymethyl lysine (CML), an important dAGE. The research divided participants into two groups: PG (breast cancer group) and CG (control group). Subgroups within PG, HER2+ (HER2−), were examined. HER2+ groups consist of breast cancer cells with HER proteins on their surfaces, while HER2− groups lack these proteins. Generally, HER2-negative breast cancer can be a slower-growing form. Analysis of serum markers in the study revealed no significant difference in serum CML and RAGE levels between PG (breast cancer group) and CG (control group). Additionally, a comparison between HER2+ (groups showed that, at the T1 (tumor not spreading to surrounding tissues) stage, despite no significant differences in energy intake, the dCML level of the HER2− group was significantly higher than that of the HER2+ group. These findings suggest that the dietary habits and serum CML levels of HER2- breast cancer patients may contribute significantly to DNA damage, inflammation, and protein oxidation effects at the T1 stage compared with HER2+ patients (Alkan et al., 2023).

3.2 Colorectal cancer

According to the WHO, colorectal cancer is the third most prevalent cancer worldwide, constituting approximately 10% of all cancer cases and ranking as the second leading cause of cancer-related deaths globally. It predominantly affects individuals over the age of 50. Colorectal cancer is characterized by the development of a tumor or abnormal tissue growth in the inner lining of the rectum or colon, which can give rise to a tumor on the rectum or colon wall if it transforms into cancer. Subsequently, it may increase the chances of metastasis to other areas by growing into blood or lymph vessels. Tumor types resulting from the growth of mucous-producing glands covering the colon and rectum are the most frequently observed pathogenic factors in this cancer, termed adenocarcinoma. Among other cancers in the colorectal region are tumors originating in hormone-producing intestinal cells, tumors arising in Cajal cells, which are colon cells, lymphomas related to the immune system in the colon or rectum, and sarcomas that typically begin in blood vessels but can rarely occur in colorectal walls. It is a prevalent disease among individuals aged 65-74, with a higher prevalence in women. Factors such as obesity, diabetes, genetic factors, sedentary lifestyle, poor dietary habits (high in fat and protein), smoking, and aging actively contribute to the development of colorectal cancer (Xi and Xu, 2021; Alzahrani et al., 2021)

Furthermore, AGEs and receptors for AGEs (RAGEs) have various effects on the initiation and progression of colorectal cancer (CRC). Several genes interacting with RAGEs are implicated in CRC formation. For example, recent studies have revealed that a protein released during inflammatory conditions limits apoptosomal caspase-9 activation. On the basis of these findings, this protein may play an apoptotic inhibitory role in colon cancer, reducing anticancer immune responses through stimulated apoptosis in immune cells. Among other examples are genes of the S100 family, including S100A8/A9, S100A4, and S100P, which are associated with colorectal cancer. Studies suggest that these proteins could serve as potential biomarkers associated with colorectal cancer (Azizian-Farsani et al., 2020).

Following the diagnosis of colorectal cancer, the consumption of high-sugar food products is associated with higher mortality. The metabolic pathways of glycolysis and fructolysis are suggested to contribute to the formation of a group of compounds known as "glycer-aldehyde-derived advanced glycation end products" (glycer-AGEs). Different types of glycer-AGEs, such as 3-hydroxy-5-hydroxy-methyl-pyridinium compounds (GLAP) and triosidine, have been identified. Glycer-AGEs are found in high-glucose and high-fructose foods (bread, corn sirup rice, etc.). Glycer-AGEs also possess pro-inflammatory and pro-oxidant properties, contributing to colorectal cancer progression and increased mortality. In addition, the binding of glycer-AGEs to RAGEs results in the release of pro-inflammatory cytokines, increased cellular damage, and elevated vascular endothelial growth factor (VEGF) expression in cancer cells. In a cohort study named EPIC conducted at Kanazawa University in Japan, serum concentrations of glycer-AGEs from 1034 colorectal cancer patients were measured using a competitive enzyme-linked immunosorbent assay (ELISA). Higher serum glycer-AGE concentrations were statistically significantly associated with higher CRC-specific and all-cause mortality (Mao et al., 2023).

3.3 Pancreatic cancer

The pancreas is a vital organ that plays a key role in digestion and metabolic processes. It produces the necessary enzymes and hormones for digestion and regulates blood sugar by facilitating the production of insulin and glucagon. Pancreatic cancer is a type of cancer that results from the uncontrolled and abnormal growth of cells in the pancreas, an organ responsible for these functions. A significant proportion of pancreatic cancer cases involve rapidly spreading ductal adenocarcinomas, typically occurring in the head of the pancreas. Because of its aggressive nature, pancreatic cancer exhibits rapid metastasis, contributing to a high mortality rate. According to studies, cancer is expected to be the second leading cause of cancer-related deaths by 2030 (Koçatakan and Ataseven, 2021).

Factors influencing the development of pancreatic cancer are similar to those of other cancers, including obesity, diabetes, alcohol consumption, smoking, substance abuse, age, and genetics. Recent studies indicate that AGEs are found in higher quantities in obese and diabetic individuals than in normal ranges, and this is identified as a factor facilitating the progression of pancreatic cancer. Menini et al. (2021) conducted studies on mice to explore the relationship between CML and pancreatic cancer. It was observed that CML acts as a substance that promotes the proliferation of cells, such as MIA PaCa-2 and PANC-1, and this effect occurs depending on the concentration and duration of exposure to CML. The more CML present and the longer the exposure, the greater the cell proliferation. In addition, even low concentrations of CML induce responses in human pancreatic duct epithelial (HPDE) cells. While these proliferations are part of the cell's life cycle, disturbances or abnormalities in these control mechanisms can lead to uncontrolled cell proliferation, increased formation of RAGEs, and potentially tumor formation (Menini et al., 2021). Another result indicates that CML treatment increases the activity of genes such as p-STAT3, NFATC1, and PIM1, and it may increase the levels and activation of NK-κB/p65 protein, potentially contributing to the development and acceleration of pancreatic cancer (Menini et al., 2021).

In another study, it was found that the protein RAGE stimulates the formation of neutrophil extracellular traps (NETs) through neutrophils in pancreatic cancer. NETs are immune products that respond to pathogens, but an excess of NETs can lead to serious damage in tissues and progression of advanced diseases such as cancer. In mice with RAGE deficiency, reductions in NET formation in pancreatic cancerous mice and decreased levels of circulating DNA are known. These findings indicate that RAGE promotes NET formation through neutrophils. Moreover, high levels of autophagy were detected in neutrophils obtained from pancreatic cancerous mice, and inhibition of autophagy (chloroquine treatment) resulted in decreased NET formation. Similarly, treatment in patients reduces serum DNA levels (Boone et al., 2015).

Hypoxia, which occurs in cancer conditions, causes RAGE to bind with oncogenic Kras (gene of cancer), which promotes tumor growth. Oncogenic Kras refers to a mutated (altered) form of the Kras gene, which has a regulatory effect on normal cell growth and division. However, RAGE deficiency inhibits Kras signaling in vitro and increases pancreatic tumor cell death under hypoxic conditions (Shahab et al., 2018).

These studies suggest significant interactions between RAGE and pancreatic cancer. Recently, particular interest and attention is the resistance of RAGE to gemcitabine, a cytotoxic drug used in the treatment of pancreatic cancer. In a study by Swami et al. (2021), RAGE inhibition was achieved in experimental mice with pancreatic cancer. This inhibition was shown to reduce autophagy induced by gemcitabine in pancreatic tumors. Autophagy is a process by which organelles and proteins within the cell are broken down. In this study, it was shown that this process increased in tumors treated with gemcitabine. TAGEs inhibition by reducing autophagy in mice treated with gemcitabine, could regulate autophagic processes within cells. This understanding emphasizes the role of RAGE in regulating apoptosis in tumor cells, highlighting its potential contribution to the development of new treatment strategies for such tumors (Swami et al., 2021)

4. Alternative Measures for Inhibiting AGEs

4.1. Nutrition

In the previous sections of this study, advanced glycation products formed because of excessive glycation in the body and the receptors of these products found in most cells in the body (RAGE) were mentioned. The excess of these products in the body and the health problems caused by this excess (especially cancer) have been addressed. AGEs must be present in some amount in the body for the natural flow of the body, but there are precautions to be taken in excess.

In this part of our review, we will discuss the measures that can be taken to prevent advanced glycation and excess of the products formed, and how these measures can be taken. Flavonoids are a group that ensure that the number of AGEs is kept under control. Flavonoids are a broad group that are usually found in fruits, plants, cereals, nuts, and vegetables and carry a phenolic or polyphenolic group. Foods containing flavonoids are considered to be the most common natural products in the human diet. In addition to inhibiting the AGEs of flavonoids, it is involved in the prevention of many chronic diseases, oxidative stress, and serious diseases such as cancer. Examples of the flavonoids most commonly found in nature are quercetin, kaempferol, myricetin, luteolin, apigenin, catechins, epicatechin, epicatechin gallate, and naringenin. Catechin, which belongs to the flavonoid group, is a powerful antioxidant. After being taken into the body, it affects the digestion of proteins during gastrointestinal digestion, regulates the release of free amino acids, and changes the particle size. The same catechin inhibits the release of AGEs during digestion with complexes formed by their combination with proteins (Ullah et al., 2020; Wu et al., 2021).

Epicatechin gallate (ECG) also inhibit AGE. In a study conducted, the interaction of ECG, a known AGE, with BSA (large flour albumin) was evaluated. Because of this evaluation, the ECG is wrapped by certain amino acid residues. Various bonds, such as pi-σ, pi-anion, alkyl, and hydrogen bonds, were formed between the ECG and these amino acid residues. Because of the formation of hydrogen bonds, the ECG–BSA complex was formed. Due to this complex, ECG inhibited BSA glycation and provided stabilization (Wu et al., 2019).

A study on one of the natural components, styrylflavonoids, found that they had an anti-inflammatory effect on inflammation linked to advanced aging. The majority of the inflammation caused by AGEs are eliminated by treatments using these and related flavonoid derivatives (Zhou et al., 2022). Except for these studies, many flavonoids inhibit or reduce the production of AGEs. Flavonoids are found in most foods and have a very important role in nutrition (Bestil and Uysal, 2023). In the table below, the nutrients and flavonoids contained are compiled (Table 1).

Another group of molecules that decrease protein glycation in the body and inhibit AGEs are vitamins. Vitamin D influences the mechanisms of aging, especially in many in vitro, animal, and human studies. Vitamin D reduces AGE levels and increases RAGE levels in cases of deficiency and pathological conditions. sRAGEs, unlike RAGEs, compete with this receptor and bind to circulating AGEs, a form that prevents intracellular adverse effects. Simultaneously, vitamin D treatment can be effective in reducing RAGE expression in some pathological conditions. The inhibitory effects of vitamin D on AGE receptors are realized through various cellular signaling pathways, such as MAPK/NF-κB and ADAM10/MMP9 (Kheirouri and Alizadeh, 2020). According to another study conducted in recent years, it is related to the effect of ascorbic acid on AGEs. According to the results of a study conducted in patients with diabetes, vitamin C supplementation added to patients' hypoglycemic medications caused a significant decrease in oxidative stress markers such as AGEs (Rabizadeh et al., 2023).

Dietary habits can affect the formation of glycation products. In particular, foods high in protein and fat content may contain high levels of AGEs. This phenomenon arises because protein and fat undergo more significant reactions during food processing procedures compared with carbohydrates. Based on this information, foods high in protein and fat content, such as meat, dairy products, cheese, and fatty foods, may contain higher levels of AGEs than those with lower carbohydrate content. At this point, our eating habits can prevent the formation of AGEs and reduce these harmful effects (Uribarri et al., 2010).

There have been different studies and research on the determination of dAGE intake over the years. For example, in Spain and France, it has been decided that the intake of CML, a known AGE, should range from 34 to 252 μg/kg of body weight per day, and excess is harmful to health. In the analysis of study outcomes, it became evident that the category with the highest AGE content comprised meats. Although fats actually tend to contain more dietary AGEs in quantity, they cause more dAGE intake because meats are presented to the consumer in a larger portion than fats. When the meat groups prepared by

the same methods were compared with each other, the highest levels of dAGEs were observed in beef, chicken, pork, and fish.

Table 1. Flavonoids obtained from foods and their AGE activities
Tablo 1. Gıdalardan elde edilen flavonoidler ve AGE aktiviteleri.

Systematic Names	Foods	Flavonoid Contents	Activity	Cited
Glycine max	Soybeans	Genistein, Daidzein, Glycitein, isoflavone	Anti-AGEs	Milkovska-Stamenova et al. (2019), Wu et al. (2020)
Cinnamomum verum	Cinnamon	Epicatechin (EC), Catechin	Anti-AGEs	Yang et al. (2018)
Allium cepa	Onion	Quercetin	Anti-AGEs	Khan et al. (2020)
Malus domestica	Apple	Phloridzin, Quercetin, Myricetin	Anti-inflammatory	Millán-Laleona et al. (2023)
Prunus dulcis	Almond	Catechin, EC, Naringenin, Kaempferol	Anti-inflammatory	Khan et al. (2020)
Camellia sinensis	Green and black tea	Catechin, Epicatechin, Theaflavin	Anti-inflammatory	He et al. (2021), Luo et al. (2022)
Spinacia oleracea	Spinach	Patuletin and Spinacetin	Anti-AGEs	Gutierrez et al. (2020)
Prunus persica	Peach	Quercetin, Genistein, Anthocyanins	Anti-AGEs	Maatallah et al. (2020), Bento et al. (2022)
Zingiber officinale	Ginger	Quercetin, gingerol, kaempferol	Anti-inflammatory	Xue et al. (2022)
Vaccinium Vitisidae	Lingonberry	Cyanidin-3-galactoside, Cyanidin-3-glucoside	Anti-CML	Račkauskienė et al. (2019), Maduma (2022)

Lamb meat, on the other hand, was ranked lower than other meats. However, what actually attracts attention in these results is that lean meats contain very high amounts of dAGEs, despite being subjected to heat treatments under dry heat. The reason for this condition is the reactive amino-lipids found in meat and reducing sugars such as fructose or glucose-6-phosphate. At the same time, high-fat foods such as butter, cream cheese, margarine, and mayonnaise are also among the foods rich in RAGE, and their intake should be restricted (Delgado and Andrade, 2016).

The AGEs content of vegetables and fruits is considered to be quite low. For example, carrots have 10 kU/100g, tomatoes have 23 kU/100g, bananas have 9 kU/100g, and cucumbers have 31 kU/100g levels of AGEs. The reason is that these foods have high water content and low protein and fat content.

In addition, vegetables and fruits are rich in antioxidants. There are studies showing that antioxidants reduce the AGE content of foods. In particular, it has been shown in recent studies that red grape peel extract (RGSE)", effectively inhibits the formation of CML because of the antioxidants it contains. For sweeteners and desserts, the results are variable. The numbers of AGEs named GO and MGO in high-fructose corn sirup commonly used in the food industry are 50.8 and 88 µg/100 g, respectively. At the same time, as another example, fruit pulps, which are an important traditional dish in Turkey, are obtained by drying the fruit puree of different fruits such as apricots, plums, and mulberries. Unfortunately, these products have a high MGO content. It is thought that the high amount of MGO in nuts containing pesticides is due to their fat and protein content (Lo et al., 2008; Jariyapamornkoon et al., 2013; Yusufoglu et al., 2020; Nowotny et al., 2018).

When considering dairy products, the situation varies. Processing methods (pasteurization, sterilization, UHT process, etc.) during the course, changes in the levels of AGEs such as CML (Ne-(carboxymethyl) lysine) and pyrroline in different dairy products were observed. For example, as dairy products go through processing processes, the amount of AGEs decreases in some cases, whereas increases are observed in others. Higher AGE levels were found in condensed products such as powdered milk and protein-rich items based on the observed results. Changes in the number of AGEs because of nutrient processing will be examined in more detail in the second part of this study (Dong et al., 2023).

4.2 Processing of foods

Besides the contents of the nutrients, the processes to which they are exposed and their packaging are also effective in changing the numbers of AGEs they contain. It is known that the number of AGEs in foods increases because of heat treatments, especially at high temperatures. The reason for this condition can be explained as an increase in the speed of the Maillard reaction, which is a stage of glycation, because of an increase in temperature. Based on this information, it can be said that the amount of AGEs in foods such as sweets, fast food, and baked goods processed at high temperatures is higher than normal. Another factor that affects the Maillard reaction is high humidity. As the humidity level increases, a decrease in the reaction rate is observed because the dilution of the reactants in the water phase is achieved. As a result, while cooking with dry heat promotes the formation of AGEs, they can be reduced by baking in an oven at high humidity. For example, an egg cooked at medium heat and in a short time contains half the AGEs compared with eggs cooked by the same method but at a high temperature. Therefore, reducing the formation of AGEs, especially during cooking, grilling, and frying, methods such as deep frying and roasting should be avoided. Instead of these methods, methods such as boiling and lean cooking should be returned. In addition to these methods, shorter cooking times and lower cooking temperatures also reduce the formation of AGEs (Sharma et al., 2015; Burak et al., 2022).

In addition, substances such as spices are also known to be effective in the formation of AGEs in foods. According to previous studies, adding fructose or lactose to meat during sterilization significantly increases CML and CEL levels in heated meat samples. These results show that sugars can react with free amino acids, which facilitates the formation of AGEs. In another study, fat, nitrite, and erythorbate were

added to minced beef meat, and the formation of CML and CEL in minced meat was examined. As a result, it has been observed that the formation of CML and CEL during cooking increases with the addition of fat and erythorbate but is inhibited by nitrite. This is because the result of fats in foods is an increase in oxidation, and in this case, it increases the formation of AGEs. The addition of salt and fat to food increases oxidation. Nitrite, on the other hand, inhibits oxidation because of its antioxidant property, which can prevent the formation of AGEs (Huang et al., 2023).

Conversely, it is inevitable that pasteurization and sterilization processes performed in dairy products affect the formation of AGE. Pasteurization is a thermal sterilization method used to kill harmful microorganisms in dairy products. A study has shown that the amount of furosine found in dairy products increases because of pasteurization, which further indicates that the heat process promotes the production of AGEs. Thermal processes in dairy products, such as heating and microwave processing, also increase the formation of AGEs. Non-thermal processing methods developed recently to prevent the formation of AGEs [pulsed electric field (PEF), carbon dioxide technology, ultraviolet light, etc.] exist, but the reliability of these technologies is insufficient. There is another system that inhibits the production of AGEs in the processing of dairy products, and this is pressure. In a previous study, it was observed that both pathogenic microorganisms and AGEs decreased because of high hydrostatic pressure (MHHP) processes with pre-incubation at moderate temperature in milk (Wu et al., 2023; Dong et al., 2023).

Yu and his colleagues (2023) noticed in a study that the amount of AGEs in pork meatballs that they kept in the freezer increased day by day (Yu et al., 2023). This indicates that myofibrillar protein oxidation increases with increasing frozen storage times of raw pork and that changes in protein structure occur. These results suggest that stored raw pork may increase CML and CEL levels in meaty balls and that these changes occur during frozen storage periods of raw pork. Huang and his friends examined the formation of CML in chicken and storage and observed that the amount of CML increased during storage in raw and boiled chicken. Considering these studies, an increase in AGE formation can be observed due to conditions such as protein and lipid oxidation, enzymatic reactions, and free amino acid formation in the storage process in general. Therefore, it is important for human health to act by following the rules required for storing food groups that tend to deteriorate quickly, such as meat and milk (Huang et al., 2022; Yu et al., 2023).

5. Conclusion

In this review, while focusing on the formation mechanisms of advanced glycation, the cellular damages it causes, and its impact on diseases, we also investigated how nutrition and food processing technologies affect the occurrence of this reaction. In particular, we delved into the detailed examination of AGEs and their influence on the formation of various cancer types, an aspect with limited prior research. The findings obtained supported the potential harmful effects of AGEs on human and societal health. Simultaneously, these findings provide evidence that nutrition and food technologies contribute to exacerbating this risk. In conclusion, starting with its broad outlines, we thoroughly evaluated AGEs and their products, aiming to elucidate the reasons for the formation and consequences of these reactions for the reader.

6. Conflict of Interest

The authors do not declare any conflicts of interest.

7.References

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Fındığın dönmeli akışlı akışkan yataklı kızılötesi ışınımlı kurutma davranışının deneysel incelenmesi ve matematiksel modellenmesi

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Özet: Bu çalışmada, kabuklu fındıkların dönmeli akışlı akışkan yataklı kızılötesi ışınımlı kurutma davranışı deneysel olarak araştırılmış ve matematiksel modellemesi gerçekleştirilmiştir. Deneysel çalışmalar, 100 g kabuklu fındık için 250 W, 500 W, 750 W ve 1000 W kızılötesi ışınım güç değerlerinde yapılmış ve zamana bağlı olarak kütle kayıpları ölçülmüştür. Boyutsuz kütle oranı, nem içeriği ve kurutma hızı gibi kurutma karakteristikleri zamana ve kütle kaybına bağlı olarak hesaplanmıştır. Kabuklu fındıkların, dönmeli akışlı akışkan yataklı kızılötesi kurutucudaki kurutma eğri denklemini belirlemek için literatürde sunulan 24 adet ince tabaka kurutma denklemi dikkate alınmıştır. En iyi modeli belirlemek için 7 farklı model uygunluk parametresi kullanılmıştır. Sonuç olarak, en iyi kurutma modelleri 250 W ve 500 W için sırasıyla Alibaş; Balbay ve Şahin; 750 W ve 1000 W için ise Geliştirilmiş Midilli-Kucuk olarak tespit edilmiştir. Ayrıca, kurutma zamanı dikkate alınarak, kabuklu fındığın kurutulması için dönmeli akışlı akışkan yataklı kızılötesi ışınımlı kurutma yönteminde ideal kızılötesi ışınım güç değerlerinin 1000 W olduğu tespit edilmiştir.

Anahtar Kelimeler: Kabuklu fındık, kızılötesi ışınımlı kurutma, akışkan yataklı kurutma, dönmeli akışlı kurutma, matematiksel modelleme

Experimental investigation and mathematical modeling of swirling flow fluidized bed infrared drying behavior of hazelnut

Abstract: In this study, swirling flow fluidized bed infrared drying behavior of shelled hazelnuts was experimentally investigated, and mathematical modeling was performed. Drying experiments were carried out at 250 W, 500 W, 750 W and 1000 W infrared power values for 100 g shelled hazelnut, and mass losses were measured depending on drying time. Drying characteristics such as dimensionless mass ratio, moisture content and drying rate were calculated based on time and mass loss. Mathematical modeling was performed to determine the thin layer drying behavior of shelled hazelnuts in swirling flow fluidized bed infrared dryer by using 24 thin layer drying equations in the literature. 7 different evaluation criteria were used to determine the best model. As a result, the best drying models were found to be Alibaş, and Balbay and Şahin for 250 W and 500 W, respectively, and Improved Midilli-Kucuk for 750 W and 1000 W. In addition, considering the drying time, it was found that the most appropriate infrared power value was 1000 W for drying of shelled hazelnut in swirling flow fluidized bed infrared drying method.

Keywords: Shelled hazelnut, infrared drying, fluidized bed drying, swirling flow drying, mathematical modeling

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1.Giriş

Tarımsal ürünlerin bozulmadan uzun süre saklanması, besin, tat ve aroma özelliklerinin korunması kurutmaya olan ihtiyacı zorunlu kılmıştır (Türkan, 2020). Kurutulacak gıdaların raf ömrünün artırtılabilmesi için, denge nemi değerine ulaşılıncaya kadar kurutma işlemi yapılmalıdır (Türkan, 2020). Kurutulacak ürünlerin kimyasal ve biyolojik özelliklerine göre kurutma yöntemi seçilmesi ürün kalitesi, enerji verimliliği ve zaman tasarrufu sağlanması açısından oldukça önemlidir (Kurtuluş, 2007). Kurutma işlemine sanayide gıda, kimya, seramik, deri, kağıt, kereste ve tekstil gibi birçok sektörde ihtiyaç duyulmaktadır. Kurutulacak ürünün yapısına ve özelliklerine göre raflı, tünel, sprey, dondurmalı, akışkan yataklı, kızılötesi ışınımla ve mikrodalga kurutucular yaygın olarak kullanılmaktadır (Kurtuluş, 2007). Yapılan literatür araştırmalarında fındık üzerine bazı kurutma çalışmalarının gerçekleştirildiği görülüştür.

Demirtas ve diğ. (1999), kabuklu fındığın tek tabaka kurutma davranışını deneysel olarak incelemiş ve modelleme için difüzyon tabanlı bir kurutma modeli kullanmışlardır. Fındık difüzivitesi, verilere en iyi uyan teorik eğriler kullanılarak 25-45°C, hava hızı 0,2-0,3 ms^{-1}, havanın %60 bağıl neminde belirlenmiştir ve kurutma havası sıcaklığı ile hızının bir fonksiyonu olarak elde edilmiştir.

Topuz (2002), fındığın akışkan yataktaki kuruma davranışını incelemiş ve matematiksel modellemesini gerçekleştirmiştir. Deneylerde, bir yıl önce hasat edilmiş, güneşte serilerek kurutulmuş fakat depolama sonucu nemlenmiş fındık ile yeni hasat edilmiş taze fındıklar kullanılmıştır. Deneysel çalışma neticesinde, yatak havası sıcaklığı artıkça kuruma hızının arttığı fakat ürünün gıda ve kimyasal bozulmaya uğramaması için sıcaklığın belli bir değeri aşmaması gerektiği ifade edilmiştir.

Aktaş (2007), ısı pompası destekli otomatik kontrollü bir kurutma fırını deney düzeneği imal etmiş ve fındığın kuruma performansını deneysel olarak incelemiştir. Deney düzeneğindeki kurutma havası sıcaklıkları 50 °C, 45 °C ve 40 °C olarak seçilmiştir. Isı pompalı kurutucuda kurutma havası sıcaklığının 50 °C olduğu durumda fındıklar 24 saatte kurutulurken 45 °C'de 27 saatte 40 °C ise 30 saatte kurutulmuştur. Kurutma havası hızları 50°C için 0,25 m/s, 45 °C için 0,32 m/s ve 40 °C için 0,38 m/s olarak belirlenmiştir. Yapılan deneysel çalışmalar sonucunda elde edilen verilere bağlı olarak ısı pompalı kurutucuya ait ısıtma tesir katsayısı COP_{wh} değeri 50 °C kurutma havası sıcaklığı için 1,70; 45 °C için 1,58 ve 40 °C için 1,40 olarak bulunmuştur.

Özdemir ve Devres (1999) kavurma sırasında fındığın ince tabaka kuruma özelliklerini 100, 120, 140 ve 160 ^oC sıcaklık değerleri için incelemişler, matematiksel modellemesini gerçekleştirmişler ve en iyi model olarak Thompson ampirik modelini belirlemişlerdir.

Acar ve diğ. (2020) tarafından güneş enerjisi destekli bir fındık kurutma sistemi tasarlanmış, kurutma performansı incelenmiş ve matematiksel modelleme çalışması gerçekleştirilmiştir. İç sıcaklığı 40 ^oC'de sabit tutmak için proses kontrol cihazı kullanılmış ve deney normal güneş ışığı koşullarında bir günde 09:00 ile 17:00 saatleri arasında gerçekleştirilmiştir. Bu kurutma süresi boyunca fındıkların 20 kg ağırlığının 17,201 kg'a düştüğü gözlemlenmiş ve Page modeli en iyi kurutma modeli olarak belirlenmiştir.

Kandemir (2019), LED teknolojisinden yararlanarak kurutma sistemi deney düzeneği imal etmiştir. Deneyde üç farklı LED sıcaklığına sahip (3000 K, 4000 K ve 6500 K) LED'ler kullanılmış ve ışınımla ısı transferinin kütle transferine etkileri incelenmiştir. LED'li fındık kurutma sisteminin, güneşte ve etüvde kurutma yöntemlerine göre daha kısa sürede fındığı denge nemine (%6) düşürdüğü belirlenmiştir. Aynı zamanda, fındıkla LED arasındaki mesafenin ve fındık boyutunun artmasıyla kuruma süresinin arttığı tespit edilmiştir.

Kızılötesi ışınımlı kurutma yöntemi kullanılarak pastırma (Batman, 2016), arı poleni (Çiftçi, 2021), Hicaz narı (Öztürk Erdem, 2018), fasulye, bulgur, esmer ve beyaz pirinç (Albayrak ve diğ., 2021), havuç dilimleri (Guo ve diğ., 2020), havuç (Doymaz, 2013), kara dut (Doymaz ve Kipcak, 2019), Mantar (Darvishi ve diğ., 2013), safran (Torki-Harchegani ve diğ., 2017), muz (Pekke ve diğ., 2013), kahverengi pirinç (Ding ve diğ., 2018), çeltik (Zare ve diğ., 2014), kivi (Özdemir ve diğ., 2017), ejder gözü meyvesi (Nathakaranakule ve diğ., 2010), soğan dilimleri (Jain ve Pathare, 2004), kabuklu fındık (Keleş ve Saçılık, 2019), nane yaprakları (Kocabıyık ve Demirtürk, 2008), yeşil fasulye (Doymaz ve diğ., 2015), nane (Demir, 2019), ayva dilimleri (Aktaş ve diğ., 2013), tatlı patates (Onwude ve diğ., 2019) ve çilek (Adak ve diğ., 2017) ürünlerinin kurutma davranışları incelenmiştir.

Akışkan yataklı kurutucuda bazı zirai ürünlerin kurutma davranışı incelenmiş ve bu kapsamda kivi meyvesi (Dağcı, 2014), odun yongası (Selbaş, 1998) patates, yeşil fasulye ve bezelye (Senadeera ve diğ., 2003), Hindistan cevizi (Niamnuy ve Devahastin, 2005), havuç (Zielinska ve Markowski, 2007), pirinç (Jaiboon ve diğ., 2009), karabiber (Promvonge ve diğ., 2011), elma (Kaleta ve diğ., 2013) ve sago çekirdeği atıkları (Rosli ve diğ., 2020) çalışılmıştır.

Dönmeli akışlı akışkan yataklı kurutma yöntemi kullanılarak literatürde buğday taneleri (Özbey ve Söylemez, 2005), yaban mersini (Gaewsondee ve Duangkhamchan, 2019), kakao çekirdekleri (Zulkarnain ve diğ., 2019), acı biber (Basrawi ve diğ., 2019), biber (Chuwattanakul ve Eiamsa-ard, 2019) ve çeltik (Sitorus ve diğ., 2021) zirai ürünlerinin kurutma davranışları çalışılmıştır.

Okur ve diğ. (2023) tarafından yeşil çayın dönmeli akışlı akışkan yataklı kızılötesi ışınımlı kurutucuda kurutma davranışı incelenmiş ve matematiksel modellemesi yapılmıştır. Kurutma, 100 W, 250 W, 500 W, 750 W ve 1000 W kızılötesi ışınım güç değerinde gerçekleştirilmiş ve boyutsuz nem oranı, nem içeriği ve kurutma hızı hesaplanmıştır. Ayrıca, kurutulan yeşil çay için su ekstraktı, toplam kül, toplam polifenol, kafein ve ham lif gibi kalite parametreleri belirlenmiştir. Sonuç olarak, proses için en uygun kızılötesi gücün 500 W ve en iyi ince tabaka kurutma modelinin Aghbashlo ve diğ. (Aghbashlo ve diğ., 2009) modeli olduğu belirlenmiş ve en yüksek su ekstraktı değeri 500 W kızılötesi güç değerinde %44,04 olarak elde edilmiştir.

Türkiye'de üretilen fındık miktarının yıllık 400.000-450.000 ton (Topçuoğlu, 2008; Kılıç, 2022) ile dünyanın ortalama %70-80'nini (Topçuoğlu, 2008) karşılamasına rağmen, bu önemli ürünün kurutulmasında halen çoğunlukla geleneksel yöntemler

kullanılmaktadır. Geleneksel kurutma yönteminde güneş altında doğal kurutma yapılması nedeniyle iklim koşullarına bağımlılığın artırması, sergi yapılacak yer sorunlarının oluşması, homojen kurutma yapılamaması gibi dezavantajlar ortaya çıkabilmektedir (Turan ve İslam, 2016). Bu çalışmanın amacı, dönmeli akış, akışkan yatak ve kızılötesi ışınım etkilerini içeren yeni bir kurutma yöntemini kullanarak kabuklu fındığın kurutma davranışını belirlemek ve bu prosesin matematiksel modelini gerçekleştirmektir.

2. Materyal ve Metot

2.1 Materyal

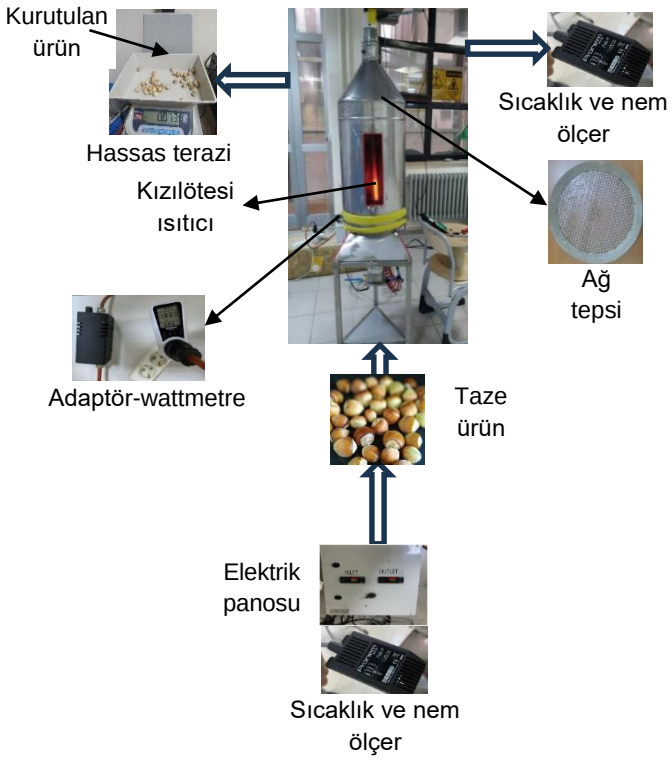
Bu çalışmada kullanılan yuvarlak şekilli fındık grubundan tombul fındık (Betulaceae familyasının Corylus cinsi) Şekil 1'de gösterilmiştir. Fındıklar Trabzon'un Sürmene ilçesinin Yemişli mahallesinden toplanmıştır. Günlük olarak toplanan taze fındıklar yeşil kabuklarından ayrılıp Kett marka 4044 model nem ölçüm cihazıyla kabuklu fındıkların nem oranları %35 olarak belirlenmiş ve 100 g olarak deneylerde kullanılmıştır. Deneyler Recep Tayyip Erdoğan Üniversitesi Makine Mühendisliği Bölümü İDEA-L İnovasyon laboratuvarında yapılmıştır.

Şekil 1. Yeşil kabuklu ve kabuklu fındık.
Figure 1. Green-husked and shelled hazelnuts.

2.2 Metot

Kabuklu fındık kurutmak için kullanılan kızılötesi ışınımlı kurutma sisteminin genel görünümü Şekil 2'de verilmiştir. Kullanılan ürün tepsisi 355 mm çapındadır ve paslanmaz çelikten imal edilmiştir. Tepsinin yüksekliği 30 mm ve tepsinin delik çapı 10 mm'dir. 301L 2B paslanmaz çelikten imal edilen kabinin çapı 400 mm ve yüksekliği 600 mm'dir.

Deneyler 250 W, 500 W, 750 W ve 1000 W kızılötesi güç değerlerinde gerçekleştirilmiştir. Ağırlıkları ölçmek için TEM marka ETEKOTER+LCD/00/00 1 g hassas terazi kullanılmıştır. Sıcaklık ve nem EMKO Pronem Mini PMI-P (-20/80°C (±0,1) ve %0-100 (±%2)) kullanılarak ölçülmüştür. Nem ve sıcaklık EMKO marka ESM-3723 dijital sıcaklık ve nem kontrol cihazı kullanılarak saptanmıştır. Kızılötesi güç seviyeleri bir adaptör kullanılarak ayarlanmış ve kızılötesi gücü okumak için WellHise marka PM-004+LCD wattmetre kullanılmıştır (Şekil 2).



Şekil 2. Dönmeli akışlı akışkan yataklı kızılötesi ışınımlı kurutma sisteminin genel görünümü.
Figure 2. General view of the swirling flow fluidized bed infrared drying system.

2.3 Matematiksel modelleme

İnce tabaka kurutma prosesine uygun olarak gerçekleştirilen deneylerden elde edilen verilerden yararlanarak yapılan matematiksel modelleme, 24 adet ince tabaka kurutma eğrisi denklemi (bkz. Tablo 1) ve 7 adet model uygunluk parametresi (bkz. Tablo 2) (Şimşek ve diğ., 2021; Küçük ve diğ., 2022; Midilli ve Kucuk, 2023) kullanılarak gerçekleştirilmiştir. Modellemede doğrusal olmayan tahmin yöntemi (Statistica) ve model uygunluk parametrelerinin hesaplanmasında Microsoft Excel programı kullanılmıştır.

Tablo 1. İnce tabaka kurutma denklemleri.
Table 1. Thin-layer drying-curve equations.

Model adı	Model denklemi	Denklem No	Kaynak
Newton (Lewis, Üstel)	$MR = exp(-kt)$	(1)	Midilli ve diğ, 2002; McMinn ve diğ, 2005; Kucuk ve diğ., 2014
Page	$MR = exp(-kt^n)$	(2)	Midilli ve Kucuk, 2003; Ghazanfari ve diğ., 2006; Kucuk ve diğ., 2014
Modifiye Edilmiş Page	$MR = exp(-(kt)^n)$	(3)	Vega-Galvez ve diğ., 2008; Kucuk ve diğ., 2014; Şimşek ve diğ., 2021
Modifiye Edilmiş Page-I	$MR = exp((-kt)^n)$	(4)	Mohamed ve diğ., 2008; Kucuk ve diğ., 2014; Midilli ve Kucuk, 2023
Modifiye Edilmiş Page-II	$MR = exp\left(-c\left(\frac{t}{L^2}\right)^n\right)$	(5)	Kumar ve diğ., 2012; Kucuk ve diğ., 2014; Okur ve diğ., 2023
Henderson ve Pabis (Tek Terimli)	$MR = a\,exp(-kt)$	(6)	McMinn ve diğ, 2005; Kucuk ve diğ., 2014; Okur ve diğ., 2023

Tablo 1. İnce tabaka kurutma denklemleri (devamı).
Table 1. Thin-layer drying-curve equations (continue).

Model adı	Model denklemi	Denklem No	Kaynak
Logaritmik (Asimptotik)	$MR = a\,exp(-kt) + c$	(7)	McMinn ve diğ, 2005; Kucuk ve diğ., 2014; Küçük ve diğ., 2022
Midilli-Kucuk	$MR = a\,exp(-kt^n) + bt$	(8)	Midilli ve diğ., 2002; McMinn ve diğ, 2005; Kucuk ve diğ., 2014
Demir ve diğ.	$MR = a\,exp(-kt)^n + b$	(9)	Kaleta ve diğ. 2013; Kucuk ve diğ., 2014; Okur ve diğ., 2023
İki Terimli	$MR = a\,exp(-k_0 t) + b\,exp(-k_1 t)$	(10)	McMinn ve diğ, 2005; Kucuk ve diğ., 2014; Küçük ve diğ., 2022
İki Terimli Üstel	$MR = a\,exp(-kt) + (1-a)\,exp(-kat)$	(11)	Chavan ve diğ., 2008; Kucuk ve diğ., 2014; Şimşek ve diğ., 2021
Verma ve diğ. (Modifiye Edilmiş İki Terimli Üstel)	$MR = a\,exp(-kt) + (1-a)\,exp(-gt)$	(12)	Ganesapillai ve d.ğ., 2008; Kucuk ve diğ., 2014; Şimşek ve diğ., 2021
Difüzyon Yaklaşımı	$MR = a\,exp(-kt) + (1-a)\,exp(-kbt)$	(13)	Kaleta ve diğ. 2013; Kucuk ve diğ., 2014; Küçük ve diğ., 2022
Modifiye Edilmiş Henderson ve Pabis (Üç Terimli Üstel)	$MR = a\,exp(-kt) + b\,exp(-gt) + c\,exp(-ht)$	(14)	McMinn ve diğ, 2005; Kucuk ve diğ., 2014; Okur ve diğ., 2023
Thompson	$t = a\,ln(MR) + b(ln(MR))^2$	(15)	Pardeshi ve diğ., 2009; Kucuk ve diğ., 2014; Midilli ve Kucuk, 2023
Wang ve Singh	$MR = 1 + at + bt^2$	(16)	McMinn ve diğ, 2005; Kucuk ve diğ., 2014; Şimşek ve diğ., 2021
Hii ve diğ.	$MR = a\,exp(-kt^n) + c\,exp(-gt^n)$	(17)	Kumar ve diğ., 2012; Kucuk ve diğ., 2014; Okur ve diğ., 2023
Basitleştirilmiş Fick Difüzyonu	$MR = a\,exp\left(-c\left(\frac{t}{L^2}\right)\right)$	(18)	Kumar ve diğ., 2012; Ruiz ve diğ., 2013; Kucuk ve diğ., 2014
Weibull	$MR = exp\left(-\left(\frac{t}{a}\right)^b\right)$	(19)	Aghbashlo ve diğ., 2009; Kucuk ve diğ., 2014; Midilli ve Kucuk, 2023
Aghbashlo ve diğ.	$MR = exp\left(-\frac{k_1 t}{1 + k_2 t}\right)$	(20)	Aghbashlo ve diğ., 2009; Kucuk ve diğ., 2014; Küçük ve diğ., 2022
Parabolik	$MR = a + bt + ct^2$	(21)	Doymaz, 2012; Kucuk ve diğ., 2014; Okur ve diğ., 2023
Balbay ve Şahin	$MR = (1-a)\,exp(-kt^n) + b$	(22)	Balbay ve Şahin, 2012; Kucuk ve diğ., 2014; Midilli ve Kucuk, 2023
Alibas (Modifiye Edilmiş Midilli-Kucuk)	$M_R = a\,exp(-kt^n) + bt + g$	(23)	Alibas, 2012; Kucuk ve diğ., 2014; Midilli ve Kucuk, 2023
Geliştirilmiş Midilli-Kucuk	$MR = a\,exp(-k_1 t^n) - exp(-k_2 t^n) - bt^n$	(24)	Midilli ve Kucuk, 2023

Tablo 2. Model uygunluk parametreleri.
Table 2. Evaluation criteria.

Model uygunluk parametreleri	Denklem	Denklem No
Korelasyon katsayısı	$r = \frac{N\sum_{i=1}^{N}(MR_{pre,i})(MR_{exp,i}) - (\sum_{i=1}^{N} MR_{pre,i})(\sum_{i=1}^{N} MR_{exp,i})}{\sqrt{\left(N\sum_{i=1}^{N} MR_{pre,i}^2 - (\sum_{i=1}^{N} MR_{pre,i})^2\right)\left(N\sum_{i=1}^{N} MR_{exp,i}^2 - (\sum_{i=1}^{N} MR_{exp,i})^2\right)}}$	(25)
Belirlilik (belirleme) katsayısı	$R^2 = 1 - \frac{SSE}{SST} = 1 - \frac{\sum_{i=1}^{N}(MR_{exp,i} - MR_{pre,i})^2}{\sum_{1}^{n}(MR_{exp,i} - MR_{avg})^2}$	(26)
Düzeltilmiş R^2	$\bar{R}^2 = 1 - (1-R^2)\frac{N-1}{N-k-1}$	(27)
İndirgenmiş ki-kare	$\chi^2 = \frac{\sum_{i=1}^{N}(MR_{exp,i} - MR_{pre,i})^2}{N-n}$	(28)
Ortalama hata kareleri karekökü	$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(MR_{pre,i} - MR_{exp,i})^2}{N}}$	(29)
İndirgenmiş hata kareler toplamı	$RSSE = \frac{\sum_{i=1}^{N}(MR_{exp,i} - MR_{cal,i})^2}{N}$	(30)
Ortalama sapma hatası	$MBE = \frac{\sum_{i=1}^{N}(MR_{pre,i} - MR_{exp,i})}{N}$	(31)

Boyutsuz nem oranı, nem içeriği ve kurutma hızı sırasıyla Denklem (32), Denklem (33) ve Denklem (34)'te verilmiştir. (Midilli ve diğ., 1999; Kucuk ve diğ., 2022).

Boyutsuz nem oranı

$$MR = \frac{M_t - M_e}{M_i - M_e} \quad (32)$$

Nem içeriği

$$MC(\%) = \frac{M_t - M_e}{M_i} \quad (33)$$

Kurutma hızı

$$DR = -\frac{dM}{dt} = -\frac{M_{t+dt} - M_t}{dt} \quad (34)$$

2.3 Deneysel hatalar ve belirsizlik analizi

Deneysel çalışma sırasında ölçüm aparatları, imalat, deneyci ve gözlemden kaynaklanan hatalar doğruluğu etkilemektedir. Bu deneysel çalışma için toplam hata oranı Denklem (35) kullanılarak hesaplanmıştır (Midilli ve diğ., 1999; Dogru ve diğ., 2002; Akpınar, 2005):

$$WR = \pm\left[\left(\frac{\partial R}{\partial x1}W_1\right)^2 + \left(\frac{\partial R}{\partial x2}W_2\right)^2 + \left(\frac{\partial R}{\partial x3}W_3\right)^2 + \cdots + \left(\frac{\partial R}{\partial xn}W_n\right)^2\right]^{1/2} \quad (35)$$

($a1$) Deneyler sırasında kütle, güç ve zaman ölçümlerinden kaynaklanan toplam hata = ± 2,001
($a2$) Kütle, güç ve zaman değerlerinin okunmasından kaynaklanan toplam hata = ± 0,01501

$$W_R = [(a1)^2 + (a2)^2 + ..]^{1/2} = \%\ 2{,}001 \quad (36)$$

3. Bulgular ve Tartışma

Dönmeli akışlı akışkan yataklı kızılötesi ışınımlı kurutucuda 250 W, 500 W, 750 W ve 1000 W olmak üzere 4 farklı kızılötesi güç değerinde kabuklu fındık kurutma deneyleri yapılmıştır.

Deneylerde giriş sıcaklığı 18 ºC ile 24,5 ºC, bağıl nem % 20 ile % 28, çevre sıcaklığı 25 ºC ile 28 ºC ve hava hızı 4 m/s ile 5 m/s arasında değişmektedir. Kütle kaybı ve zamana bağlı olarak boyutsuz nem oranı, nem içeriği ve kurutma hızı hesaplanmıştır.

Deneylerden elde edilen veriler kullanılarak Tablo 1'de sunulan tek ya da ince tabaka kurutma eğrisi denklemleri elde edilmiştir. Oluşturulan modellemeler Tablo 2'te verilen model uygunluk parametrelerine göre değerlendirilmiş ve en iyi 5 model belirlenmiştir. En iyi 5 model ve deneysel verilerden elde edilen boyutsuz nemin (MR) zamana bağlı grafikleri sunulmuş ve en iyi model için kurutma eğrisi denklemi elde edilmiştir (Bkz. Şekil 3) Ayrıca, kurutma hızının ve nem içeriğinin zamana bağlı değişimleri sırasıyla Şekil 4 ve Şekil 5'te gösterilmiştir.
En iyi beş model r, R^2, $\bar{R}^2$, χ^2, RMSE, RSSE ve MBE model uygunluk parametreleri dikkate alınarak seçilmiş ve bu parametrelerin değerleri 250 W, 500 W, 750 W ve 1000 W için Tablo 3'te verilmiştir. En iyi model, 1'e en yakın r, R^2, $\bar{R}^2$ değerleri ile 0'a en yakın χ^2, RMSE, RSSE ve MBE değerleri bulunarak belirlenmiştir.

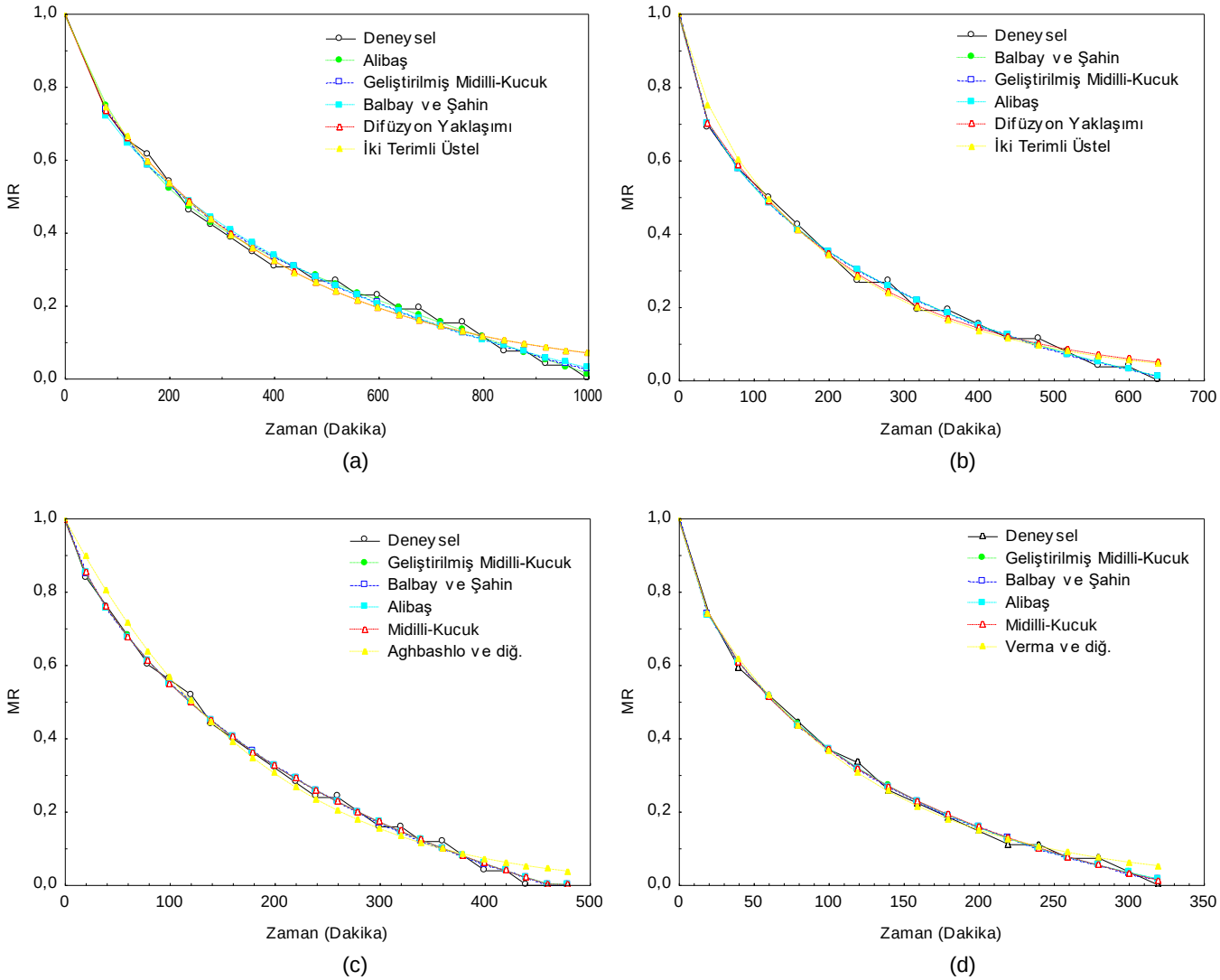
Bu modellerdeki model sabitleri, dönmeli akışlı akışkan yataklı kızılötesi ışınımlı kurutma sisteminin işletme şartlarından etkilenmektedir. Bunlar, hava sıcaklığını artıran kızılötesi güç, akışkan yatak şartını sağlayan hava hızı ve dönmeli akıştır.

Tablo 3. Model uygunluk parametreleri verileri.
Table 3. Data for the evaluation criteria.

Model adı	r	R^2	χ^2	$\bar{R}^2$	RMSE	RSSE	MBE
Kızılötesi Işınım gücü							
250 W							
İki Terimli Üstel	0,99428	0,99531	0,00073	0,99488	0,02590	0,00067	0,00260
Difüzyon Yaklaşımı	0,99436	0,99536	0,00075	0,99470	0,02577	0,00066	0,00236
Balbay ve Şahin	0,99707	0,99764	0,00040	0,99717	0,01838	0,00034	0,00001
Geliştirilmiş Midilli-Kucuk	0,99753	0,99801	0,00036	0,99749	0,01687	0,00028	0,00003
Alibas (Modifiye Edilmiş Midilli-Kucuk)	**0,99833**	**0,99866**	**0,00024**	**0,99830**	**0,01386**	**0,00019**	**0,00008**
500 W							
İki Terimli Üstel	0,99582	0,99555	0,00069	0,99491	0,02474	0,00061	0,00351
Difüzyon Yaklaşımı	0,99742	0,99728	0,00045	0,99665	0,01933	0,00037	0,00287
Alibas (Modifiye Edilmiş Midilli-Kucuk)	0,99864	0,99865	0,00026	0,99803	0,01365	0,00019	0,00002
Geliştirilmiş Midilli-Kucuk	0,99864	0,99864	0,00026	0,99803	0,01365	0,00019	0,00001
Balbay ve Şahin	**0,99864**	**0,99865**	**0,00024**	**0,99819**	**0,01365**	**0,00019**	**0,00000**
750 W							
Aghbashlo ve diğ,	0,99554	0,99545	0,00084	0,99503	0,02778	0,00077	0,00651
Midilli-Kucuk	0,99915	0,99922	0,00016	0,99907	0,01147	0,00013	0,00001
Alibas (Modifiye Edilmiş Midilli-Kucuk)	0,99915	0,99923	0,00016	0,99902	0,01146	0,00013	0,00003
Balbay ve Şahin	0,99917	0,99924	0,00015	0,99909	0,01136	0,00013	0,00000
Geliştirilmiş Midilli-Kucuk	**0,99918**	**0,99925**	**0,00016**	**0,99905**	**0,01126**	**0,00013**	**0,00010**
1000 W							
Verma et al,	0,99789	0,99779	0,00039	0,99728	0,01803	0,00033	0,00307
Midilli-Kucuk	0,99920	0,99922	0,00015	0,99896	0,01074	0,00012	0,00001
Alibas (Modifiye Edilmiş Midilli-Kucuk)	0,99923	0,99925	0,00016	0,99890	0,01054	0,00011	0,00002
Balbay ve Şahin	0,99922	0,99924	0,00015	0,99899	0,01058	0,00011	0,00000
Geliştirilmiş Midilli-Kucuk	**0,99923**	**0,99924**	**0,00016**	**0,99890**	**0,01054**	**0,00011**	**0,00000**

Şekil 3, dönmeli akışlı akışkan yataklı kızılötesi ışınımlı kurutma sisteminde kurutulan kabuklu fındığın boyutsuz nem oranının kurutma süresine bağlı değişimini göstermektedir. En yüksek nem kaybının sırasıyla ilk, orta ve son kurutma periyotlarında meydana geldiği ve nem kaybının son kurutma periyodunda oldukça düşük olduğu görülmüştür. Şekil 3'te görüldüğü gibi kurutma eğrisi genel kurutma eğrisi karakteristiğine uygun olarak üstel değişim göstermiştir. Dönmeli akış, akışkan yatak ve kızılötesi ışınım etkilerinin olduğu bu kurutma sisteminde kuruma süresi ve boyutsuz nem

oranının kızılötesi ışınım güç değerinin artmasıyla azaldığı görülmektedir. Kurutma süresi 250 W, 500 W, 750 W ve 1000 W güç değerleri için sırasıyla 1000 dakika, 640 dakika, 480 dakika ve 320 dakika olarak belirlenmiştir. Bu kurutma süreleri sonunda ürünün son nem değerleri sırasıyla %9, %9, %10 ve %8 olarak elde edilmiştir. Keleş ve Saçılık (2019) kızılötesi ışınımlı ısıtıcı ve hava kurutucu destekli bir sistemde fındığı %6 nem değerine 8-14 saatte düşürmüşlerdir.



Şekil 3. Boyutsuz nem oranının zamanla değişimi (a) 250 W, (b) 500 W, (c) 750 W, (d) 1000 W.
Figure 3. Variation of dimensionless moisture ratio over time a) 250 W, (b) 500 W, (c) 750 W, (d) 1000 W.

En iyi kurutma modelleri 250 W ve 500 W için sırasıyla Alibaş; Balbay ve Şahin; 750 W ve 1000 W için ise Geliştirilmiş Midilli-Kucuk olarak tespit edilmiş ve model denklemleri sırasıyla Denklem (37), Denklem (38), Denklem (39) ve Denklem (40)'da verilmiştir. Okur ve diğ. (2023) yeşil çay yapraklarının dönmeli akışlı akışkan yataklı kızılötesi ışınımlı kuruma isteminde en iyi modelleri 250 W ve 1000 W için Parabolik, 500 W ve 750 W için sırasıyla Aghbashlo ve diğ. ve Wang ve Singh olarak belirlemişlerdir.

$$M_R = 0{,}490748\,exp(-0{,}004908t^{1{,}075147}) - 0{,}000493t + 0{,}505994 \quad (37)$$

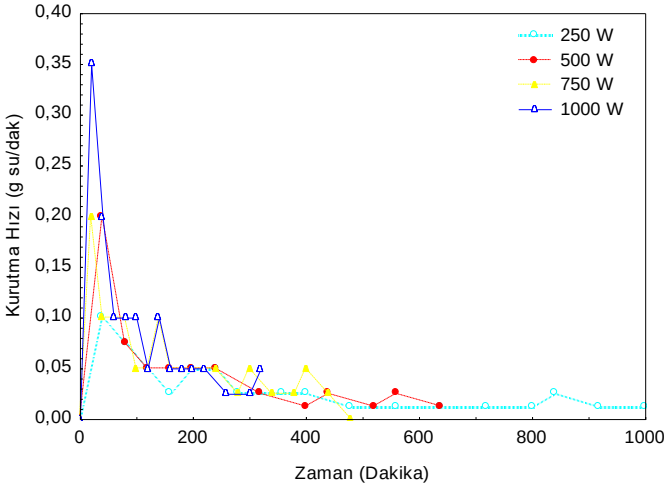
$$MR = (1 + 0{,}359177)\,exp(-0{,}027189t^{0{,}59847}) - 0{,}359983 \quad (38)$$

$$MR = 1{,}99809\,exp(-0{,}042689t^{0{,}636555}) - exp(-0{,}070353t^{0{,}636555}) - 0{,}004197t^{0{,}636555} \quad (39)$$

$$MR = 2{,}000\,exp(0{,}086339t^{0{,}38005}) - exp(-0{,}290611t^{0{,}38005}) - 0{,}473938t^{0{,}38005} \quad (40)$$

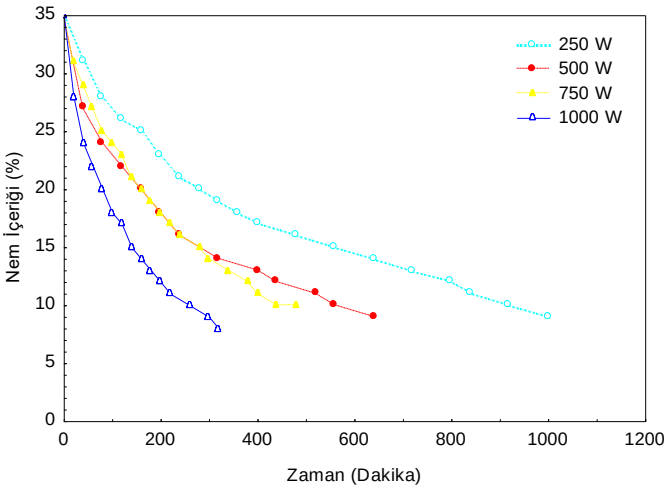
Şekil 4, dönmeli akışlı akışkan yataklı kızılötesi ışınımlı kurutma sisteminde kurutulan kabuklu fındık için 250 W, 500 W, 750 W ve 1000 W güç değerlerinde zamana bağlı kurutma hızı değişimini göstermektedir. Kızılötesi ışınım güç değeri arttıkça kurutma zamanının önemli ölçüde azaldığı ve özellikle ilk kurutma periyodunda kurutma hızının ise önemli ölçüde arttığı görülmektedir. Bu yöntemde, kızılötesi ışınım doğrudan kabuklu fındıkların yüzeyine etki eder ve böylece ısı üretilir ve nemin ürünlerin içinden daha hızlı buharlaşması gerçekleşir.

Giriş havasında herhangi bir ısıtma yoktur ve deneyler sırasında giriş havasının sıcaklığı 18 ºC ile 24,5 ºC arasında değişmektedir. Kurutma havasının ortalama çıkış sıcaklığı 250 W, 500 W, 750 W ve 1000 W için sırasıyla 28,7 ºC, 35 ºC, 41ºC ve 42,6 ºC olarak ölçülmüştür. Enerji, üründeki nemi buharlaştırmak için kızılötesi ışınım kaynağı tarafından kızılötesi ışınım yoluyla doğrudan kabuklu fındıklara iletilir. 250 W, 500 W, 750 W ve 1000 W kızılötesi ışınım güç değerlerinde Denklem (34)'ten hesaplanan kurutma hızının maksimum değerleri sırasıyla 0-40 dakikalık kurutma periyodunda 0,1 g su/dakika, 0-40 dakikalık kurutma periyodunda 0,2 g su/dakika, 0-20 dakikalık kurutma periyodunda 0,2 g su/dakika ve 0-20 dakikalık kurutma periyodunda 0,35 g su/dakika'dır.



Şekil 4. Kurutma hızının zamanla değişimi.
Figure 4. The variation of drying rate over time.

Şekil 5, Denklem (33) kullanılarak hesaplanan kabuklu fındığın nem içeriğinin zamanla değişimini göstermektedir. Şekil 5'te gösterildiği gibi, nem içeriği kurutma zamanının artmasıyla azalmaktadır. Ayrıca, kızılötesi ışınım güç değeri arttığında kabuklu fındıklara kızılötesi ışınım yoluyla transfer edilen enerji miktarının arttığı ve böylece kurutma süresinin önemli ölçüde azaldığı tespit edilmiştir. En hızlı nem kaybı 1000 W kızılötesi ışınım güç değerinde meydana gelirken en yavaş nem kaybı ise 250 W güçte meydana gelmiştir. 500 W ve 750 W güç değerlerinde ise nem içeriğindeki değişimin birbirine yakın olduğu görülmüştür. Benzer değişimler bu güç değerlerinde kurutma hızında da görülmüştür (Bkz. Şekil 4). Kabuklu fındığın başlangıç nem içeriği %35 iken, son nem içeriği 250 W, 500 W, 750 W ve 1000 W kızılötesi ışınım güç değerleri için sırasıyla %9, %9, %10 ve %8 olarak tespit edilmiştir. Olgun ve Rzayev (2000) fındığın açık havada 82 saatte, kabinet tipli kurutucuda ise ek ısıtıcı kullanıldığında 28 saatte, ek ısıtıcı kullanılmadığında 50 saatte, çadır tipli kurutucuda 73 saatte ve ek ısıtıcı kullanılmayan dolap tipli kurutucuda ise 72-76 saatte kuruduğunu belirlemişlerdir.



Şekil 5. Nem içeriğinin zamanla değişimi.
Figure 5. The variation of moisture content over time.

4. Sonuç

Bu çalışma, 250 W, 500 W, 750 W ve 1000 W kızılötesi ışınım güç değerleri için kabuklu fındıkların dönmeli akışlı akışkan yataklı kızılötesi ışınımlı kurutma davranışı deneysel olarak araştırmaya ve matematiksel modellemesini yapmaya odaklanmıştır. Bazı önemli sonuçlar aşağıda verilmiştir:

- ✓ Kabuklu fındığın kurutulması için dönmeli akışlı akışkan yataklı kızılötesi ışınımlı kurutma yönteminde ideal kızılötesi ışınım güç değeri 1000 W'tır.
- ✓ En iyi kurutma modelleri 250 W ve 500 W için sırasıyla Alibaş; Balbay ve Şahin; 750 W ve 1000 W için ise Geliştirilmiş Midilli-Kucuk olarak tespit edilmiştir.
- ✓ Kurutma hızı kızılötesi güç değerinin artmasıyla artar.
- ✓ Kuruma süresi kızılötesi güç değerinin artmasıyla önemli ölçüde azalır.

Dönmeli akışlı akışkan yataklı kızılötesi kurutma teknolojisinin ilk kez fındığın kurutulmasında kullanılmış olması çalışmanın bilimsel özgünlüğünü göstermektedir. Ayrıca, elde edilen verilerin fındık meyvesi ile ilgilenen işletmeler, üreticiler, politika geliştiriciler, araştırmacılar ve sanayiciler için kaynak teşkil edeceği gerçeği çalışmanın endüstriyel ve teknolojik önemine işaret etmektedir.

5. Teşekkür ve Bilgi

Yazarlar bu çalışmaya teknik destek sağladığı için Recep Tayyip Erdoğan Üniversitesi'ne teşekkür eder.

6. Çıkar Çatışması

Yazarlar çıkar çatışması beyan etmemektedir.

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Biyokontrol Yaklaşımı ile Küflerin Kontrolü

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Özet: Dünya nüfusunun artmasıyla birlikte küresel gıda talebini karşılayabilmek amacıyla gıda üretimi de artış göstermektedir. Artan bu gıda talebi; özellikle tarımsal gıda üretimi üzerinde büyük bir baskı oluşturmakta, dolayısıyla tarım alanlarının daha verimli ve etkili biçimde kullanılmasını gerekli kılmaktadır. Tarım alanlarındaki verim kayıplarını azaltmak amacıyla yakın geçmişte üzerinde en çok çalışma yürütülen konulardan birisi olan biyokontrol yaklaşımı sayesinde tarımsal gıda üretiminde gerçekleştirilen kayıpların azaltılması, böylece hem sürdürülebilir hem de gıda güvenliği açısından uygun kabul edilen üretim proseslerinin entegrasyonunun sağlanması hedeflenmektedir. Biyokontrol yaklaşımı, çeşitli mikroorganizmaların bitki patojenlerini kontrol etmek amacıyla, insan, hayvan ve bitki sağlığı üzerinde toksik etkileri görülen kimyasal pestisitler yerine kullanımını içermektedir. Bu çalışma kapsamında zirai ürünlerde küf gelişimini kontrol etmek amacıyla bakteri, küf ve mayaların kullanımı ve etki mekanizmaları incelenmiştir.

Anahtar Kelimeler: Biyokontrol, küf, hasat sonrası, meyve, mikroorganizma.

Control of Molds with Biocontrol Approach

Abstract: With the world's population increasing day by day, food production increases in direct proportion to meet the global food demand. This demand for food generates great pressure on agricultural production, thus making it necessary to expand farm land and use it more efficiently and effectively. In order to reduce yield losses in farming areas, the biocontrol approach, one of the most studied topics in the recent past, aims to reduce losses in agricultural food production, thus ensuring the integration of production processes that are considered both sustainable and appropriate regarding food safety. Biocontrol approach refers to using various organisms to control plant pathogens instead of chemical pesticides that have toxic effects on human, animal and plant health. The substances used in this approach are called biocontrol agents. In this study, the prominent mechanisms of biocontrol agents on plant pathogens will be mentioned, and then the biocontrol agents of bacteria, mold and yeast used against molds will be emphasized.

Keywords: Biocontrol, mold, post harvest, fruit, microorganism.

Derleme

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1.Giriş

Geride bıraktığımız yarım yüzyıl boyunca, dünya nüfusuiki katına çıkmış olmasına rağmen gıda üretimi alanındaki ilerlemeler sayesinde aç nüfus oranında dikkate değer miktarda azalma gözlemlenmiştir (Godfray ve diğ., 2010). Bununla birlikte bugün dünyadaki her yedi insandan birinin yeterli beslenemediği bilinmektedir (Evans, 2009). Birleşmiş Milletler Gıda ve Tarım Örgütü (Food and Agriculture Organization - FAO) verileri incelendiğinde 2021 yılında dünyada açlıktan etkilenen insan sayısının 2019 yılındaki 250 milyon kişiden 828 milyon kişiye yükselmiş olması da bu bilgiyi destekler niteliktedir (FAO ve diğ., 2023). Ayrıca 21. yüzyılın ortasına gelindiğinde dünya nüfusunun 9 milyar kişiye ulaşacağı öngörüldüğünden küresel gıda üretiminin %70-100, tarımsal gıda üretiminin ise %50 oranında artış göstereceği belirtilmiştir (Godfray ve diğ., 2010; Raymaekers ve diğ., 2020).

Bununla birlikte artan gıda üretimi başta sürdürülebilirlik ile ilgili olmak üzere çeşitli sorunlara da yol açmaktadır. Gıda üretiminin artması; Birleşmiş Milletler'in de belirttiği üzere gezegen, hayvan sağlığı ve biyolojik çeşitlilik kavramlarının olumsuz etkilenmesine yol açmakta ve böylece sürdürülebilirlik konusundaki sorunları tetiklemektedir (Garvey, 2022). Bu alanda Birleşmiş Milletler, sürdürülebilir süreçlerin uygulanması amacıyla belirlediği Sürdürülebilir Kalkınma Amaçları (SKA) arasında gıda güvenliği ve iklim koşullarının korunumunu içeren sıfır açlık hedefini (SKA-2) de benimsemiştir. "Sıfır açlık" hedefi sürdürülebilir tarımsal gıda çalışmalarının önemi sebebiyle belirlenen Birleşmiş Milletlerin belirlediği 17 Sürdürülebilir Kalkınma Amacı içinde önemli bir konuma sahiptir (Fenibo ve diğ., 2021). Kesintisiz olarak artan üretim faaliyetleri sırasında gıda üretiminin burada belirtilen hususlara göre gerçekleştirilmesi öngörülmektedir.

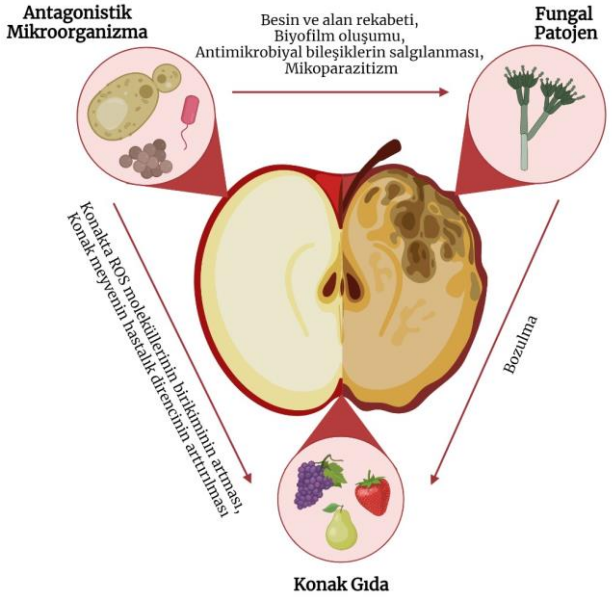
Bu bağlamda tarımsal gıda üretimi büyük önem taşımaktadır çünkü, bitkisel kaynaklar insanlara ihtiyaç duydukları kalori miktarının %90'ını ve benzer şekilde protein miktarının %80'ini sağlayabilen tek doğrudan kaynaktır (Jaiswal ve diğ., 2022). Özellikle tarımsal gıda üretimi alanında; hızla büyüyen dünya nüfusunun daha yüksek miktarda gıda ihtiyacını ve talebini beraberinde getirmesi tarımsal üretim üzerinde ciddi bir baskı oluşturmakta ve dolayısıyla açık üretim alanları ve seralara ayrılan alanların daha efektif ve verimli kullanılması konusunda yapılan yatırımların arttırılması gerekliliğini doğurmaktadır. Ancak günümüz şartlarında bile tarımsal gıda üretimi için dünyadaki kara alanının yaklaşık %34'ü kullanım halindedir (Guzmán-Guzmán ve diğ., 2023; Smetana ve diğ., 2020). Artan gıda talebi ve ek tarım alanlarının sınırlı mevcudiyeti göz önüne alındığında, verimi artırmak ve kayıpları azaltmak önem kazanmaktadır (Raymaekers ve diğ., 2020). Günümüz şartlarında verim kayıplarını azaltmaya odaklanan birçok farklı çalışma yapılmaktadır. Örneğin hastalık ve zararlıların temel ürünlerden beşi (buğday, pirinç, mısır, patates ve soya fasulyesi) üzerinde küresel bağlamda %17-30 oranlarında ürün kaybına neden olması bu çalışmalara ihtiyaç nedeni olarak açıklanabilir (Savary ve diğ., 2019). Daha genel bir kapsamda değerlendirilme yapıldığında ise gerçek ürün kayıplarının tüm üretimin %40'ına kadar ulaşabildiği görülmüştür (Deutsch ve diğ., 2018). Gıda üretimindeki kayıpların tehlikeli düzeylere ulaştığı ve gıda güvenliği alanında da ciddi sorunlara yol açtığı söylenebilir (Hough ve diğ., 2022). Tarımsal ürünlerdeki önemli bozulma nedenlerinden olan fungal kaynaklı bozulmaların etkili bir şekilde kontrol edilmesinin küresel olarak artan gıda talebine önemli ölçüde katkı sağlayacağı açıktır. Bahsedilen gıda kayıplarını önlemek ve bitkilerin sağlığını korumak amacıyla kullanılan uygulamalardan ilk akla gelen kimyasal kaynaklı pestisitlerin kullanımıdır. Kimyasal pestisitler; tarım alanlarında mahsul koruma ve verim artışını sağlama amacıyla oldukça yaygın olarak kullanılmaktadır. Pratik uygulamalara dayalı olarak, pestisitler; insektisitler, herbisitler, fungisitler, bakterisitler, mitisitler, nematisitler, mollusisitler, rodentisitler ve ahşap koruyucular olarak sınıflandırılmaktadırlar (Marican ve Durán-Lara, 2018). Pestisitlerin aşırı kullanımı çevre ve ekosistem üzerinde olumsuz bir etkiye sahip olmasının yanında aynı zamanda insan sağlığı için zararlı sonuçlar ortaya çıkarabilir. Pestisitler sebebiyle çevre kirliliğinin oluşması da tüm dünyada önemli bir endişe kaynağıdır (Shaik ve diğ., 2023). Ayrıca tarımsal gıda üretimi üzerindeki olumlu etkilerine rağmen bu kimyasalların büyük bir çoğunluğu bozunmaya dirençli, çevrede birikmeye yatkın ve doğal besin zincirini bozucu özellikler taşımaktadır. Bu nedenle olması gerekenden sık, uygunsuz ve yüksek miktarlarda kullanımı; biyoçeşitlilikte azalma ve hedef organizmalarda direnç gelişmesine de sebebiyet vermektedir (Souza ve diğ., 2023). Bu süreçte hedef organizmaların mevcut pestisitlere karşı direnç kazanması sonucu uygulanan pestisit miktarının arttırılması ve alternatif etki mekanizmalarına sahip kimyasal pestisitlerin kullanımı ile sorunlar giderilmeye çalışılmıştır. (Bruce ve diğ., 2017). Ancak 2007 yılından beri yapılan daha sıkı yasal düzenlemeler ve kamuoyu baskısıyla pestisit kullanımı sınırlandırılmıştır (Hough ve diğ., 2022). Yasal düzenlemelere örnek olarak Avrupa Parlamento'sunun 91/414/EEC numaralı yönergesi verilebilir. Bu yönerge kapsamında 1993 ve 2011 yılları arasında piyasaya sunulması planlanan 1000 yeni üründen 750 adedinin geri çekilmesi ve yalnızca 180 tanesinin kabul edilmesi, yasal düzenlemelerin getirdiği sınırlamaların ciddiyetini kanıtlar niteliktedir (Chapman, 2014). Bu yönergeye ek olarak, etkili bir diğer uygulama ise yine Avrupa Parlamentosunun yürürlüğe soktuğu 'Entegre Zararlı Yönetimi' (Integrated Pest Management) programıdır. Program, tarımsal ekosistemlerde imkanlar dahilinde mümkün olan en sağlıklı mahsullerin yetiştirilmesini ve doğal yollarla yapılan zararlı kontrolünü teşvik etmektedir (Peshin ve Zhang, 2014). Belirtilen tüm bu hususlar sebebiyle kimyasal pestisitlere alternatif olarak daha sürdürülebilir tarımsal kontrol mekanizmalarının uygulamaya alınması şart haline gelmiştir. Kimyasal pestisit kullanımı, daha yeşil alternatif zirai kontrol yöntemlerinin teşvik edilmesiyle birlikte giderek kısıtlanmaktadır. Sonuç olarak, biyokontrol ajanları gibi alternatif daha güvenli seçeneklerin uygulanmasına acil bir ihtiyaç vardır (Garvey, 2022).

Öne çıkan avantajları nedeniyle pestisitlere alternatif olarak biyokontrolün (biyolojik kontrol) kullanılması umut verici çözümlerden biridir. Biyokontrol kısaca, bitki hastalıklarını önlemek veya zararlıları kontrol etmek için doğal olarak oluşan madde veya var olan mikroorganizmaların kullanılması anlamına gelir (Raymaekers ve diğ., 2020). Biyokontrol uygulamalarında kullanılan madde ve organizmalara biyokontrol ajanları adı verilir. Uluslararası Biyokontrol Üreticileri Derneği (International Biocontrol Manufacturers Association-IBMA) biyokontrol ajanlarını makroorganizmalar, mikroorganizmalar, doğal ürünler ve böceklerin davranışları üzerinde etki sağlayan semiyokimyasallar olarak dört grupta kategorize etmiş, bu gruplardan en önemlisinin mikroorganizmalar olduğu belirtilmiştir (Vedamurthy ve diğ., 2021). Biyokontrol ajanları olarak adlandırılan organizmalar doğada yaygın olarak bulunur ve mikroorganizmalar grubunda; bakteriler, mantarlar, mayalar, virüsler ve protozoalar yer almaktadır. Kimyasal pestisitlere alternatif olarak kullanılan organizmaların kendileri ve buna ek olarak ürettikleri metabolitleri çeşitli canlılar tarafından bitkilerde oluşan hastalıkları önlemede ve bitki sağlığını yönetmede rol sahibidir (Vedamurthy ve diğ., 2021). Ancak çevresel faktörler, uygulama zamanı, mevsim, uygulama tekniği ve sıklığı gibi faktörlere bağlı olarak biyokontrol ajanının etkinliği değişkenlik gösterebilir (Thambugala ve diğ., 2020).

Bu çalışmada öncelikle biyokontrol ajanlarının bitki patojenleri üzerindeki etki mekanizmalarından, ardından günümüzde yaygın olarak biyokontrol ajanı olarak çalışılmış bakteri, küf ve mayalar ve ürün uygulamaları hakkındaki çalışmaların derlenmesi amaçlanmaktadır.

2. Biyokontrol Mekanizmaları

Biyokontrol ajanlarının bitki patojenleri üzerinde kontrol sağlayabilmesi çeşitli mekanizmalar aracılığıyla gerçekleşir. Bu mekanizmalar; doğrudan, dolaylı ve karmaşık yollu olanlar şeklinde gruplandırılmıştır. Doğrudan kontrol mekanizmaları antagonizm, mikoparazitizmi; dolaylı olanlar parazitizm, besin öğeleri ve yer için rekabeti ve konakçı bitkinin bağışıklık sisteminin indüklenmesini; karmaşık yollu olanlar ise uçucu organik bileşiklerin salgılanması, antibiyoz ve litik enzimlerin üretilmesini içerir (Şekil 1) (El-Wakeil ve diğ., 2020).



Şekil 1. Biyokontrol ajanların mekanizması.
Figure 1. Mechanisms of biocontrol agents.

2.1 Mikoparazitizm

Mikoparazitizm, biyokontrol ajanlarının fungal patojeninin hiflerine bağlanarak hücre duvarını parçalayan enzimler salgılayarak fungal patojenlerle beslenmesi olgusunu ifade eder. Özellikle besin yetersizliği durumunda, biyokontrol ajanları patojenik hücrelerden besini absorblar ve bu hücrelerin ölümüne yol açar (Zhang ve diğ., 2020).

2.2 Antibiyoz

Antibiyoz genellikle bir biyokontrol ajanının değişken bir hedef spektrumuna sahip yayılabilir veya uçucu antibiyotik bileşiklerinin üretimi yoluyla başka bir mikroorganizmanın büyümesini engelleme veya öldürme özelliği olarak kabul edilir. En yaygın antibiyotikler doğal kökenlidir ve yıllar içinde yeni moleküller keşfedilmiştir. İnsanlar için potansiyel olarak zararlı olan mikrobiyal türlerde antibiyotik direncinin olası başlangıcıyla ilgili sorunlar nedeniyle antibiyotik üreten ajanların kullanımı hala tartışılmaktadır. Mevcut stratejiler temel olarak, bitkinin toprak üstü kısımlarında ve özellikle yenilebilir kısımlarında kullanılmak üzere antibiyotik üretmeyen ajanların seçilmesini amaçlamaktadır (Palmieri ve diğ., 2022).

2.3 Uçucu Organik Bileşiklerin Salgılanması

Uçucu organik bileşikler, bakteriler, mayalar ve küfler tarafından birincil ve ikincil metabolizmaları sırasında üretilen düşük moleküler ağırlıklı bileşiklerdir. Uçucu organik bileşiklerin üretimi türe özgüdür ve hücreler arasında kimyasal bir iletişim sinyali, karbon salınım mekanizması ve mikrobiyal gelişmenin destekleyicisi veya inhibitörü olarak işlev görürler. Biyokontrol ajanı olarak kullanılan mikroorganizmalar tarafından üretilenler birçok bitki patojenini inhibe etme kapasitesine sahiptir (Contarino ve diğ., 2019).

2.4 Besin Öğeleri ve Yer için Rekabet

Hem bitki patojenleri hem de biyokontrol ajanları çoğalmak için besin ve alana ihtiyaç duyar. Bu nedenle, besin ve alan için rekabet, antagonistik mayaların patojenleri baskıladığı birincil mekanizma olarak kabul edilmiştir. Çoğu biyokontrol ajanı hasarlı meyvenin yüzeyiyle temas ettiğinde, hasarlı dokuyu işgal ederek besin maddelerini hızla tüketmektedir. Bundan sonra, bitki patojenlerini kontrol etmek için diğer etki mekanizmaları iş birliği içinde devreye girmektedir (Zhang ve diğ., 2020).

2.5 Litik Enzimlerin Üretimi

Biyokontrol ajanlarının fitopatojenlere karşı antagonistik en önemli mekanizmalarından biri, fungus hücre duvarının farklı bölgelerine etki ederek hücre lizisine ve ölümüne neden olan glukanazlar, kitinazlar ve proteazlar gibi litik enzimlerin üretilmesidir (Hernandez-Montiel ve diğ., 2021).

2.6 Konakçı Bitkinin Bağışıklık Sisteminin İndüklenmesi

Antagonistik mikroorganizmalar bitkilerde direnci indükleyebilir ve böylece geniş bir bitki patojeni spektrumuna karşı sistemik direnç sağlayabilir. Patojenik olmayan mikroorganizmaların uygulanmasıyla önceden uyarılan bitkilerde biyotik ve abiyotik hastalıklar ve hatta bazı durumlarda böcekler ve nematodların neden olduğu zararlar azaltılabilir. Bitki savunması, patojenik ve patojenik olmayan mikroorganizmalar tarafından mikroorganizmayla ilişkili moleküler yapılar veya bazı doğal veya sentetik kimyasal bileşiklerle indüklenebilir. Patojenik olmayan mikroorganizmalar bitkilerde sistemik direnci indükleyebilir, bu da bitkilerin birden fazla bitki patojenine karşı savunma kapasitelerini artırabilir (El-Wakeil ve diğ., 2020).

3. Antifungal Biyokontrol Ajanları

3.1 Bakteriler

Biyokontrol ajanları içinde en yaygın olarak kullanılanı bakterilerdir. Hedeflerinin çoğu böcekler olmakla birlikte; çeşitli bakteri ve küf türleri gibi birçok patojenin inhibisyonunu da sağlamaktadır. *Bacillus, Lactobacillus, Enterobacter, Erwinia, Microbacterium, Pseudomonas, Rhizobium, Serratia, Stenotrophomonas, Streptomyces ve Xanthomonas, Azospirillum, Flavobacterium, Pantoea* bakterilerinin biyokontrol ajanı olarak kullanıldığı bilinmektedir. Bu bakteriler; bitki patojenlerinin gelişimini sınırlandırmak amacıyla birden fazla mekanizmayı uygulayarak çoklu etki sağlayabilmektedir. Bu mekanizmalar enfeksiyon bölgelerinin kolonizasyonu, patojenin rekabete dayalı olarak dışlanması, antibiyotikler veya hücre duvarı litik enzimleri gibi oldukça aktif antimikrobiyallerin salgılanmasına dayalı antagonistik aktivite artışı ve bitki direncinin indüklenmesi olarak sıralanabilir (Bonaterra ve diğ., 2022). Tablo 1' de biyokontrol ajanı olarak bakterilerin küf gelişimini kontrolü ve ürün uygulama çalışmaları özetlenmiştir.

3.1.1 *Bacillus* spp.

Bacillus türleri, biyopestisit olarak en çok kullanılan bakteriler arasındadır. Toprak ve bitki yüzeyleri gibi çeşitli habitatlarda yaygın olarak bulunurlar ve olumsuz çevre koşullarına dirençli endospor oluşturma yeteneğine sahiptirler. Çeşitli fungal bitki patojenlerine karşı antagonizma geliştirebilirler. *Bacillus* spp.'nin en dikkat çekici özelliği, antimikrobiyal aktiviteye sahip, yüzey aktif ve bitki savunma yanıtlarının

indüksiyonunda yer alan metabolitler dahil olmak üzere tarımsal uygulamalar için değerli çeşitli biyoaktif bileşikler üretme yeteneğidir (Bonaterra ve diğ., 2022).

Bacillus türlerinin biyokontrol uygulamalarındaki en öne çıkan özellikleri bitkilerde fitopatojenlerin gelişimini kitinaz ve beta-1,3 glukanaz salgılayarak inhibe etmeleri, bitki gelişimini teşvik etmeleri, toprakta yer alan besleyici öğelerin bitkiler tarafından kullanımına uygunluğunu arttırmaları, toprağın mikrobiyal çeşitliliğini olumlu yönde değiştirmeleri, biyotik ve abiyotik strese karşı bitkilere direnç kazandırmaları ve hasat sonrası kaliteyi arttırmalarıdır. Biyokontrol uygulamalarında en yaygın şekilde kullanılan *Bacillus* türlerine örnek olarak *Bacillus subtilis, Bacillus thuringiensis, Bacillus cereus, Bacillus velezensis* ve *Bacillus amyloliquefaciens* sayılabilir (Islam ve diğ., 2016; Wang ve diğ., 2022).

Tablo 1. Mikroorganizmaların antifungal ajan olarak kullanımı.
Table 1. The use of microorganism as antifungal agent.

Antagonistik Mikroorganizma	Uygulama Alanı	Hedef Küf	Referans
	Bakteri		
***Bacillus amyloliquefaciens* BAF1**	Karpuz	*Fusarium incarnatum*	Liu ve diğ., 2023
***Bacillus velezensis* YW17**	Ginseng	*Fusarium oxysporum*	Wei ve diğ., 2023
***Bacillus mojavensis* YLRY0310**	Elma	*Penicillum expansum*	Ding ve diğ., 2023
***Bacillus subtilis* HS93**	Biber	*Phytophthora capsici*	Chen ve diğ., 202028.03.2024 13:44:00
***Bacillus subtilis* CL2**	Fıstık	*Aspergillus flavus*	Ling ve diğ., 2022
***Bacillus velezensis* OEE1**	Armut	*Erwinia amylovora*	Medhioub ve diğ., 2022
***Bacillus velezensis* QSE21**	Elma	*Botrytis cinerea*	Xu ve diğ., 2021
***Pseudomonas* sp. 4L**	Mısır	*Fusarium oxysporum*	Chavéz-Díaz ve diğ., 2022
***Pseudomonas* sp. 11L**	Mısır	*Fusarium sambucinum*	Chavéz-Díaz ve diğ., 2022
***Pseudomonas chlororaphis* ZL3**	Kiraz	*Botrytis cinerea*	Wang ve diğ., 2021
***Pseudomonas poae* JSU-Y1**	Elma	*Penicillum expansum*	Ren ve diğ., 2021
***Pseudomonas fluorescens* VUPF506**	Patates	*Rhizoctonia solani*	Fathi ve diğ., 2021
***Pseudomonas fluorescens* ZX**	Turunçgiller	*Penicillum italicum*	Wang ve diğ., 2021
***Lactobacillus plantarum* 17215**	Tahıl Çeşitleri	*Aspergillus niger*	Quattrini ve diğ., 2018
***Lactobacillus plantarum* 17215**	Tahıl Çeşitleri	*Aspergillus flavus*	Quattrini ve diğ., 2018
Lactobacillus fermentum	Mısır	*Fusarium verticillioides*	Kharazian ve diğ., 2017
Lactobacillus paralimentaris	Mısır	*Penicillum aphani*	Kharazian ve diğ., 2017
***Lactobacillus plantarum* CKXP13**	Turunçgiller	*Penicillum digitatum*	Chen ve diğ., 2021
Lactobacillus mesenteroides T3Y6b	Tahıl Çeşitleri	*Fusarium proliferatum*	Mateo ve diğ., 2023
***Pediococcus pentosaceus* M9MM5b**	Tahıl Çeşitleri	*Fusarium verticillioides*	Mateo ve diğ., 2023
***Streptomyces* sp. M4**	Yaprak Yüzeyleri	*Alternaria brassicicola*	Sharma ve diğ., 2020
***Streptomyces* sp. CX3**	Yaban Mersini	*Botryosphaeria dothidea*	Wang ve diğ., 2022
***Streptomyces* sp. HSL-9B**	Mango	*Collectotrichum gloeosporiodies*	Zhou ve diğ., 2022
***Streptomyces* sp. H4**	Çilek	*Colletotrichum fragariae*	Li ve diğ., 2021
	Küf		
***Trichoderma harzianum* Tha739**	Elma	*Collectotrichum gloeosporioide*	Zhang ve diğ., 2022
***Trichoderma harzianum* 3636**	Fıstık	*Fusarium solani RC386*	Erazo ve diğ., 2021
***Trichoderma koningii* MTCC796**	Pamuk	*Rhizoctonia solani*	Gajera ve diğ., 2016
***Trichoderma viride* NBAII**	Pamuk	*Rhizoctonia solani*	Gajera ve diğ., 2016
***Trichoderma atrovide* T17**	Turunçgiller	*Guignardia citricarpa*	Lima ve diğ., 2016
Trichoderma asperellum	Elma	*Alternaria spp.*	Matas-Baca ve diğ., 2022
	Maya		
***Saccharomyces cerevisiae* ACB-K1**	Turunçgiller	*Colletotrichum acutatum*	Lopes ve diğ., 2015
***Saccharomyces cerevisiae* CCMA 0159**	Kahve Çekirdekleri	*Aspergillus carbonarius*	Neves ve diğ., 2021
***Saccharomyces cerevisiae* GA-8**	Üzüm	*Colletotrichum gloeosporioides*	Liu ve diğ., 2018
***Saccharomyces cerevisiae* ACB-K1**	Turunçgiller	*Colletotrichum acutatum*	Lopes ve diğ., 2015
***Candida oleophila* I-182**	Kivi	*Botrytis cinerea*	Li ve diğ., 2023
***Candida oleophila* I-182**	Kivi	*Penicillium expansum*	Li ve diğ., 2023
***Candida oleophila* P-316**	Armut	*Alternaria alternata*	Nie ve diğ., 2019
***Aureobasidium pullulans* L-1**	Şeftali	*Monilinia laxa*	Di Francesco ve diğ., 2017
***Aureobasidium pullulans* ACBL-77**	Turunçgiller	*Geotrichum citri-aurantii*	Klein ve Kupper, 2018
***Aureobasidium pullulans* S-2**	Domates	*Alternaria* sp.	Shi ve diğ., 2022
***Metschnikowia pulcherrima* MP-30**	Elma	*Botrytis cinerea*	Fernandez-San Millan ve diğ., 2021
Metschnikowia fructicola	Elma	*Alternaria* spp.	Biasi ve diğ., 2021
***Metschnikowia* spp.**	Limon	*Penicillium* spp.	Oztekin ve Karbancioglu-Guler, 2021

3.1.2 *Pseudomonas* spp.

Biyokontrol ajanı olarak kullanılan bir başka bakteri cinsi ise *Pseudomonas*'dır. *Pseudomonas* türlerinin bitki hastalıklarını azaltmadaki verimliliğini ve güçlü bir biyokontrol ajanı olarak kabul edilmesini sağlayan çeşitli özellikleri vardır. Bu özellikler; yüksek ekolojik uyumluluk ve adapte olabilme kapasitesi, bazı bitki patojenlerine karşı güçlü antagonistik etkiler gösterebilme yeteneği ve bitkilerin bağışıklık sistemini indükleyici gücü ile ilgilidir (Belgium ve Höfte, 2021). *Pseudomonas* türlerini iyi bir biyokontrol ajanı yapan nitelikleri; hızlı çoğalma kapasitesi sayesinde ticari kullanım amacıyla laboratuvarda hızlı ve yüksek miktarlarda üretilebilmesi, bitkilerin kökleri ve

üzerlerinde kolayca gelişip çoğalabilmesi, antibiyotikler gibi biyoaktif bileşikleri üretme kapasitesine sahip olması, ortamdaki besinler için hedef organizmalar ile rekabet edebilmesi, çevresel baskılara hızlı ve kolay adapte olabilmesi ve son olarak yerleştiği bölgede hızla çoğalarak varlık gösterebilmesi şeklinde sıralanabilir (Panpatte., 2016; Weller ve diğ., 2002). Biyokontrol ajanı olarak genellikle fungusit olarak kullanılırlar (Bonaterra ve diğ., 2022). Bu cinse mensup olan bakterilerden günümüzde biyokontrol uygulamalarında en yaygın olarak tercih edilenleri; *Pseudomonas protegens, Pseudomonas chlororaphis, Pseudomonas corruguta, Pseudomonas fluorescens, Pseudomonas koreensis, Pseudomonas mandelii, Pseudomonas putida* ve *Pseudomonas aeruginosa*'dır (Belgium ve Höfte, 2021).

3.1.3 Laktik asit bakterileri

Lactococcus lactis, *Lactobacillus rhamnosus* GG, *Lactobacillus plantarum* ECGC, *Lactobacillus casei*, *Lactobacillus fermentum* gibi laktik asit bakterileri ABD Gıda ve İlaç İdaresince GRAS statüsünde tanımlandığından ve aynı zamanda Avrupa Gıda Güvenliği Otoritesi (EFSA) tarafından yayınlanan Nitelikli Güvenlik Karinesi (Qualified Presumption of Safety -QPS) listesinde yer aldıklarından dolayı güçlü biyokontrol ajanı adayı olarak kabul edilmekte ve birçok meyve ve sebze türünde kullanılabilmektedir (Trias ve diğ., 2008). Laktik asit bakterilerinin biyokontrol ajanı olarak kullanılma potansiyeli bir veya daha fazla ikincil metabolit üretimlerinden kaynaklanmaktadır. İçerdiği organik asitlere (laktik, asetik, formik, propiyonik asitler gibi) ek olarak ortamın pH'sını düşürerek de etkileri artar. Diğer etken maddeler arasında yağ asitleri, asetoin, hidrojen peroksit, diasetil, antifungal bileşikler (propionat, fenil-laktat, hidroksifenil-laktat, döngüsel dipeptidler ve 3-hidroksi yağ asitleri), bakterisitler (nisin, reuterin, reuterisiklin, pediosin, laktisin, enterosin ve diğerleri) ve bakterisit benzeri inhibitör maddeler (BLIS) bulunur (Favaro ve diğ., 2015).

3.1.4 *Streptomyces* spp.

Streptomyces spp., bitki patojenlerini inhibe eden biyoaktif bileşikler ürettikleri için en çok araştırılan bakterilerdendir ve çeşitli bakteriyel ve fungal bitki hastalıklarının kontrolünde etkilidir. Bu metabolitlere örnek olarak makrolid benzokuinonlar, aminoglikozidler, polienler ve nükleosidler bulunur. *Streptomyces* suşları ayrıca fungal hücre duvarı parçalanmasında etkili olan ekstraselüler enzimler üretme yetenekleriyle de bilinir (Bonaterra ve diğ., 2022). Günümüzde en çok öne çıkan *Streptomyces* türleri *Streptomyces lydicus, Streptomyces griseoviridus, Streptomyces avermitilis* ve *Streptomyces chattnoogensis* olarak sayılabilir (Devi ve diğ., 2022).

3.2. Küfler

Bitki hastalıklarını kontrol etme amacıyla derinlemesine araştırılan mikroorganizmalar arasında küfler de bulunmaktadır. Küflerin biyokontrol ajanı olarak yaygın kullanıma sahip olmaları, biyokontrol uygulamalarında kullanılan izolatların ortam koşullarında oluşum miktarını arttırmalarını sağlayan metabolik aktiviteleri, hedef organizmaları baskılamadaki etkinlik seviyeleri ve çevresel açıdan güvenli sayılabilmelerinden kaynaklanmaktadır (Baron ve diğ., 2019).

Bitki patojenlerine karşı yüksek gelişme hızı ve kısa jenerasyon süreleri nedeniyle küflerin uygulama potansiyeli önemli derecede artmıştır (Thambugala ve diğ., 2020). Birçok küf türü, bitkileri patojenik fungusların neden olduğu hastalıklardan etkili bir şekilde korumalarına izin veren mekanizmalara sahiptir. Karşılaşılan çoğu durumda patojenlerin kontrolü, küfler ve bitkiler arasında var olan doğrudan iletişimi içerir. Bu durumlarda küfler çeşitli antibiyotikler ve amonyak, siyanür, alkol, ester, keton gibi uçucu bileşenler üreterek, çevredeki mineral kaynakları için rekabet oluşturarak, mikoparazitizm uygulayarak ve bitkilerin kendi sistemik direncini indükleyerek patojenlere karşı antagonizm oluştururlar (Gohel ve diğ., 2022). Ayrıca buna ek olarak konakçının yokluğunda, parazitik modlarını saprotrofizme çevirerek çevrede hayatta kalabilirler ve böylece sürdürülebilirliği sağlarlar (Asad, 2022). Thambugula ve diğ. (2020), günümüzde fungusit olarak en yaygın kullanıma sahip fungal biyokontrol ajanlarına örnek olarak *Trichoderma* spp., *Gliocladium* spp., *Ampelomyces quisqualis, Coniothyrium minitans, Ulocladium oudemansii*'yi göstermektedir.

Trichoderma türleri uzun zamandır bitki hastalıklarına sebebiyet veren küflere karşı kullanılan antagonistik bir biyokontrol ajanıdır. Tropikal ve ılıman iklim koşullarına sahip bölgelerdeki toprakların mikrobiyal popülasyonunun önemli bir kısmını oluşturan, kendi içerisinde çok fazla tür barındıran bir cins olma özelliğine de sahiptir (El-Wakeil ve diğ., 2020). *Trichoderma* cinsine mensup olan küfler, bitki patojenlerine karşı faaliyetleri nedeniyle en çok çalışılan küf cinsidir. Bu cinsin üyeleri hızlı büyüme gösterir ve doğadaki başlıca rolleri birincil ayrıştırıcılardır. Ayrıca *Trichoderma* türleri, antibiyotik ve çeşitli enzimler üretme yetenekleri ve biyokontrol ajanları olarak potansiyelleri nedeniyle çalışmaların hedefi olmuş ve ticari olarak kullanılmıştır. Bu küfler, bitki direncini indükleyerek veya antagonist, mikoparazit veya rakip olarak doğrudan patojene karşı hareket ederek fitopatojenik mantarların büyümesini engelleyebilmektedir (Baron ve diğ., 2019). *Trichoderma* spp. bulunduğu ortamda genellikle *Rhizoctonia, Penicillum, Endothia, Helminthosporium, Botrytis, Fusarium, Pythium* gibi küfleri inhibe eder (Asad, 2022). Yaklaşık olarak 20 farklı *Trichoderma* türü toprak kaynaklı patojenlere karşı biyokontrol ajanı olarak kullanılabilir. *Trichoderma harzianum, Trichoderma koningii, Trichoderma viride, Trichoderma atroviride, Trichoderma pseudokoningii, Trichoderma longibrachiatum, Trichoderma hamatum ve Trichoderma polysporum* biyolojik kontrol ajanı olarak kullanılan en yaygın türlerdir (Gohel ve diğ., 2022).

Trichoderma spp.'ye ek olarak küflere karşı *Gliocladium* spp., *Ampelomyces quisqualis, Coniothyrium minitans, Ulocladium oudemansii* gibi fungusit biyokontrol ajanları da mevcuttur. Yapılan bir çalışmada *Gliocladium* türlerinden *Gliocladium virens*'in ağaç köklerinde çürümeye neden olan *Chaetomium globosum* ve *Chaetomium cupreum* küflerinin biyokontrolü amacıyla kullanılabileceği ifade edilmiştir (Vanshree ve diğ., 2022). Bir başka çalışmada ise *Ampelomyces quisqualis* CPA-9 suşunun kabak bitkisi yapraklarının ve çiçek tomurcuklarının alt yüzeylerinde beyaz renkli un benzeri görünüşlü bir tabaka oluşturan *Podosphaera fuliginea*'ya karşı etkili bir koruma sağlayabildiği kanıtlanmıştır (Carbó ve diğ., 2020).

Ayrıca *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. gibi küflerin de biyokontrol amaçlarıyla kullanımları mümkündür (Thambugala ve diğ., 2020). Bunlarla ilgili yakın geçmişte yürütülen farklı çalışmalar vardır ve bu bağlamda yapılan araştırmaların sayısı gün geçtikçe artmaktadır (Bennett ve diğ., 2023). *Fusarium verticillioides*'in atoksijenik bir suşu olan 302-A6, mısırda yüksek miktarda fumonisin üreticisi *Fusarium verticillioides* suşlarının gelişmesi ve fumonisin üretimine etkisi değerlendirilmiştir. Yapılan çalışmanın sonucunda atoksijenik suşun toksijenik suşların ortamdaki miktarında ve fumonisin birikiminde azalmaya neden olduğu belirlenmiş ve bu suşun biyokontrol potansiyeli kanıtlanmıştır. Benzer olarak aflatoksin üreticisi olmayan *Aspergillus flavus*'un aflatoksin üreticisi diğer suşlara karşı antagonistik etki gösterdiği de rapor edilmiştir (Alaniz Zanon ve diğ., 2016).

3.3. Mayalar

Mayalar, çoğu tek hücreli olan ve tomurcuklanma yoluyla çoğalan bir grup ökaryotik fungustur. Biyokontrol uygulamalarında kullanılan mayalar, diğer isimleriyle antagonistik mayalar, özellikle küfler olmak üzere bitki patojenlerinin, gelişmesini, üremesini veya aktivitesini engelleyebilen veya bunlara müdahale edebilen maya veya maya benzeri yapıya sahip olan fungusları ifade eder (Zhang ve diğ., 2020). Antagonistik bir mayanın sahip olması gereken özellikler; genetik olarak kararlı olma, basit beslenme gereksinimlerine sahip olma ve kolayca çoğaltılabilme, düşük konsantrasyonlarda bile birden fazla farklı bitki patojenine karşı etkili kontrol sağlayabilme, konakçı organizmalara karşı patojenik özellik göstermeme, çevre ve insan sağlığına zararlı olmama olarak sıralanabilir (Hernandez-Montiel ve diğ., 2021). Antagonistik mayalara biyokontrol kapasitesi sağlayan mekanizmalar, genel anlamda biyokontrol ajanlarının sahip olduğu birçok farklı mekanizmayı içermektedir (Freimoser ve diğ., 2019). Bunlar, besin öğeleri ve yer için patojen organizmalarla rekabet etme; bitkilerin bağışıklık sistemlerini çeşitli patojenlere karşı indükleme; kitinaz, glukanaz ve lipaz gibi enzimler salgılayarak hedef patojenleri degradasyona uğratma; çeşitli patojenler üzerinde etkili olan bazı toksinleri üretme; uçucu organik bileşikler üreterek bu sayede patojenlerin aktivitelerini inhibe edebilme ve nadir görülmesine rağmen mikoparazit özellik gösterme olarak sıralanabilir (Freimoser ve diğ., 2019). Antagonistik maya cinslerinden *Candida, Metschnikowia, Aureobasidium, Saccharomyces, Cryptococcus, Picha, Rhodotorula* ve *Kloeckera*'nın birincil olarak küfler ve çeşitli başka patojenlere karşı etkili olduğu rapor edilmiştir. Bu mikroorganizmalardan *Candida oleophila, Candida sake, Metschnikowia fructicola, Aureobasidium pullulans, Saccharomyces cerevisiae, Cryptococcus albidus* günümüzde ticari amaçlarla kullanılmaktadır (Zhang ve diğ., 2020). Antagonistik mayalar genellikle elma, şeftali, çilek, üzüm, mango ve turunçgillerde *Botrytis, Aspergillus, Alternaria, Penicillium, Rhizopus* spp.gibi fungal patojenlere karşı kullanılırlar (Hernandez-Montiel ve diğ., 2021). Biyokontrol ajanı olarak kullanılabilen antagonistik maya çalışmaları Tablo 1 'de özetlenmiştir.

3.3.1. *Saccharomyces cerevisiae*

Saccharomyces cerevisiae; uzun yıllardır gıda sektöründe bira yapımında, hem içilebilir hem de endüstriyel alkol üretiminde, şarap ve ekmek yapımında ve ayrıca tedavi amaçlı uygulamalarda kullanılmaktadır. Mayalar içinde tartışmasız en çok üzerinde çalışma gerçekleştirilen tür olma özelliği taşır. Üzerinde bu kadar fazla miktarda çalışma gerçekleştirilmesinin sebebi olarak ökaryotik hücrelere ilişkin temel araştırmalar için önemli bir model sistem olmasından kaynaklanmaktadır (Shi ve diğ., 2022). Gıda sektöründeki kullanım alanlarına ek olarak *Saccharomyces cerevisiae,* çeşitli küf yapısındaki bitki patojenlerine karşı önemli bir biyokontrol ajanı olduğu da yapılan çalışmalarla belirtilmiştir (Di Canito ve diğ., 2021). Lopes ve diğ., (2015)'nin *Saccharomyces cerevisiae*'nın küflere karşı biyokontrol ajanı olarak kullanılmasını sağlayan mekanizmaları antifungal bileşikler salgılama, ortamdaki besin öğeleri için rekabet etme, glukanaz ve kitinaz gibi litik enzimler salgılayarak ortamdaki küfleri ve toksinlerini inhibe etme kapasitesi olarak sıralanmıştır.

3.3.2. *Candida oleophila*

Antagonistik mayalar arasında *Candida oleophila*'nın elma, kivi, greyfurt, muz, papaya ve armut gibi birçok meyve bünyesinde küflerin aktiviteleri sonucu gerçekleşen çürümeye karşı oldukça etkili bir biyokontrol ajanı olduğu bildirilmiştir (Li ve diğ., 2023; Sui ve diğ., 2020). Bu türün biyokontrol yeteneğini test etme amacıyla yapılan araştırmaların büyük bir çoğunluğu *Botrytis cinerea, Alternaria alternata, Penicillium expansum* ve *Diaporthe* türleri üzerinedir (Gao ve diğ., 2021). Biyokontrol ajanı olarak kullanılan bu mayanın mekanizmasında öne çıkanlar yer ve besin öğeleri için rekabet, süper oksit anyon üretimi, mikoparazitizm, inhibe edici antifungal bileşiklerin salgılanması ve glukanaz gibi litik enzimleri salgılama yeteneği olarak sıralanabilir (Droby ve diğ., 2002; Pu ve diğ., 2014).

3.3.3. *Aureobasidium pullulans*

Aureobasidium pullulans, karasal ve sucul birçok farklı habitatta doğal olarak bulunan ve küf kaynaklı bitki hastalıklarına karşı biyokontrol ajanı olarak kullanılan bir fungustur (Roberti ve diğ., 2019). Diğer biyokontrol ajanlarında da olduğu gibi bu tür de küflerin biyokontrolünde antagonistik mayalarda sıklıkla görülen farklı kontrol mekanizmalarını kullanır. Belirtilen bu tür kapsamında en önemli görülen mekanizmalar, bitkinin bağışıklık sistemini patojenlere karşı indüklelme gücü; yer ve besin öğeleri için ortamda rekabet oluşturma; termostabil antifungal bileşikler salgılama; proteaz, ekzokitinaz, glukanaz ve benzeri litik enzimleri salgılama, uçucu hücre dışı bileşikleri oluşturup ortama yayma, patojenlere karşı öldürücü etki gösteren toksinler oluştma ve aureobasidin antibiyotiğini üretme kapasitesidir (Klein ve Kupper, 2018). *Aureobasidium pullulans'*ın biyokontrol ajanı olarak genellikle turunçgiller, domates, elma, kiraz ve şeftali gibi pek çok meyvede *Botrytis cinerea, Penicillium expansum* ve *Monilinia* cinsine mensup küfleri inhibe etmek için kullanıldığı belirtilmiştir (Di Francesco ve diğ., 2017).

3.3.4. *Metschnikowia* spp.

Metschnikowia cinsi; çoğunlukla küresel olarak dağılmış filosfer ve nektar mayası türlerinden oluşur. Bu türlerin arasından biyokontrol açısından üzerinde en çok çalışma yürütülmüş olanlar *Metschnikowia fructicola* ve *Metschnikowia pulcherrima*'dır. Bu türler patojen küf kaynaklı bitki hastalıklarını hasat sonrası dönemde inhibe etme özelliği gösterir. Günümüzde tanınmış en güçlü antagonistik mayalardan ikisi *Metschnikowia fructicola* ve *Metschnikowia pulcherrima*'dır (Freimoser ve diğ., 2019). Bu cinse mensup olan türlerin fungal patojenlerin inhibisyonunu sağlayan biyokontrol mekanizmaları arasında besin öğeleri ve alan için rekabet, savunma sinyalinde yer alan genlerin aşırı ekspresyonu yoluyla konakçı bitkinin bağışıklık sisteminin indüklenmesi, demir sekestrasyonu için pulcherrimin üretimi, kitinaz ve benzeri litik enzimlerin sentezi ve süper oksit anyonların üretimi en öne çıkanlardır (Zhimo ve diğ., 2021). Bu biyokontrol ajanları genellikle turunçgiller ve elmada *Alternaria, Aspergillus, Comoclatris, Penicillium, Nigrospora* ve *Podosphaera* gibi küflere karşı kullanılır (Biasi ve diğ., 2021).

4. Biyokontrol ajanlarının birlikte uygulamaları

Farklı biyokontrol ajanlarının bitki patojenlerine karşı birlikte kullanımı ve ayrıca biyokontrol ajanlarının çeşitli maddelerle veya proseslerle birlikte uygulanmaları üzerine yürütülen çalışmalar günümüzde önem kazanmaktadır. Antagonist mikroorganizmaların küf kontrolünde etkinliğini arttırmak amacıyla farklı maddelerle veya proseslerle birlikte uygulama çalışmaları yaygın olarak gerçekleştirilmektedir. Cheng ve diğ. (2023) yaptıkları çalışmada, *Meyerozyma guilliermondii* ve UV-C uygulamasının kivide *B. cinerea*'nın kontrolünde, tek başına uygulanmasından daha etkili olduğunu göstermişlerdir. Başka bir çalışmada, kivide *B. cinerea*'nın neden olduğu küflenmenin kontrolünde oligogalakturonidin ve *Candida*

oleophila birlikte uygulanmış, küf gelişimini kontrol etmesinin yanında kivide savunma enzimleri olan peroksidaz ve fenilalanin amonyak-liyazın gen ekspresyonunu ve enzim aktivitesini de indüklemiştir (Gao ve diğ., 2021). Buna ek olarak, *Hanseniaspora uvarum* ve β-aminobutirik asit kombinasyonu, her iki ajanın da tek başına uygulanmasıyla karşılaştırıldığında kivide önemli düzeyde hasat sonrası hastalık kontrolünü sağlamıştır.

Portakalda *Penicillium digitatum*'un neden olduğu yeşil küflenmenin kontrolünde fosfatidilkolin (soya fasulyesi ekstraktı) kullanılmasının *H. uvarum*'un biyokontrol etkinliğini kalite özelliklerinde bir değişime neden olmadan arttırdığı gözlemlenmiş ve portakalda hasat sonrası küflenmenin önlenmesinde kullanılabileceği belirtilmiştir (Li ve diğ., 2016). Üzümde *B. cinerea*'ya karşı *H. uvarum*'un tek başına veya salisilik asit veya sodium bikarbonat ile kombinasyonu çalışılmıştır. Kombine uygulama ile üzüm görünümünü, sertliğini, toplam çözünür katı miktarını ve titre edilebilir asitliğini korurken, bozulma oranını ve ağırlık kaybını önemli ölçüde azaltmıştır (Qin ve diğ., 2015).

Yapılan başka bir çalışmada ise *Pichia cecembensis* ile birlikte UV-C işlemi, kavunda *Fusarium oxysporum* ve *Alternaria alternata* kaynaklı hasat sonrası bozulmanın kontrolü amacıyla uygulanmıştır. Uygun dozajlarda UV-C uygulamasının meyve konakçılarında savunma tepkisini ortaya çıkarabildiği ve biyolojik kontrol ile sinerjistik etkiye katkıda bulunabildiği söylenebilir (Huang ve diğ., 2015). Ou ve diğ., (2016), ananasta hasat sonrası meydana gelen küf kaynaklı bozulmaların kontrolü için ise UV-C uygulamasını *Candida tropicalis* uygulaması ile entegre etmiştir. Biyokontrol etkinliğinin arttırılmasına ek olarak, pektin metilesteraz, poligalakturonaz ve selülozun daha düşük aktivitelerine karşılık gelen UV-C/maya uygulamasına tabi tutulan meyvelerde, meyve sertliği daha iyi korunmuş ve meyve perikarpının hücre duvarı parçalanmasını geciktirdiği ve depolama sırasında toplam çözünür katıların ve indirgeyici şekerlerin yüksek içeriğini koruduğu gözlemlenmiştir. UV-C uygulamasının meyvede oluşan yaralarda maya gelişimini etkilemediği ifade edilmiştir. Domates meyvesinde *C. laurentii* ve UV-C kombinasyonun β-1,3-glukanaz, fenilalanin amonyak-liyaz, peroksidaz ve süperoksit dismutazın aktivitesini arttırdığı belirtilmiştir. Ayrıca UV-C'nin biyokontrol etkinliğini arttırmasındaki mekanizmasının savunma tepkisinin ortaya çıkmasıyla ilişkili olabileceği de vurgulanmıştır. Zhang ve diğ., (2013), mandalinada *P. digitatum* kaynaklı bozulmaların kontrolü için *Hanseniaspora uvarum*, *Meyerozyma guilliermondii* ve *Metschnikowia aff. pulcherrima* P01A016'nın tek başına ve birlikte uygulanması ile etkisini incelemişlerdir. Bu üç mayanın birlikte uygulaması ile birlikte *in vitro* ve *in vivo* çalışmalarda en yüksek biyokontrol etkinliği gözlemlenmiş, sinerjistik etki saptanmıştır.

Bir başka çalışmada ise Canonico ve diğ. (2023), şarap üretimi sırasında biyokontrol ajanı ve aynı zamanda aroma belirginleştirici olarak *Metschnikowia pulcherrima* ilebirlikte *Saccharomyces cerevisiae* suşlarının kullanımı değerlendirilmiştir. u Bu araştırma kapsamında; şarap üretimi sırasında ortamda doğal olarak bulunan ve diğer suşlarla kıyaslandığında az miktarda sülfit sentezleyen *Saccharomyces cerevisiae* DİSVA 708 izolatı ile *Metschnikowia pulcherrima* DİSVA 269 analiz edilmiştir. Sonuç olarak bu iki mayanın birlikte kullanımının başarılı bir biyokontrol aktivitesi gösterdiği ve az miktarda sülfit içeren, istenen aromatik ve duyusal özelliklere sahip ürün üretimine olanak sağladığı belirtilmiştir.

5. Sonuç

Bu çalışmada biyokontrol ajanı olarak kullanılan mikroorganizmaların patojenik küfler üzerinde etki mekanizmaları ve biyokontrol ajanı olarak bakteri, küf ve maya kullanımına yönelik uygulamalar incelenmiştir. Günümüzde biyolojik kontrol uygulamaları tarımsal üretimde yaygın olarak kullanılan kimyasal pestisitlere karşı doğal, etkili ve sürdürülebilir bir alternatif yöntem olarak önem kazanmaktadır.

6. Çıkar Çatışması

Yazarlar herhangi bir çıkar çatışması beyan etmemektedir.

7. Teşekkür

Şekil 1, lisanslı BIORENDER (biorender.com) programı kullanılarak çizilmiştir.

8. Kaynaklar

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Determination of Total Phenolic Compounds and Antioxidant Activity of Turkish Propolis Extracted by Different Methods

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Abstract: Propolis, also known as bee glue, has a wide range of biological activities and physiological properties. Propolis is a product collected by bees during honey making, and due to its resinous structure it cannot be consumed in its raw form and can be made suitable for consumption by separating unwanted substances. Many methods can be used to extract bioactive compounds from raw propolis, but since there may be differences between these methods in terms of extraction efficiency, an effective method should be used. In this study, the total phenolic content (TPC) and antioxidant activity of propolis samples extracted by microwave assisted ethanolic extraction (MAE), ultrasonic assisted ethanolic extraction (UAE), and supercritical fluid (SCF) extraction methods were investigated. In the experiments, TPC was analyzed by Folin-Ciocalteu method and antioxidant activity tests were performed using DPPH radical scavenging and CUPRAC methods. As a result of the analyses, the TPC of the samples was found in the range of 42.83-83.88 mg GAE/g sample, DPPH radical scavenging activity was in the range of 22.97-37.30 mg TE/g sample and antioxidant capacity obtained by CUPRAC method was in the range of 143.83-259.69 mg TE/g sample. In line with the values obtained, even though MAE and UAE were determined to have the highest phenolic content and antioxidant capacity among the propolis extracts, no significant difference was found between them, but as a result of the literature information, it was understood that the content of propolis varies according to climatic conditions and geographical origin and that MAE and UAE can be preferred in order to increase the extraction efficiency, while the procedures of the methods should be given more importance.

Keywords: Propolis, phenolics, extraction, antioxidant activity, ultrasound.

Farklı Yöntemler ile Ekstrakte Edilmiş Propolis Örneklerinin Toplam Fenolik Madde ve Antioksidan Aktivitelerinin Belirlenmesi

Özet: Arı tutkalı olarak da bilinen propolis, çok çeşitli biyolojik aktivitelere ve fizyolojik özelliklere sahiptir. Propolis, reçinemsi yapısı nedeniyle arılar tarafından bal yapımı sırasında toplanan, ham haliyle tüketilemeyen ve istenmeyen maddelerin ayrıştırılmasıyla tüketime uygun hale getirilebilen bir üründür. Ham propolisten biyoaktif bileşiklerin ekstraksiyonu için birçok yöntem kullanılabilir ancak bu yöntemler arasında ekstraksiyon verimi açısından farklılıklar olabileceğinden etkili bir yöntem kullanılmalıdır. Bu çalışmada, mikrodalga destekli etanolik ekstraksiyon (MAE), ultrasonik destekli etanolik ekstraksiyon (UAE) ve süperkritik sıvı (SCF) ekstraksiyon yöntemleri ile ekstrakte edilen propolis örneklerinin toplam fenolik içeriği (TPC) ve antioksidan aktivitesi araştırılmıştır. Deneylerde, Toplam Fenolik madde içeriği Folin-Ciocalteu yöntemi ile analiz edilmiş ve antioksidan aktivite testleri ise DPPH radikal süpürme ve CUPRAC yöntemleri kullanılarak gerçekleştirilmiştir. Analizler sonucunda örneklerin toplam fenolik madde içeriği 42.83-83.88 mg GAE/g örnek aralığında, DPPH radikal süpürme aktivitesi 22.97-37.30 mg TE/g örnek aralığında ve CUPRAC yöntemi ile elde edilen antioksidan kapasitesi 143.83-259.69 mg TE/g örnek aralığında bulunmuştur. Elde edilen değerler doğrultusunda propolis ekstraktları arasında MAE ve UAE'nin en yüksek fenolik madde içeriğine ve antioksidan kapasiteye sahip olduğu belirlense de aralarında anlamlı bir fark bulunamamış ancak literatür bilgileri neticesinde propolis içeriğinin iklim koşullarına ve coğrafi kökene göre değişkenlik gösterdiği ve ekstraksiyon veriminin arttırılması için MAE ve UAE'nin tercih edilebileceği, yöntemlerin prosedürlerine ise daha fazla önem verilmesi gerektiği anlaşılmıştır.

Anahtar Kelimeler: Propolis, fenolik madde, ekstraksiyon, antioksidan aktivite, ultrason.

Article

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1.Introduction

Propolis, also called bee glue, is a resinous and strongly adhesive natural substance collected by honey bees (*Apis mellifera* L.) from leaves, plant nectar and buds of trees and plants, mixed with enzymes secreted by bees and pollen, and used by bees to smooth the inner walls of the hive and protect the hive as well as to keep the temperature inside the hive constant (Kalogeropoulos et al., 2009, Pasupuleti et al., 2017). At the same time, its antiseptic properties prevent microbial infection of larvae, honey combs and combs and its antibiotic properties protect the health of the hive from disease in a bee, despite the large number of bees in a cramped environment (Kuropatnicki et al., 2013.). Propolis, which have been used as a herbal treatment method since ancient times and can be obtained from many different plant sources, are found in various chemical compositions with the effect of geographical features, vegetation and seasonal changes, and more than 300 compounds have been identified in their content (Ozdal et al., 2019; Potkonjak et al., 2012). In general, its structure consists of 50-60% resin and wax, 30-40% beeswax, 5-10% essential oils, 5% pollen grains, microelements and vitamins (Rufatto et al., 2017). Several studies have shown that propolis extracts have antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, anti-tumor, anti-viral and other biological activity properties (Göç et al., 2018; Altuntas et al., 2023). Especially these properties are attributed to the phenolic acids and alcohols, flavanoids, terpenes and sesquiterpenes found in propolis (Machado et al., 2015; Oldoni et al., 2015). Propolis has limited consumption in its raw form due to its resinous structure, needs to be extracted to make it consumable (Keskin et al., 2018). For this reason, the substances in raw propolis should be removed by extraction and the polyphenolic fractions that contribute the most to its therapeutic properties should be preserved (Erdogan et al., 2011). Especially ethanol extracted propolis is produced and used as raw material in antioxidant capsules, throat sprays, cosmetics and toothpastes and as antibacterial, antiviral, antioxidant, anticancer and anti-inflammatory agents (Aliyazıcıoğlu et al., 2013). Propolis can be extracted by various methods to obtain its substances effectively. In this direction, microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE) and supercritical fluid extraction (SCFE) methods can be applied to compare with simple ethanolic extraction (Jang et al., 2009; Haminiuk et al.,2012; Machado et al., 2015). Traditional methods include extraction by simple steam distillation, vacuum distillation, but these methods require high temperature and energy consumption and may result in loss of desired compounds (Tylkowski et al., 2010). Although ethanol extraction is the most widely used method, more and more people are allergic to ethanol, which may limit its use (Biscaia and Ferreira, 2009). However, as Trusheva et al. (2007) indicated that, it is generally accepted to use 70% ethanol as solvent for the extraction of phenolics, as in most commercial products, and microwave-assisted extraction and ultrasonic extraction methods have been developed for faster and more efficient extraction of organic substances. Even though ethanol is the first choice solvent due to its chemical properties in relation to the matrix, ethyl ether, water, methanol and chloroform can also be used for the extraction of certain compounds, while alternative methods such as supercritical fluid extraction can be used in addition to traditional methods because they show the desired properties and can adjust the solvent strength and process selection (Machado et al., 2015).

For solvent selection, reconstituted ethanol was indicated as a better choice for the extraction of phenolic compounds, especially flavanoids, from crude propolis, and it was also proved that ethanol concentration also affected the extraction efficiency, with extracts using 70% ethanol having higher flavanoid and phenolic acid content than those using 96% ethanol (Woźniak, et al., 2019). In addition, in order to increase the extraction efficiency, ethanol-treated samples were extracted and filtered for 15 and 30 minutes under a microwave producing 2450 MHz with a maximum wattage of 800 watts, and for 3 hours with ultrasound as another method (Jang et al., 2009). Furthermore, in microwave-assisted extraction, the propolis solution was microwaved 2 or 3 times for 10 seconds each to reduce the loss due to high temperature, while for ultrasonic-assisted extraction, an ultrasonic bath was used for 10 and 30 minutes to maintain a constant temperature (Trusheva, 2007). In a study, supercritical extraction method was carried out by passing CO_2 at a flow rate of 1 g/minute and 0,5, 10 and 15% ethanol as co-solvent at 150, 200 and 250 bar pressures 3 times for 30 minutes at temperatures of 20, 35 and 50°C, respectively (Paviani et al., 2012). Due to its resinous structure, raw propolis dissolves best in ethanol and is offered to consumers with many products, but the use of propolis extracts obtained with ethanol is limited due to the harmful effects of alcohol consumption and the pungent taste and odor it gives to the final product (Keskin et al., 2019). In addition, pressurized liquid extraction is considered as a rapid analysis method because of its positive impact on the environment due to high input, automation and low solvent consumption, and because pressurized liquids remain liquid at boiling points and allow extraction at high temperatures (Erdogan et al., 2011). The supercritical fluid method, which can be used instead of traditional methods in propolis extraction, is a clean technology and has promising properties such as its capacity to preserve antioxidant properties due to its low temperature (Paviani et al., 2012).

The aim of the present study was to determine the amount of phenolic substances and antioxidant activity of propolis extracts obtained by different methods and to determine the effectiveness of various extraction methods. In this direction, ethanol extraction, microwave assisted extraction, ultrasonic assisted extraction and supercritical CO_2 extraction methods. The antioxidant activities of the obtained propolis extracts were determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method and copper (II) ion reduction based antioxidant capacity method (CUPRAC). In addition, the total phenolic content was determined and all the results were statistically compared and evaluated.

2. Materials and Methods

2.1 Materials

In this experiment, crude propolis sample obtained from local producers from Istanbul, Çatalca region was used in ground form stored in a closed form at -20°C. Ethanol, Folin Ciocalteau reactant, Sodium carbonate (Na_2CO_3), Gallic acid; DPPH (1,1-diphenyl-2-picrylhydrazyl), methanol, Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Copper (II) chloride dihydrate ($CuCl_2.2H_2O$), Neocuproin (2,9-

dimethyl 1,10-phenanthroline) and Ammonium acetate (NH_4Ac) were purchased from Sigma Aldrich company (Germany).

2.2 Methods

2.2.1. Preparation of propolis extracts

Firstly, the propolis sample stored at -20°C and stuck together due to moisture was pulverized again with a grinder. For ethanolic, microwave-assisted and ultrasonic-assisted extractions, 3 grams of each were weighed and extracted with 25 mL ethanol/water (70/30) solution for 24 hours at room temperature under sealed conditions. Then, 3 of the extracts were filtered with prepared filter paper and transferred to a falcon tube for the extraction with stirring; 3 were treated in a microwave oven for 10 seconds 3 times at 30 second intervals and then filtered with filter paper and the remaining 3 extracts were subjected to ultrasonic stirring for 10 min each and then filtered with filter paper. The collected extracts were placed in falcon tubes and kept overnight at -20°C and centrifuged at 10000 rpm for 10 minutes to remove the remaining sediment. The extracts were stored sealed at -20°C until analysis was performed. For supercritical extraction, 10 grams of the ground sample was exposed to a 6 g/min flow of CO_2 at 50°C and 5% ethanol/water (70/30) solution in this flow and extracted at initial pressures of 150, 250 and 350 bar for a total of 2 hours 30 minutes.

2.2.2. Determination of total phenolic compound

Spectrophotometric assay was performed using the Folin-Ciocalteu method according to the Altuntas et al. (2023). Samples obtained by 4 different extraction techniques, each with 3 parallels, were diluted 1:50 with ethanol/water (70/30) solution. Then, 200 µL of diluted sample, 1.5 mL of Folin-Ciocalteu reagent diluted 1:10 with distilled water and 1.2 mL of Na_2CO_3 solution (7.5 g Na_2CO_3/100 mL distilled water) were added to each test tube with an automatic pipette. The mixture in the tubes was quickly homogenized by vortex and incubated in the dark for 45 minutes. At the end of 45 minutes, 300 µL of sample was placed in the wells and the absorbance measured at 765 nm. For the preparation of the calibration graph, gallic acid solutions in the concentration range of 0.08-0.2 mg/mL were substituted for the sample in the same way and the calibration curve was obtained and given in terms of standard gallic acid equivalent (GAE).

2.2.3. Determination of DPPH radical scavenging activity

The antioxidant content in propolis extracts was determined by using DPPH method according to the Apak et al. (2004). Samples obtained by 4 different extraction techniques, each with 3 parallels, were diluted 1:50 with ethanol/water (70/30) solution. Then, 100 µL of diluted sample and 2 mL of DPPH solution (3.943 mg/100 mL methanol) were added to each test tube. The mixture in the tubes was quickly homogenized with a vortex and incubated in the dark for 30 min. At the end of 30 minutes, 300 µL of sample was added to the wells and the absorbance was measured at 517 nm. For the preparation of the calibration graph, Trolox solutions in the concentration range of 0.08-0.2 mg/mL were substituted for the sample in the same way and the calibration curve was obtained and expressed in terms of standard Trolox Equivalent (TE).

2.2.4. Copper (II) ion reduction based antioxidant capacity method (CUPRAC)

The determination of antioxidant substances in propolis extracts was carried out using the CUPRAC method according to the Altuntas et al (2023). Samples obtained by 4 different extraction techniques, each with 3 parallels, were diluted 1:50 with ethanol/water (70/30) solution. Then, 100 µL of diluted sample, 1 mL of $CuCl_2.2H_2O$ solution (0.1748 g/100 mL distilled water), 1 mL Neocuproin (0.156 g/100 mL ethanol) and 1 mL NH_4Ac (7.708 g/100 mL distilled water) were added to each test tube. The mixture in the tubes was quickly homogenized by vortexing and incubated in the dark for 30 minutes. At the end of 30 minutes, 300 µL of sample was placed in the wells and absorbance was measured at 450 nm. For the preparation of the calibration graph, Trolox solutions in the concentration range of 0.08-0.2 mg/mL were substituted for the sample in the same way and the calibration curve was obtained and expressed in terms of standard Trolox Equivalent (TE).

2.2.5. Statistical analysis

Antioxidant activation and phenolic substance results obtained from the analyses were compared using one-way analysis of variance (ANOVA) with Minitab® 18 statistical software program and the accuracy and significance of the results were examined. Tukey's comparison test was used to examine the differences between the results at 95% confidence interval. Regression analysis was also performed using Microsoft Excel 2016 program.

3. Results and Discussion

3.1. Total Phenolic Compound

The amounts of total phenolic compound (TPC) of propolis extracts obtained by various methods were expressed in terms of Gallic acid equivalent (GAE) per gram sample. The TPC of the samples were calculated as the average and standard deviation value and is shown in Table 3.1.

Table 3.1. Results of total phenolic content.
Tablo 3.1. Toplam fenolik fadde içeriği sonuçları.

Extraction method	TPC (mg GAE/g sample)
Ethanolic Extraction	71.64±1.2
MA Extraction	83.88±2.3
UA Extraction	83.62±3.9
SCF Extraction	42.83±10.3

According to the results, TPC of the samples was found to be 71.64, 83.88, 83.62 and 42.83 mg GAE/g sample for ethanolic extraction, microwave assisted extraction, ultrasonic assisted extraction and supercritical CO_2 extraction, respectively. The amount of phenolic substances was found to be close between the methods and relatively low in the sample with supercritical CO_2 extraction. According to ANOVA one-way analysis of variance, there was no significant difference between the propolis extracts in TPC. When the results were analyzed with Tukey test at 95% confidence interval, the differences between the samples were not statistically significant ($p<0.05$). The results of the ANOVA test analysis of variance are given in Table 3.4. The lack of significant differences between the methods can be explained by the fact that no precautions were taken to keep the temperature constant during the experiments and the loss of antioxidant and phenolic substances due to the sensitivity of the

compounds in propolis to heat and therefore the yield of microwave and ultrasonic assisted extractions did not lead to a more significant difference. As observed by Jang et al. (2009), some degradation of some substances may have occurred in this study as a result of exposing the sample to excessive heat, such as the lower concentration of phenolics after 14 days of extraction than after 12 hours, which may have been due to dissolution or degradation of phenolic substances.

Differences between supercritical extraction conditions and other extraction conditions should also be taken into account when comparing the results. According to Silva et al. (2012), the phenolic content of hydro-alcoholic (80% ethanolic) extracts varied from 87.15±4.80 to 277.17±7.5 mg/g from region to region and these values were lower in methanolic and aqueous extraction methods. The values found in this study were between 42.83 and 83.88 mg/g and considering the geographical factors, the phenolic content values of the samples prepared in 70% ethanol were relatively lower. On the other hand, in another study, the total phenolic content of propolis samples extracted with pure ethanol was found to be 10.673 mg/g (Temizer et al., 2017). In line with these data, it can be stated that the results obtained as higher are not completely shaped by error sources but stand out due to the effect of regional differences.

In another study conducted to measure the effectiveness of ultrasonic (UAE) and microwave-assisted extractions (MAE), the total phenolic content varied between 35.9-52.0 mg/g and 24.4-40.4 mg/g, respectively, while the extraction with 70% ethanol was 43.0-44.0 mg/g, moreover, it was observed that the amount of phenolics in the samples exposed to MAE for a longer time was lower (Trusheva et al., 2007). Therefore, it can be said that the data obtained with ultrasonic and microwave-assisted extractions are at an acceptable level. In a study conducted with supercritical extraction, the total phenolic amount varied between 62.21-80.3 mg/g depending on the amount of ethanol passed as a co-solvent and it was determined that the yield of 1% ethanol was higher than 2% ethanol (Machado, 2015). Even though the values found in this study are relatively low, they are acceptable because the amount of phenolics varies depending on the solvent ratio, pressure and temperature.

3.2. DPPH Radical Scavenging Activity

In the determination of antioxidant activity, DPPH radical scavenging method was used and the required calibration graph was created using Trolox standard. Antioxidant activity values of propolis extracts were given in terms of Trolox equivalent (TE). The results of the samples were given as average values and strandard deviation, and antioxidant activities were indicated according to the extraction method as seen in Table 3.2.

Table 3.2. Results of the DPPH radical scavenging activity.
Tablo 3.2. DPPH radikal süpürme aktivitesi sonuçları.

Extraction method	DPPH (mg TE/g sample)
Ethanolic Extraction	37.30±4.2
MA Extraction	35.65±2.3
UA Extraction	35.31±0.4
SCF Extraction	22.97±4.8

The antioxidant activity determination with the concentrations calculated from the absorbances measured by removing DPPH radical was found as 38.30, 35.65, 35.31 and 22.97 mg TE/g sample for ethanolic extraction, microwave assisted extraction, ultrasonic assisted extraction and supercritical extraction, respectively. Among the methods, DPPH radical scavenging antioxidant activity was found at close values and relatively low in the sample subjected to supercritical CO_2 extraction. There was no significant difference in antioxidant capacity between propolis extracts according to ANOVA one-way analysis of variance with $p>0.05$. When the results were analyzed by Tukey test at 95% confidence interval, the differences between the samples were not statistically significant as in the determination of total phenolic matter. Anova test analysis of variance results are given in Table 3.4.

The reasons for the lack of a significant difference between the findings obtained in the determination of antioxidant activity, as in the analysis of total phenolic matter, can be attributed to uncontrollable reasons such as losses due to high temperature, inability to use the extraction methods in the most effective way and other environmental conditions. In a study in which the extraction was carried out with 60% ethanol and homogenized every day in a dark environment for 6 days, DPPH radical scavenging antioxidant activities were found as 135, 151 and 454 mg TE/g in different propolis samples from Turkey (Yesiltas, 2014). The fact that these values were much higher than the values obtained in the study gave an idea about the efficiency of the extraction and it was observed that a longer extraction in a controlled environment could increase the efficiency. As a result of the analysis of ethanolic extracts of propolis, DPPH radical scavenging capacity was found to be 0.33-1.11 mmol TE/g in samples obtained from various regions (Kalogeropoulos et al., 2009). When the values obtained from this study were converted to mmol TE/g, an antioxidant capacity between 0.14-0.17 was determined and it was understood that lower results were obtained.

3.3. Copper (II) Ion Reduction Based Antioxidant Capacity Method (CUPRAC)

The average values of the samples for CUPRAC method based on copper (II) ion reducing activity were taken and the antioxidant activities were shown in Table 3.3 according to the extraction method.

Table 3.3. Results of the CUPRAC tests.
Tablo 3.3. CUPRAC testi sonuçları.

Extraction method	CUPRAC (mg TE/g sample)
Ethanolic Extraction	239.37±16.7
MA Extraction	250.41±7.3
UA Extraction	259.69±3.5
SCF Extraction	143.83±4.9

Antioxidant activity determination with concentrations measured using the CUPRAC method was found to be 239.37, 250.41, 259.69 and 143.83 mg TE/g sample for EE, MAE, UAE and SCFE, respectively. Among the methods, the antioxidant activity determined by CUPRAC method was found to be relatively low in the samples subjected to supercritical fluid extraction. There was no significant difference in antioxidant activity between propolis extracts according to ANOVA one-way analysis of variance with $p>0.05$. When the results were analyzed by Tukey test at 95% confidence interval, the differences between the samples were not statistically significant as in the determination of TPC and DPPH method. The results of the analysis of variance

Anova test are given in Table 3.4.

The CUPRAC method, which generally gives higher results among antioxidant analysis methods, gave a higher result than the other method in this study. In a study conducted with extracts from two different regions, antioxidant activities measured by CUPRAC method were found to be 12-35 mM TE/100 mL (Daraban et al., 2019). In order to compare the studies, the values obtained in this study were converted to mM TE/100 mL and found to be between 38-41 and it was observed that the values were relatively higher. In a study in which TPC was determined between 143-380 mg GAE/g, the antioxidant capacity determined by CUPRAC method was found to be 575-1433 mg TE/g (Yeşiltaş, 2014). In this study, the amount of phenolic substances obtained was found to be 42.83-83.88 mg GAE/g and antioxidant capacity values were found as 143.83-259.37 mg TE/g, although the amount of antioxidant capacity was found to be low, there was no discrepancy between the data.

In our study, although there were quantitative differences in the extraction of phenolic compounds with different polarity ethanol and carbon dioxide, there was no statistically significant difference. The results of Haminiuk et al. (2014), showed that higher contents of phenolic compounds were not obtained either with the most or the least polar solvents where ethanol, methanol and water used (Haminiuk et al., 2014). In line with the previous studies examined, phenolic substances and antioxidant activities were found to be at acceptable values in propolis, although it is understood that they may be at higher amounts, and a linearly increasing relationship was observed between the amount of phenolic substances and antioxidant activity. The antioxidant activity in propolis, which removes unstable free radicals that cause a number of diseases such as aging and immune system disorders in the human body, was determined by removing free radicals added to a certain extent, and the beneficial effect of propolis on health was once again understood.

3.4. Relationship Between Total Phenolic Compounds and Antioxidant Activity Results

In order to examine the relationship between total phenolic compounds and antioxidant activities of the extracts, regression analysis was performed separately for DPPH and CUPRAC methods and the data obtained are summarized in Table 5.4. In addition, the results of the analysis of variance (ANOVA) test are also given in Table 3.4.

The regression analysis results between TPC and DPPH method; TPC and CUPRAC method, respectively, are shown in Table 5.4. The high r^2 values obtained as a result of the comparison of the antioxidant activity methods with the TPC prove the accuracy of the relationship between the experiments.

4. Conclusion

In this study, the total amount of phenolic substances and antioxidant activities of propolis extracts obtained by ethanolic extraction, UAE, MAE and SCFE methods were investigated.

Table 3.4. Relationship between TPC and antioxidant activity results.
Tablo 3.4. Toplam fenolik madde içeriği ile antioksidan aktivite sonuçları arasındaki ilişki.

Extraction Method	TPC (mg GAE/g sample)	DPPH (mg TE/g sample)	CUPRAC (mg TE/g sample)
Ethanolic Extraction	71.64±7.2	37.30±4.2	239.37±16.7
MA Extraction	83.88±2.3	35.65±2.3	250.41±7.3
UA Extraction	83.62±3.9	35.31±0.4	259.69±3.5
SCF Extraction	42.83±0.3	22.97±4.8	143.83±14.9
P value obtained from ANOVA	*0.707*	*0.081*	*0.561*
Regression coefficient (r^2)	-	0.83	0.97

The effectiveness of the methods was compared by determining the content of phenolic substances. In line with the experiments, it was observed that there was no significant difference between the phenolic substance and antioxidant values obtained by extraction in four different methods and their activities were statistically similar. It was revealed that ultrasonic and microwave assisted extractions gave higher values in terms of phenolic matter and antioxidant capacity among the methods. It is understood that various extraction techniques, especially ultrasonic and microwave assisted extraction techniques can be used for the most effective use of these properties.

The efficiency of the four different extraction methods used in this study generally covered the determination of phenolic substances. However, the fact that the yield did not change significantly as a result of ultrasonic and microwave assisted extractions, although it increased, showed that further research should be carried out for these two methods and the conditions affecting the propolis content should be carefully examined and extraction processes should be carried out in accordance with these conditions. In addition, for a more effective and accurate comparison, the amount of solvent, temperature and similar conditions used for samples obtained by supercritical extraction should be applied in the same way for simple and conventional extractions and the differences between them should be indicated.

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6. Conflicts of Interest

The authors declare no conflict of interest.

7. References

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