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Genel Yayın Yönetmeni
Şakir SARIÇAY
info@akademikgida.com
ssaricay@gmail.com

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Research Paper / Araştırma Makalesi

Anthocyanin-Based Natural Food Colorant from Fresh Waste Carnation Flower Petals: Effect of pH, Temperature, and Drying Method on its Degradation Kinetics and its Use in Ice Cream

Ecem Vural[1], Ayhan Topuz[2]

[1]Akdeniz University, Institute of Natural and Applied Sciences, 07058 Antalya, Turkey
[2]Akdeniz University, Faculty of Engineering, Department of Food Engineering, 07058 Antalya, Turkey

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Corresponding author (Yazışmalardan Sorumlu Yazar): atopuz@akdeniz.edu.tr (A. Topuz)
+90 242 310 2447 +90 242 310 6306

ABSTRACT

The carnation flowers (*Dianthus caryophyllus* L.) with broken stems or overgrown buds remain in the greenhouse and are discarded after all cutting operations are completed. Waste flowers are also separated while bouquets are being prepared. Therefore, this study aimed to utilize these fresh waste flowers in the production of natural food colorants. Anthocyanin extract was obtained from waste carnation flowers and turned into powder products via freeze- and spray-drying. Various properties of the powders were analyzed and statistically compared. Since degradation parameters of anthocyanins should be taken into consideration during the planning of the materials to which anthocyanin-based colorant will be added, the kinetic parameters of carnation anthocyanins were calculated at different pH (2.6, 4.0, and 6.0) and temperature (70, 80, and 90°C) values. Except for the liquid extract, the activation energies of all samples sharply decreased when the pH changed from 2.6 to 6.0. The most and least susceptible samples to the temperature elevation were freeze-dried samples at pH 2.6 and spray-dried samples at pH 6.0, respectively. Across all the data, the activation energies of the liquid extract sample at pH 2.6 and pH 4.0 were not significantly different from each other ($p<0.05$). Moreover, the produced colorant was tested in ice cream as a model food. There was no statistically significant difference between the total color differences of ice creams prepared with the commercial colorants and carnation flowers-based colorants.

Keywords: Anthocyanin, Carnation flower, Colorant, Degradation kinetics, Stability

Taze Atık Karanfil Çiçeği Taçyapraklarından Üretilen Antosiyanin Bazlı Doğal Gıda Renklendiricisi: Bozunma Kinetiği Üzerine pH, Sıcaklık ve Kurutma Yönteminin Etkisi ile Dondurmada Kullanılması

ÖZ

Karanfil çiçeği (*Dianthus caryophyllus* L.) üretimi sırasından kısa boylu, kırık saplı veya tomurcuğu fazla açılmış çiçekler serada kalmakta ve tüm kesim işlemleri tamamlandıktan sonra sökülüp atılmaktadır. Kesimi yapılan çiçeklerin buket haline getirilmesi aşamasında da yine atık çiçekler ortaya çıkmaktadır. Bu nedenle mevcut çalışmada bu taze atık çiçeklerin doğal gıda renklendiricisi üretiminde değerlendirilmesi amaçlanmıştır. Atık karanfil çiçeklerinden antosiyanin ekstraktı elde edildikten sonra donuk ve püskürterek kurutma yöntemleriyle toz ürün formuna getirilmiştir. Tozların çeşitli fiziksel özellikleri analiz edilmiş ve istatistiksel olarak karşılaştırılmıştır. Antosiyanin bazlı renklendirici ilave edilecek malzemelerin planlanmasında antosiyaninlerin bozunma parametrelerinin dikkate alınması gerektiğinden karanfil antosiyaninlerinin farklı pH (2.6, 4.0 ve 6.0) ve sıcaklık (70, 80 ve 90°C) değerlerinde kinetik parametreleri hesaplanmıştır. pH 2.6'dan 6.0'a yükseldiğinde, sıvı ekstrakt hariç tüm örneklerin aktivasyon enerjileri

keskin bir şekilde azalmıştır. Sıcaklık artışına en çok ve en az duyarlı numuneler sırasıyla pH 2.6'daki donuk kurutulmuş numuneler ve pH 6.0'daki püskürtülerek kurutulmuş numuneler olmuştur. Tüm veriler incelendiğinde, sadece sıvı ekstrakt örneğinin pH 2.6 ve pH 4.0'daki aktivasyon enerjileri birbirinden önemli ölçüde farklı olmadığı görülmüştür ($p<0.05$). Ayrıca elde edilen renklendiriciler, model gıda olarak dondurmada test edilmiştir. Ticari renklendiricilerle hazırlanan dondurmalar ile karanfil çiçeği bazlı renklendiricilerle hazırlanan dondurmaların toplam renk farklılıkları arasında istatistiksel olarak anlamlı bir fark bulunmamıştır.

Anahtar Kelimeler: Antosiyanin, Karanfil çiçeği, Renklendirici, Bozunma kinetiği, Stabilite

INTRODUCTION

The consumption of processed food is on the rise in many countries. The color of a food item may alter during processing or due to the formulation it underwent. A food item that does not have the expected color can affect the taste perception and purchasing preference. In that case, manufacturers add colorants to attain the market demand, retain quality and gain enough income [1].

Synthetic colorants are cheaper and easier to produce in addition to higher stability during food processing. Although fewer amounts are enough for the desired coloring, safety concerns about synthetic colorants are increasing. The number of legally permitted colorants is decreasing due to their negative biological effects or side effects. Thus, natural colorants are once again becoming the center of attention [2]. These natural colorants can be used in foods, cosmetics, and textiles, especially child clothes. They are safe for such applications where non-toxicity is a must. Recent trends in health and wellness provide new opportunities to use agricultural crops as renewable resources to partially replace synthetic components in food [3].

Pigments, which have different chemical and physical properties, bless natural colors to fruits, vegetables, and flowers. Many are affected by pH, temperature, presence of oxygen and light. While, some are soluble in water, others are soluble in oil or organic solvents [4]. To coup this situation, producers of natural food colorants have focused on three main categories: processing technology, formulation, and alternative pigment sources. Although natural food colorants have a limited color range and stability, they can be produced from herbal, animal, microbial and mineral sources using appropriate technologies [2, 4].

Anthocyanins are the most important group of commercial natural food colorants. They are soluble in water and turn into pink, red, violet, blue, or purple depending on pH of the media. Anthocyanidins are phenolics and constitute the aglycone part of anthocyanins. One or more sugar moieties can be linked to an anthocyanidin with glycosidic bonds. Anthocyanins often exist in the acylated form due to acylation with organic acids. Furthermore, they are highly reactive as it lacks an electron in their flavylium nucleus [5, 6].

Due to high reactivity, anthocyanins easily degrade and turn into unwanted brown-colored or colorless compounds. Stability of anthocyanins depends on their structures and copigmentation. While the number of methoxyl groups in the B ring increases stability, the number of hydroxyl groups decreases it. In acidic pH, four different anthocyanin structures (flavylium cation, quinoidal- base, carbinol pseudo-base and chalcone) are in equilibrium, although their relative amounts may vary. Anthocyanins are generally more stable in acidic media. Intermolecular copigmentation can also increase their stabilities. Aqueous extracts of fruits, vegetables and flowers have a composition that can include various compounds acting as copigments. However, if there are free sugar molecules, they turn into furfurals after the Maillard reaction and tend to speed up the degradation of anthocyanins. The degradation rate is correlated with the type of sugar moiety [6-8].

Worldwide, anthocyanin-based commercial colorants are commonly produced from wine production waste and black carrot. Anthocyanins have been extracted from various plants such as red cabbage, grape, black carrot, radish, jamun, sweet potato and purple corn [6, 9-12]. It is also possible to extract anthocyanin pigment from flowers that are edible and non-toxic.

Edible flowers are a good source of natural food colorants, and they contain high amount of acylated anthocyanins. They may be also consumed owing to their special taste and impressive colors [3, 13]. Cut flower industry is a large industry worldwide that has attractive outcomes in sense of trade and product variations. Carnation (*Dianthus caryophyllus* L.) is a member of the Caryophyllaceae family, whose homeland is the Mediterranean region, and is classified as an edible flower. Compared to the anthocyanins obtained from fruits or vegetables, anthocyanins extracted from carnation are stable at different pH and high temperatures owing to their highly acylated structure. Specifically, anthocyanins acylated with malic acid have been reported only in species within the Dianthus genus, such as *D. barbatus, D. caryophyllus, D. chinensis and D. deltoides* [14]. According to TÜİK (Turkish Statistical Institute) 2021 data, the carnation flower had a share of 35.5% out of total cultivation of ornamental plants in Türkiye. During the production of carnation flowers in the greenhouse, flowers with suitable heights and bud openings are collected. Flowers with short, broken stems or too open buds are discarded. While the cut flowers are used to prepare bouquets, a second batch of waste flowers come out. These fresh wastes are only used as animal feed or fertilizer.

Among the literature regarding the extraction of anthocyanin, limited information is available to obtain the food colorants from petals [13, 15-17]. Furthermore,

no study using carnation flower was found as a source of natural food colorant.

During the production of carnation flowers, the flowers with the appropriate size and bud opening are mostly selected. The flowers with broken stems or overgrown buds remain in the greenhouse and are discarded after all cutting operations are completed. Waste flowers are also separated while bouquets are being prepared. Therefore, this study was aimed to utilize these fresh waste cut flowers in the production of natural food colorants. Since degradation parameters of anthocyanins should be taken into consideration during the planning of the materials to which anthocyanin-based colorant will be added, it was also aimed to calculate the kinetic parameters of carnation anthocyanins at different pH and temperature values Moreover, the produced colorant was tested in ice cream as model food.

MATERIALS and METHODS

Materials

Fresh waste carnation flowers, cultivars of "Osiris", were obtained from a local carnation producer. The chemicals were purchased from Sigma-Aldrich and Merck (Darmstadt, Germany).

Extraction of Anthocyanins

The petals were cut by a sharp knife from the flowers and mixed in the acidified ethanol solution (80%, diluted with 0.1N HCl solution) in 1/20 solid-liquid ratio. The mixture was blended (Ultra-Turrax, IKA T18 Digital, Staufen, Germany) at 13,000 rpm for 5 seconds thrice. Anthocyanins were extracted in a shaking water bath (Digital precise shaking water bath, WSB-30, Daihan Scientific Co. Ltd., Gangwon-Do, South Korea), maintained at 30°C and 178 rpm for 20 minutes [18]. The obtained mixture was filtered through 4-7 µm filter paper using Buchner funnel equipped with a vacuum pump (BFC, BF-S2500 Diaphragm vacuum/pressure pump, Shanghai, China). The filtered extract was concentrated to 10°Bx by using a rotary evaporator (IKA RV-10, Staufen, Germany) at 53 rpm rotation speed, 50°C water bath temperature, and 153 mbar absolute pressure to remove the residual ethanol [19].

Encapsulation of Anthocyanins

Concentrated anthocyanin extract was diluted with distilled water until 5°Bx to obtain the standard extract (E). Subsequently, maltodextrin (DE-12) was added in such an amount that the final concentration was 20°Bx. The mixture was stirred with a magnetic stirrer (Wisestir MSH-20A, Daihan Scientific Co. Ltd., Gangwon-Do, South Korea) for 10 min to completely dissolve maltodextrin and homogenized with a disperser (Ultra-Turrax, IKA T18 Digital, Staufen, Germany) at 10000 rpm for 15 min at ambient temperature [19, 20]. The final mixture was then divided into two parts for encapsulation processes (spray-drying (S) and freeze-drying (F))

Encapsulation by Spray-Drying

Spray-drying was carried out using a lab-scale dryer (Büchi B-290, Essen, Germany) under the following operating conditions: drying air inlet temperature, 180°C; nozzle air flow rate, approximately 500 L/h; and aspiration, 70%. The outlet temperature was held constant at 90°C by adjusting the liquid feed volumetric flow rate (from 100 to 500 mL/h) [20]. The powder was immediately transferred into an amber glass container, closed, and stored at -18°C until analyses.

Encapsulation by Freeze-Drying

Lyophilization was carried out on a lab-scale freeze dryer (Operon, FDU-7003, Gyeonggi-do, South Korea). The final mixture was poured into trays that belong to the freeze-dryer and frozen at -76°C for 2 h in an ultra-freezer (Ultra-low temperature freezer, U410-86, New Brunswick Scientific, Hertfordshire, England). Then, the trays were placed into a freeze-dryer under the following drying conditions: -76°C and 0.38-0.40 mmHg absolute pressure. The sample was left to dry for 48 hours [19]. Dried sample was ground and immediately transferred into an amber glass container, closed, and stored at -18°C until analysis.

Powder Analyses

Powder Yield

Powder yield was gravimetrically calculated as the ratio of the dry matter of powder to the total dry matter of the feed solution.

Water Activity and Moisture Content

The water activity and the moisture content of the powders were measured with a water activity meter (Aqualab 4TE: Decagon Devices, Pullman, WA, USA) and a moisture analyzer (Kern DBS 60-3, Balingen, Germany), respectively.

Color Measurement

The color values of the powdered samples were determined using a colorimeter (UltraScan VIS HunterLab, Reston, VA, USA). Colorimeter was calibrated with black and white calibration plates prior to measurements, respectively. Then, the sample was filled to the vessel of the device. The measurement was carried out at three different points. The color of the samples was recorded as L* (darkness-lightness), a* (greenness-redness), b* (blueness-yellowness). Hue angle and chroma value were calculated from the a* and b* values using Equations 1 and 2 [21]:

$$Hue\ angle = \frac{180}{\pi} * arctan\frac{b^*}{a^*} \quad (1)$$

$$Chroma = \left(\sqrt{a^{*2} + b^{*2}}\right) \quad (2)$$

Total color difference (TCD) was also computed from the L*, a* and b* values using Equation 3:

$$TCD = \sqrt{(L_F^* - L_S^*)^2 + (a_F^* - a_S^*)^2 + (b_F^* - b_S^*)^2} \quad (3)$$

while L_F^*, a_F^* and b_F^* are color values of freeze-dried powder, whereas L_S^*, a_S^* and b_S^* are color values of spray dried powder [22].

Solubility

Solubility was determined with the method used by [23] with slight modifications. For this purpose, 0.5 g of the powder (dry basis) was solved in 50 mL of distilled water by stirring in a beaker for 5 min by using a magnetic stirrer (Wisestir MSH-20A, Daihan Scientific Co. Ltd., Gangwon-Do, South Korea) at 600 rpm. Solution was poured into a tube and centrifuged at 3000×g for 5 min. Then, 10 mL of the supernatant was poured into a pre-weighed Petri dish and dried in an oven at 70°C till the weight became constant. Then the solubility (%) was calculated by difference of weight.

Bulk Density

Bulk density of the powders was measured according to the method performed by [24] with slight modifications. The amount of 0.5 g powder was filled in a 10 mL graduated cylinder. Then the final volume was read from the scale of the cylinder. Bulk density was determined as the ratio of powder mass to the volume.

Turbidity

Turbidity of aqueous solutions prepared with the powders was measured by a turbidimeter (Hach 2100 N, Loveland, CO, USA). Results were described as nephelometric turbidity units (NTU).

Determination of Total Monomeric Anthocyanin Content

Total monomeric anthocyanin (TMA) contents were determined using the pH-differential method [25]. The samples were individually diluted with buffer solutions. The dilution factors were predetermined to be into the linear region of Lambert–Beer law. The absorbances of the solutions were recorded against distilled water at 513 (λ_{max}) and 700 nm. The λ_{max} is the wavelength of the highest absorbance in the spectrum. The net absorbances and TMA contents (as cyanidin-3-glucoside equivalents/L) of the solutions were calculated using Equation 4 and 5:

$$A = (A_{\lambda vis-max} - A_{700\,nm})_{pH1.0} - (A_{\lambda vis-max} - A_{700\,nm})_{pH4.5} \quad (4)$$

$$TMA\ (mg/L) = \frac{A*MW*DF*1000}{\varepsilon*L} \quad (5)$$

where, A=absorbance, MW=449.2 g/mol (molecular weight of cyanidin-3-glucoside), DF=dilution factor, 1000=conversation from gram to milligram, ε=26900 L/mol·cm (molar extinction coefficient for cyanidin-3-glucoside) and L=1 cm (path length).

Thermal Degradation Kinetics of Carnation Anthocyanins

Thermal stability of the extract (E) and the powders (S and F) was determined at different pH (2.6, 4.0, and 6.0) and temperatures (70, 80 and 90°C). Citrate-phosphate buffer was used to maintain the determined pH. Certain amounts of samples (E, S and F), containing approx. 4 mg/L TMA, were dissolved in citrate-phosphate buffer solutions at different pH. Aliquots of 10 mL solution were transferred into test tubes, and they were tightly closed with screw caps. The tubes were incubated in a water bath (Digital precise shaking water bath, WSB-30, Daihan Scientific Co. Ltd., Gangwon-Do, South Korea) operated at selected temperature. Two tubes were sampled per hour throughout the incubation period and instantly cooled in an ice bath. TMA content of the tubes was analyzed. Earlier studies showed that thermal degradation of anthocyanins followed a first-order reaction [2, 26, 27]. Following equations were used to determine the thermal degradation behavior of the anthocyanins at different conditions:

$$\ln(\frac{C_t}{C_0}) = -kt \quad (6)$$

$$t_{\frac{1}{2}} = -\frac{\ln(0.5)}{k} \quad (7)$$

The temperature-dependence of the degradation rate constant was determined by using the Arrhenius equation:

$$k = k_0 * e^{-Ea/RT} \quad (8)$$

The decimal reduction time (D-value), which is the required time for a decuple decrease of the initial concentration at a particular temperature, was calculated by using Equation 9:

$$D = \frac{\ln(10)}{k} \quad (9)$$

The temperature coefficient (Q_{10}) was calculated using Equation 10:

$$Q_{10} = e^{(\frac{Ea}{R})(\frac{10}{T_2*T_1})} \quad (10)$$

where (in Eqs: 6-10) C_0 is the initial value of TMA content, C_t is the TMA content after t minute heating at the determined temperature, t is time (min) $t_{1/2}$ is the half-life (min), k is the first order kinetic rate constant (min^{-1}), k_0 is the frequency factor (min^{-1}), E_a is the activation energy (kJ/mol), D is the decimal reduction time, Q_{10} is the temperature coefficient, R is the universal gas constant (8.314 J/mol·K) and T is the absolute temperature (Kelvin) [9, 28].

Pesticide Residue Analysis

Pesticide residue in the extract was determined according to AOAC 2007.01 method via LC-MS/MS by Akdeniz University Food Safety and Agricultural Research Center [29].

Application in Food Model System

The carnation-based colorant was tested in a model food, ice cream. The ice cream mix was prepared in accordance with the formulation specified in the study by [30], and divided into four equal portions. Citric acid (5%) and certain amounts of colorants (black carrot anthocyanin-based commercial liquid colorant, E, S, and F), containing the same amount of TMA, were added to each one. The colored ice cream mixtures were frozen with a kitchen-type ice cream machine and stored at -20 °C. Color values (L*, a*, b*) of ice creams were measured during storage.

Statistical Analyses

The data were subjected to the analysis of variance using the SAS 9.0 (SAS Institute, Cary, NC, USA) software package, significant differences were revealed by the Duncan's Multiple Range Test and Independent Sample T-Test at a confidence level of 5%.

RESULTS and DISCUSSION

Powder Properties

Results of powder analyses were given in Table 1. All properties of the samples were found significantly (p<0.05) different from each other, except solubility and turbidity. In general, physical properties of the powder sample obtained via freeze-drying technique were superior in quality than those obtained *via* spray-drying technique. There is a similar report where freeze-drying provided efficient results than spray-drying for different type products [20].

Table 1. Physical properties of powdered samples*

Source	Freeze dried powder (F)	Spray dried powder (S)
Powder yield (%)	91.56^{a}±0.22	61.30^{b}±0.18
Moisture (%)	4.34^{b}±0.01	6.33^{a}±0.03
Water activity	0.24^{b}±0.02	0.41^{a}±0.00
Color		
*L**	47.07^{b}±0.04	55.38^{a}±0.00
*a**	37.03^{b}±0.20	46.71^{a}±0.02
*b**	5.66^{b}±0.03	6.28^{a}±0.01
h°	8.69^{a}±0.00	7.65^{b}±0.00
*C**	37.46^{b}±0.20	47.12^{a}±0.02
Solubility (%)	81.01^{a}±0.17	80.39^{a}±0.06
Bulk density (kg/m^3)	500.10^{a}±0.10	244.19^{b}±5.91
Turbidity (NTU)	14.95^{a}±0.25	14.85^{a}±0.15

*: Results are the mean ± standard error; values within a row with different superscript letters are significantly ($p < 0.05$) different *according to* T-test.

Yield of the freeze-dried powder (91.56%) was much higher than the spray dried powder (61.30%). Similar results were also reported by Laokuldilok and Kanha (2015). Major reason of low powder yield for spray-drying is probably the stickiness problem on the drying chamber surface. According to [31] spray drying process can be considered successful if the product yield is >50%.

The moisture content of a powdered food is often affected by its composition, quality, and stability, that could further affect storing, packing, and processing (Tonon et al., 2010). Moisture content is different from a_w, as the moisture content shows the amount of water in a food system whereas a_w expresses the presence of free water that is usable for any biochemical reactions. As water activity increases, the rate of microbial spoilage and degradation of chemical components accelerates, thereby shortening the shelf-life of the product [32]. Moisture contents of the freeze-dried and the spray dried powders in this study were found 4.34% and 6.33%, respectively. Similar range of moisture contents were reported by [23] for freeze- and spray-dried blackberry powders (6.11% and 3.7%, respectively) and by [20] for freeze- and spray-dried black glutinous rice (*Oryza sativa* L.) bran powders (7.63% and 2.47%, respectively). The average water activities of the freeze- and the spray-dried powders in this study were determined as 0.24 and 0.41, respectively. These values can be deemed microbiologically safe and stable when the water activity (a_w) is below 0.6, as it prevents hydrolytic and enzymatic degradation [20].

Color is an important quality parameter, particularly for the kind of products which are produced as colorants. Since color values substantially vary by the raw materials, comparing powders produced from different raw material could be misleading. For this reason, the obtained color results were compared with each other. The color values of the powders revealed that the freeze-dried powder was darker (lower L* value) and had a lighter red color (lower a* value) than those of spray dried powder with the higher L* and a* values. The color of the spray dried powder was close to intense red (0°) with lower hue angle and higher chroma values according to the CIELAB color space diagram. TCD was calculated as 12.77 between the freeze-dried powder and the spray dried powder. According to [22], color differences can be analytically classified as very distinct

(TCD>3), distinct (1.5<TCD<3), and slightly distinct (TCD<1.5). In this case, the color difference between the freeze- and the spray-dried powders was categorized as "very distinct" in this study.

Bulk density is related to the size, shape, and surface properties of particles. Powders with a smooth and uniform surface have higher bulk density than those with a rough and non-uniform surface. A decrease in particle size allows less void space between neighboring particles, hereby, the bulk density increases as there are more particles in a certain volume. The aim is to obtain high bulk density powder to reduce shipping and packaging costs. Furthermore, bulk density also affects flowability and solubility of powders [33]. Bulk density of the freeze-dried powder (500.10 kg/m^3) was two times higher than that of the spray-dried powder (244.19 kg/m^3). These results concurred with several reports like [32] for spray dried black mulberry juice powder (350-550 kg/m^3); by [24] for spray-dried açai juice (390 kg/m^3); by [20] for freeze- and spray- dried of black glutinous rice (*Oryza sativa* L.) bran (350 and 240 kg/m^3, respectively); and by [23] for freeze- and spray-dried blackberry powders (450 and 430 kg/m^3, respectively).

Solubility is an essential property especially for powdered additives. In addition to the raw materials and carrier materials used, the moisture content of the obtained powder, the particle size and the physical state of the particles have a significant effect on the solubility [33]. No significant difference was found between solubilities of the freeze- and spray-dried powders (81.01 and 80.39%, respectively), probably due to the fact that same carrier material (maltodextrin) was used in both cases, and they were amorphous. These results show coherence with the findings of several studies [20, 23, 32].

Stability of Carnation Anthocyanins

Stability of carnation anthocyanins was investigated depending on different pH (2.6, 4.0, and 6.0) and temperature (70, 80 and 90 °C) values to evaluate the potential of liquid and powder samples as natural food colorant. This step was carried out to determine the degradation kinetics of the anthocyanins for each sample. R^2 values (0.886-0.997) indicated that the first order model was appropriate to predict anthocyanin content as a function of time. Thus, data were fitted to the first order model equations. Calculated parameters were shown in Table 2. Previous studies have also shown that thermal degradation of monomeric anthocyanins followed a first-order reaction [28, 34, 35].

According to Table 2, degradation rate constants (k values), especially the general mean of temperature, showed that the thermal stability of the anthocyanins decreased with increasing temperature for each sample. The lowest k value was observed at the extract pH 4.0 and 70°C. Furthermore, highest k values were determined at pH 2.6 and 90°C for each sample. There was no significant difference between extract and spray-dried powder, contrast to freeze-dried powder that has the highest general mean for the k value. Moreover, there was a significant difference between general means of temperature contrary to general mean of pH. The results indicated that temperature had a stronger influence on the stability of the anthocyanins.

As expected, degradation rate increased at higher temperatures while shortening the half-life ($t_{½}$) (Table 2). The longest half-life was estimated for the extract at pH 4.0 and 70°C. Furthermore, the shortest half-lives were determined at pH 2.6 and 90°C for each sample. Anthocyanins in the freeze-dried powder were more vulnerable to high temperatures in comparison to the others. A significant difference between only the mean of temperature among other general mean values was determined. According to the general mean value of temperature, it was found that an increase in temperature shortened the half-life extremely. There are several reports stating that anthocyanin loss accelerates at higher temperatures [6, 28].

Activation energy (E_a) is the energy of reaction required to reach the transition state. High E_a value indicates strong temperature dependence, which means that the reaction carries out slowly at low temperatures, but relatively fast at high temperatures [28]. The E_a values computed from experimental data were presented in Table 2. Except of liquid extract sample, the E_a values of all samples sharply decreased when pH value changed from 2.6 to 6.0. The most and least susceptible samples to the temperature elevation were freeze-dried at pH 2.6 and spray-dried at pH 6.0, respectively. Moreover, across all the data, the activation energies of the liquid extract sample at pH 2.6 and pH 4.0 were not significantly different from each other. According to general mean of pH values, pH 2.6 and pH 6.0 values were statistically different from each other but not from pH 4.0 values. The general mean of spray-dried powder samples was observed lower than the others. Thus, spray-dried powder is comparatively less susceptible to temperature elevation.

The behavior of temperature coefficient (Q_{10}) was like E_a when the effect of temperature on anthocyanin degradation was analyzed. This coefficient is a ratio between two rate constants that belong to temperatures having 10°C difference in between. It can also be calculated via E_a without experimental data [28]. The Q_{10} values calculated from E_a are presented Table 2. The highest Q_{10} values were determined when temperature was changed from 70 to 80°C at pH 2.6 for all samples. In addition, there was a significant ($p<0.05$) difference between general mean of pH while samples at pH 2.6 had the highest mean. According to the general mean of sample, mean of freeze-dried sample was statistically lower comparatively. Thereby, the rate constant of the freeze-dried sample was less susceptible to temperature change of 10°C.

Table 2. Effect of temperature, pH and, drying methods on the degradation kinetics parameters*

Degradation rate constants (k, k x10^3 min^{-1})

Temperature (°C)	pH	Sample: Extract (E)	Sample: Freeze Dried Powder (F)	Sample: Spray Dried Powder (S)	General mean of temperature
70	2.6	0.402^{F}	0.405^{G}	0.453^{F}	0.513^{C}
	4.0	0.354^{cF}	0.603^{aF}	0.490^{bF}	
	6.0	0.506^{cE}	0.776^{aE}	0.625^{bE}	
80	2.6	0.905^{bC}	1.184^{aD}	0.938^{bD}	1.050^{B}
	4.0	0.786^{bD}	1.503^{aC}	0.667^{cE}	
	6.0	0.927^{bC}	1.607^{aC}	0.936^{bD}	
90	2.6	2.339^{bA}	3.178^{aA}	2.150^{cA}	2.291^{A}
	4.0	1.967^{bB}	3.028^{aA}	1.563^{cB}	
	6.0	2.274^{bA}	2.628^{aB}	1.489^{cC}	
General mean of sample		1.162^{b}	1.657^{a}	1.035^{b}	
General mean of pH	2.6	1.328			
	4.0	1.218			
	6.0	1.308			

Half-lives ($t_{1/2}$, h)

Temperature (°C)	pH	Extract (E)	Freeze Dried Powder (F)	Spray Dried Powder (S)	General mean of temperature
70	2.6	28.77^{B}	28.53^{A}	25.50^{A}	23.82^{A}
	4.0	32.61^{aA}	19.18^{cB}	23.59^{bB}	
	6.0	22.85^{aC}	14.92^{cC}	18.48^{bC}	
80	2.6	12.77^{aE}	9.76^{bD}	12.35^{aE}	11.85^{B}
	4.0	14.71^{bD}	7.69^{cE}	17.32^{aD}	
	6.0	12.51^{aE}	7.2^{bE}	12.34^{aE}	
90	2.6	4.94^{bF}	3.64^{cF}	5.37^{aG}	5.36^{C}
	4.0	5.87^{bF}	3.82^{cF}	7.39^{aF}	
	6.0	5.08^{bF}	4.40^{cF}	7.76^{aF}	
General mean of sample		15.57	11.01	14.46	
General mean of pH	2.6	14.62			
	4.0	14.69			
	6.0	11.73			

Activation energies (E_a, kJ/mol)

pH	Extract (E)	Freeze Dried Powder (F)	Spray Dried Powder (S)
2.6	91.12^{bA}	106.73^{aA}	80.60^{cA}
4.0	88.69^{aA}	83.70^{bB}	59.84^{cB}
6.0	77.71^{aB}	63.31^{bC}	44.91^{cC}
General mean of sample	85.84^{a}	84.58^{a}	61.78^{b}
General mean of pH 2.6	92.81^{A}		
4.0	77.41^{AB}		
6.0	61.98^{B}		

Temperature coefficients (Q_{10})

Temperature (°C)	pH	Extract (E)	Freeze Dried Powder (F)	Spray Dried Powder (S)	General mean of temperature
70-80	2.6	2.47^{bA}	2.88^{aA}	2.22^{cA}	2.18
	4.0	2.41^{aB}	2.29^{bD}	1.81^{cD}	
	6.0	2.16^{aF}	1.87^{bG}	1.56^{cG}	
80-90	2.6	2.35^{bC}	2.72^{aB}	2.13^{cB}	2.09
	4.0	2.30^{aD}	2.19^{bE}	1.75^{cE}	
	6.0	2.07^{aG}	1.81^{bH}	1.52^{cH}	
90-100	2.6	2.24^{bE}	2.58^{aC}	2.04^{cC}	2.01
	4.0	2.20^{aEF}	2.10^{bF}	1.70^{cF}	
	6.0	1.99^{aH}	1.75^{bI}	1.49^{cI}	
General mean of sample		2.24^{a}	1.80^{b}	2.24^{a}	
General mean of pH	2.6	2.40^{A}			
	4.0	2.08^{B}			
	6.0	1.80^{C}			

Decimal reduction times (D-value, h)

Temperature (°C)	pH	Extract (E)	Freeze Dried Powder (F)	Spray Dried Powder (S)	General mean of temperature
70	2.6	95.54^{B}	94.75^{A}	84.72^{A}	79.14^{A}
	4.0	108.30^{aA}	63.71^{cB}	78.37^{bB}	
	6.0	75.90^{aC}	49.54^{cC}	61.39^{bC}	
80	2.6	42.41^{aE}	32.41^{bD}	41.03^{aE}	39.36^{B}
	4.0	48.87^{bD}	25.55^{cE}	57.52^{aD}	
	6.0	41.55^{aE}	23.92^{bE}	40.98^{aE}	
90	2.6	16.42^{bF}	12.08^{cF}	17.85^{aG}	17.82^{C}
	4.0	19.51^{bF}	12.69^{cF}	24.55^{aF}	
	6.0	16.88^{bF}	14.62^{cF}	25.78^{aF}	
General mean of sample		51.71	36.59	48.02	
General mean of pH	2.6	48.58			
	4.0	48.79			
	6.0	38.95			

* Results are the mean ± standard error; values within a row with different superscript lower cases and values within a column with different superscript upper cases are significantly ($p < 0.05$) different *according to Duncan's Multiple Range Test*.

Table 2 shows decimal reduction time (D-value), which indicates the time required for decay 90% of total anthocyanin. The extract at pH 4.0 showed the highest D-value at 70°Cwhile the lowest D-values were determined at 90°C and for the samples at all pH values for freeze-dried sample. There was a significant (p<0.05) difference between general means of temperature on the contrary to general means of sample and pH. Temperature elevation had a strong influence on D-value.

Stability of anthocyanins obtained from various plants such as poppy, tulip, rose, rosella, black carrot, grape and some berries has been investigated by many researchers [9, 13, 16, 34, 36, 37]. However, to the best of our knowledge, there has been no report about the degradation kinetics of carnation flower anthocyanins. Our results were found in agreement with those of the previous studies with slight differences arose due to the source and/or processing conditions.

In the present study, degradation rates (k) of carnation extract were found between $0.354x10^{-3}$ and $2.339x10^{-3}$ min^{-1} under the experimental conditions (Table 2). The degradation rate of carnation anthocyanins, observed in this study, was relatively higher than poppy anthocyanins [16] and quite close to black carrot anthocyanins [37] under the same thermal conditions. Comparison of the current results with the reported ones show that the degradation rate of carnation anthocyanins was lower than the other potential colorant sources such as red tulips, roses, roselles [16], tulips [13, 34] and grapes [9]. At 90°C, degradation rate of the anthocyanins was pH 2.6>6.0>4.0, respectively. Similar results about correlation between the anthocyanin degradation and pH was reported by [38] who studied degradation of the purple-fleshed sweet potato anthocyanins and by [34] who studied tulip petal as a novel natural food colorant source.

It is well known that as the temperature increases, degradation rate also increases while a decrease occurred in half-life. In this study, half-lives ($t_{1/2}$) of carnation extract were found between 4.94 and 32.61 h as shown in Table 2. These data indicate that the carnation anthocyanins were more stable anthocyanins compared to other samples obtained from different sources [9, 13, 16, 34], except poppy [16] and black carrot [37]. The higher stability can be related with acylation and/or co-pigmentation with phenolic acids, minerals etc. [1, 2].

E_a values of the carnation anthocyanins, ranged from 77.71 to 91.12 kJ/mol (Table 2), were also consistent with the literature results [9, 13, 16, 34]. Temperature dependency of the anthocyanin obtained from different sources was at proximate level under similar experiment conditions. As contrary, it was observed that poppy [16] and black carrot [37] anthocyanins were slightly outside this range i.e. 114.13 and 42.0 kJ/mol E_a, respectively.

Q_{10} values of the carnation anthocyanins were found in range of 1.99 to 2.47 (Table 2). These values were also consistent with literature results [9, 26, 37]. Similar with [9]'s results, Q_{10} values decreased with temperature elevation. This result may depend on decomposition of phenolics in extract with increasing temperature. The polymerization reaction of anthocyanins mostly occurs by reacting the monomeric anthocyanins with other phenolic compounds [27]. Thus, the observed result for the reaction could be explained by substrate limitation.

D-values of carnation extract were determined in the range of 16.42 and 108.30 h (Table 2). D- values of the carnation anthocyanin were higher, thus, time required for decay of 90% of total anthocyanin was longer than values reflected by Juçara, 'Italia' grape [9], purple-flesh potato and grape at the same temperatures [26].

Pesticide Residue Results

The widespread usage of pesticides in agriculture poses the residue problem in the final product. The extract was analyzed for 300 different pesticides. All of them were found to be below the standard maximum residue limits (MRL) according to Regulation (EC) NO 396/2005 (Data were not shown.). It can be said that pesticides either degraded prior to harvesting or decomposed during the extraction treatment at high temperature and acidic medium [39].

Storage Test of Ice Cream

The carnation-based colorant was successfully applied in the food model system. During storage, it was observed that the total color difference values of all ice cream samples were close to each other (Figure 1) and the hue angle (h°) values of all samples remained almost constant. Considering the initial hue angle values, it was determined that the hue angle value of the sample prepared with commercial colorant was lowest (Figure 2).

According to the Table 3, there was no difference in total color difference values between the samples prepared with liquid extract and the samples prepared with commercial colorant, and also, between the samples prepared with commercial colorant, spray-dried powder, and freeze- dried powder.

It is thought that the obtained colorants can be successfully used in ice cream by adding appropriate amounts. In the literature studies on similar model foods, color values are measured, and the results are interpreted, and it is stated that the addition of anthocyanin-based extract obtained from grapes at a rate of 0.3-0.5% by weight in dairy products creates an impressive color [1]. In a similar study, black carrot, red cabbage and grape skin extracts added to the ice cream mixture at the amount of 3 mg/100 g increased the phenolic content of the ice creams and was also liked sensory [30]. In another study, yogurt, and sour cream; prepared with extracts (5 mg/100 g) from different sources such as red radish, red cabbage and black carrot; it has been reported that red radish and black carrot give the product a pleasant red color, while red cabbage creates an attractive purple color. It has been stated that desired colors can be obtained by adding at

least 5% microcapsules to yoghurt products [5]. Considering that the a few weeks shelf life of dairy products, it is stated that natural anthocyanin sources can be used successfully in such products [2]. In another study, cornelian cherry was used for the production of a novel acidic non-carbonated red beverage with attractive red and stable color [40].

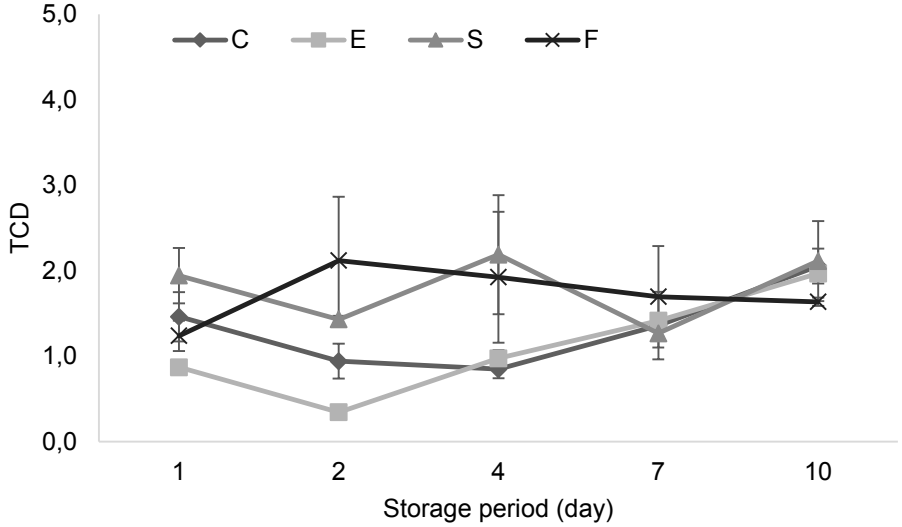

Figure 1. Total color difference values (TCD) of ice cream samples (C: Ice cream prepared with commercial liquid colorant, E: Ice cream prepared with extract, S: Ice cream prepared with spray-dried powder, F: Ice cream prepared with freeze-dried powder)

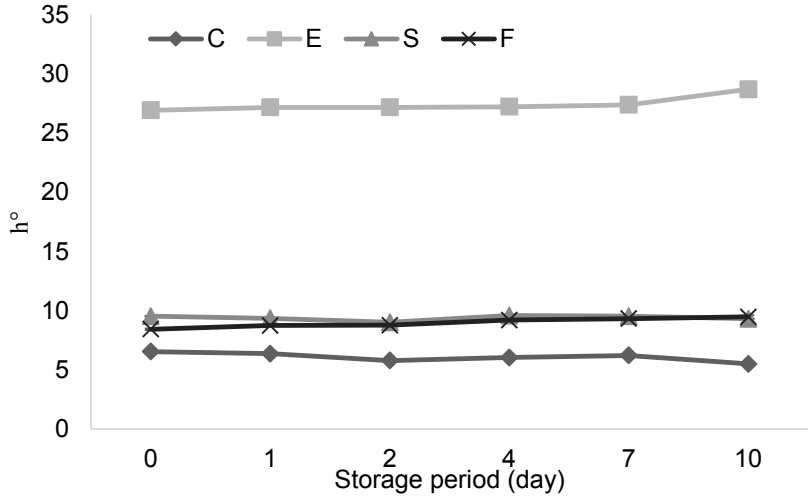

Figure 2. Hue angle (h°) values of ice cream samples (C: Ice cream prepared with commercial liquid colorant, E: Ice cream prepared with extract, S: Ice cream prepared with spray-dried powder, F: Ice cream prepared with freeze-dried powder)

Table 3. Storage analysis results of ice creams prepared with different colorants*

Source	TCD	
Colorant	Commercial liquid colorant (C)	1.33^{ab} ± 0.53
	Extract (E)	1.11^{b} ± 0.58
	Spray-dried powder (S)	1.79^{a} ± 0.58
	Freeze-dried powder (F)	1.72^{a} ± 0.66
Storage period (day)	1	1.38 ± 0.49
	2	1.21 ± 0.81
	4	1.48 ± 0.84
	7	1.43 ± 0.42
	10	1.94 ± 0.34

**: Results are the* mean ± standard error*; values within a column with different superscript upper letters are significantly ($p < 0.05$) different* according to Duncan's Multiple Range Test.

CONCLUSION

This study evaluated the potential of carnation flower as a novel source of natural food colorant. Colorants obtained in liquid and powder forms were investigated for their stabilities under different temperature and pH conditions. Generally, the physical properties of the powder sample obtained by freeze-drying were found more efficient than the ones obtained by spray-drying. According to kinetic data, the degradation behaviors of the liquid and the powder colorants were significantly different. This difference might be caused by the percentage of acylated-anthocyanins and/or by the chemistry of anthocyanins at different pH. Moreover, pesticide analysis indicated that carnation flowers were safe from the perspective of pesticide residues and may be a novel and safe source of natural food colorants. By way of conclusion, anthocyanin-based colorant, derived from carnation, can be either used or improved for various purposes such as coloring foods and textile materials as they are stable under different pH and temperature conditions.

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Conflict of Interests

The authors declare no conflict of interests.

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Research Paper / Araştırma Makalesi

Mineral and Bioactive Component Contents of Rosehip (*Rosa canina* L.) Seed Powder

Sati Gamze Çürük, Muath Njjar, Denizcan Köseoğlu, Abdullah Akdogan

Department of Chemical Engineering, Faculty of Engineering, Pamukkale University, TR-20017 Denizli, Türkiye

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Corresponding author (Yazışmalardan Sorumlu Yazar): akdogan@pau.edu.tr (A. Akdogan)
+90 258 296 3081 +90 258 296 2338

ABSTRACT

This study determined the bioactive components of rosehip (*Rosa canina L.*) extract obtained via supercritical carbon dioxide extraction. The total phenolic content of its extract was 214.4 mg gallic acid equivalent/kg, with the total flavonoid content of 21.1 mg quercetin equivalent/kg. The antioxidant activity of the extract, which was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, was 64.8 µmol Trolox® equivalent antioxidant capacity (TEAC)/g. Gas chromatography-mass spectrometry identified 15 bioactive components in the extract. Additionally, pre- and post-processing heavy metal analyses were conducted on rosehip powder and seeds using inductively coupled plasma mass spectrometry (ICP-MS). Results showed that metal concentrations ranged from 0.064 to 9134.1 mg/kg in rosehip powder and from 0.143 to 1929.5 mg/kg in seeds, with the concentrations of potassium and magnesium as the highest. Despite the limited uses of rosehip products, this study indicated that wild rosehips are rich in functional components with potential health benefits.

Keywords: Rosehip powder, *Rosa canina* L., Heavy metal, Supercritical carbon dioxide extraction, Bioactive component

Kuşburnu (*Rosa canina* L.) Tohumu Tozunun Mineral ve Biyoaktif Bileşen İçerikleri

ÖZ

Bu çalışma, süperkritik karbon dioksit ekstraksiyonu yoluyla elde edilen kuşburnu (*Rosa canina* L.) ekstraktının biyoaktif bileşenlerinin analizine odaklanmıştır. Toplam fenolik içeriğin 214.4 mg gallik asit eşdeğeri/kg, toplam flavonoid içeriğinin ise 21.1 mg kuersetin eşdeğeri/kg olduğu bulunmuştur. DPPH (2,2-difenil-1-pikrilhidrazil) yöntemi ile belirlenen antioksidan aktivitenin 64.8 µmol TEAC/g olduğu tespit edilmiştir. Gaz kromatografisi kütle spektrometresi, özütte 15 biyoaktif bileşeni tanımlamıştır. Ayrıca kuşburnu tozu ve tohumları üzerinde, indüktif eşleşmiş plazma kütle spektrometresi (ICP-MS) kullanılarak işlem öncesi ve sonrası ağır metal analizleri yapılmıştır. Sonuçlar, kuşburnu tozunda 0.064 ila 9134.1 mg/kg ve tohumlarda 0.143 ila 1929.5 mg/kg arasında değişen konsantrasyonları göstermiştir, potasyum ve magnezyum en yüksek konsantrasyonları göstermiştir. Çalışma yabani kuşburnunun potansiyel sağlık faydalarına sahip fonksiyonel bileşenler açısından zengin olduğunu vurgulamaktadır.

Anahtar Kelimeler: Kuşburnu tozu, *Rosa canina* L., Ağır metal, Süperkritik karbondioksit ekstraksiyonu, Biyoaktif bileşen

INTRODUCTION

Rosa canina L., commonly known as rosehip or dog rose, is the fruit of plants belonging to the *Rosaceae* family, specifically the *Rosaideae* subfamily. The term "rosehip" refers to the fruit of the rose plant. In Latin, it is referred to as Fructus Rosae [1]. Globally, there are more than 100 species of rosehip distributed across different geographic regions of Europe, Asia, the Middle East, and North America [2]. Turkey, specifically, is home to 27 of these species. Additionally, rosehip includes 5 subspecies, 2 varieties, and 15 hybrids. Sixteen of these rosehip species are particularly located in the Eastern Black Sea region [3].

The utilization of rosehip throughout history underscores its cultural and historical importance. In Mediterranean countries during ancient times, rosehip was used as a symbol of purity and cleanliness. The Romans, for instance, utilized rosehip flowers as medicine for abdominal pain and made wine, jam, and cakes from their fruits. Throughout history, rosehip has been a valuable plant used for various purposes in different cultures. The recognition of rosehip as an edible natural resource further emphasizes its significance within the broader context of non-wood forest products.

The rosehip fruit is one of the richest fruits in nature, especially in terms of anthocyanins, proanthocyanidins, catechins, quercetin, gallic acid, ellagic acid, flavonoids, and other polyphenolic compounds. Additionally, it contains essential nutrients such as organic acids, essential fatty acids (omega-3 and omega-6), tocopherols (a form of vitamin E), carotenoids (precursors of vitamin A), vitamin C, phenolics, and sugars [4]. These components collectively contribute to the antioxidant [5], anti-inflammatory [6], antiproliferative [7], anti-obesity, anti-diabetic activity [8], and overall positive health effects of rosehip. Rosehips also provide other essential vitamins and minerals, including vitamin P, K, E, B1, B2, provitamin A, calcium, zinc, potassium, iron, magnesium, manganese, sodium, and phosphorus, making them a highly beneficial fruit for overall health [9].

During the processing of rosehips, the pulp of the rosehip is the main utilized part, while the remaining seeds are generally considered as waste. However, the seeds also have significant applications. The economic value of rosehips, coupled with the soothing properties of the seeds, enhances their importance. Additionally, an experiment conducted with mice fed with rosehip seeds has raised the possibility of using rosehip seeds as a component in dietary human foods [10,11]. Rosehip seeds are abundant in unsaturated fatty acids (FAME's) and boast a higher vitamin E content when compared to rosehip pulp. They serve as an excellent source of omega-6 FAME's and exhibit significant antioxidant capacity [12]. The most abundant FAME's found in the seed oil are linoleic acid (50.08%), arachidic acid (20.00%), and oleic acid (19.31%) [11,13].

The nutritional richness of rosehip, coupled with its versatility in culinary applications and potential health-promoting properties, makes it a valuable resource. However, processing and consumption methods can influence its content levels. An ideal extraction method should be fast, simple, and cost-effective, and no additional steps should be required to prepare it for analysis. Traditional extraction methods involve the use of exhaustive solvent extraction for long periods. While these methods yield high amounts of analytes, they also involve the overuse of harmful solvents, which can violate environmental and health guidelines. For this reason, there is an urgency to develop cost-effective and environmentally friendly extraction processes that may compete advantageously with the industrially established method. These processes should preserve the nutritional, functional, and biological properties of analytes while ensuring high extraction yields.

Among these alternative processes, supercritical fluid extraction (SCFE), which was developed in the mid-1980s, has been suggested as a promising solution to the challenges in the extraction process. This process is highly effective due to the low polarity of CO_2, which is ideal for extracting non-polar compounds such as essential oils, FAME's, tocopherols, and carotenoids from plant material. This method eliminates the need for hazardous organic solvents and prevents thermal degradation of thermosensitive compounds. Supercritical fluids have the unique ability to diffuse into solid matrices like a gas while simultaneously solubilizing certain compounds of interest in the solid material. The utilization of carbon dioxide in SFE-CO_2 provides additional benefits, given its affordability, non-toxicity, recyclability, chemically inert, non-inflammability, abundant availability, and non-polar nature. Moreover, the efficient separation of CO_2 through depressurization at the end of the process enhances overall extraction efficiency [14].

This study evaluated the total phenolic content, total flavonoid content, and antioxidant activity of an extract made from rosehip powder using the Supercritical Carbon Dioxide Extraction (SFC-CO_2) method. In addition, a gas chromatography-mass spectrometry (GC-MS) examination was conducted to determine whether the extract contained any bioactive ingredients. This study also investigates the fruit's heavy metal contents both before and after the extraction process.

MATERIALS and METHODS

Plant Material

Commercially purchased rosehip flour, labelled as 100%, was stored in its original packaging in a refrigerator set at 4°C until used in the experiment

Chemicals

Methanol (CH_3OH, purity>99.8%), heptane (HPLC, ≥99%), and hexane (C_6H_{14}, purity>99.99%) were purchased from IsoLab (Eschau, Germany). Additionally, high-purity gallic acid, Folin-Ciocalteu reagent, DPPH, Trolox (±-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), sodium chloride,

boron trifluoride and sodium carbonate were obtained from Sigma Aldrich. Solutions were prepared and diluted using ultra-pure deionized water with a resistance of 18.2 MΩ cm^{-1} that was acquired from a reverse osmosis system (Human Corp. Seoul, South Korea).

Apparatus

All weighing in the experiments were performed on an analytical balance with a sensitivity of 0.1 mg (Denver Instrument, APX-200 model, USA). The mixing processes were carried out using a vortex device (Velp Scientifica, ZX Classic model, Italy). For the separation process, a centrifuge device (EBA 20, Hettich, Zentrifugen, Tuttlingen, Germany), was used. Laboratory materials and extractor bags were dried using a hot air sterilizer (Nüve, FN 055 Model, Istanbul, Turkey). A UV-Vis spectrophotometer with 8 cells (EMC-11, Duisburg, Germany) was used to determine the total phenol contents.

Supercritical Fluid Extraction

After placing 150 g of ground rosehip powder (Figure 1a) into special extraction bags (Figure 1b), they were loaded into the extraction cell. The extraction took place in a 0.5 L stainless steel vessel (Figure 1c). Optimal extraction conditions were determined to be as follows: 40°C extractor temperature, 120°C restrictor temperature, 50°C separator temperature, 300 bar pressure (using CO_2 for pressurization),CO_2 flow rate of 50 g/min and a working time of 180 minutes, including a 20 minute static extraction time. The extracted rosehip powder after the extraction process is shown in (Figure 1d). Finally, the extract collected in the vial after extraction (Figure 1e) was stored at +4°C for the determination of antioxidant capacity, total phenolic content, total flavonoid content, and bioactive component quantities.

The efficiency of the supercritical fluid extraction process for rosehip powder is defined as the yield, expressed as a percentage (% yield), calculated by dividing the mass of the extract (g extract) by the mass of the initial dried powder (g dry powder). The amount of phenolic extracts obtained at the end of the extraction process was found to be 3.5% using the following equation;

$$\%Yield = \frac{m_{extract}}{m_{initial\,powder}} \cdot 100 \qquad (1)$$

Where; $m_{extract}$ is the amount of extract (g), and $m_{initial\ powder}$ is the initial amount of dry rosehip powder (g).

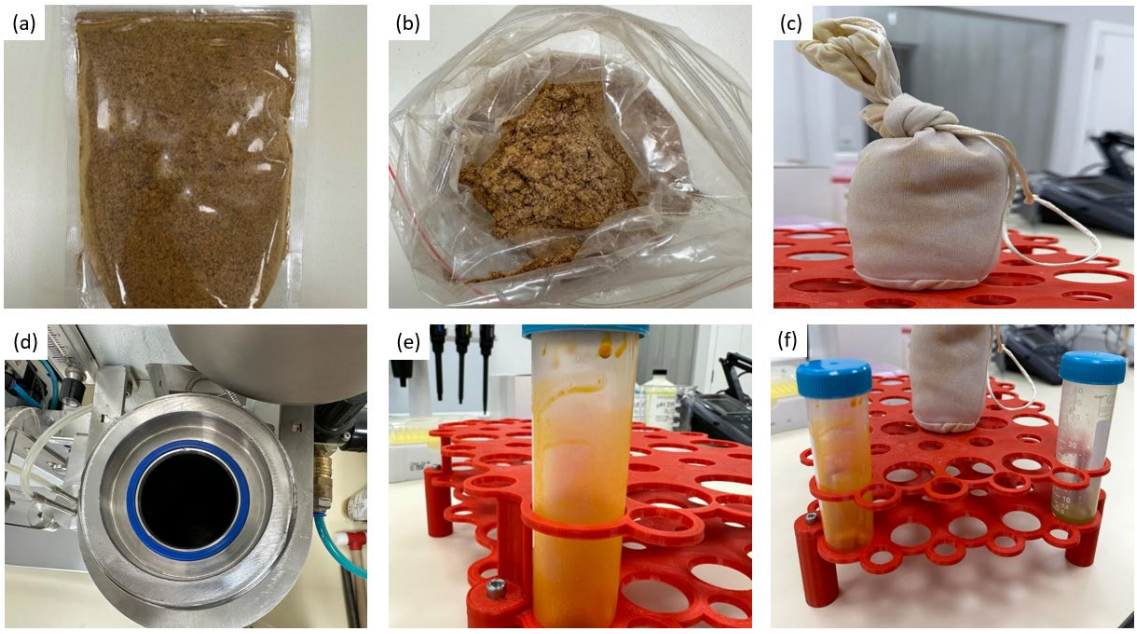

Figure 1. a) Rosehip powder. b) Rosehip powder after the extraction. c) Extraction sac. d) Extraction cell. e,f) Extract collected in vial

FAME's Extraction Method

Transesterification was carried out by boiling 0.1 g of rosehip SFE extract in ca. 4 mL of methanolic KOH for several minutes. Methanolic BF3 (14% w/v, 5 mL) was then added, and the mixture boiled further for 2 minutes. Finally, 5 mL of heptane was added, the vial was shaken and the mixture boiled for 1 minute. To a cooled solution, 15 mL of saturated NaCl (aq) was added and the vial was shaken vigorously (1 min). A 1 mL aliquot of the supernatant heptane layer was transferred into a GC vial for analysis.

DPPH Radical Scavenging Assay

The DPPH· radical scavenging activity of rosehip powder was determined using the DPPH· antioxidant activity method reported in [15]. DPPH· solution is prepared using methanol, and its absorbance is adjusted to 1.1 at 515 nm. A mixture of 150 µL of the sample and 2850 µL of DPPH· is prepared and left in dark environment at room temperature for 1 hour. After the incubation period, the change in absorbance of the samples is analyzed at 515 nm using a UV spectrometer. The average of triplicates was calculated and the obtained values were expressed in mmol Trolox® equivalent (mmol TE) per 100 mL based on the equation derived from the Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard curve. The antioxidant capacity of the samples is determined using the calibration curve ($y = 0.0132x + 0.3419$, $R^2 = 0.9821$) obtained from Trolox® standard solutions (10, 20, 30, 40, and 50 µL).

Total Phenolic Content

The total phenolic content of rosehip powder extract was determined using the Folin-Ciocalteu method, as reported by Singleton et al. [16]. This method is based on a redox reaction where phenolic compounds reduce the Folin-Ciocalteu reagent and convert it to an oxidized form. The steps of the method are as follows. 100 µL is drawn from the prepared extract, followed by the addition of 4.5 mL of distilled water. Subsequently, in the second phase, 100 µL of Folin-Ciocalteu reagent (1 N) is introduced and thoroughly mixed, followed by the addition and thorough mixing of 300 µL of 2% sodium carbonate solution. The resultant mixture is then left in dark environment for a waiting period of 2 hours. Moving on to the fourth step, spectrophotometric analysis is conducted after the waiting period, measuring the absorbance at a wavelength of 760 nm using a UV-visible spectrophotometer. For the blank test, 0.5 mL of methanol is utilized instead of the sample. Finally, under consistent conditions, analysis is carried out using 50-250 mg/L gallic acid standard solutions. The absorbance values obtained are then applied to a calibration curve, and the final result is expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of the sample.

The determination of total phenolic compound content in the samples is expressed in terms of gallic acid equivalents, and this quantification is derived from the calibration curve ($y = 0.0099x + 0.0806$, $R^2 = 0.9999$). The average of three replicated measurements was calculated.

Total Flavonoid Content Analysis

According to the spectroscopic method applied by Zhinsen et al.[17], the total flavonoid content was determined. In this analysis, 1 mL of the sample was taken and transferred to a test tube, and 4 mL of distilled water was added and mixed. Then, 0.3 mL of a 5% $NaNO_2$ solution was added to the tube, and it was homogenized with the help of a vortex. After waiting for 5 minutes, 0.6 mL of a 10% $AlCl_3.6H_2O$ solution was added to the mixture, and after waiting for an additional minute, 2 mL of a 1 mol/L NaOH solution was added. The total volume in the tube was completed to 10 mL with distilled water. The resulting mixture was vortexed once again, and the absorbance value against water was measured at a wavelength of 510 nm. In this way, the total flavonoid content was determined.

The total flavonoid content was calculated based on the quercetin standard curve prepared in concentration intervals with three repeated experiments. The standard curve ($y = 0.0009x + 0.0001$, $R^2 = 0.9975$) was plotted using quercetin solutions of 20, 40, 60, 80, and 100 mg/L. The measurements were made at a wavelength of 510 nm to create the standard curve. Using the generated standard curve, the total flavonoid content in the samples was calculated in terms of milligrams of quercetin per gram of dry weight.

Bioactive Compound Content and FAME's Analysis

For the analysis of the bioactive compound content in the extract, a gas chromatography-mass spectrometer (GC-MS) device was employed. The extract was diluted 50-fold in hexane prior to analysis. The chromatographic system used in this study a Shimadzu QP-2020NX model gas chromatograph-mass spectrometer, incorporating a split-splitless injector system (AOC 20i model autoinjector with AOC 20s automatic sampler). The GC column used was an Rtx-5MS (Restek) column (30 m x 0.25 mm i.d. x 0.25 µm, coated with 95% dimethylpolysiloxane and 5% diphenyl thin film). The carrier gas used was hydrogen with a purity of 99.9999%, generated from ultra-pure water using the Peak Scientific Precision H2 450 model gas generator. The maximum flow rate from the generator is 450 mL/min, and the gas outlet pressure is balanced to 5 bar. The carrier gas (hydrogen) is passed through a molecular sieve and oxygen traps from Shimadzu GLC Ltd. before being sent to the system. All analyses in the device were conducted in the 70 eV electron impact (EI) mode.

The full scan (SCAN) mode was utilized. Following the analysis, qualitative identification was carried out using library scans and similarity indices. GC-MS injector and detector temperatures were 250°C and 300°C, respectively. A split ratio of 10:1 was used to inject 1 µL of sample. Hydrogen carrier gas was used at a flow rate of 1.2 mL min^{-1}. The ion source was kept at 230°C. Solvent delay was set at 1.4 minutes. The column temperature was increased from 40 to 250°C at 3°C min^{-1}.

FAME's were analyzed by GC-FID prior to area normalization-based quantification. A Shimadzu GC-2030 was used. The system was equipped with an Rx-5SilMS column (30 m × 0.25 mm × 0.25 µm). A split ratio of 70:1 was used to inject 1 µL of sample. Hydrogen carrier gas was used at a flow rate of 2.0 mL min-1. The temperature program was as follows: 50°C (1 min hold) to 140°C at 10°C min-1, then to 260°C at 3°C min-1, and held for 5 min. Injector and detector temperatures were 260°C and 280°C, respectively.

Microwave Method

Rosehip powder samples were dissolved using the microwave digestion method. Approximately 0.500 ±0.001 g of rosehip powder is precisely weighed and placed into pressurized Teflon tubes. To aid in the processing, 4 mL of HNO_3 and 1.0 mL of $HClO_4$ are introduced into each tube, followed by sealing with Teflon disks. Subsequently, the tubes were positioned in an oven, and the oven program conditions, set according to the manufacturer's recommendations as outlined in Table 1, were applied. Once the process was complete, the tubes were allowed to cool before being opened under a fume hood. The extracted solutions were then transferred into 50 mL volumetric flasks and the volume was adjusted with distilled water. The resulting solutions for analysis are stored in the refrigerator at +4°C.

Table 1. Microwave oven program

Step	Heating ramp (min)	Time/min	Temperature, °C
1	5	5	145
2	3	5	170
3	2	18	190
4	1	1	75
5	1	1	75

Mineral Content

The mineral content analysis of the rosehip powder sample was conducted at the Advanced Technology Application and Research Center of Pamukkale University. The rosehip fruit, in its non-powdered form, and a commercially purchased sample were simultaneously analyzed through service procurement. For this purpose, both samples were initially dissolved using the CEM brand Mars6 iPrep model microwave dissolution system. The dissolved samples were then analyzed using the Perkin Elmer Nexion2000 model (USA) Inductively Coupled Plasma Mass Spectrometer (ICP-MS). The system uses argon gas as the primary gas and helium as the collision gas for interference removal. With a dual-mode quadrupole, the system has a mass measurement range of 5-279 amu.

RESULTS and DISCUSSION

Total Phenolic Content and Antioxidant Activity

The extract obtained from rosehip powder using the supercritical fluid extraction method was analyzed for its total phenolic content, with concentrations determined using a calibration curve of standard gallic acid solutions ranging from 50 to 250 mg/L ($y = 0.009x + 0.080$, $R^2 = 0.999$). Experimental absorbance values were fitted to this curve to determine their corresponding concentrations. The total phenolic content in the rosehip sample was found to be 214.4 mg GAE/kg.

For the calculation of the total flavonoid content in R. canina powder extractions, the calibration curve provided was $y = 0.000x + 0.0004$, $R^2 = 0.997$. According to the experimental results, the total flavonoid content in rosehip extracts was determined to be 21.1 mg quercetin equivalent/kg.

Calibration curves for antioxidant capacity measurements in rosehip extractions using the DPPH method were given as $y=0.013x + 0.341$, $R^2 = 0.982$. The antioxidant capacity of rosehip extracts, based on DPPH results, was determined to be 64.8 µmol TEAC/kg.

These results indicate that the total phenolic content, flavonoid content, and antioxidant capacities in the fruits of different rosehip species can vary. It was observed that rosehip had a lower total phenolic content compared to other species, but its total flavonoid content and antioxidant capacity were higher.

When examining the results of various extractions of rosehip, as shown in Table 2, it is evident that our research findings align with other studies, indicating that the supercritical carbon dioxide extraction method yields the highest amounts of total phenolic content and total flavonoids in rosehip fruits. However, the antioxidant activity content in supercritical carbon dioxide extraction was found to be lower in comparison to other extractions.

These findings suggest that the choice of extraction method can significantly impact the phytochemical composition of rosehip extracts, highlighting the importance of considering different factors when selecting an extraction method for obtaining specific bioactive compounds.

Bioactive Component Content and FAME's Analysis

In the chromatographic analysis, the sample was directly analyzed without undergoing any preprocessing steps, resulting in the identification of fewer components than expected. The list of these components is provided in Table 3, including their names, CAS numbers, mass spectral information, and similarity index values. Compounds with a similarity index of 70% or higher were identified using the NIST11 (National Institute of Standards and Technology), GCorganic acid, and FFNSC3 (Flavour and Fragrance Natural and Synthetic Compounds) libraries. Figure 2 presents the GC-MS chromatogram of the extract. It was observed that preprocessing steps were necessary for determining the FAME's and sterol composition of the extract. Figure 3 shows the GC-FID chromatogram of FAME's of rosehip seed extract, and the list of these components is provided in Table 4.

Table 2. Total phenolic, total flavonoid, and antioxidant activity content in rosehip powder extract and comparison with the literature

Extraction method	Total phenolic content (mg gallic acid equivalent/kg)	Total flavonoids (mg quercetin equivalent/kg)	Antioxidant (DPPH) activity (mmol Trolox® equivalent/kg)	References
Supercritical carbon dioxide extraction	214.4 ±22.6	2.1±0.2	64.8 ±5.9	This Study
Solid-liquid extraction (50% acetone)	5.09±0.14	-	379±2.81	[18]
Solid-liquid extraction (80% methanol)	2.59±0.14	-	190±4.81	[18]
Solid-liquid extraction (methanol)	424.6±1.8	23.6±4.2	-	[19]
Solid-liquid extraction (aqueous)	74.6±3.08	1.22±0.02	32.7±1.54	[20]
Solid-liquid extraction (methanol)	50.9±3.60	0.65±0.03	21.7±2.04	[20]
Solid-liquid extraction (70% acetone)	21.5±0.33	-	-	[21]
Solid-liquid extraction (90% methanol)	21.2±0.38	-	-	[21]
Solid-liquid extraction (80% ethanol)	16.3±0.31	-	-	[21]
Solid-liquid extraction (methanol)	10.74±3.09	3.43±1.44	25.03±4.91*	[22]
Solid-liquid extraction (methanol+water+formic acid)	31.08±0.19	9.48±0.94	278.90±5.60*	[23]
Solid-liquid extraction (methanol+1% HCL)	6.298±116.7	-	-	[24]
Solid-liquid extraction (methanol)	225.65±2.50	2.02±0.03	-	[25]
Solid-liquid extraction (ethanol)	76.26	-	457.2 ±626.2	[26]
Supercritical carbon dioxide extraction (ethanol)	118 ±13.7	-	-	[27]

* All values are in mg/mL

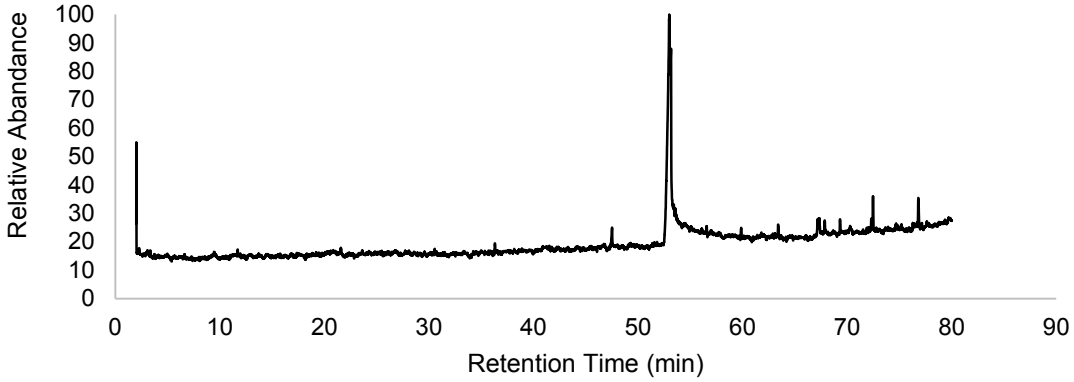

Figure 2. GC-MS chromatogram of bioactive component of rosehip seed extract

Table 3. Bioactive components in rosehip flour extract: GC–MS analysis

#	Compound	t_R	CAS Number	%Area	%Height	Similarity index
1	n-Hexadecanoic acid (palmitic acid)	47.51	57-10-3	4.03	1.42	93
2	Linoleic acid	52.97	60-33-3	14.77	56.91	95
3	Mentha-1(7),8-diene	53.09	13837-95-1	4.34	13.42	81
4	Oleic acid	53.15	112-80-1		11.1	91
5	Hexadec-(11E)-en-1-ol	56.54	61301-56-2	4.13	0.53	85
6	Bis(2-ethylhexyl) adipate	59.86	103-23-1	3.95	0.76	78
7	Pelargol	63.40	106-21-8	4.0	0.89	75
8	9,12-Octadecadienoic acid (Z,Z)- (2-hydroxy-1-(hydroxymethyl)ethyl ester)	67.18	3443-82-1	5.52	1.65	87
9	Tetradec-(7Z)-enal	67.31	65128-96-3	5.69	1.69	84
10	Hexacosane	67.84	630-01-3	3.87	0.91	92
11	9-Octadecenoic acid (Z), methyl ester	69.31	301-02-0	3.15	0.81	88
12	Eicosene	72.31	27400-78-8	4.51	0.99	92
13	Hexacosane	72.47	630-01-3	3.57	2.31	97
14	Tricos-(9Z)-ene	76.28	27519-02-4	5.00	0.65	79
15	Dotriacontane	76.80	544-85-4	4.74	2.64	92

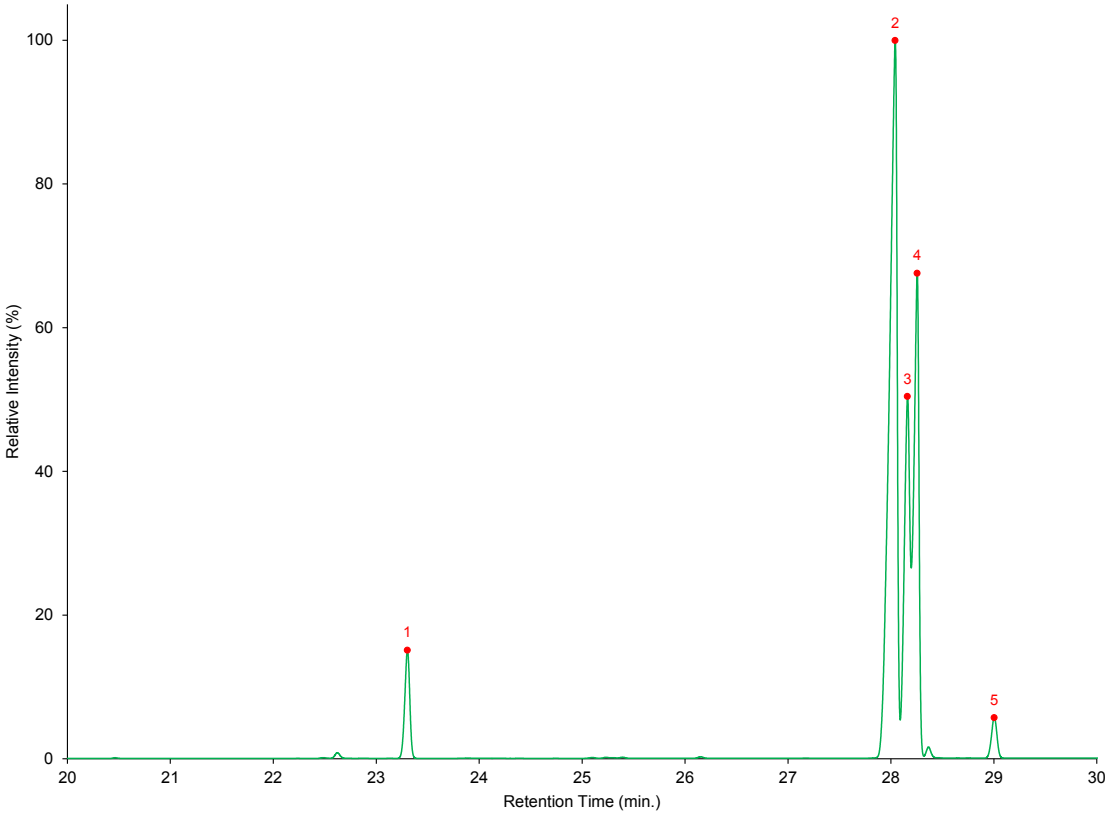

Figure 3. GC-FID chromatogram of FAME's of rosehip seed extract

Table 4. Bioactive components in rosehip flour extract: GC–MS analysis

Peak ID	Name	FID		
		RT (min)	Area	Area %
1	Palmitic acid (C16:0)	23.303	231654	4.97
2	Linoleic acid (C18:2w6)	28.041	2368444	50.83
3	alpha-Linolenic acid (C18:3w3)	28.161	903240	19.39
4	Oleic acid (C18:1w9)	28.255	1060018	22.75
5	Stearic acid (C18:0)	29.004	96011	2.06

Heavy Metal Content

The concentrations of heavy metals and mineral substances in both the powder and the seeds of the plant were determined. The findings obtained are presented in Table 4, expressed in milligrams per kilogram (mg/kg).

Table 4. Concentrations of heavy metals in rosehips (mg/kg)

Metals	Rosehip powder	Seed	ICP-MS LOD concentrations (µg/L)
Li	<LOD*	<LOD	0.179
B	<LOD	<LOD	2.180
V	0.064	0.143	3.592
Se	<LOD	<LOD	0.380
Mo	<LOD	<LOD	1.096
Na	<LOD	<LOD	2.044
Mg	1639.1	1929.5	1.3331
Al	21.5	62.7	0.024
K	9134.1	1006.4	0.014
Ca	622.8	747.3	0.334
Tl	<LOD	<LOD	0.001
Pb	<LOD	10.5	0.003
Bi	<LOD	<LOD	0.086
Si	92.4	317.7	0.204
As	<LOD	0.895	0.004
Be	<LOD	<LOD	0.041
Ti	2.4	2.4	0.047
Mn	36.9	77.5	0.014
Fe	45.5	97.8	0.781
Hg	<LOD	0.010	0.025
Co	<LOD	<LOD	0.003
Ni	0.590	3.753	0.018
Cu	10.5	4.5	0.842
Zn	5.7	25.8	4.610
Ga	3.1	2.5	0.019
Sr	22.2	92.8	0.004
Ag	<LOD	<LOD	0.009
Cd	<LOD	<LOD	0.008
In	<LOD	<LOD	0.001
Cr	<LOD	6.7	0.243
Ba	12.3	10.4	0.007
Sb	<LOD	<LOD	0.004

*LOD: Limit of detection

CONCLUSION

In this research, the characteristics of bioactive components in rosehip fruit powder were investigated through supercritical carbon dioxide extraction, including the analysis of total phenolic content, antioxidant activity (DPPH radical scavenging activity), and total flavonoid content of ground rosehip wild fruit along with its seeds. Additionally, the heavy metal content of the extract was analyzed using gas chromatography-mass spectrometry. The findings were compared with data from similar studies.

According to the research results, the characteristics of bioactive components in rosehip powder were generally found to be higher than those reported by various domestic and foreign researchers. However, differences observed in some parameters were attributed to the extraction method used.. This research demonstrates that supercritical carbon dioxide extraction on rosehip fruit powder is an effective method for revealing and characterizing the bioactive components of the fruit.

The findings of this study indicate that rosehip fruit is a significant source of potential health benefits and functional properties. The wild-grown fruit, which is economically undervalued, has been shown to contain important food components that can positively impact consumer health. These findings support the idea that rosehip fruit could be considered a functional food, and its consumption could be increased by incorporating it into various new food products. It is believed that this fruit, which can be grown in almost every region of the country, could contribute significantly to the food industry and the national economy. Therefore, further research is needed to expand the areas of utilization for rosehip fruit and to increase its consumption. Such research could highlight the health benefits of the fruit and lead to the development of new products. In conclusion, given the importance and potential of rosehip fruit, it is essential to encourage its consumption and explore new evaluation methods. This way, this fruit with positive effects on human health could reach a wider audience.

In the study, the content of elements present in different parts of the rosehip plant was analyzed. Element and heavy metal analyses in rosehip powder samples are important for identifying potential harmful substances in this plant and taking precautions for food safety. Such analyses are a crucial tool for protecting consumer health and determining the level of environmental pollution in foods.

Conflict of Interest Statement

The authors declare no conflict of interest.

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Research Paper / Araştırma Makalesi

Effects of Humic Acid and Bromide on Trihalomethane Formation during Water Disinfection with Chlorine

Yakup Sedat Velioğlu[1], Rukiye Akdoğan[1,2], Zehra Baloğlu[3]

[1]Ankara University, Faculty of Engineering, Department of Food Engineering, Golbaşı, Ankara, Türkiye
[2]Present Address: Güvenlik Cad. No: 37, Aşağıayrancı, Ankara, Türkiye
[3]Ministry of Health, General Directorate of Public Health, Department of Public Health Reference Laboratories, Cankaya, Ankara, Türkiye

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Corresponding author (Yazışmalardan Sorumlu Yazar): velioglu@ankara.edu.tr (Y.S. Velioğlu)
+90 312 203 3300 /3619 +90 312 317 8711

ABSTRACT

Chlorination is one of the most important methods used in water disinfection. Chlorine reacts with natural organic substances in water and causes the formation of disinfection byproducts that might cause health problems. The predominant by-product of chlorination is trihalomethanes. Humic substances, which make up the majority of natural organic substances, are the primary precursors of trihalomethanes. In this study, the effect of different doses of chlorine on the formation of chloroform, bromodichloromethane, dibromochloromethane and bromoform in the presence of natural organic matter and bromide in drinking water was evaluated. Artificial raw water samples prepared with the addition of 2, 3 and 5 mg/L humic acid representing natural organic matter were subjected to chlorination at doses of 1, 2 and 3 mg/L and analysed on the 0th, 3rd and 7th day. The only trihalomethane formed was chloroform with a concentration of 20.52-131.13 µg/L. Increased humic acid and chlorine levels resulted in increased chloroform content. Free chlorine in the water caused chloroform formation to continue even on the 7th day. Accordingly, the amount of chloroform formed increased with the contact time. While the chlorine dose was constant, increased humic acid resulted in decreased free chlorine. To evaluate the effect of bromide on trihalomethane formation, 200 µg/L bromide was added to 2 mg/L humic acid containing water, and 1 mg/L and 2 mg/L chlorination was applied. At the end of the chlorination process in bromide-free waters, only 23.46-41.90 µg/L of chloroform was formed. In the presence of bromide, chloroform, bromodichloromethane, dibromochloromethane and bromoform were formed and the total trihalomethane level increased to 50.03-85.59 µg/L. While the ratio of brominated trihalomethane increased, the amount of chlorinated species decreased.

Keywords: Water, Disinfection, Chlorine, Bromide, Trihalomethanes, Humic acid

Suların Klorla Dezenfeksiyonunda Trihalometan Oluşumuna Hümik Asit ve Bromürün Etkisi

ÖZET

Suların dezenfeksiyonunda kullanılan en önemli yöntemlerden biri klorlamadır. Klor, sudaki doğal organik maddelerle reaksiyona girerek sağlık sorunlarına neden olabilecek dezenfeksiyon yan ürünlerinin oluşmasına neden olur. Klorlamanın baskın yan ürünü trihalometanlardır. Doğal organik maddelerin çoğunluğunu oluşturan hümik maddeler, trihalometanların birincil öncülleridir. Bu çalışmada farklı düzeylerde uygulanan klorun doğal organik madde ve hümik asit varlığında kloroform, bromodiklorometan, dibromoklorometan ve bromoform oluşumuna etkisi incelenmiştir. Denemede kullanılan su örnekleri, doğal organik maddeyi temsilen deiyonize suya 2, 3 ve 5 mg/L hümik asit eklenmesiyle hazırlanmış ve 1, 2 ve 3 mg/L düzeyinde klorlanarak 0., 3. ve 7. günlerde analiz edilmiştir. Oluşan tek trihalometan 20.52-131.13 µg/L düzeyindeki kloroform olmuştur. Artan hümik asit ve klor düzeyleri kloroform artışına yol açmıştır. Suda bulunan serbest formdaki klor 7. günde bile kloroform oluşturmuştur, dolayısıyla temas süresi de

artışa etki etmiştir. Sabit klor dozunda artan hümik asit miktarı serbest kloru azaltmıştır. Trihalometan oluşumuna bromür iyonunun etkisini anlamak üzere 2 mg/L hümik asit içeren suya 200 µg/L bromür eklendikten sonra 1 mg/L ve 2 mg/L düzeyinde klor uygulanmıştır. Bromür içermeyen sularda klorlama işlemi sonunda sadece 23.46-41.90 µg/L düzeyinde kloroform oluşmuştur. Bromür varlığında ise kloroform, bromodiklorometan, dibromoklorometan ve bromoform oluşarak toplam trihalometan düzeyi 50.03-85.59 µg/L'ye yükselmiştir. Bromlu trihalometan oranı yükselirken klorlu türlerin miktarı azalmıştır.

Anahtar Kelimeler: Su, Dezenfeksiyon, Klor, Bromür, Trihalometanlar, Hümik asit

INTRODUCTION

Disinfection which is the process of removing pathogenic organisms, is a necessary water treatment step for food industry, and avoid infectious diseases transmitted with potable water [1]. The disinfectants used in the disinfection of water, while inactivating the microorganisms that cause diseases, react with the organic and inorganic substances in the water and cause the formation of disinfection byproducts (DBPs) with toxic effects [2-4]. Different DBPs can be formed depending on the type and amount of the disinfectant used and the organic and inorganic substances in the water. Chlorine, chloramine, chlorine dioxide, ozone and UV are widely used in the disinfection of water [5-6]. Chlorine, which is widely used worldwide due to its microbial efficiency, low cost, ease of supply and application, and protection against microbial contamination that may occur in pipelines, causes the formation of trihalomethanes (THMs). Gaseous chlorine (Cl_2), sodium hypochlorite (NaClO) or calcium hypochlorite ($Ca(ClO)_2$) is widely used in the disinfection of water with chlorine [4]. The greatest advantage of chlorination is that chlorine remains in water as residual chlorine after dosing, and disinfection activity continues in distribution and storage systems. Adding chlorine as a disinfectant to water causes the formation of hypochlorous acid (HOCl) and hypochlorite ions (OCl^-), expressed as free chlorine, have disinfectant properties and the ability to react with organic substances [5]. The chlorine balance of water depends on pH, and the pH value is a critical parameter that can affect both disinfection efficiency and the formation of DBPs in different manners [7].

After the discovery of halogenated DBPs in disinfected waters, researchers have conducted many studies focusing on the toxicity, formation mechanisms, precursors, speciation, control and removal technologies of DBPs [8-10]. DBPs are secondary pollutants formed as a result of the oxidation of natural organic matter (NOM) and inorganic compounds with disinfectants. NOMs, the precursors of DBPs, are primarily responsible for imparting taste, color and odor to water. NOMs, which also affect water quality, are a heterogeneous mixture of carbohydrates, proteins, lignin, and organic acids of various origins and types [6,11].

DBPs consist of a complex mixture having more than 700 defined and many more undefined types [4]. The key factors controlling the formation of DBPs are the character of the raw water source and characteristics of the disinfectant. The spectrum of NOM and halogenated molecules in water, pH, temperature, contact time, distribution system, disinfectant and concentration are variables that affect the formation of DBPs [12-13]. Many countries and international organizations have introduced legal regulations that determine the maximum levels of the most common DBPs due to the potential health risks that may arise from DBPs [14]. DBPs that occur because of chlorination vary according to raw water and disinfection conditions, but the dominant fraction is THMs. Haloacetic acids (HAAs), halonitromethanes, haloacetonitriles (HANs), chloramines, chlorophenols, halofuranones and chloral hydrate can be formed with THMs [12]. It has been shown that humic species (humic acid and fulvic acid) constitute a large part of natural organic substances, which are the precursors of DBPs; these structures are effective in the formation of THM and humic acid (HA) exhibits higher chlorine reactivity than fulvic acid [6]. Humic materials in the surface water originated from the degradation of plants, animal remains and/or soil runoff [11].

THMs are compounds formed by the replacement of active groups in organic compounds in water with halogens such as chlorine, bromine, and iodine and formulated as CHX3 [15]. These include chloroform (TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM), bromoform (TBM), dichloroiodomethane, bromodichloroiodomethane, chlorodiiodomethane, dibromoiodomethane, bromodiiodomethane and iodoform compounds [16]. The main THM compound formed is chloroform, and the presence of bromine in water leads to the formation of bromine and chlorine-based THM compounds such as BDCM and DBCM together with TBM [17]. Since there is little iodine in surface waters, the TCM, BDCM, DBCM and TBM compounds are expressed as total THM (TTHM or THM4), and limitations have been made by conducting many studies on them [16]. Many countries and international organizations have set a maximum limit for THMs, which is 100 µg/L in Europe, Canada and Turkey and 80 µg/L in the USA [12]. Since THMs are a hazard to human health, understanding the effects of humic acid on water disinfection with chlorine in order to control their formation is important for safe drinking water supply [11]. Chloroform and bromodichloromethane are included in the 2B group in the cancer classification made by the International Agency for Research on Cancer (IARC) and are listed as substances that possibly carcinogenic to humans. Dibromochloromethane and bromoform are among the compounds (Group 3) that not classifiable as to its carcinogenicity to humans [18].

Inorganic components in water affect the formation of byproducts during disinfection, and one of the most important inorganic components studied extensively is bromide, the precursor ion of TBM formation [3]. Bromide ions are found in aquatic environments worldwide and are generally at low levels in freshwater. However, depending on the water pollution, salt water intake and geology, higher levels can be found in waters that are close to salt deposits, estuarine waters and waters where urban-industrial wastewater discharges are made [19-20].

Bromide concentrations in natural waters are higher in oceans but generally vary between 4 and 1000 µg/L [21]. On the other hand, its concentration in natural spring waters in Europe varies between 30-200 µg/L, sometimes up to 500 µg/L [22]. The critical value of 50 µg/L bromide in waters is environmentally important. For this reason, the bromide concentration should be checked before disinfection in water [21]. During the disinfection of water containing bromide with chlorine, the bromide ion reacts with HOCl/OCl^- and oxidizes to hypobromous acid (HOBr), which is much more reactive than HOCl. HOBr then reacts with THM precursors to form brominated THMs [23]. Brominated DBPs are known to be more toxic than chlorinated analogues [15, 24]. It has been reported in the literature that bromide ions are approximately 20 times more active against organic substances than chlorine. Therefore, besides the total amount of by-products, species distribution is also a major concern [7].

The most basic method for the control of THMs is the optimization of the chlorine dose. However, reducing the chlorine dose should not result in inadequate disinfection. The use of alternative disinfectants is also one of the methods. The formation of other by-products should be taken into account when choosing an alternative disinfectant. Other approaches to prevent the presence of THM in drinking water: (i) Source control; that is, reducing the precursors, especially humic substances, in the water before disinfection (ii) Removing the formed THMs from the water with appropriate technologies. The coagulation/flocculation process applied during treatment eliminates some natural organic matter. In addition, the use of additional techniques such as adsorption, membrane filtration or ion exchange resin before chlorination increases NOM removal. Air stripping process, adsorption by granular activated carbon, membrane process or UV-based processes are applied to remove the formed THMs [17].

Since chlorine is widely used in the disinfection of water, many studies have focused on the THM precursor HA. In this study, by applying different levels of chlorine to water, the formation of THM compounds, e.g., TCM, BDCM, DBCM and TBM, was investigated. In this context, humic acid was added to deionized water disinfected with UV to represent the real raw water to ensure that the experiments were carried out under controlled conditions, and the effect of bromide ions (Br^-) on THM formation was also examined.

MATERIALS and METHODS

Materials

Artificial raw waters were prepared using deionized water (18.2 MΩ·cm ultrapure water produced by a Sartorius-Arium 611VF model pure water device) disinfected with UV. The stock (5000 mg/L) humic acid solution was prepared using humic acid sodium salt (Sigma-Aldrich H16752) and kept in the dark at 4°C for a maximum of 20 days.

Chlorine solution was freshly prepared at a concentration of 1% (= 10000 mg/L) using 7% stock sodium hypochlorite (Merck 1056142500) solution.

A dosing solution at a concentration of 10 mg/L was prepared from a stock solution containing 1000 µg/mL Br^- (Bromide, High-Purity Standards) in water.

Certified standard solutions: 1000 µg/mL Br^- in water (Bromide, High-Purity Standards).

Certified reference material: SM 137. 1 mg/L bromide, 10 mg/L chloride, 1 mg/L fluoride, 1 mg/L nitrate, 5 mg/L phosphate and 10 mg/L sulphate in water (SM-137-801, High-Purity Standards).

Certified standard solution: 200 µg/mL TCM, 200 µg/mL BDCM, 200 µg/mL DBCM and 200 µg/mL TBM in methanol (THM mix, High-Purity Standards).

Internal Standard (IS): 2000 µg/mL fluorobenzene in methanol (AccuStandard).

Experimental Design

Water samples containing 2 mg/L, 3 mg/L and 5 mg/L humic acid were prepared using stock humic acid solution in deionized water. The water samples at different concentrations were subjected to chlorination at doses of 1 mg/L, 2 mg/L and 3 mg/L. The disinfected water samples were kept in sealed amber-coloured glass bottles under room conditions to prevent the loss of volatile byproducts, and samples were analysed on the 0^{th}, 3^{rd} and 7^{th} day. All experiments, including blind ones, were performed in triplicate at 18.5±3°C to minimize the effect of temperature variation.

In order to investigate the effect of bromide ion, 200 µg/L Br^- was added to water containing 2 mg/L humic acid, and 1 mg/L and 2 mg/L chlorination was performed.

Disinfection by Chlorination

For 1 and 3 liter volumes, necessary amounts of chlorine solution (for 1, 2 and 3 mg/L) were added to amber-coloured bottles. For sufficient effectiveness of chlorine, a 30-min contact time was given, and afterwards, the analyses were performed. While the stock chlorine solution was kept in the dark at 4±2°C, the dosing solution was freshly prepared before disinfection.

Analysis of pH, Conductivity, Temperature and Colour

Determinations of pH and conductivity were made according to ISO 10523 [25] and EN 27888 [26], respectively, with a Thermo Orion Star 215 Model pH and conductivity measuring device. Temperature was determined with the thermometer included in the same device and colour was measured at 465 nm as a PtCo unit by using a Hach Lange DR 2800 spectrophotometer, respectively [27-28].

Chlorine Analysis

The amounts of free chlorine and bound chlorine in the water samples were determined titrimetrically according to the ISO 7393-1 method [29]. This method is suitable for the determination of total chlorine in the range of 0.03 - 5 mg/L. In the assay pH 6.5 buffer solution, N,N-diethyl-1,4-phenylenediamine sulphate (DFD) solution, ammonium iron (II) sulphate titration solution and potassium iodide (KI) were used. All solutions were kept in the dark at +4°C and used considering the times specified in the method. Bound chlorine was calculated by subtracting the free chlorine content from the total chlorine content.

Bromide Analysis

Bromide analyses were performed by liquid chromatography according to the EN ISO 10304-1 method [30]. The working conditions were as follows:

Device: Thermo Dionex ICS-5000+ Ion Chromatography; pump unit: isocratic pump (DP); eluent generator: EGC III KOH (produces KOH for anion analysis); detector: conductivity (DC); column: Dionex AS19 Analytical column - 4x250 mm; front column: Dionex AG19 Guard column - 4x50 mm; suppressor: anion suppressor, CSRS 4 mm; autosampler: AS-DV; injection volume: 25 µL; firmware: Chromelon; mobile phase: produced automatically from deionized water with an eluent generator. The retention time was 14.45 min, and the limit of quantification (LOQ) was 20 µg/L.

Calibration: Calibration standards were prepared in deionized water at concentrations of 0.020-0.050-0.125-0.25-0.5-1 mg/L. Standard solutions were prepared in PE bottles and kept at 4 °C in the dark. The calibration graph ($y = 1.0072x-0.0363$, $R^2 = 0.9999$) was drawn with 6 points.

Trihalomethane Analysis

Total THM analyses were performed on a purge and trap (P&T) gas chromatography-mass spectrometry (GC-MS) system according to the EPA method [31]. The purge and trap extraction method does not require any preparation. Water samples were filled to the brim in 40 mL glass vials so that no air gap was left and then injected directly into the device.

The working conditions were as follows:

P&T terms: purge and trap unit: Teledyne Tekmar/Atomx (Serial No: US13302007); injection volume: 5 mL, transfer line temperature: 140 °C, valve oven temperature: 140 °C, and purge flow rate: 40 mL/min.

Trap10 (tenax): purge time: 10 min, trap temperature: 180°C, dry purge time: 1 min, desorp preheating temperature: 245°C, desorp temperature: 250°C, desorp time: 2 min, bake temperature: 260°C, and bake time: 2 min.

GC-MS conditions: device: Agilent Technologies 7890A GC System (Serial Number: CN12481071).

Inlet temperature: 180°C, split ratio: 5:1, flow rate: 1.2 mL/min, column: Agilent DB-624, 30 m x 0.25 mm x 1.40 µm, detector: Agilent Technologies 5975C inert XL MSD with Triple-Axis Detector, acquisition mode: SIM, and runtime: 19.79 min.

Oven: The oven temperature program is single ramp. After being held constant at 40°C for 4 minutes, it increases by 10°C per minute until reaching 220°C, where it is held steady for 2 minutes at this temperature.

In all analyses, an internal standard solution (fluorobenzene) prepared at a concentration of 125 mg/L and loaded in the device was used. Samples were placed in 40 mL screw septum-capped glass vials.

Calibration: TCM, BDCM, DBCM, and TBM working standards were prepared as mixtures from the intermediate stock solution diluted at a concentration of 2 mg/L in methanol (Sigma-Aldrich 24229) in the range of 0.2 - 100 (0.2-0.5-1-5-10-25-50-100) µg/L using deionized water. The calibration curve was plotted using 25 µg/L fluorobenzene as an internal standard. To determine the high concentrations of TCM, a curve in the range of 5 - 200 (5-10-25-50-100-200) µg/L was also drawn. Data (curve equation, linearity, LOQ, retention time and ions) for the tested THMs are given in Table 1.

Statistical Analysis

The effect of different chlorine doses applied to water samples with different concentrations of humic acid on trihalomethane formation was evaluated using variance analysis in three replicate factorial patterns. According to the results of variance analysis, the differences between groups were evaluated using the One-way-ANOVA test. The possible difference caused by chlorine doses in the effect of bromide ions on by-product formation was evaluated by one-way analysis of variance (ANOVA). Open source R statistics software [32] and SPSS package program were used in the statistical calculations.

Table 1. Limit of quantification (LOQ), retention time, fragmentation ions and linearity data for trihalomethanes

Trihalomethane	Equation (y=ax+b)	Linearity	LOQ (µg/L)	Retention time (min)	Ions (m/z)
TCM	y=0.9760x -0.0034	R^2=0.9993	0.2	10.26	83, 85, 47
BDCM	y=0.9619x+0.0031	R^2=0.9992	0.2	11.56	83, 85, 129
DBCM	y=0.9369x+0.0221	R^2=0.9988	0.2	12.67	129, 127, 131, 79
TBM	y=0.9141x+0.0385	R^2=0.9986	0.2	13.73	173, 171, 175, 105
Fluorobenzene (IS)	-	-	-	10.92	96, 70, 95, 97

RESULTS and DISCUSSION

Effects of Different Chlorine and Humic Acid Doses on THM Formation

According to the results, the chlorination process led to the formation of only TCM in water samples containing different amounts of HA. Other THM compounds were not formed. The amount of TCM formed depending on the chlorine and HA doses and analysis day is shown in Table 2. When the chlorination dose is constant, the amount of TCM formed is directly related to the amount of HA in water. As the amount of HA increased, the amount of TCM formed increased. At the same time, when the amount of HA was constant, the formed TCM amount increased as the applied chlorine dose increased.

Table 2. Effects of various chlorine and humic acid doses on chloroform formation [a, b, c]

Chlorine (mg/L)	Humic acid (mg/L)	Chloroform (µg/L)		
		Day 0	Day 3	Day 7
Control (0)	2	0	0	0
	3	0	0	0
	5	0	0	0
1	2	20.52±1.25^{aA}	21.02±1.56^{aA}	20.37±1.42^{aA}
	3	29.50±3.95^{aA}	32.35±3.59^{aA}	34.50±3.97^{aB}
	5	43.73±5.68^{aB}	46.64±5.46^{aB}	45.84±3.57^{aC}
2	2	28.98±0.95^{aA}	33.70±2.04^{abA}	39.30±4.23^{bA}
	3	57.22±7.53^{aB}	60.59±8.25^{aA}	60.73±6.71^{aA}
	5	108.59±11.81^{aC}	108.22±11.44^{aB}	106.92±10.92^{aB}
3	2	36.18±1.15^{aA}	38.85±1.91^{abA}	45.19±3.27^{bA}
	3	71.47±8.69^{aB}	76.05±9.48^{aB}	83.15±8.99^{aB}
	5	120.42±7.31^{aC}	124.79±7.08^{aC}	131.13±6.70^{aC}

[a]: Values shown in the table are average values of three replicates (±standard deviation). [b]: For the same humic acid amount, different letters (a-b) in the same line indicate statistically significant differences ($p<0.05$). [c]: For the same chlorine amount, different letters (A-C) in the same column indicate statistically significant differences ($p<0.05$).

Contact time, which is also responsible for the formation of THMs, is a critical factor for inactivating microorganisms. Many studies have shown that THM levels increase with increasing contact time. It has been reported that THM formation is faster in the beginning and slows down over time [17].

THM formation, which starts with disinfection in the water treatment process, can continue in the distribution system due to the release of residual free chlorine in the water. In a study, it was observed that by increasing the contact time from 30 min to 24 hours, there was a two- to three-fold increase in the total THM and haloacetic acid concentration. DBPs can continue to form as long as residual disinfectant and precursor are present, and the rate of formation varies greatly according to the properties of organic matter [13]. In another study showing the effect of contact time, the concentrations of total THMs and individual compounds increased with a longer contact time, but the concentration of haloacetic acid showed little change with the contact time. The greatest change in concentration with contact time was obtained for TCM. The amount of TCM, which was 0.2 - 2 µg/L in one hour of contact time, increased to 2.5 - 4 µg/L after 24 hours [33]. In another study, the concentration of THMs in the distribution system increased by an average of 36% [34]. The available information shows that THMs occur with a rapid onset formation during chlorination, followed by a formation that continues with a slower increase rate over time. During chlorination, both the reactive group and free chlorine are reduced, which reduces the reaction rate and inhibits the formation of THMs [7].

In our study, when the chlorine dose was constant, the amount of TCM formed increased linearly with the contact time. The presence of free chlorine in the environment caused TCM formation to continue. Since the samples were kept closed, increased levels of TCM were detected even at the end of the 7th day. The EU limit is 100 µg/L for THMs in drinking waters. The TCM content was determined to be 20.52 - 131.13 µg/L in this study. As a result of chlorination of 5 mg/L HA with 2 mg/L and 3 mg/L Cl_2, it was observed that the amount of TCM formed at the end of day 0, day 3 and day 7 varied

between 106.92 and 131.13 µg/L, which exceeded the legislative limit.

Effect of Bromide Ion on THM Formation

After 1 and 2 mg/L chlorine treatment, only TCM was formed in no bromide-free samples, while BDCM, DBCM, TBM and TCM were formed in waters containing bromide. It has been determined that Br^- ions are more reactive to NOMs than chlorine and that the type of THM changes due to the presence of bromide. When the formation of brominated THM increases, the amount of chlorinated THM species decreases. The applied chlorine dose was effective on the amount of TCM formed. The amounts of TTHMs formed at the end of the 1 mg/L and 2 mg/L chlorination processes were 50.03 µg/L and up to 85.60 µg/L, respectively (Fig. 1).

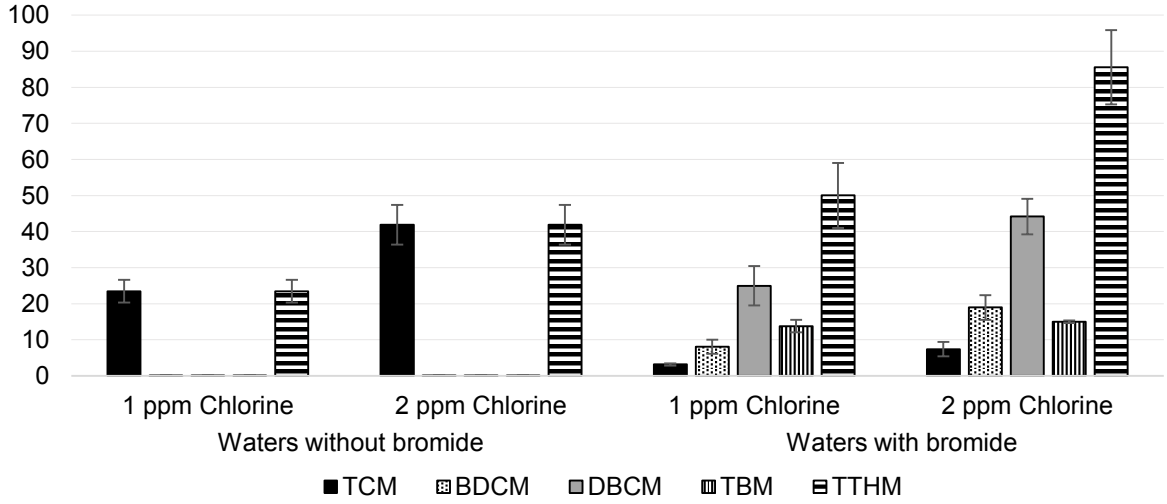

Figure 1. Effects of bromide on THM formation (µg/L) with different chlorine doses. [a]TCM: Trichloromethane (chloroform); BDCM: Bromodichloromethane; DBCM: Dibromochloromethane; TMB: Tribromomethane (bromoform); TTHM: Total THMs. [b]THMs were not formed in control (0 mg/L chlorine) samples

In a study conducted by Sakai et al [8], TBM could not be determined in the chlorination of waters containing different levels of total organic carbon (1.45 - 6.84 mg/L) and Br^- (32-222 µg/L), while the highest TCM was formed among the four tested THM types, followed by BDCM. While the DBCM content showed a high correlation with bromide, no relationship was observed between organic matter and DBCM formation.

In our study, when waters containing 200 µg/L Br^- were subjected to a 1 mg/L chlorination process, the order of formed compounds was found to be DBCM> TBM> BDCM> TCM THM. When the amount of chlorine is increased to 2 mg/L, there is a shift from brominated DBPs to chlorinated byproducts, as chlorine can compete with the more reactive Br^-. By observing an increase in the amount of BDCM, the order changed to DBCM> BDCM> TBM> TCM (Fig. 1). This finding shows the importance of bromide and chloride levels in determining the distribution of THMs formed. In a study investigating the effect of bromide ion amount on THM formation during chlorination [35], the order of DBCM> TBM> BDCM> TCM in the presence of 200 µg/L Br^-, 9.61 µg/L DBCM, 4.49 µg/L TBM, 4.47 µg/L BDCM and 3.65 µg/L TCM were observed.

In a study on chlorination of different concentrations of Br^-containing waters, the order of the amount of THMs formed at low bromide ion contents was TCM> DBCM> BDCM> TBM. With increasing Br^-, the content of TCM decreased sharply, and BDCM and DBCM levels decreased after rising in the first stage, while the TBM concentration increased continuously. At high bromide ion concentrations (>500 µg/L), TBM becomes the main component [24]. In another study where similar results were obtained, TCM was the main component, with a proportion of 40 - 98% in spring water forming 14.2 - 143 µg/L TTHM, followed by BDCM [34].

Padhi et al [13] found 38.3 µg/L THMs in sea water, 18.8 µg/L in river water and 21.5 µg/L in tank water at a 1 mg/L Cl_2 dose and 30 min contact time. TBM has been observed to be the only dominant species constituting approximately 90% of the total THMs in seawater containing high amounts of bromide. It has been reported that the distribution of THM species in river water is in the order of TCM ~ DCBM> CDBM> TBM, and the distribution between chloro and bromo analogues of THMs primarily depends on the bromide concentration in raw water.

The bromine atom has a higher molecular weight than chlorine. Therefore, the mass concentration of by-products increases upward with increasing Br^- level [17]. In our study, it was found that the presence of 200 µg/L bromide caused an average 108.76% increase in the amount of TTHM in chlorination processes. The amount of TTHM, which was 23.46 µg/L in the 1 mg/L chlorination process, increased to 50.03 µg/L in the presence of bromide. Likewise, for 2 mg/L chlorination, these values increased from 41.90 µg/L to 85.59 µg/L. Chlorination of water typically yields chloroform from

total THM compounds. Chlorination of bromine-containing waters resulted in the formation of Br-THM, and the presence of Br and chlorinated by-product species shifted towards mixed brominated-chlorinated THMs.

Effect of Treatments on Free, Total and Bound Chlorine Levels

The effects of HA content and chlorine dose on the amount of free chlorine, total chlorine and bound chlorine are given in Table 3.

Table 3. The effects of humic acid content and chlorine dose on the amount of free chlorine, total chlorine and bound chlorine [a, b, c]

Residue chlorine	Humic acid (mg/L)	Chlorine (mg/L)			
		0	1	2	3
Free chlorine	2	0^{a}	0.73 ± 0.03^{bA}	1.50 ± 0.04^{cA}	2.31 ± 0.01^{dA}
	3	0^{a}	0.62 ± 0.03^{bA}	1.47 ± 0.03^{cA}	2.29 ± 0.06^{dA}
	5	0^{a}	0.54 ± 0.09^{bA}	1.290 ± 0.16^{cA}	1.64 ± 0.18^{cB}
Total chlorine	2	0^{a}	0.73 ± 0.03^{bA}	1.51 ± 0.05^{cA}	2.38 ± 0.02^{dA}
	3	0^{a}	0.64 ± 0.02^{bA}	1.49 ± 0.04^{cA}	2.36 ± 0.08^{dA}
	5	0^{a}	0.55 ± 0.09^{bA}	1.31 ± 0.17^{cA}	1.65 ± 0.18^{cB}
Bound chlorine	2	0^{a}	0.006 ± 0.006^{aA}	0.006 ± 0.003^{aA}	0.07 ± 0.02^{bA}
	3	0^{a}	0.02 ± 0.01^{abA}	0.02 ± 0.01^{abA}	0.07 ± 0.03^{bA}
	5	0^{a}	0.01 ± 0.01^{aA}	0.01 ± 0.01^{aA}	0.006 ± 0.006^{aA}

[a]: Values shown in the table are average values of three replicates (±standard deviation). [b]: For the same humic acid amount, different letters (a-c) in the same line indicate statistically significant differences ($p<0.05$). [c]: For the same chlorine amount, different letters (A-B) in the same column indicate statistically significant differences ($p<0.05$).

According to the results, while the chlorine dose was constant, the increase in the amount of HA caused a decrease in the amount of free chlorine. When the amount of HA was constant, the amount of free chlorine increased as the chlorine dose increased. The term "residual disinfectant" is used for the concentration of chlorine detected in the water after the disinfectant has been applied in the disinfection process. The effectiveness of chlorine depends on the amount applied and the residual disinfectant concentration. The stability of the disinfectant under the application conditions and the presence of chlorine-requiring substances in the disinfection environment greatly affect the residual disinfectant level. Natural organic matter, PAHs, pesticides, and metals in water react with chlorine and therefore increase the demand for disinfectants in practice. Residual disinfectants, i.e., free chlorine, can significantly affect disinfection and by-product formation [36-37]. Accordingly, the results show that increasing the amount of HA increases the need for disinfectants.

Effects of Treatments on pH, Electrical Conductivity and Colour

The effects of the different chlorine and HA doses on pH, electrical conductivity and colour are given in Table 4. The increase in the chlorine dose caused an insignificant increase in the pH. It has been reported that pH is effective for DBP formation and THM formation increases with increasing pH [17]. In a study, it was observed that increasing the pH from 5.0 to 9.0 increases the formation of THM during chlorination [2]. However, the effect of pH on THM formation was not investigated in our study. It was observed that the increased active chlorine and HA content resulted in increased conductivity. While increasing the HA dose increased the colour, some colour loss occurred with oxidation caused by chlorine.

Table 4. The effects of humic acid content and chlorine dose on several analytical parameters [a, b, c]

Analyte	Humic acid (mg/L)	Chlorine (mg/L)			
		0	1	2	3
pH	2	5.67 ± 0.16^{aA}	6.31 ± 0.18^{bA}	6.87 ± 0.01^{cA}	7.18 ± 0.09^{cA}
	3	6.21 ± 0.27^{aA}	6.34 ± 0.09^{aA}	6.94 ± 0.08^{bA}	7.32 ± 0.12^{bA}
	5	6.50 ± 0.32^{aA}	6.95 ± 0.41^{aA}	7.21 ± 0.28^{aA}	7.34 ± 0.30^{aA}
Conductivity (µS/cm)	2	1.69 ± 0.07^{aA}	8.99 ± 0.1^{bA}	16.73 ± 0.11^{cA}	24.40 ± 0.22^{dA}
	3	2.19 ± 0.29^{aA}	9.11 ± 0.54^{bA}	16.39 ± 13.46^{cA}	23.75 ± 0.93^{dA}
	5	3.47 ± 0.55^{aB}	16.09 ± 2.91^{bB}	23.74 ± 2.92^{cB}	26.40 ± 0.99^{cA}
Colour (PtCo)	2	33.00 ± 2.08^{aA}	24.33 ± 0.67^{bA}	23.33 ± 1.20^{bA}	20.67 ± 0.33^{bA}
	3	47.33 ± 3.71^{aA}	34.33 ± 4.70^{abA}	31.00 ± 4.00^{bA}	29.67 ± 3.93^{bA}
	5	94.67 ± 6.64^{aB}	75.67 ± 4.63^{bB}	68.67 ± 2.40^{bB}	64.67 ± 3.33^{bB}

[a]: Values shown in the table are average values of three replicates (±standard deviation). [b]: For the same humic acid amount, different letters (a-d) in the same line indicate statistically significant differences ($p<0.05$). [c]: For the same chlorine amount, different letters (A-B) in the same column indicate statistically significant differences ($p<0.05$).

Effects of Bromine on Free, Total and Bound Chlorine and Several Water Quality Parameters

The effects of chlorine dose and bromine addition at a constant HA dose of 2 mg/L on the amount of free chlorine, total chlorine and bound chlorine and several other water quality parameters are given in Table 5. The bromide ion did not have a significant effect on pH, electrical conductivity, free chlorine or colour. pH along with bromide ions is an important factor in the formation of THM. Therefore, by-product formation should be considered together with factors that can act simultaneously, such as bromide, pH, chlorine dose, amount of HA, and contact time. It was shown that an alkaline pH resulted in increased THM formation [7]; however, in our study, the effect of bromide ions on pH was not evaluated.

Table 5. The effect of bromide presence on some water quality parameters and residual disinfectant levels with various chlorine (chl.) treatments [a, b]

Analyte	2 mg/L humic acid			2 mg/L humic acid and 200 µg/L Br^-		
	Chlorine (mg/L)					
	0	1	2	0	1	2
pH	5.90±0.26^{A}	6.62±0.21^{B}	7.03±0.17^{C}	6.35±0.16^{B}	6.59±0.15^{B}	6.99±0.13^{C}
Conductivity (µS/cm)	1.64±0.04^{A}	8.97±0.05^{B}	16.66±0.03^{C}	1.73±0.13^{A}	8.37±0.53^{B}	15.46±0.72^{C}
Temperature (°C)	18.80±1.6^{B}	18.40±1.1^{B}	18.40±1.2^{B}	16.40±0.1^{A}	17.30±0.2^{AB}	17.70±0.7^{AB}
Colour (PtCo)	30.00±1.0^{C}	23.00±1.0^{B}	22.00±2.0^{B}	28.00±2.0^{C}	19.00±0.0^{A}	17.00±1.0^{A}
Bromide (µg/L)	-	-	-	150.00±17.32^{B}	28.00±1.16^{A}	26.00±2.00^{A}
Free chl. (mg/L)	-	0.63±0.03^{A}	1.52±0.06^{B}	-	0.58±0.01^{A}	1.38±0.04^{B}
Total chl. (mg/L)	-	0.65±0.02^{B}	1.55±0.05^{C}	-	0.58±0.01^{A}	1.39±0.04^{C}
Bound chl. (mg/L)	-	0.02±0.01^{A}	0.03±0.02^{A}	-	0.00±0.00^{A}	0.01±0.00^{A}

[a]: Values shown in the table are average values of three replicates (±standard deviation). [b]: For each of the analytes, different letters (A-C) indicate statistically significant differences ($p<0.05$), and at least one common letter indicates that the difference is nonsignificant ($p>0.05$).

CONCLUSION

Increasing the amount of HA and chlorine increased the formation of THM, which has negative effects on health. In the European Union, the limit value for the total amount of THM in drinking water is 100 µg/L. Chlorination of 5 mg/L HA-containing waters with 2 mg/L and 3 mg/L Cl_2 resulted in TCM contents between 106.92 µg/L and 131.13 µg/L at the end of days 0, 3 and 7. All of these values exceeds the legislative limit. This means that chlorination of high HA-containing waters with 2 or 3 mg/L chlorine is not suitable for such waters. In this type of water, the amount of HA should be reduced by treatment techniques such as coagulation and activated carbon adsorption, or alternative disinfection methods such as ozonation should be evaluated.

When the chlorine dose was constant, it was found that increased contact time (0, 3 and 7 days) increased TCM formation. The presence of free chlorine in water samples caused continuous TCM formation. This shows that THM formation, which starts with disinfection, can continue in the distribution network when residual free chlorine is found in the water.

As a result of chlorination, only TCM was formed in water samples containing different amounts of HA representing natural organic substances. In the presence of bromide in water, bromine-containing halomethanes are formed as a result of chlorine treatment. DBCM occurred as the dominant species.

The presence of bromide ions in water increased THM formation from 23.46 - 41.90 µg/L to 50.03 - 85.59 µg/L and changed the THM diversity. Therefore, the presence and amount of bromide in the water is a critical factor to consider when choosing the disinfection method.

When the effects of chlorine and HA applications on free, total and bound chlorine levels and pH, electrical conductivity and colour are examined, an increase in the chlorine dose generally caused an increase in the amount of free, total and bound chlorine, pH and electrical conductivity, and a decrease in colour. As the content of HA increased, the electrical conductivity and colour increased, while the amount of free, total and bound chlorine decreased.

Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Research Paper / Araştırma Makalesi

Effect of Dietary Fiber Enrichment on Quality Characteristics and Consumer Acceptance of Fruit Snacks

Özge Taştan

Yeditepe University, Faculty of Engineering, Department of Food Engineering, 34755 Ataşehir, İstanbul

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Corresponding author (Yazışmalardan Sorumlu Yazar): ozge.tastan@yeditepe.edu.tr (Ö. Taştan)
+90 507 792 3463 +90 216 578 0400

ABSTRACT

In this study, different fiber sources such as inulin, peas and carrots were used to produce fruit snacks enriched with dietary fiber. The effect of these fiber sources on the proximate composition, pH, titratable acidity, water activity, color, texture, total phenolic content, microbial load, and sensory acceptability of fruit snacks was determined. Results showed that fruit snacks enriched with inulin (5.0%) had the highest content of total dietary fiber while snacks enriched with carrot fiber (5.0%) had the highest total phenolic content. The moisture content of fruit snacks with different fibers decreased in comparison to control snacks as fiber was added into their formulation. Moreover, the water activity values of fruit snacks (a_w) were lower than 0.7, indicating a low risk for bacterial growth and affirming a favorable shelf life. Compared to control snacks, increasing the dietary fiber addition from 2.5 to 5.0% significantly increased the hardness, gumminess and chewiness values of fruit snacks. The microbiological analysis of fruit snacks indicated that snacks were safe. Additionally, results showed that fruit snacks (2.5%) enriched with inulin and pea fiber were found to have higher sensory acceptability scores than others.

Keywords: Fruit pastes, Snack, Inulin, Pea fiber, Carrot fiber, Quality properties

Meyveli Atıştırmalıkların Diyet Lifiyle Zenginleştirilmesinin Kalite Özellikleri ve Tüketici Kabulüne Etkisi

ÖZ

Bu çalışmada, diyet lifi ile zenginleştirilmiş meyveli atıştırmalıklarının üretilmesi amacıyla inülin, bezelye ve havuç lifi gibi farklı lif kaynakları kullanılmıştır. Farklı lif kaynaklarının meyve atıştırmalıklarının temel bileşimi, pH, titre edilebilir asitlik, su aktivitesi, renk, tekstür, toplam fenolik madde içeriği, mikrobiyal yük ve duyusal kabul edilebilirliği üzerindeki etkileri incelenmiştir. Sonuç olarak, inülin ile zenginleştirilmiş meyve atıştırmalığının (%5.0) en yüksek toplam diyet lifi içeriğine sahip olduğu belirlenirken, diğer yandan havuç lifi (%5.0) ile zenginleştirilmiş meyve atıştırmalığı ise en yüksek toplam fenolik madde içeriğindedir. Formülasyona diyet lifi ilave edildiğinde, kontrol grubuna göre meyve atıştırmalığı örneklerinin nem içeriği azalmıştır. Ayrıca, örneklerin su aktivitesi değerlerinin 0.70'in altında olduğu belirlenmiş olup, bu da bakteriyel gelişme riskinin düşük olduğunu ve raf ömrünün de uzun olduğunu göstermektedir. Kontrol grubuna kıyasla, meyve atıştırmalıklarına ilave edilen lif içeriğinin %2.5'ten %5.0'a çıkarılması durumunda, örneklerin sertlik, sakızımsılık ve çiğnenebilirlik değerlerini önemli ölçüde artmıştır. Meyve atıştırmalıklarının mikrobiyal analiz sonuçlarına göre, örneklerin mikrobiyolojik olarak güvenli olduğunu belirlenmiştir. Ayrıca, bu çalışmada elde edilen sonuçlara dayanarak, inülin ve bezelye lifi ile zenginleştirilmiş meyve atıştırmalıklarının (%2.5) diğerlerine göre daha yüksek duyusal kabul edilebilirliğe sahip olduğu görülmüştür.

Anahtar Kelimeler: Meyve ezmesi, Atıştırmalık, İnülin, Bezelye lifi, Havuç lifi, Kalite özellikleri

INTRODUCTION

Many consumers frequently enjoy snack products available on the market, often including chocolates, chips, and wafers that are high in fat, refined sugar, and calories. Manufacturers of these snacks frequently emphasize taste over nutritional content. However, in recent times, there has been a notable increase in the demand for healthier snack alternatives that are rich in vitamins, minerals, and dietary fiber while containing minimal amounts of oil. This shift in consumer preferences is driven by a heightened awareness of the health impact of their dietary choices [1].

Fruits are essential for sustaining a balanced and nourishing diet because they provide a wealth of energy, dietary fiber, minerals, and vitamins. Fruit snacks are concentrated fruit-based products including the mixture of various fruit pastes and nuts, which have high nutritional value with long shelf life and can be classified as confectionery [2]. A fruit snack such as fruit bar, ball, freeze-dried fruits, impregnated fruits serve as a convenient option for enjoying fruits even when they are out of season, and it offers concentrated nutritional value as it is made from dried fruits. The production of fruit snacks can vary significantly among different manufacturers, depending on their unique formulation and processes. Ingredients like fruit pulp, both fresh and dried fruits, sugars (such as sucrose, maltodextrin, glucose syrup, and fruit juice concentrates), binding agents (like pectin, glycerol, and various carbohydrates especially fibers), and additional components like colorants, flavors, and acids can all be utilized in the manufacturing of these tasty snacks [3]. Consumption of fruit-based snacks has increased the intake of nutrients and phytochemicals, resulting in beneficial health effects and potentially aiding individuals in achieving the recommended daily intake.

Food fortification or enrichment is the process of adding essential nutrients to a food product, regardless of whether these nutrients are naturally found in the food, in order to prevent or address deficiencies within the population. Fruit-based bars are economical and convenient functional foods that can replace fresh fruits and vegetables as a source of various essential nutrients. As consumer demand for healthy, natural, and convenient food choices continues to increase, fruit-based snacks are becoming increasingly popular and can serve as excellent options for delivering health benefits to consumers [4].

Dietary fibers (DF) comprise the edible portions of fruits and vegetables that resist digestion by human enzymes and absorption in the small intestine, resulting in a fermentation process carried out by gut microbiota in the large intestine. DF exhibits diverse physiological properties, influenced by its solubility, physicochemical characteristics, and two fractions: insoluble fiber (IF) and soluble fiber (SF). On one hand, IF primarily enhances fecal bulk due to its porosity and low density, stimulates intestinal motility, increases defecation frequency, thus reducing transit time, and traps toxins. On the other hand, SF is more prone to fermentation by colonic bacteria, influencing gut microbiota and generating short-chain fatty acids, regulates satiety, forms gels that lower blood glucose and moderate plasma cholesterol levels, and enhances viscosity, reducing glycemic response and limiting the absorption of cholesterol and free fatty acids [5, 6].

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) advise a daily intake of over 25 grams of DF, primarily sourced from consuming 400 grams of fruits and vegetables. Furthermore, insufficient DF intake is linked to diseases such as obesity, diabetes mellitus, inflammatory bowel disorders, various types of cancer, constipation, and diverticulosis. To combat these health issues, the food industry is actively developing DF-enriched foods to encourage greater DF consumption. In pursuit of this goal, the food industry is exploring new DF sources [6, 7].

While processing fruits and vegetables like apples, oranges, carrots, peas, etc. various by-products are generated, containing valuable compounds. Notably, the fiber component stands out for its significant potential in creating functional foods. Plant fibers exhibit specific functional attributes, including their ability to retain water and expand in volume. Key features of commercially available plant fibers include a dietary fiber content exceeding 50%, moisture content below 9%, minimal lipid content, low calorie level, and a neutral flavor profile [8].

Due to the health benefits of dietary fiber enrichment for fruit-based snacks, there is a tendency to make a nutrition claim in the development of fruit snacks. According to the Food and Drug Administration (FDA), to have a food product with a ‘‘high in fiber’’ or “excellent source of fiber” and ‘‘good source of fiber’’ claim, it must contain, 20% or more fiber and 10-19% of fiber of the recommended daily value for dietary fiber in a serving size, respectively [5]. In the Turkish Food Codex, the food must contain at least 6 g of fiber in 100 g of product to make ‘‘high in fiber’’ or “excellent source of fiber” claim [9].

Fiber-enriched fruit snacks offer a combination of health benefits, convenience, and taste, making them an important component of a balanced and nutritious diet. In addition, high-fiber snacks can contribute to a feeling of fullness, reducing overall calorie intake and supporting weight management efforts. The fiber-enriched fruit snacks provide a convenient and portable way to incorporate healthy foods into a busy lifestyle. They are easy to pack and consume, making them suitable for various settings, including work, school, or travel. The popularity of fiber-enriched fruit snacks can drive innovation in the food industry, leading to the development of new, healthier snack options. Therefore, the food industry is currently exploring alternative plant fiber sources to increase total fiber content of fruit snacks while maintaining the quality characteristics and consumer acceptability of enriched fruit snacks.

Currently, there is a lack of research regarding the enrichment of various dietary fibers in fruit snacks produced by date and apricot paste which are highly nutritious containing minerals, vitamins, and digestible carbohydrates. Thus, the objective of this study was to assess the fiber enrichment at levels of 2.5% and 5% of plant fibers impacts the chemical, physical, microbial, and sensory characteristics of these snacks. In addition, making fiber claim on the label of enriched fruit snacks will also be evaluated.

MATERIALS and METHODS

Materials

Pasteurized fruit pastes (apricot and date) were supplied from a local company in İzmir, Türkiye. Different fibers as inulin (Vegrano, Belgium), pea fiber (Miller, Turkey), and carrot fiber (Unipektin, Germany) were used for enrichment studies. Sunflower oil (Yudum, Türkiye) was used in the formulation of fruit snacks. All chemicals used for analysis was purchased from Sigma Aldrich with analytical grade (Darmstadt, Germany). Peptone and mediums for microbiological analysis also purchased from Merck (Darmstadt, Germany).

Methods

Production of Fruit Snacks

Fruit snacks were prepared according to the formulations given in Table 1. After weighing the ingredients, all materials without sunflower oil were blended using a mixer for 5 min until a homogeneous mixture was obtained. Then, the pieces were taken as 10 ± 0.5 g and manually shaped as ball. The sunflower oil was used in shaping step to prevent sticking the snacks. After that, fruit snacks were rested at room temperature (20 ± 2 °C) for 24 h before packaging. 100 g of fruit snacks were packed in polyethylene bags and stored at room temperature for further analysis.

Table 1. Formulations of fruit snacks enriched by various plant fibers

Code	Sample name	Type of dietary fiber added	Formulation
C	Control	-	50% apricot paste, 49.5% date paste, 0.5% sunflower oil
I-1	Inulin enriched fruit snack-1	Inulin	50% apricot paste, 47% date paste, 2.5% inulin, 0.5% sunflower oil
I-2	Inulin enriched fruit snack-2	Inulin	50% apricot paste, 44.5% date paste, 5% inulin, 0.5% sunflower oil
PF-1	Pea fiber enriched fruit snack-1	Pea fiber	50% apricot paste, 47% date paste, 2.5% pea fiber, 0.5% sunflower oil
PF-2	Pea fiber enriched fruit snack-2	Pea fiber	50% apricot paste, 44.5% date paste, 5% pea fiber, 0.5% sunflower oil
CF-1	Carrot fiber enriched fruit snack-1	Carrot fiber	50% apricot paste, 47% date paste, 2.5% carrot fiber, 0.5% sunflower oil
CF-2	Carrot fiber enriched fruit snack-2	Carrot fiber	50% apricot paste, 44.5% date paste, 5% carrot fiber, 0.5% sunflower oil

General Composition Analysis and Energy Content

The proximate composition including moisture, ash, protein, fat, and total dietary fiber of fruit snacks was determined by AOAC [10]. The total carbohydrates (%) were calculated by differences from the protein, fat, moisture, ash, and crude fiber contents (Eq. 1). Total energy content of fruit snacks was calculated by Equation 2. All measurements were performed in triplicate.

$$\text{Total Carbohydrate (\%)} = [100 - (\text{Protein} + \text{Fat} + \text{Moisture} + \text{Ash} + \text{Fiber})] \quad \text{(Eq.1)}$$

$$\text{Energy (kcal/100 g)} = \text{Protein} * 4 + \text{Carbohydrate} * 4 + \text{Fat} * 9 \quad \text{(Eq.2)}$$

pH, Total Titratable Acidity and Water Activity

The pH of the fruit snacks was measured in a suspension obtained from a mixture of 5 g of sample with 50 mL of deionized water, using a pH meter (Radiometer Analytical, PHM210, France). Total titratable acidity was determined by potentiometric titration method with 0.1 N NaOH and the results were reported as g of citric acid/100 g of sample. Water activity of fruit snacks was determined using a water activity meter (Rotronic, HygroPalm AW, Switzerland) at 25 °C.

Color

The L*, a* and b* values of fruit snacks were determined using a Chroma meter CM-5 (Konica Minolta, Tokyo, Japan), which had been calibrated using a standardized white plate. Color difference (ΔE*) and chroma (C*) of fruit snacks compared to control snack, which has no fiber addition, was calculated using Equation 3 and 4, respectively.

$$Color\ difference\ (\Delta E^*) = \sqrt{\left(L^* - L^*_{ref}\right)^2 + \left(a^* - a^*_{ref}\right)^2 + \left(b^* - b^*_{ref}\right)^2} \quad \text{(Eq. 3)}$$

$$Chroma\ (C^*) = \sqrt{a^{*2} + b^{*2}} \quad \text{(Eq. 4)}$$

Texture

Textural properties such as hardness, adhesiveness, cohesiveness, gumminess, and chewiness are important features of fruit snacks. To assess the texture profile analysis of the fruit snacks, a texture analyzer (TA-XT2 Plus, Stable Microsystems, UK) equipped with a 36 mm cylindrical probe and 5 kg of load cell was employed. The analysis involved the use of 8 different fruit snacks. The sample underwent compression to a depth of 30% at a pre-test speed of 1 mm/s, followed by testing at a speed of 3 mm/s, and finally, post-testing at a speed of 10 mm/s [3].

Total Phenolic Content

The total phenolic content of fruit snacks was determined as described by Singleton & Rossi [11], using the Folin-Ciocalteu method. In order to prepare the fruit extract before analysis, 5 grams of the fruit snack homogenized in 50 mL of ethanol (80%) at 10,000 rpm for 2 minutes. Afterward, the mixture was gently stirred at 200 rpm in a water bath maintained at a temperature of 40°C for 2 hours. Finally, the extract was obtained by filtering the mixture through a filter paper [1]. a 0.50 mL aliquot of the diluted sample was combined with 2.5 mL of a Folin–Ciocalteu reagent diluted at a 1:10 ratio. Following a 5-minute incubation at room temperature, 2 mL of a saturated Na_2CO_3 solution (75 g/L) was added to the mixture. After allowing the mixture to incubate for 2 hours at room temperature in dark, its absorbance was measured at 760 nm by a UV-VIS spectrophotometer (Thermo Scientific, Genesys-10S). Gallic acid used as the reference standard, and the results were expressed as milligrams of gallic acid equivalents per 100 g of fruit snack (mg GAE/100 g).

Total Aerobic Mesophilic Bacteria and Yeast and Mold Count

Total aerobic mesophilic bacteria (TAMB) and yeast & mold counts (YM) of fruit snacks were determined according to FDA's Bacteriological Analytical Manual [12]. For microbiological analysis, 25 g of fruit snack was put inside a stomacher bag and 225 mL of buffered peptone water (0.1%) was added. Then, the mixture was homogenized in a stomacher (Interscience, Bag Mixer 400). Serial dilutions were made in sterile peptone water and used for enumeration of microorganisms. TAMB and YM counts were determined by pour plate method onto PCA (Plate Count Agar) and spread plate method onto DRBC (Dichloran Rose Bengal Chloramphenicol Agar), respectively. Petri plates were incubated at 35°C for 48 h and 25°C for 5 days, respectively for TAMB and YM counts. The mean values were obtained by conducting the tests in triplicate and the results were given as log CFU/g.

Sensory Analysis

Sensory analysis was conducted by 20 untrained panelists, according to Otunola et al. [13]. Panelists were willing consumers of fiber-enriched fruit snacks. Samples were portioned, coded using 3-digit numbers and served on plastic plates at ambient temperature. Panelists evaluated odor, color, flavor, texture, appearance, overall acceptance, and FACT (willingness to eat the product if it was available on the market) values using a 7-point scale, going from "1 - only if forced, I would eat this fruit ball" to 7 - "I would eat this fruit ball every time I had the chance".

Statistical Analysis

The statistical differences between the fruit snacks were evaluated by one-way analysis of variance (ANOVA) (SPSS 20, New York). The multiple comparison test as Duncan's Multiple Range test were used to measure specific differences between pairs of means ($P < 0.05$), and results were expressed as mean ± standard deviation.

RESULTS and DISCUSSION

pH, water activity, acidity, and total phenolic content of control and fiber-enriched fruit snacks were given in Table 2. pH of control group and fiber enriched fruit snacks were determined in the range of 4.86-5.04. Compared to the control group, pH values of fiber-enriched snacks were significantly different ($p<0.05$) except for fruit snack enriched with 2.5% of pea fiber (PF-1). The pH values of samples I-2 and PF-2 were found to be similar, and likewise, there was no statistically significant difference in the pH values between samples I-2 and CF-2 ($p>0.05$). The increase in pH observed particularly in groups where 5% dietary fiber was added compared to the control group could be due to the neutral pH characteristics of plant fibers and the quantitative decrease in date paste in the formulation, resulting in an increase in the pH value of the fruit snack. In a study, the pH of fruit bar formulated by date and apple pulp was 4.30-5.28 [14]. It was observed that consistent results in line with the literature had been obtained.

Water activity measurements play a crucial role in predicting the textural attributes, stability, and shelf life of food products. As seen in Table 2, it is observed that the water activity values of fruit snacks enriched with dietary fiber show a decrease, ranging between 0.61 to 0.69. The highest water activity value was observed in the control group (C), while the lowest water activity was observed in CF-2. Compared to the control group, the changes in water activities of fiber-enriched samples were found to be statistically significant. The water

activity values of I-1, I-2, and PF-1 samples were found to be similar ($p>0.05$). a_w values were well below 0.7, indicating a low risk of microbial proliferation, potential pathogenic spoilage, and affirming a favorable shelf life [15]. Similarly, a study reported that the water activity of snack bar produced by date paste was 0.613±0.005 [16]. In another study, the moisture content and water activity levels of the formulated date bars, ranging from 33.59% to 34.67% and 0.60 to 0.65, respectively, were consistent with the established moisture (20-40%) and water activity (0.70-0.90) criteria for intermediate moisture (IM) foods [17]. In this study, the reason for the decrease in water activity values in fruit snacks enriched with dietary fiber is thought to be the decrease of date paste from the formulation and the addition of powdered fiber with very low moisture content.

Acidity is a critical factor in determining the quality of fruit, impacting not just how we perceive its tartness but also its sweetness. Titratable acidity stands out as the primary indicator of acidity, closely tied to our perception of sourness. Furthermore, pH levels also play a role in shaping our perception of acidity. Sensory analysis experiments using synthetic acid solutions have confirmed that the perception of acidity is strongly linked to titratable acidity, with pH having a somewhat less pronounced effect on this perception [18]. Furthermore, the sensory appeal of fruit is greatly influenced by its acidity, primarily owing to the abundance of malic and citric acids, which are the dominant organic acids in most mature fruits.

The effect of plant fiber addition to the formulation on the acidity of fruit snacks can be seen in Table 2. In general, the titratable acidity values of fruit snacks vary between 0.64% and 0.72%, and the addition of dietary fiber to fruit snacks has resulted in a reduction in acidity ($p<0.05$). An increase in pH values is observed to correspond with a decrease in acidity values in the samples. While the acidity values of the control and I-1 samples were found to be similar ($p>0.05$), an increase in inulin content to 5% (I-2) led to a significant reduction in acidity ($p<0.05$). Additionally, the acidity values of the control sample and CF-1 sample were found to be statistically different ($p<0.05$). It can be considered that the reason for fruit snacks enriched with carrot fiber having the lowest acidity values compared to other samples may be due to the pH of the added carrot fiber being approximately between 5 and 5.5 in the formulation. A study reported by Akhtar et al. [14], the titratable acidity of fruit bar produced by date and apple pulp as citric acid equivalent was found to be 0.41-0.52%. In another study on guava and orange fruit bar, the titratable acidity varied between 0.32 and 0.64% [19]. It was also reported that high acidity present in fruits serves a dual function by inhibiting the growth of microorganisms and supporting the preservation of the fruit's color and flavor [19].

Table 2. pH, water activity, acidity, and total phenolic content of fiber-enriched fruit snacks

Sample code	pH	Water activity	Titratable acidity (%)	Total phenolic content (mg GAE/100 g)
C	4.86±0.01^{e}	0.69±0.01^{a}	0.72±0.01^{ab}	595.1±9.5^{ab}
I-1	4.90±0.01^{d}	0.66±0.02^{b}	0.70±0.02^{bc}	583.5±4.8^{abc}
I-2	4.95±0.02^{bc}	0.64±0.00^{bc}	0.68±0.02^{cd}	571.9±10.6^{c}
PF-1	4.84±0.01^{e}	0.65±0.01^{bc}	0.73±0.01^{a}	581.0±13.5^{bc}
PF-2	4.93±0.01^{cd}	0.63±0.02^{cd}	0.69±0.01^{c}	566.9±7.6^{c}
CF-1	5.04±0.02^{a}	0.63±0.00^{cd}	0.64±0.02^{e}	597.3±5.8^{ab}
CF-2	4.98±0.03^{b}	0.61±0.01^{d}	0.66±0.00^{de}	601.4±12.3^{a}

$^{a-e}$ Different letters in same column indicates significant difference between the groups ($p<0.05$).

Dried fruits are globally recognized as essential nutritious snacks, representing a condensed version of fresh fruits with reduced water content. Common examples of traditional dried fruits, without added sugars, include apples, apricots, dates, figs, mulberries, peaches, pears, prunes, and raisins. The quantity of polyphenolic compounds found in these fruits is influenced by factors such as the fruit variety, environmental factors (such as soil quality, fertilization, temperature, and cultivation methods), storage and transportation conditions, as well as the specific processing techniques employed [20, 21].

The total phenolic content of fruit snacks was given in Table 2, and ranging from 566.9 to 601.4 mg GAE/100 g. The total phenolic content of samples I-1, CF-1, and CF-2 was found to be similar to that of the control group ($P>0.05$). However, samples I-2 and PF-2 exhibited a significantly lower total phenolic content compared to the other samples ($p<0.05$). The total phenolic content of dried apricots and dates were reported as 549 mg GAE/100 g and 661 mg GAE/100 g, respectively [22, 23]. In this study, in order to make a meaningful the comparison of the obtained results with the literature, a mixture of apricot and date at a ratio of 50:50 (w/w) was considered, resulting in a total phenolic content of 605 mg/100 g. Therefore, the obtained results were found to be in accordance with the literature [22, 23]. In another study, it was reported that the total phenolic content of fruit bar produced date paste was 224.3-240.3 mg GAE/100 g [24].

The composition and energy values of all fruit snacks were presented in Table 3. Moisture content of samples were changed between 22.8 to 23.8%, higher moisture level was observed for fiber enriched snacks compared to the control group ($p<0.05$). The moisture contents of fruit snack samples I-1 and CF-1 were found to be similar ($p>0.05$), and there was also no statistically significant difference found between the samples of I-2

and CF-2 (p>0.05). According to Parn et al. [24], the moisture content of fruit bar produced by date paste varied between 24.5 to 26.3. Moreover, it was reported that the moisture content of freshly prepared date paste was 19.12% [17]. In another study by Sharma et al. [25], the moisture content of apricot bar was 18.9-20.9%. In addition, it was determined that the moisture content of dried dates and apricots were 25% and 35%, respectively [26].

Table 3. General composition and energy values of fiber-enriched fruit snacks

Sample code	Moisture (%)	Ash (%)	Total fat (%)	Protein (%)	Carbohydrate (%)	Total dietary fiber (%)	Energy (kcal/100 g)
C	23.8±0.10^{a}	5.60±0.12^{b}	0.65±0.13^{a}	2.90±0.10^{de}	60.3±0.45^{a}	6.8±0.05^{e}	258.5±0.43^{ab}
I-1	23.3±0.02^{c}	5.62±0.07^{b}	0.69±0.05^{a}	2.68±0.04^{f}	58.9±0.14^{c}	8.8±0.04^{b}	252.6±0.11^{c}
I-2	22.8±0.04^{e}	5.63±0.02^{b}	0.68±0.02^{a}	2.80±0.02^{e}	57.3±0.07^{d}	10.8±0.06^{a}	246.5±0.38^{d}
PF-1	23.4±0.05^{b}	5.70±0.10^{ab}	0.70±0.01^{a}	3.30±0.10^{b}	59.7±0.19^{b}	7.2±0.01^{d}	258.3±0.31^{ab}
PF-2	23.1±0.01^{d}	5.60±0.05^{b}	0.68±0.05^{a}	3.84±0.05^{a}	59.1±0.02^{c}	7.7±0.08^{c}	257.8±0.73^{b}
CF-1	23.3±0.04^{c}	5.70±0.10^{ab}	0.71±0.03^{a}	3.00±0.04^{d}	60.1±0.18^{ab}	7.2±0.10^{d}	258.6±0.41^{a}
CF-2	22.8±0.06^{e}	5.81±0.02^{a}	0.73±0.02^{a}	3.15±0.01^{c}	59.9±0.08^{ab}	7.6±0.02^{c}	258.8±0.46^{a}

$^{a-f}$ Different letters in same column indicates significant difference between the groups (p<0.05).

Results of the ash contents of fruit snacks, which represents the overall mineral content, were ranged between 5.60 and 5.81% (Table 3). There was no significant difference observed for the ash content of fruit snacks except for CF-2. As a result, addition of plant fiber did not affect the total ash content of fruit snacks significantly compared to the control group. A study on vegetable dietary fiber concentrates, the ash content of carrot fiber was found to be 5.03% [6]. It was reported that dried fruits had 2.4-3.5% of total ash content [27]. Moreover, a study on apricot snack bar including 69.4% of apricot paste and 30.6% of other ingredients (corn flour, skim milk, almond, pistachio, coconut powder, chickpea, and brown sugar) reported the total ash content as 3.08% [28]. Furthermore, the ash content of cereal bar including oat flakes, dried fruits (grapes, apricots), nuts, butter, glycerol and honey was 1.35-1.51% [3].

The total fat content of control group and fiber enriched fruit snacks were determined in the range of 0.65-0.73%. There was no statistically significant difference observed between the groups (p>0.05). In a study by Singh et al. [17] reported the total fat content of date paste was 0.76%. In another study by Drougoudi et al. [29] found that the dried apricot had 3.39 g of protein, 2.57g of ash, 0.51g of fat per 100 g of fruit. In addition, it was determined that the fat content of dried dates and apricots were 0.17% and 0.33-1.20%, respectively [26].

The protein content of fruit snacks was presented in Table 3. The protein content of control group was 2.90%, and fiber enriched fruit snacks had the protein between the range of 2.68-3.84%. While the total protein content of I-1 was the lowest, PF-2 showed the highest protein content. I-1 and CF-1 had similar protein content with control group (p>0.05), and the other snacks were found to be statistically different (p<0.05). Although the protein content of samples I-1 and CF-1 is similar to that of the control group (p>0.05), the protein contents of other fruit snacks were found to be statistically different from the control group (p<0.05). In a study by Singh et al. [17] determined that date paste had 1.75% of protein. Another study by Munir et al. [16] reported that the protein content of date paste was 2.38%. In addition, the protein content of date bars prepared by different varieties of date fruit was found to be 2.22-4.06% [24]. Moreover, the protein content of carrot fiber was determined as 6.73% [6]. Furthermore, the protein content of date bar was found to be 1.90% [17]. In a study, the protein content of cereal bar including oat flakes, dried fruits (grapes, apricots), nuts, butter, glycerol and honey was 8.64% [3]. In addition, it was determined that the protein content of dried dates and apricots were 1.8-2.5% and 2.5%, respectively [26].

The carbohydrate content of fruit snacks was shown in Table 3, ranging from 57.3 and 60.3%. The lowest carbohydrate content was determined in I-2 and the highest carbohydrate was observed in control group. PF-1, CF-1, and CF-2 had similar total carbohydrate content with control group (p>0.05). In addition, there is no significant differences found in the carbohydrate content of I-1 and PF-2 (p>0.05). Compared to the control, the carbohydrate content of fruit snacks enriched with inulin and pea fiber was found to be different (p<0.05). In a study reported by Parn et al. determined the carbohydrate content of date bar produced by different varieties of date fruit as 56.8-72.65% [24]. Moreover, it was reported that the total carbohydrate of dried apricots was 62.6% [29]. Another study reported by Munir et al. [28], total carbohydrate content of apricot bar was found to be 67.3%. In addition, it was determined that the carbohydrate content of dried dates and apricots were 67.5% and 62.5%, respectively [26]. Obtained results from this study was in line with the previous literature research.

Total dietary fiber content of control group (without plant fiber addition) was found as 6.8%, indicating that apricot and date paste (1:1, w/w) had good source of fiber content as expected. The total dietary fiber content of fruit snacks enriched with plant fibers ranged from 7.2% to 10.8%. In comparison to the control group, the total dietary fiber content of all fruit snacks was found to be statistically different (p<0.05). The total fiber content of PF-2 and CF-2 samples was found to be similar (p<0.05). Furthermore, there was no significant difference in fiber content between PF-1 and CF-1 samples (p>0.05). Among all samples, the highest fiber content was observed in sample I-2. According to the Turkish Food Codex & the Regulation on Nutrition and Health Claims, since the formulated fruit snacks contain more than 6 grams of total dietary fiber per 100 grams, it

can be labeled as 'high in fiber' or 'excellent source of fiber' on the packaging.

In a study by Singh et al. [17] determined that date paste had 2.30% of crude fiber. In addition, it was reported that apricot-based snack bar had the fiber content as 8.33% [28]. In another study by Munir et al. [16], total fiber content of date-based snack bar determined as 2.9%. Moreover, the total fiber content of date bars were found to be 4.5-5.6% [24]. In another study, total fiber content of fruit-based functional snack bars was 2.54-5.42% [15]. It was reported that dried dates and apricots had 7.5% of dietary fibers [26].

The energy values of fruit snacks range from 246.5 to 258.8 kcal/100 g (Table 3), and the energy value for the control group has been determined as 258.5 kcal/100 g. The lowest calorie value was calculated in sample I-2, while the highest calorie value was observed in sample CF-2. While the energy values of samples C, PF-1, CF-1, and CF-2 were found to be similar to each other ($P>0.05$), the energy values of samples I-1 and I-2 were found to be statistically different ($p<0.05$). In a study on nutritional composition on dried fruits, the energy values of date and dried apricot were found as 300 kcal/100 g and 250 kcal/100 g [26].

Table 4. Texture of fiber-enriched fruit snacks

Sample code	Hardness (g)	Adhesiveness (g*sec)	Cohesiveness	Gumminess	Chewiness
C	4000.3±115^{b}	-162.04±87.6^{b}	0.29±0.05^{c}	1153.4±21.1^{bc}	544.07±12.1^{de}
I-1	2530.8±29^{g}	-1041.1±21.3^{e}	0.42±0.04^{ab}	1059.2±15.9^{c}	741.9±17.5^{c}
I-2	3040.4±58.2^{e}	-86.6±31.8^{a}	0.44±0.01^{a}	1322.8±25.9^{b}	781.8±18.6^{c}
PF-1	2671.9±25.3^{f}	-678.3±14.4^{d}	0.38±0.02^{b}	1035.2±129.2^{c}	591.5±88.25^{d}
PF-2	3798.3±40.9^{c}	-116.4±15.5^{ab}	0.45±0.01^{a}	1739.4±210.2^{a}	1148.85±31.5^{a}
CF-1	3527.4±38.5^{d}	-268.6±11.3^{c}	0.31±0.02^{c}	1100.4±109.8^{c}	480.9±42.7^{e}
CF-2	4322.2±37.7^{a}	-131.1±17.0^{ab}	0.40±0.01^{ab}	1753.55±14.3^{a}	1001.1±10.45^{b}

$^{a-g}$ Different letters in same column indicates significant difference between the groups ($p<0.05$).

The texture of a product plays a crucial role in influencing consumers' willingness to embrace a new offering. Textural properties of fruit snacks enriched with different fibers (hardness, adhesiveness, cohesiveness, gumminess, and chewiness) are presented in Table 4. Among the textural parameters, hardness is a key parameter to effect consumer acceptability of fruit snacks [16]. In this study, minimum hardness was measured for I-1, while maximum value was observed for CF-2. Compared to control group, the addition of dietary fiber was significantly increased the hardness values of fruit snacks ($p<0.05$). The adhesiveness values of the samples range from -86.6 to -1041.1 g*sec. Samples I-2, PF-2, and CF-2 exhibited similar adhesiveness values ($P>0.05$). Overall, it is observed that the addition of 5% fiber to the samples resulted in a decrease in adhesiveness values. The cohesiveness values of fruit snacks range from 0.29 to 0.45, and the cohesiveness values of samples I-1, I-2, PF-2, and CF-2 were found to be similar ($p>0.05$). The gumminess values of the samples vary between 1035.2 and 1753.6, with the gumminess value of the control sample being 1153.5. In groups where 2.5% fiber was added, the gumminess value decreased compared to the control group, while in groups where 5% fiber was added, the gumminess value increased. The gumminess values of samples I-1, PF-1, and CF-1 were found to be similar to the control group ($p>0.05$). The chewiness values of the samples range from 480.9 to 1148.9, with the control group having a chewiness value of 544.1. There was no statistically significant difference in chewiness values between samples I-1 and I-2, with the highest chewiness value observed in sample PF-1. A study reported that the hardness, adhesiveness, cohesiveness, gumminess and chewiness values of fruit bar produced by date paste were 1572-2189, -733.8-793.9, 0.36-0.61, 836.5-1334.6, 772.90-1319.1 g/s, respectively [24]. Furthermore, it was reported that hardening of fruit bars after the addition of dietary fiber may be linked to the transfer of water between the carbohydrate and the protein components. Carbohydrate ingredients, such as dietary fibers added for moisture retention and texture modification, could effectively prevent moisture loss to the surrounding environment [15].

Color is the most important parameter affecting consumer acceptability of snack foods. The color parameters (L*, a*, and b*), color difference compared to control group, and Chroma values were shown in Table 5. The L* values of fruit snacks range from 23.1 to 34.1, the a* values range from 9.7 to 12.3, and the b* values range from 14.6 to 19.6. It was observed that when the dietary fiber content was increased for all groups, L* values also increased while a* decreased ($p<0.05$). In addition, the L* values of fiber-enriched fruit snacks increased with increasing concentration of dietary fibers from 2.5 to 5%. Considering the a* value, the addition of 2.5% of carrot fiber to the snack formulation was found similar to control group which has no fiber enrichment ($p>0.05$). b* values of samples were found significantly different for fiber-enriched fruit snacks except for PF-1.

Color difference (ΔE^{*}) values were calculated as 5.73-11.31 (Table 5). ΔE^{*} values below 1 suggest that color variances were not easily detectable by the human eye. When ΔE^{*} falls between 1 and 3, the differences in color were not readily noticeable to human perception. On the other hand, when ΔE^{*} surpasses 3, it indicates distinct and easily recognizable color differences as perceived by the human eye [30]. In this study, ΔE^{*} values were found as >5 meaning that the addition of 2.5 and 5% of fiber to the formulation of fruit snacks changed the color and this change was easily noticeable. These results showed that color changes occur in the preparation of fruit snacks, and addition of different type of fiber might have contributed to the observed color changes. Chroma (C*) indicates the intensity of color with ranging from 0 to 100, which is considered as a quantitative

measure of color [31]. Chroma (C*) values of fruit snacks were given in Table 5, ranging between 17.53 to 22.10. The C* values of CF-1 was similar to control group (p>0.05), while the highest color intensity was observed in CF-2. As stated by Sharma, a primary factor in determining color differences is attributed to lightness, and due to the reduced sensitivity of human eyes to changes in both lightness and chroma [32]. In other words, a small C* can be easier to differentiate than a high ΔE* [32]. In a study by Parn et al., the color values of date bar were determined as L* of 40.37-43.89, a* of 9.85-12.15, and b* of 29.03-30.45 [24].

Table 5. Color values of fiber-enriched fruit snacks

Samples	L*	a*	b*	Color difference (ΔE*)	Chroma (C*)
C	23.1±1.10^{e}	12.3±1.06^{a}	16.5±1.24^{c}	-	20.62±0.36^{b}
I-1	28.2±0.60^{d}	10.5±1.28^{bc}	15.1±0.62^{d}	5.73±0.12^{d}	18.40±1.24^{cd}
I-2	30.3±0.45^{bc}	9.7±0.08^{c}	14.6±0.04^{d}	7.89±0.45^{b}	17.53±0.08^{d}
PF-1	31.5±0.09^{b}	11.2±1.02^{abc}	15.8±0.06^{cd}	8.54±0.06^{b}	19.38±0.64^{c}
PF-2	34.1±0.04^{a}	10.4±0.06^{bc}	14.9±1.05^{d}	11.31±0.10^{a}	18.18±0.83^{cd}
CF-1	29.5±1.12^{c}	11.6±1.34^{ab}	18.4±0.15^{b}	6.83±0.87^{c}	21.77±0.59^{ab}
CF-2	30.7±0.03^{bc}	10.2±0.05^{bc}	19.6±0.05^{a}	8.47±0.04^{b}	22.10±0.07^{a}

$^{a-e}$ Different letters in same column indicates significant difference between the groups (p<0.05).

Table 6. Total aerobic mesophilic bacteria (TAMB), yeast and mold counts of fiber-enriched fruit snacks

Samples	TAMB (log CFU/g)	Yeast and Mold (log CFU/g)
C	3.32^{d}	1.96^{c}
I-1	3.30^{e}	2.05^{b}
I-2	3.37^{c}	1.97^{c}
PF-1	3.24^{g}	2.08^{b}
PF-2	3.28^{f}	2.13^{a}
CF-1	3.47^{b}	2.16^{a}
CF-2	3.48^{a}	2.08^{b}

$^{a-g}$ Different letters in same column indicates significant difference between the groups (p<0.05).

Table 6 indicates the total aerobic mesophilic bacteria (TAMB) and yeast & mold counts (YM) of control and fiber-enriched fruit snacks. The TAMB and YM counts of control group were 3.32 and 1.96 log CFU/g, respectively. The TAMB of fruit snacks were found between 3.24 to 3.48 log CFU/g and there were significant differences observed as the type of fiber changed in the formulation of fruit snack (P<0.05). In terms of YM counts, I-2 sample was similar to control group (P>0.05). In addition, there was no statistically important differences found in I-1, PF-1, and CF-2 (P>0.05). According to the Turkish Food Codex Microbiological Criteria Regulation, only mold and yeast counts (n:5, c:2, m: 10^4, M: 10^5) are considered as criteria for dried fruits. In this study, all analyzed samples of fruit snacks fall within the acceptable limits defined by the Turkish Food Codex Microbiological Criteria Regulation [33] in terms of mold and yeast counts.

The effect of addition of different plant fibers on the sensory properties of fruit snacks is presented in Table 7. When the odor values of the samples were considered, scores between 4 and 6.2 were obtained, and all samples except CF-2 showed similar characteristics to the control group (p>0.05). In terms of color evaluation of the fruit snacks, scores ranging from 4.9 to 6.55 were obtained, and all samples except CF-1 and CF-2 exhibited similar characteristics to the control group (p>0.05). The flavor values of the samples ranged from 3.90 to 6, with the control sample being the most liked and CF-2 being the least liked. The textural properties of the samples were found to be similar (p>0.05), except for CF-2, with CF-2 being the least liked in terms of texture. Appearance values for the samples were scored between 4.75 and 6.55, with sample I-1 being the most preferred. Except for CF-2, all other samples were statistically similar to the control group. The overall acceptability values of the samples ranged from 4.15 to 6.55, and the fruit snacks enriched with inulin and pea fiber showed similar overall acceptability to the control group (p>0.05). The lowest overall acceptability value was observed in the CF-2 sample (p<0.05). The FACT values of the samples ranged from 3.80 to 6.40, and the samples enriched with inulin and pea fiber exhibited similar characteristics to the control group (p>0.05). According to the sensory analysis results, the consumer acceptability of fruit snacks enriched with carrot fiber was found to be low.

Numerous research studies support the notion that increasing dietary fiber intake enhances feelings of satiety, reduces hunger, and promotes a sense of satisfaction. Foods rich in dietary fiber often have a high volume and a low calorie density, contributing to a sense of fullness and playing a role in regulating energy balance [34]. In the future, there is an expectation that research will persist in exploring the health impacts of industrially or functionally improved foods rich in dietary fiber. It is anticipated that irrespective of dietary preferences, these foods will assume a more significant position in the dietary patterns of numerous individuals [35]. Thus, it is important to produce high in fiber fruit snacks with high quality and consumer acceptability for daily diet.

Table 7. Sensory properties* of fiber-enriched fruit snacks

Sample code	Odor	Color	Flavor	Texture	Appearance	Overall acceptance	FACT
C	6.20±0.89^{a}	6.55±0.60^{a}	6.00±0.65^{a}	6.35±0.59^{a}	6.40±0.68^{a}	6.55±0.60^{a}	6.40±0.50^{a}
I-1	5.95±1.05^{a}	6.40±0.60^{a}	5.70±0.57^{a}	6.45±0.51^{a}	6.55±0.60^{a}	6.45±0.51^{a}	6.50±0.51^{a}
I-2	5.85±0.90^{a}	6.10±0.72^{a}	5.80±0.62^{a}	6.35±0.49^{a}	6.35±0.75^{a}	6.31±0.74^{a}	6.42±0.60^{a}
PF-1	5.65±0.88^{a}	6.20±0.62^{a}	5.55±0.76^{ab}	6.20±0.52^{a}	6.10±0.72^{a}	6.25±0.79^{a}	6.35±0.49^{a}
PF-2	5.55±0.83^{ab}	6.25±0.55^{a}	5.00±0.97^{abc}	5.90±0.55^{a}	6.15±0.67^{a}	5.75±0.72^{ab}	6.05±0.60^{a}
CF-1	5.30±0.73^{ab}	5.45±0.50^{b}	4.30±0.73^{bc}	5.75±0.55^{a}	5.30±0.57^{ab}	4.90±0.45^{bc}	4.50±0.69^{b}
CF-2	4.00±0.79^{b}	4.90±0.65^{c}	3.90±0.72^{c}	3.95±0.76^{b}	4.75±0.72^{b}	4.15±0.67^{c}	3.80±0.41^{b}

*: 1-point for least undesirable and 7-point indicating for the most desirable. $^{a-c}$: Different letters in same column indicates significant difference between the groups ($p<0.05$).

CONCLUSION

The enrichment of fruit snacks with dietary fiber holds significant importance in promoting overall health and well-being. Fiber is an essential component of a balanced diet, playing a crucial role in maintaining digestive health and regulating blood sugar levels. Incorporating fiber into fruit snacks not only enhances their nutritional value but also contributes to satiety, helping individuals feel fuller for longer periods. In this study, fruit snack formulations based on dates and apricots were enriched with different plant-based dietary fibers (inulin, pea, and carrot fiber) at 2.5% and 5.0% ratios, and the changes in quality attributes and consumer acceptability were investigated. The addition of plant fiber to the formulation resulted in a decrease in the acidity and moisture contents of the fruit snacks compared to the control. All samples had a dietary fiber content of >6 g/100 g, allowing them to be labeled as "high in fiber" on the packaging. When the added fiber content was increased from 2.5% to 5.0%, it was determined that the hardness, gumminess, and chewiness values of the fruit snacks increased. Moreover, the addition of fiber significantly affected the color difference of the fruit snacks compared to the control. All produced fruit snacks were found to be microbiologically safe. Fruit snacks enriched with inulin and pea fiber were well-liked by the panelists in terms of sensory attributes, and their consumer acceptability was higher than the fruit snacks enriched by carrot fiber. It is recommended that future studies focus on developing high-nutrient and highly consumer-accepted fruit snack formulations using different plant fiber sources, investigation of quality changes during storage, and conducting *in vitro* digestibility studies of fiber-enriched fruit snacks.

CONFLICT OF INTEREST

The author declared no conflict of interest.

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Research Paper / Araştırma Makalesi

Effect of Persimmon (*Diospyros kaki* Thunb.) Powder and Quince (*Cydonia oblonga*) Seed Mucilage on Physical, Chemical, Textural and Sensory Properties of Turkish Noodles

Ülgen İlknur Konak[1], Rahime Dilruba Kaya[1], Yasemin Yavuz Abanoz[2]
Mine Aslan[3], Sultan Arslan Tontul[4]

[1]Faculty of Engineering and Architecture, Department of Food Engineering, Avrasya University, 61250 Trabzon, Türkiye
[2]Coordinating Office of Tea Specialization, Recep Tayyip Erdoğan University, 53020 Rize, Türkiye
[3]Faculty of Engineering, Department of Food Engineering, Necmettin Erbakan University, 42090 Konya, Türkiye
[4]Faculty of Agriculture, Department of Food Engineering, Selçuk University, 42130 Konya, Türkiye

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Corresponding author (Yazışmalardan Sorumlu Yazar): ulgen.ilknur.konak@avrasya.edu.tr (Ü.İ. Konak)
+90 462 335 5000 +90 462 335 5001

ABSTRACT

In this study, persimmon powder (PP) was substituted in concentrations of 0, 5 and 10% per 100 g of einkorn flour (EF) in Turkish noodle production. Quince seed mucilage (QSM) was used as an egg replacer at levels of 20, 30 and 40%. The cooking properties, chemical composition, color values, texture characteristics, and sensory properties of Turkish noodles were determined. The lowest cooking time (8.33 min) was found in Turkish noodles substituted with 10% PP. When 40% QSM was added to the formulation, both volume increase and water absorption values increased. Turkish noodles produced with a higher concentration of PP resulted in increased ash, crude fiber, mineral contents, especially in potassium, and total phenolic content. The lowest firmness and work of shear were obtained when PP and QSM substitutions were increased up to 10% and 40%, respectively. The sensory evaluation indicated that Turkish noodles substituted with 10% PP were greatly appreciated by the panelists in terms of taste, odor, and overall acceptability.

Keywords: Turkish noodle, Einkorn, Total phenolic, Mineral

Eriştelerin Fiziksel, Kimyasal, Tekstürel ve Duyusal Özellikleri Üzerine Trabzon Hurması (*Diospyros kaki* Thunb.) Tozu ve Ayva Çekirdeği (*Cydonia oblonga*) Müsilajının Etkisi

ÖZ

Bu çalışmada, erişte üretiminde 100 g siyez unu (SU) %0, 5 ve 10 konsantrasyonlarında Trabzon hurması tozu (THT) ile ikame edilmiştir. Ayva çekirdeği müsilajı (AÇM) yumurta ikame maddesi olarak %20, 30 ve 40 seviyelerinde kullanılmıştır. Eriştelerin pişme özellikleri, kimyasal bileşimleri, renk değerleri, tekstürel ve duyusal özellikleri değerlendirilmiştir. En düşük pişirme süresi (8.33 dakika) %10 THT ikame edilen eriştelerde bulunmuştur. Formülasyona %40 oranında AÇM ilave edildiğinde hem hacim artışı hem de su absorpsiyonu değerleri artmıştır. Yüksek konsantrasyonda THT ile üretilen eriştelerde kül, ham lif, mineral madde (özellikle potasyum) ve toplam fenolik madde içeriğini artmıştır. En düşük sertlik ve kesme kuvveti, THT ve AÇM ikameleri sırasıyla %10'a ve %40'a kadar artırıldığında elde edilmiştir. Duyusal değerlendirme, %10 THT ile ikame edilen eriştelerin tat, koku ve genel kabul edilebilirlik açısından panelistler tarafından büyük ölçüde beğenildiğini göstermiştir.

Anahtar Kelimeler: Erişte, Siyez, Toplam fenolik, Mineral

INTRODUCTION

Turkish noodles (erişte) are traditional cereal products that contain soft wheat flour, salt, and egg. Mixing of ingredients, sheeting of the dough, cutting, and drying are the main production steps of Turkish noodles, respectively [1]. Traditional noodles are generally rich in starch but deficient in bioactive compounds, protein, vitamins, and minerals. Various studies have reported the use of different types of flour such as buckwheat flour [1], oat flour [2], quinoa flour [2], legume flour [3], and fruit seed flour [4], as well as other ingredients like legume hull [5], cereal bran [6], fiber [7], and vegetables [8, 9] to develop Turkish noodles.

Persimmon (*Diospyros kaki* Thunb.) is rich in ascorbic acid, dietary fiber, minerals, carotenoids, and polyphenols. Studies have shown that persimmon has antiatherogenic, antiobesity, antidiabetic, and antioxidant effects. The fruit is widely cultivated in both the Mediterranean and Black Sea regions of Türkiye [10, 11]. The antioxidant activity of cookies and rice cakes produced with the persimmon leave powder was evaluated by Kim et al. [12] and Lim and Lee [13], respectively. Moreover, immature persimmon juice and flour obtained from persimmon juice coproducts have been used to enrich spaghetti [11], rice noodles [14], and cakes [15].

Quince (*Cydonia oblonga*) seeds produce a sticky and tasteless liquid (mucilage) when soaked in water. The liquid has a gel structure formed by easily hydrolyzable polysaccharides [16]. In several studies, mucilage has been employed as a thickener, gelling agent, stabilizer, or edible film coating component. Researchers have focused on the addition of mucilage in yogurt [17], ice cream [18], and edible films [19]. This study attempts to enrich Turkish noodles with freeze-dried persimmon. Moreover, the investigation of the possibility of using quince seed mucilage instead of egg in Turkish noodle production was aimed. Thus, proximate compositions, cooking attributes, texture parameters, and sensory properties of the noodles were investigated.

MATERIALS and METHODS

Raw Materials and Chemicals

Einkorn flour (EF, Doğalsan, Türkiye) and table salt (Billur, Türkiye) were used in Turkish noodle production. The EF had the following characteristics: moisture content of 9.73 g/100 g, protein content of 22.03 g/100 g, lipid content of 2.99 g/100 g, ash content of 2.42 g/100 g, and fiber content of 1.94%. Persimmon and quince seeds were supplied by the local markets in Trabzon and Adana, respectively. All the chemicals were of high-purity grade and supplied by Sigma-Aldrich (Steinheim, Germany).

Preparation of Persimmon Powder and Quince Seed Mucilage

After the removal of the peel and seeds, the homogenized fruit flesh was frozen at -20°C for 12 h. Then, the homogenized flesh was freeze-dried (-90°C, 6x10-3 torr) to a water content of 10%. The dried samples were ground by a spice grinder (SCM 2934, Sinbo, Türkiye) and passed through a 0.15-mm sieve. Quince seed mucilage (QSM) was extracted according to Jouki et al. [16] with some modifications. First, 10 g of quince seeds were placed in a 1 L beaker and washed with the triple weight of ethanol solution (96% w/v) by stirring at 1000 rpm for 5 min. Afterwards, the solution was removed and the wet seeds were dried at 45±1°C. Then, distilled water was added in 30:1 proportion (v:w) and stirred at 1000 rpm and 45±1°C for 45 min. Later, centrifugation was performed at 4500 rpm and 26±1°C for 15 min.

Preparation of Noodles

Turkish noodle production was performed according to Bilgiçli [1] with some modifications. The control sample was produced with 100 g of einkorn wheat flour, 30 g of distilled water, 30 g of whole egg, and 1 g of salt. The formulation of the Turkish noodles is given in Table 1. The raw materials were mixed by a mixer (Prochef XI, Schafer, Germany) for 5 min at medium speed and then rested in a polypropylene bag for 15 min at room temperature (26±1°C). Afterward, the dough was passed through two rollers (Atlas 150, Marcato, Italy) by reducing the sheeting gap gradually to get dough sheets 2 mm in thickness. The sheeted dough pieces were folded in half and sheeted twice at each stage to achieve homogeneity. Then, the sheet was cut into 6 mm in width and 4 cm in length. The Turkish noodle strips were dried for 17 h at 40°C in a drying oven (KD-200, Nüve, Türkiye). Later, the dried Turkish noodles were cooled to room temperature (26±1°C) for further analysis.

Table 1. Turkish noodle formulation with einkorn flour (EF), persimmon powder (PP), and quince seed mucilage (QSM)*

Sample Code	EF (g)	PP (g)	QSM (g)
1	100	0	20
2	100	0	30
3	100	0	40
4	95	5	20
5	95	5	30
6	95	5	40
7	90	10	20
8	90	10	30
9	90	10	40

*EF: einkorn flour; PP: persimmon powder; QSM: quince seed mucilage

Cooking Quality

Cooking time, cooking loss, water absorption, and volume increase were determined according to the AACC method 66-50.01 [20]. Turkish noodles (25 g) were cooked in boiling distilled water (250 mL) and cooking time was determined by crushing the cooked Turkish noodles between a pair of glass plates until the opaque central core in the Turkish noodle strand disappeared. Cooking loss was determined by drying 50 mL of cooking water at 105°C until a constant weight was obtained. Cooking loss was expressed as the percentage of dried solids in cooking water to the weight of uncooked Turkish noodles. Water absorption was calculated after cooked Turkish noodles were drained for 5 min to remove excess water. Water absorption was expressed as the weight ratio of cooked Turkish noodles (drained) to uncooked Turkish noodles. The volume increase was calculated by measuring the increase in water level after cooked and uncooked Turkish noodles were put into a graduated cylinder filled with a certain amount of distilled water. The volume increase was expressed as the percentage of difference in the volume of cooked and uncooked Turkish noodles.

Color Measurement and pH

Finely ground uncooked Turkish noodles (10 g) were mixed with 100 mL of distilled water and stirred at 1000 rpm for 5 min. The pH of the filtrate was measured using a pH meter (Jenco 6173, USA) [21]. Color values (L*: lightness; a*: redness; b*: yellowness) of two uncooked Turkish noodle strands were measured using a chroma meter (CR-400, Konica Minolta, Japan). Three readings were taken on each side of the uncooked Turkish noodles [22].

Proximate Composition

Moisture (ICC, 1976), ash (ICC, 1990), crude protein (ICC, 1994), fat (ICC, 1984), and crude fiber (ICC, 1972) contents were determined by ICC methods [23]. The oven drying at 100ºC (method 110/1), dry combustion at 550°C (method 104/1), Kjeldahl method (method 105/2), Soxhlet method (method 136) and gravimetric method (method 113) were applied to determine moisture, ash, crude protein, fat and crude fiber content of raw materials and uncooked Turkish noodles, respectively.

Total Phenolic Content

Total phenolic content was determined according to Menga et al. [24]. Finely ground uncooked Turkish noodles (1 g) were extracted with a mixture of methanol/distilled water/HCl (8 mL; 80:19:1 v/v/v) with stirring at 1000 rpm and 26±1°C for 30 min. Then, the extract was centrifuged at 4000 rpm for 15 min. After that, 200 µL of the supernatant was added to 1.5 mL of Folin-Ciocalteu reagent (10-fold diluted) and allowed to stand for 5 min at 26±1°C. Then, 1.5 mL of sodium carbonate solution (6%) was added and the mixture was allowed to stand for 90 min at 26±1°C. Later, the absorbance was measured at 725 nm. The acidified methanol solution was used as a blank. The results were expressed as mg gallic acid/g dry matter.

Mineral Content

Finely ground Turkish noodle samples (0.5 g) were digested by a microwave digestion system (Speedwave, Berghof, Germany) using a mixture of 4 mL of HNO_3 (65%), 1 mL of H_2O_2 (30%), and 3 mL of deionized water. Mineralization was carried out at 170°C for 30 min. After digestion, the samples were cooled to room temperature (26±1°C) and diluted up to 25 mL with deionized water. Mineral content (magnesium (Mg), potassium (K), iron (Fe), and zinc (Zn)) was determined by ICP-OES (Optima 7000 DV, Perkin Elmer, USA) [25].

Texture Analysis

The firmness and work of shear of the cooked Turkish noodles were determined according to the AACC Standard Method (66-50.01) by a texture analyzer (TA-XTPlus, UK) with a Knife Blade (A/LKB-F) probe and a 5 kg load cell. The texture analysis was carried out within 15 min after cooking and each Turkish noodle was cooked for the optimum cooking time. The test speed was 0.17 mm/s, the post-test speed was 10 mm/s, the distance was 4.5 mm, and the trigger type was button [20].

Sensory Evaluation

Sensory analysis was performed with 30 semi-trained panelists (15 females and 15 males, aged between 20 and 50) at Avrasya University. Cooked Turkish noodles were evaluated for color, appearance, taste, odor, hardness, chewiness, and overall acceptability using a five-point hedonic scale (1: very bad and 5: very good). The Turkish noodles were presented to panelists with three-digit codes in random order on white plastic dishes. Water was used as a palate cleanser between samples.

Statistical Analysis

The data was analyzed using SAS System Software (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) followed by Duncan's Multiple Range test was performed to evaluate significant differences ($p<0.05$) observed in the mean values of the results.

RESULTS and DISCUSSION

Cooking Quality

The cooking time, cooking loss, water absorption, and volume increase of the control were 15.5 min, 7%, 124%, and 139%, respectively. Generally, lower cooking losses and higher weight and volume gains are crucial attributes for high-quality noodles. The effects of PP incorporation and QSM addition on the cooking properties of the Turkish noodles are presented in Table 2. The addition of an increasing amount of PP resulted in a shorter cooking time ($p<0.05$). The authors reported

the same results for the cooking time of noodles enriched with terebinth (*Pistacia Terebinthus*) fruit [26]. It could be due to the weakening of the network between gluten and starch due to higher fiber content [11]. The addition of PP and QSM at different ratios significantly affected ($p<0.05$) cooking loss, water absorption, and volume increase parameters. An increase in the PP content resulted in an increased cooking loss. This fact might be due to the high fiber content of the noodles that was responsible for weakening the starch network in the noodle strings [27]. Similar results were observed by Köten and Ünsal [26]. Moreover, both water absorption and volume increase values decreased as the PP level increased. These values were in agreement with the results reported by Lucas-González et al. [11] that persimmon flour decreased water absorption in spaghetti samples. The results indicated that QSM had a significant effect ($p<0.05$) on the cooking properties except for cooking time. The Turkish noodles produced with mucilage instead of egg showed significantly higher ($p<0.05$) cooking loss than the control sample. However, the cooking loss of the Turkish noodles considerably decreased as the concentration of QSM decreased. Additionally, when the interaction between PP incorporation and QSM addition was considered, the lowest cooking loss was observed as the Turkish noodles were produced with 0% PP and 20% QSM. These findings were similar to studies on adding mucilaginous seeds into noodle or pasta formulations [27-29], whereas noodles produced with cassava mucilage had lower cooking loss [30]. A significant increase ($p<0.05$) in both the weight and volume values of the Turkish noodles was observed with increasing QSM concentration. Similar results were reported by Kasunmala et al. [27], Kishk et al. [28], and Naji-Tabasi et al. [29].

Table 2. Effects of PP and QSM levels on cooking time (min), cooking loss (%), water absorption (%), and volume increase (%) of Turkish noodles

Cooking time	EF:PP	100:0	95:5	90:10
		11.17^{a}±0.17	9.67^{b}±0.21	8.33^{c}±0.21
Cooking loss	EF:PP	90:10	95:5	100:0
		12.22^{a}±0.24	8.70^{b}±0.68	6.50^{c}±0.23
	QSM	40	30	20
		10.13^{a}±1.03	9.13^{b}±1.13	8.16^{c}±1.09
Water absorption	EF:PP	100:0	95:5	90:10
		127.17^{a}±0.60	124.67^{b}±2.19	123.33^{c}±0.56
	QSM	40	30	20
		126.67^{a}±0.92	125.83^{b}±1.14	122.67^{c}±1.75
Volume increase	EF:PP	100:0	95:5	90:10
		155.33^{a}±2.77	151.33^{b}±0.62	136.33^{c}±4.29
	QSM	40	30	20
		155.50^{a}±2.74	142.83^{b}±5.35	142.67^{b}±5.29

*EF: einkorn flour; PP: persimmon powder; QSM: quince seed mucilage. **Results are presented as the mean ± standard deviation result of ANOVA performed on data obtained by analysis of duplicate samples taken from two replications. ***Means not sharing a common letter within the same row are significantly different at $p<0.05$.

Color Measurement and pH

The L*, a*, and b* values of the control sample were 52.25, 8.62, and 20.96, respectively. We observed that the L*, a*, and b* values of the uncooked Turkish noodles were lower than those of the control sample (data not shown). L* values ranged from 50.69 to 51.55, a* values ranged from 7.02 to 7.09, and b* values ranged from 17.49 to 18.04 depending on the PP concentration. Lucas-González et al. [11] reported that persimmon flour addition decreased L* values but increased a* and b* values in spaghetti samples produced with durum wheat semolina. Similar results were also observed by Han et al. [14] in rice noodles. However, in our study, there was no significant difference between the Turkish noodles produced with different PP and QSM concentrations. This fact is due to the darker flour color of einkorn instead of durum wheat or rice which could cause a suppression of PP during dough formation. The effects of PP incorporation on the pH value of the Turkish noodles are given in Table 3. The pH values of EF, PP, QSM, and the control sample were 6.55, 6.08, 5.40, and 5.80. In the presence of PP, the pH value of the Turkish noodles increased from 5.95 to 6.12 ($p<0.05$) due to the pH value of PP.

Proximate Composition

The moisture, ash, protein, lipid, and crude fiber content of the control sample were 8.81%, 2.96%, 23.30%, 5%, and 2.57%, respectively. Moreover, PP had 10.76% moisture, 2.20% ash, 5.98% protein, 0.18% lipid, and 3.04% crude fiber, respectively. The QSM addition into the noodle formulation did not show significant differences among the Turkish noodles in terms of proximate composition. The effects of PP incorporation on the proximate composition of the Turkish noodles are presented in Table 3. Ash and crude fiber contents of the Turkish noodles increased ($p<0.05$) as the amount of PP increased. In contrast, the protein content of the Turkish noodles containing PP was found to be lower ($p<0.05$) than those of the control sample. The protein content of the Turkish noodles ranged from 17.00% to 18.78%, but PP incorporation at different levels did not influence the protein content of the Turkish noodles significantly. As expected, a decrease in the lipid content of the Turkish noodles was observed related to

the lower lipid content of PP. Similar results were observed for cake [15] and spaghetti [11] production with persimmon.

Total Phenolic Content

The total phenolic content (TPC) of EF, PP, QSM, and the control sample was 1.18 mg GAE/g, 9.83 mg GAE/g, 0.05 mg GAE/mL, and 0.82 mg GAE/g, respectively. As shown in Table 3, the substitution of PP provided a significant increase ($p<0.05$) in the TPC value of the Turkish noodles (maximum 32%). Similar results were observed by Yeşilkanat and Savlak [15], Lucas-González et al. [31], Abdallah et al. [32], and Hosseininejad et al. [33] for cake, spaghetti, cupcake, and muffin production with persimmon, respectively. Furthermore, persimmon was utilized for the improvement of non-cereal-based foods such as beer [34], yogurt [35], and ice cream [36] with enhanced phenolic content. Several studies have shown that persimmon is rich in antioxidants including phenolic compounds and carotenoids [31, 37, 38].

Mineral Content

The mineral composition of the control sample in terms of Mg, K, Fe, and Zn was 0.169 g/100 g, 0.569 g/100 g, 0.129 g/100 g, and 0.082 g/100 g, respectively. The Mg, K, Fe, and Zn contents of PP were 0.065 g/100 g, 1.062 g/100 g, 0.005 g/100 g, and 0.012 g/100 g, whereas those of EF was 0.212 g/100 g, 0.544 g/100 g, 0.123 g/100 g, and 0.105 g/100 g, respectively. The results indicated that the highest increase among minerals was observed in the K content of the Turkish noodles (max 59%) due to a rich source of persimmon in terms of K [39] (Table 3). In addition, Mg content increased by 26% with the addition of persimmon to the Turkish noodle formulation. Similar results were observed when persimmon was used to enrich cake [15], beer [34], yogurt [35], and ice cream [36].

Table 3. Effects of PP levels on proximate composition (%), pH, total phenolic (mg GAE/g), and mineral (mg/100 g, dry basis) contents of uncooked Turkish noodles

Dry matter	EF:PP	100:0	95:5	90:10
		$91.88^{a}\pm0.13$	$91.04^{b}\pm0.30$	$89.88^{c}\pm0.21$
Ash	EF:PP	90:10	95:5	100:0
		$3.09^{a}\pm0.03$	$2.90^{b}\pm0.19$	$2.83^{b}\pm0.05$
Lipid	EF:PP	100:0	95:5	90:10
		$2.25^{a}\pm0.09$	$2.16^{ab}\pm0.07$	$1.91^{b}\pm0.05$
Crude fiber	EF:PP	90:10	95:5	100:0
		$2.26^{a}\pm0.05$	$2.14^{a}\pm0.09$	$1.64^{b}\pm0.05$
pH	EF:PP	90:10	95:5	100:0
		$6.12^{a}\pm0.12$	$5.97^{b}\pm0.10$	$5.95^{b}\pm0.12$
Total phenolic content	EF:PP	90:10	95:5	100:0
		$1.09^{a}\pm0.03$	$1.00^{b}\pm0.04$	$0.83^{c}\pm0.02$
Mg	EF:PP	90:10	95:5	100:0
		$0.254^{a}\pm0.011$	$0.229^{b}\pm0.017$	$0.202^{c}\pm0.002$
K	EF:PP	90:10	95:5	100:0
		$0.834^{a}\pm0.027$	$0.686^{b}\pm0.057$	$0.524^{c}\pm0.011$
Fe	EF:PP	100:0	95:5	90:10
		$0.139^{a}\pm0.003$	$0.126^{b}\pm0.000$	$0.121^{c}\pm0.001$
Zn	EF:PP	90:10	95:5	100:0
		$0.091^{a}\pm0.001$	$0.080^{b}\pm0.003$	$0.075^{c}\pm0.001$

*EF: einkorn flour; PP: persimmon powder; Mg: magnesium; K: potassium; Fe: iron; Zn: zinc. **Results are presented as the mean ± standard deviation result of ANOVA performed on data obtained by analysis of duplicate samples taken from two replications. ***Means not sharing a common letter within the same row are significantly different at $p<0.05$.

Texture Analysis

The firmness and work of shear values of the Turkish noodles are also shown in Table 4. The firmness and work of shear values of the control sample were 1233.72 g and 126.90 g.cm, respectively, which were higher than the Turkish noodles produced with PP and QSM. The texture of the cooked noodles is one of the most important attributes in evaluating the noodle quality. A firm texture was obtained in the egg noodles due to egg albumin protein [40]. Texture parameters were significantly affected ($p<0.05$) by both PP incorporation and QSM addition. As shown in Table 5, a significant reduction in firmness and work of shear was observed when the level of PP increased. Texture attributes are primarily influenced by the structural network between starches and glutens. These may either weaken or strengthen the formation of hydrogen bonds within the noodle structure network [41]. Additionally, Solta Civelek [42] reported that higher fiber content in pasta had a destructive effect on the protein matrix; therefore, the weakened protein structure caused the pasta to have a softer texture. In this study, lower firmness and work of shear in Turkish noodles produced with PP incorporation indicated that PP could not have a function to fortify the network structures of the noodles. Texture parameters were also significantly affected ($p<0.05$) by QSM levels in the formulation. Turkish noodles with the highest level of QSM showed the lowest firmness and work of shear. These findings were similar to studies reported by Kasunmala et al. [27] and Charles et al. [30] that found cassava, *Neolitsea*

cassia, and *Dillenia retusa* mucilage addition resulted in lower firmness of noodles.

Table 4. Effects of PP and QSM levels on firmness (g) and work of shear (g.cm) of cooked Turkish noodles

Firmness	EF:PP	100:0	95:5	90:10
		1007.94^{a}±37.95	870.93^{b}±32.69	797.62^{c}±39.70
	QSM	20	30	40
		987.78^{a}±41.16	882.95^{b}±46.82	805.77^{c}±37.67
Work of shear	EF:PP	100:0	95:5	90:10
		94.52^{a}±3.57	71.95^{b}±3.59	69.01^{b}±5.24
	QSM	20	30	40
		88.06^{a}±2.81	74.00^{b}±8.19	73.42^{b}±5.35

*EF: einkorn flour; PP: persimmon powder; QSM: quince seed mucilage. **Results are presented as the mean ± standard deviation result of ANOVA performed on data obtained by analysis of duplicate samples taken from two replications. ***Means not sharing a common letter within the same row are significantly different at p<0.05.

Sensory Evaluation

The results of the sensory evaluation performed by the panelists are presented in Figure 1. According to the sensory analysis, as the level of PP increased, higher scores in sensory attributes were given by the panelists. Color values were recorded as 4.25 for the control sample and 4.33 for the Turkish noodles containing 10% PP. Additionally, there was no significant difference between the Turkish noodles produced with and without PP in terms of appearance. Among the Turkish noodles, the noodles containing 10% PP received the highest taste score (3.73) followed by the noodles containing 5% PP (3.45), whereas the lowest score (3.03) was recorded in the control sample. Similar to our findings, Hosseininejad et al. [33] reported that the addition of persimmon flour caused a significant increase ($p < 0.05$) in sweetness, fruity taste, and caramel taste in gluten-free muffins. A higher ratio of sugars to organic acids in the fruit composition is responsible for the sweet taste of the fruit which plays an important role in terms of sensory acceptability [43]. The odor scores were in accordance with the taste scores. Turkish noodles with an increasing level of PP had higher firmness and chewiness scores with significant differences ($p<0.05$) compared to the control sample or the Turkish noodles produced without PP. No significant differences were observed in the overall acceptability between the control sample (3.48) and the Turkish noodles produced without PP (3.51). Additionally, overall acceptability was positively influenced ($p<0.05$) by the increasing PP level in the Turkish noodles. These results were in accordance with those reported by Dipti et al. [44] where the addition of Persimmon sauce resulted in improved sensory evaluation of the cake. Further, Abdallah et al. [32] reported that cupcakes containing 33.3% persimmon puree were perceived the highest scores in terms of sensory parameters.

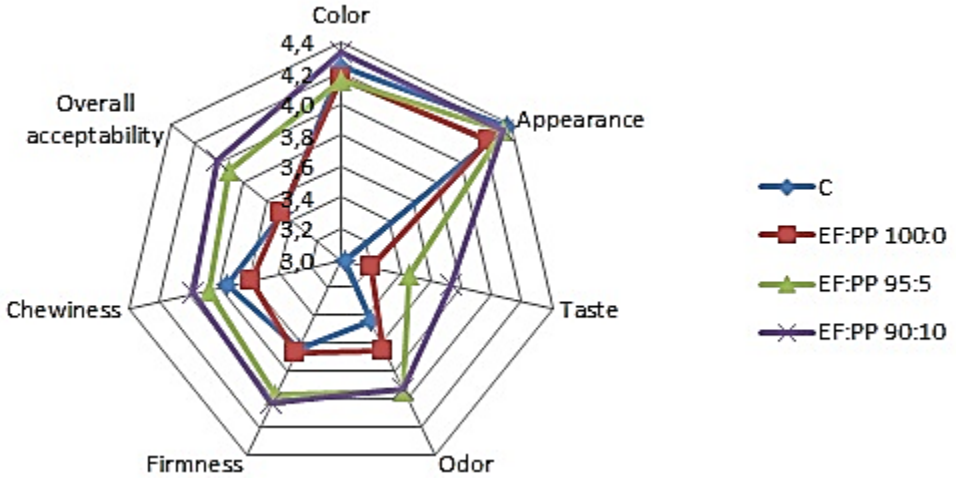

Figure 1. Spider plot of sensory evaluation of cooked Turkish noodles (C: control sample; EF: einkorn flour; PP: persimmon powder; QSM: quince seed mucilage)

CONCLUSION

In conclusion, it was observed that PP substitution enriched the nutritional quality of the Turkish noodles in terms of ash, dietary fiber, minerals (Mg, K, and Zn), and phenolic content. However, increasing the PP substitution level resulted in inferior quality in terms of cooking properties. The Turkish noodles produced with increasing level of QSM resulted in higher weight and volume gain. Moreover, the cooking loss of the Turkish noodles produced with 20% QSM was found to be similar to that of the control sample. Therefore, QSM could be used as an egg replacer in Turkish noodle production. The Turkish noodles containing high PP had

a softer texture with a lower work of shear. The highest scores in sensory attributes were obtained with 10% PP substitution. Further studies can be conducted to investigate the antidiabetic effect of Turkish noodles produced with persimmon and quince seed mucilage.

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Research Paper / Araştırma Makalesi

Presence of Carotenoid Gene in Lactic Acid Bacteria Isolated from White Cheese

Aslı Polat[1], Ceren Özbağcı[1], Dicle Dilara Akpınar[1], Ömer Şimşek[2]

[1]Yıldız Technical University, Institute of Science, Department of Food Engineering, TR-34210 İstanbul, Türkiye
[2]Yıldız Technical University, Faculty of Chemistry and Metallurgy, Department of Food Engineering, TR-34210 İstanbul, Türkiye

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Corresponding author (Yazışmalardan Sorumlu Yazar): omers@yildiz.edu.tr (Ö. Şimşek)
+90 212 383 45 45 +90 212 383 45 71

ABSTRACT

Carotenoids are organic pigments with antioxidant properties that are commonly found in nature. Various types of carotenoids are produced by microorganisms. In this study, we aimed to determine the microorganisms with potential carotenoid production and yellow-orange pigment production during the storage of white cheese below 10°C. Five different white cheeses with pigmentation problems were obtained from the provinces of Istanbul and Kocaeli. Colonies with a typical yellow-orange color and morphological differences were selected on MRS and M17 media. The presence of carotenoid genes in 136 selected colonies was determined by agarose gel electrophoresis using colony PCR, and carotenoid genes were detected in 6 colonies. According to the 16S rRNA sequence results, one of the 6 bacterial colonies carrying the carotenoid gene was *Lactococcus lactis*, another was *Enterococcus faecium*, and the rest were *Lactobacillus plantarum*. In addition to genotypic identification, Gram-staining was performed to determine the phenotypic characteristics of bacteria carrying the carotenoid gene, and it was found that six bacteria had Gram-positive and bacilli morphology. These results showed that some carotenoid producer strains existed in the microbioata of cheeses during cold storage.

Keywords: White Cheese, Carotenoid, *Lactococcus lactis*, *Lactobacillus plantarum*, *Enterococcus faecium*

Beyaz Peynirden İzole Edilen Laktik Asit Bakterilerinde Karotenoid Gen Varlığı

OZ

Karotenoidler antioksidan özellik gösteren ve doğada yaygın olarak bulunan organik pigmentler olarak bilinmektedir. Çeşitli karotenoid türleri mikroorganizmalar tarafından üretilmektedir. Bu çalışmada, beyaz peynirin 10°C'nin altında depolanması sonucu ortaya çıkan, potansiyel karotenoid üreticisi olup sarı-turuncu pigment oluşturan mikroorganizmaların belirlenmesi amaçlanmıştır. İstanbul ve Kocaeli illerinden toplamda 5 farklı sari pigmentasyon olan beyaz peynir temin edilmiştir. Peynirlerden MRS ve M17 besiyerleri üzerinden tipik sarı-turuncu renkli ve morfolojik bakımdan farklılık gösteren koloniler seçilmiştir. Seçilen 136 adet kolonide karotenoid geni varlığı koloni PZR yapılarak agaroz jel elektroforezde incelenmiş ve sonuca göre 6 adet kolonide karotenoid geni bulunduğu belirlenmiştir. Karotenoid geni varlığı tespit edilen kolonilerin 16S rRNA gen bölgesi PZR ile çoğaltılarak sekansa gönderilmiştir. 16S rRNA sekans sonucuna göre karotenoid geni taşıyan 6 adet bakteri kolonisinden bir tanesinin *Lactococcus lactis*, diğerinin *Enterococcus faecium* ve kalan diğerlerinin ise *Lactobacillus plantarum* olduğu tespit edilmiştir. Genotipik tanımlamanın yanında, karotenoid geni taşıyan bakterilerin fenotipik özelliklerinin belirlenmesi için gram boyama yapılmış ve 6 adet bakterinin Gram-pozitif ve çubuk hücre yapısında olduğu belirlenmiştir. Tüm bu sonuçlar peynirin soğuk muhafazasında muhtemel karatenoid üreticisi suşların varlığını göstermiştir.

Anahtar Kelimeler: Beyaz peynir, Karotenoid, *Lactococcus lactis*, *Lactobacillus plantarum*, *Enterococcus faecium*

INTRODUCTION

Milk is an important food source in terms of essential amino acids, fats, mineral substances, lactose, and vitamins. Milk is a perishable food owing to its rich nutritional content. This situation necessitates the application of heat treatments, such as pasteurization or sterilization of milk or converting milk into various fermented foods [1], [2]. Cheese, a product obtained by milk fermentation, preserves the main components of milk and is produced in various aroma, flavor, and textural forms [3]. Cheese is obtained by coagulating the milk protein casein with rennet, separating the whey, and salting the curd obtained by shaping or keeping it in brine [4]. There are more than 1000 types of cheese worldwide, of which 193 are produced in Turkey [3]. In Turkey, where there is a wide variety of cheese production, white cheese, cheddar cheese, and Tulum cheese are preferred among the people. In recent years, laited, curd, cottage, herby, and mihalic cheeses have attracted a great deal of attention [5].

White cheese is defined in the Turkish Food Codex No. 29261 as brined cheese with characteristic features, which can be defined as fresh or ripened according to the differences in production stages, produced by processing curd obtained by coagulating the raw material using rennet [6]. Studies have shown that *Lactococcus lactis* is the most dominant species in the microbiota of white cheese, followed by *Enterococcus feacalis, Enterococcus faceium, Lactiplantibacillus plantarum, Lacticaseibacillus casei, L. brevis, Leuconostoc lactis* and *Leu. Mesenteroides* ssp. *Dextranicum* species. It has also been shown that the microflora of white cheese changes during the ripening process. As cheese ripening progresses, a decrease in *Lactococcus* species and an increase in Lactobacillus species are observed [7]. At the end of maturation, *Lacticaseibacillus casei* and *Lactiplantibacillus plantarum* species were dominant [8]. In Turkey, white cheese is produced from milk pasteurized at 72°C for 20 s, and *Lactococcus lactis* subsp. and *Lactococcus lactis* subsp. *cremoris* strains are considered suitable [9]. White cheese is a semi-hard cheese obtained by mixing cow, sheep, goat, or milk in an appropriate ratio [10]. To provide the desired quality characteristics in white cheeses, they are matured in cold stores for at least 3 months and 6-12 months and presented to the consumer [11]. White cheese, which initially has a soft texture, is called semi-hard or semi-soft after it has matured in brine for 3 months [12].

For cheese production, raw milk is standardized and pasteurized for 2-3 seconds at 80-85°C, 30 min at 63°C, or 5 min at 65°C. After the temperature of the cooled milk after pasteurization reached 32°C, it was transferred to the fermentation tank. Starter culture and $CaCl_2$ are added to the pasteurized milk and left for incubation for 30 minutes, and at the end of this period, rennet is added. to 30-45 minutes after the yeast is added, the milk starts to take its gel form and after 75-90 minutes it becomes a firmer curd. The cheese pieces obtained by cutting the curd into cubes were kept in whey for 5-10 minutes. After soaking, the whey was removed and the cheese curd was transferred to stainless steel molds. After the transfer, a cloth was laid on the surface of the cheese and pressure was applied to the cheese at room temperature by placing a weight on it to tighten the curd. This process continues for 3-6 hours until the whey reached low levels. When the weight was lifted, the cloth was opened, and the cheese mass was cut into 7 × 7 × 7 or 7 × 7 × 10 cm^3 pieces and kept in brine at 15-16°C for 6-12 hours. Cheese molds were placed under the cans and filled with 14-16% brine to the brim, and the mouths of the cans were closed. Cheeses kept in tin cans at 12-15°C mature within 1-2 months and are ready for consumption [5]. During the ripening stage, various biochemical reactions occur in the cheese as a result of physical, chemical, and enzymatic interactions. These reactions can cause undesirable odor, flavor, and color changes as well as release components that have positive effects on flavor and aroma [13].

Carotenoid pigments, commonly found in milk, are important. Carotenoids are pigments synthesized in plant and animal cells, as well as in fungi and photosynthetic and non-photosynthetic microorganisms. It plays an important role in reducing oxidation reactions by removing free radicals with conjugated double bonds in Gram-positive bacteria [14]. It is necessary to determine the components that cause color changes during food spoilage and to reveal the factors that cause this change. Therefore, effective preventive methods should be developed. Microorganisms should be kept in the first place in deteriorations that cause color changes [13].

Color change is one of the leading quality issues in food. This study was carried out to identify the microorganisms that cause color changes in white cheese and carry a potential carotenoid gene. In this context, five white cheeses were collected from Istanbul and Kocaeli provinces. Dilutions were prepared from cheese samples and inoculated onto M17 and MRS media. Strains with a typical yellow-orange color and thought to differ in colony morphology due to incubation were selected. The aim of this study was to determine whether the selected strains carried the carotenoid gene. Genotypic and phenotypic identification of the strains carrying this gene was then performed.

MATERIALS and METHODS

Materials

Five unpackaged cheeses with yellow pigmentation problems were collected from the Istanbul and Kocaeli district bazaars. White cheese samples were stored at 4°C until further analysis (Figure 1).

Methods

Microbiological Analyses of White Cheese Samples

In this study, the counts of Total Aerobic Mesophilic Bacteria (TAMB), yeast mold, and Lactic Acid Bacteria (LAB) in cheese samples were determined using standard microbiological enumeration methods [15]. Appropriate dilutions were prepared from the cheese

samples brought to the laboratory, and microbiological inoculations were performed using the spread plate method on Plate Count Agar (PCA, Neogen, NCM0010A) for TAMB, Dichloran Rose Bengal Chloramphenicol (DRBC, Neogen, NCM0082A) for yeast mold, De Man, Rogosa and Sharpe (MRS, Neogen, NCM0079A) for Lactobacilli, and M17 (Liofilchem, 610505) for *Lactococcus*. The prepared Petri dishes were incubated at 37°C for 48 h for TAMB, 20-25°C for 5-7 days for yeast mold, and 37°C for 48 h for LAB.

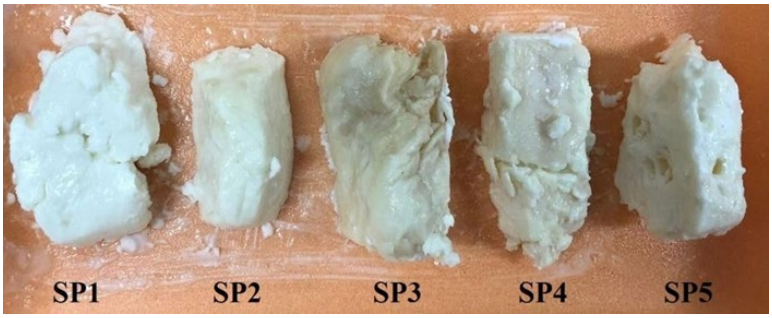


Figure 1. White cheese samples and their codes used in this study

pH and Titratable Acidity Values of Cheese Samples

The pH was determined using a compound electrode digital pH meter (PL-700PV, model pH meter). (Mettler Toledo, USA) [16]. For titratable acidity values, filtrate obtained from the cheese sample was titrated with 0.1 N sodium hydroxide solution using phenolphthalein indicator, and the titratable acidity of the cheese was expressed as percent lactic acid [17].

Isolation of Bacterial Strains

Physiological saline solution (90 mL, 0.85% NaCl) was added to 10 g of cheese sample and homogenized in a stomacher device. Dilutions of up to 10^6 were prepared from homogenized samples. For white cheese samples, MRS agar was used for *Lactobacillus* spp., and M17 agar was used for *Lactococcus* spp. Cells were cultured in MRS and M17 agar at 37°C for 48-72 hours. Typical yellow and orange colonies growing on M17 and MRS media, and colonies with different morphological features were selected.

Phenotypic Identification of Selected Strains by Gram-staining

Gram-staining was performed using strains selected from the colonies grown on MRS and M17 media.

Identification of Selected Colonies by Molecular Methods

Detection of Strains Carrying the *crt* Gene by Colony PCR

Glycerol stocks of the isolated colonies were plotted on MRS and M17 Agar media. The cells were then incubated for 24 h at 37°C. Colony PCR was performed to detect the presence of carotenoid genes in selected colonies. PCR reactions were performed on a PCR BIO-RAD T100™ Cycler using the FIREPol MasterCard Mix enzyme mixture, by preparing the carotenoid gene forward primer 5'-CGCGGAATTC TGAAGCAAGT TCGATTATTGGC and reverse primer-3' and 5'-GATCGAATTCTTAAGCCTCCTTAAGGGCTAGTTC-3, respectively. The PCR conditions for amplification of the carotenoid gene were as follows: initial denaturation at 95°C for 3 min, denaturation at 95°C for 30 s, adhesion at 58°C for 30 s, and elongation at 72°C for 150 s. Amplification, from denaturation to elongation, was repeated for 30 cycles. PCR reactions were loaded on a gel containing 1% agarose and carried out for approximately 90 min by applying a 100 Volt current. At the end of the run, the gel was kept in an ethidium bromide solution for approximately 1 h and then visualized on the BIO RAD GelDoc™ XR+ device under UV light. Colonies carrying the carotenoid gene were selected and genomic DNA was isolated.

Identification of strains using 16S rRNA

The genomic DNAs of the isolated strains was isolated using the Gene MATRIX Tissue & Bacterial RNA Purification kit. Because the 16S rRNA identification method was used for bacterial strain identification, the 16S rRNA region was chosen as the target region. For this purpose, 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-AAGGAGGTGATCCAGCCGCA-3' were used as forward and reverse primers, respectively. Amplification of the 16S rRNA gene region was performed using a BIO RAD T100™ Thermal Cycler device. The PCR conditions for amplification of the 16S rRNA gene were as follows: initial denaturation at 95°C for 3 min, denaturation at 98°C for 30 s, adhesion at 58°C for 30 s, and elongation at 72°C for 150 s. Amplification, from denaturation to elongation, was repeated for 30 cycles. 16S rRNA reactions were loaded onto a gel containing 1% agarose and carried out for approximately 90 min by applying a 100 Volt current. At the end of the run, the gel was kept in an ethidium bromide solution for approximately 1 h, and the result was visualized on the BIO RAD GelDoc™ XR+ device under UV light.

RESULTS and DISCUSSION

In white cheese samples, *Lactococcus* spp. counts varied between $1.17x10^6$ and $9.9x10^7$, while *Lactobacillus* spp. counts varied between $2.1x10^5$ and $1.69x10^7$ (Table 1). This indicates that white cheese is a rich source of lactic acid bacteria.

Table 1. Microbiological analysis results (CFU/g) of white cheese samples

Sample code	*Lactococcus* spp.	*Lactobacillus* spp.	Yeast-Mold	Total Aerobic Mesophilic Bacteria
SP-1	$9.90x10^7$	$1.69x10^7$	$5.00x10^4$	$7.60x10^6$
SP-2	$1.92x10^7$	$1.60x10^7$	$1.00x10^2$	$1.68x10^7$
SP-3	$1.17x10^6$	$2.10x10^5$	0	$6.60x10^5$
SP-4	$7.00x10^2$	$2.90x10^5$	0	$2.90x10^5$
SP-5	$9.90x10^5$	$1.60x10^6$	0	$9.70x10^4$

The titratable acidity values of the five cheeses analyzed varied between 1.05% and 2.96% in terms of lactic acid. The average value was found to be 1.80%. The pH values of the analyzed white cheese samples varied between 4.55 and 5.97, with an average value was found to be 5.20 (Table 2). The cheese samples complied with the standards in terms of pH and titratable acidity.

Table 2. pH and titratable acidiy values of white cheese samples

Sample code	pH	Titration Acidity (% lactic acid)
SP 1	4.55	1.70
SP 2	5.97	1.05
SP 3	4.64	1.93
SP 4	5.91	1.43
SP 5	5.07	2.96

A total of 136 yellow-colored colonies with different colony morphologies were selected from white cheese samples sold in Istanbul and Kocaeli provinces and cultured on M17 and MRS agar medium. Examples are named SP1, SP2, SP3, SP4, and SP5. After screening, 62 colonies from MRS medium and 74 colonies from M17 medium were selected: 30 from SP1, 25 from SP2, 33 from SP3, 25 from SP4, and 23 from SP5. Colony PCR was performed to determine whether the selected colonies harbored the carotenoid gene. PCR analysis revealed that six strains carried the carotenoid gene (Figure 2).

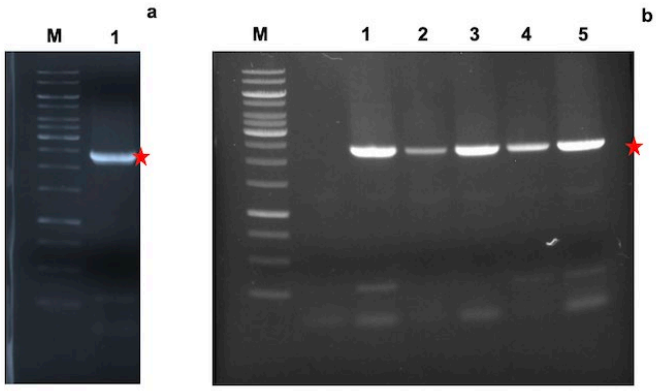
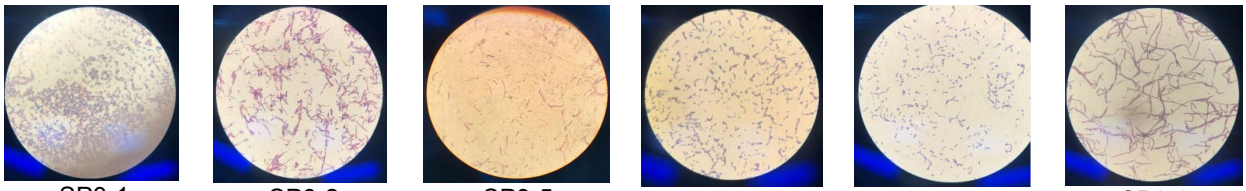


Figure 2. Colony PCR results of carotenoid gene screening of strains isolated from cheese samples. M: Ladder, a) First gel of Colony PCR, 1: SP3-1, b) Second gel of Colony PCR, 1: SP3-2, 2: SP3-3, 3: SP3-5, 4 : SP3-7, 5: SP3-9. The expected carotenoid gene length of 2379 bp is indicated with an asterisk.

Gram-staining was performed to determine phenotypic characteristics of the bacteria. Under the microscope, blue violet-colored cells were evaluated as Gram-positive and pink-colored cells as Gram-negative. All the strains found to carry the carotenoid gene were Gram-positive and bacil-positive (Figure 3).



Cheese Code

Figure 3. Gram-staining images of microorganisms

16S rDNA sequence analysis was used to identify bacterial strains. Sequence results were analyzed with the help of a plasmid editor program and converted to the FASTA format. Sequence data were analyzed using Nucleotide BLAST: nucleotide databases were searched using a nucleotidequery (nih.gov) [18]. According to the blast scan of the 16S rDNA sequence results for the identification of the strains carrying the carotenoid gene; It was determined that 1 strain (SP3-7) was *Lactococcus lactis*, 1 strain (SP3-3) was *Enterococcus faecium* and 4 strains (SP3-9, SP3-5, SP3-1, SP3-2) were *Lactobacillus plantarum* (Table 3).

Table 3. Blast scan results

JobTitle	ScientificName	MaxScore	TotalScore	QueryCover	E value	Percent Identification
SP3-1	*Lactobacillus plantarum*	2108	2108	%100	0.0	97.67
SP3-2	*Lactobacillus plantarum*	2071	2071	%99	0.0	98.64
SP3-3	*Enterococcus faecium*	1142	1142	%98	0.0	87.96
SP3-5	*Lactobacillus plantarum*	2217	2217	%99	0.0	98.57
SP3-7	*Lactococcus lactis*	2194	2194	%99	0.0	98.18
SP3-9	*Lactobacillus plantarum*	1988	1988	%98	0.0	97.20

Similar to our findings, Garrido-Fernández et al. [19] reported that 18 strains of *Lactobacillus plantarum* and *S. aureus* isolated from olive fermentation were carotenoid producers. Colonies isolated on MRS agar showed a dark yellow pigmentation. PCR studies have shown that *Lactobacillus plantarum* carries *crtM* and *crtN* genes in its genome.

In a study by Turpin et al. [20], it was determined that among 158 bacteria cultured in MRS medium, 36 strains produced carotenoid-like compounds. Among these strains, the carotenoid-producing bacterium *Lactobacillus plantarum* WCFS1 was also present, along with *Limosilactobacillus fermentum* and *Pediococcus acidilactici*, which are known to produce carotenoid-like compounds.

In a study by Kim et al. [21], 79 strains forming yellow colonies from various fermented foods were screened, and the 16S rDNA gene was identified by PCR amplification. According to the identification results obtained through 16S rDNA sequencing analysis, it was determined that among the identified bacterial strains, the *Lactilactobacillus plantarum* strain also carried the carotenoid gene.

In a study by Nam et al. [22], bottom portion of mevalonate partway of *Enterococcus faecium* VTCC-B-935 was transferred to *E. coli* and it was found that the biosynthesis of β-carotene increased 3 times.

In this study, we determined that the strains producing carotenoids were *Lactobacillus plantarum*, *Enterococcus faecium*, and *Lactococcus lactis*. A review of the literature led to the conclusion that *Lactobacillus plantarum* and *Enterococcus faecium* strains are carotenoid producers. However, whether *Lactococcus lactis* possesses the carotenoid gene was inconclusive. In this study, based on the identification of 16S rDNA, it was established that the *Lactococcus lactis* strain possesses the carotenoid gene.

This study highlights potential strategies involving the use of *Lactobacillus plantarum*, *Enterococcus faecium*, and *Lactobacillus plantarum* strains to meet the demand for carotenoid pigments in the food industry. The ability of these bacteria to produce carotenoids should be considered in future research to address the demand for natural coloring agents and to enhance the value of food products.

CONCLUSION

Five samples of white cheese were inoculated into MRS and M17 media. A total of 136 colonies with distinct yellow-orange color and morphological characteristics were selected, and their carotenoid gene was determined through colony PCR. Subsequently, six strains bearing the carotenoid gene were identified through 16S rRNA sequence analysis. The strains *Lactobacillus plantarum, Enterococcus faecium,* and *Lactococcus lactis* were found to carry the carotenoid gene. This finding provides an explanation for the yellow pigmentation observed in the cheese samples during cold storage, which is likely due to the stress response of certain strains within the microbiota.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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Araştırma Makalesi / Research Paper

Gül (*Rosa damascena* Mill.) Uçucu Yağının *Pseudomonas aeruginosa*'da Biyofilm Oluşumu ve Kayma Hareketi Üzerine Etkisi

Halime Çevikbaş, Seyhan Ulusoy

Süleyman Demirel Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, Isparta

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Yazışmalardan Sorumlu Yazar (Corresponding author): seyhanulusoy@sdu.edu.tr (S. Ulusoy)
0246 211 4068 0246 211 4399

ÖZ

Pseudomonas aeruginosa, bağışıklığı baskılanmış hastalarda akut ve kronik enfeksiyonlara sebep olan Gram-negatif, fırsatçı bir patojendir. *P. aeruginosa*, virülens faktörlerinin üretimi ve biyofilm oluşturma özelliklerini bir çeşit hücreler arası iletişim sistemi olan çevreyi algılama (Quorum sensing, QS) haberleşme sistemi ile kontrol eder. Bu haberleşme sisteminin farklı sentetik veya doğal moleküller ile engellenmesi veya yönlendirilmesiyle patojen bakterilerin kontrolünü konu alan çalışmalar yapılmaktadır. İçerdiği aktif moleküller sayesinde antibakteriyel, antifungal ve antiviral aktivitelere sahip olan bitkisel uçucu yağlar bu anlamda büyük potansiyel taşımaktadır. Bu çalışmada gül uçucu yağının, gül uçucu yağının temel bileşenlerinin (sitronellol, geraniol ve nerol) ve bu üç bileşenin karışımının (CGN) *Pseudomonas aeruginosa* PA01 suşu için hücrelerarası iletişim (QS) sistemi üzerine engelleyici etkisi araştırılmıştır. Çalışma sonucunda *P. aeruginosa* PA01 suşu için kayma hareketini; gül uçucu yağının %83, sitronellol, geraniol, nerol ve karışım CGN'nin, %61-75 oranında engellediği belirlenmiştir. *P. aeruginosa* PA01 suşu için biyofilm oluşumunu, gül uçucu yağı %54-68, sitronellol, geraniol, nerol ve karışım CGN %10-15 oranında baskılamıştır. Gül uçucu yağının *P. aeruginosa*'nın kayma hareketini ve biyofilm oluşumunu gül yağının temel bileşenlerinden daha yüksek oranda inhibe etmesi önemlidir. Bu çalışmanın sonuçları, sitronellol, nerol, geraniol ve CGN'nin *P. aeruginosa* suşu için anti-QS aktivitesine sahip olduğunu, ancak gül uçucu yağının çeşitli uygulamalarda kullanılabilecek potansiyelinin bulunduğunu göstermektedir.

Anahtar Kelimeler: Gül uçucu yağı, Biyofilm, *P. aeruginosa,* Kayma hareketi

Effect of Rose (*Rosa damascena* Mill) Essential Oil on Biofilm Formation and Swarming Motility on *Pseudomonas aeruginosa*

ABSTRACT

Pseudomonas aeruginosa is a Gram-negative, opportunistic pathogen that causes acute and chronic infections in immunocompromised patients. *P. aeruginosa* controls the production of virulence factors and biofilm formation properties with the Quorum sensing (QS) communication system, which is a kind of intercellular communication system. Studies have been carried out on the control of pathogenic bacteria by blocking or directing this communication system with different synthetic or natural molecules. Herbal essential oils, which have antibacterial, antifungal and antiviral activities because of their active molecules, have great potential in this sense. In this study, the inhibitory effects of rose essential oil, its major components (citronellol, geraniol, and nerol), and a combination of these three components (CGN) on the intercellular communication (QS) system of *Pseudomonas aeruginosa* PA01 strain were investigated. As a result of the study, it was determined that rose essential oil inhibited the swarming motility for the *P. aeruginosa* PA01 strain by 83%, and the inhibition by citronellol, geraniol, nerol, and a mixture CGN was in the range of 61-75%. For *P. aeruginosa* PA01 strain, biofilm formation was suppressed by rose essential oil by 54-68%, citronellol, geraniol, nerol, and mixture CGN by 10-15%. It was important that rose essential oil inhibited the swarming motility and biofilm formation of *P. aeruginosa* at a higher rate than the essential components of rose oil.

The results of this study showed that citronellol, nerol, geraniol, and CGN had anti-QS activity for *P. aeruginosa* strain, but rose essential oil had a potential to be used in various applications.

Keywords: Rose essential oil, Biofilm, *P. aeruginosa*, Swarming motility

GİRİŞ

Pseudomonas aeruginosa, akut ve kronik solunum yolu enfeksiyonları, göz ve yanık yaraları gibi birçok enfeksiyona neden olan Gram-negatif fırsatçı bir patojen bakteridir [1-3]. *P. aeruginosa*, bu enfeksiyonlara oluşturmak için hareketlilik, çeşitli proteazlar ve toksinler gibi çok çeşitli virülans faktörlerini içeren donanıma sahiptir. Ayrıca dezenfektanlara ve antibiyotiklere karşı yüksek direnç seviyeleri nedeniyle *P. aeruginosa'*nın sebep olduğu enfeksiyonlar en önemli sağlık sorunları arasında kabul edilmektedir [4-7].

P. aeruginosa'da virülans faktörlerinin üretilmesi, biyofilm oluşumu gibi özelikler QS iletişim sistemi tarafından düzenlenmektedir. *P. aeruginosa*'da bu sistemi oluşturan las, rhl, Pseudomonas kinolon sinyal (PQS) ve entegre QS (IQS) olmak üzere dört temel QS yolağı rapor edilmiştir [8-12]. Bu iletişim sistemi, *P. aeruginosa*'da biyofilm oluşumu, piyosiyanin, elastaz ve ramnolipid üretimi ve virülans genlerinin ekspresyonunu düzenlemek için birbirine bağlıdır [13]. *P. aeruginosa'nın* biyofilm oluşturma yeteneği, geleneksel antibiyotiklere direnç sağlaması nedeniyle hastalar ve sağlık sektörü için ciddi bir sorun oluşturmaktadır. *P. aeruginosa*'nın sebep olduğu biyofilm enfeksiyonlarının tedavisi oldukça zor ve maliyetlidir. Ayrıca bu enfeksiyonlar yüksek morbidite ve mortalite oranlarına sebep olmaktadır [14].

Son yıllarda, antibiyotik dirençli suşların ortaya çıkışını azaltmak için virülans faktörlerinin ve düzenleyici mekanizmaların (QS) hedeflenmesi, Pseudomonas'ın sebep olduğu enfeksiyonların kontrolü için umut verici yaklaşımlardan biridir [15, 16]. Doğada bitkisel uçucu yağlar antimikrobiyal özellikleri sayesinde bitkilerin korunmasında, önemli bir rol oynamaktadır. Çeşitli patojenlere karşı seçici toksisiteye sahip olmaları ve mikroorganizmalarda direnç gelişimini önleyen farklı etki mekanizmalarına sahip bileşenler içermesi, uçucu yağların dikkat çekici özelliklerindendir [17]. Ayrıca uçucu yağların QS sistemi üzerine etkilerini inceleyen araştırmalar mevcuttur [18-22].

Gül yağının, antioksidan, antimikrobiyal, antikanser, antifungal, probiyotik, antipiretik etkiler, anti Human Immunodeficiency Virus (HIV), antiülser etkileri çeşitli çalışmalarla rapor edilmiştir [23-30]. Ancak gül yağının ve onun başlıca bileşenlerinin *P. aeruginosa* için biyofilm oluşumuna etkisi ilk defa bu çalışma ile araştırılmıştır.

MATERYAL ve METOT

Bakteri Suşu, Ortam, Kültür Şartları ve Kimyasallar

Bu çalışmada *Pseudomonas aeruginosa* PAO1 suşu (*P. aeruginosa* PAO1) kullanılmıştır. *P. aeruginosa* PAO1 suşunu geliştirmek için Luria-Bertani (LB) besin ortamı kullanılmış [31] ve 24 saat 37°C'de inkübe edilmiştir. Gül (*R. damascena*) uçucu yağı Sebat Ltd. Şti'den (Keçiborlu, Isparta) temin edilmiştir. Sitronellol, geraniol ve nerol, Sigma Aldrcih'den (St. Louis, ABD) satın alınmış ve etanol ile çözülmüştür.

GC-MS Analizi

Gül uçucu yağının bileşen analizi Shimadzu QP5050 (Kyoto, Japonya) marka GC-MS ile Süleyman Demirel Üniversitesi Deneysel ve Gözlemsel Araştırma Uygulama Merkezinde yapılmıştır. Analiz Varian marka CP WAX 52 kapiler kolon (50m x 0,32 mm), taşıyıcı gaz olarak helyum kullanılarak gerçekleştirilmiştir. Enjeksiyon hacmi 1 µL dir.

Antibakteriyel Etkilerin Araştırılması

Sitronellol, geraniol, nerol ve gül uçucu yağının farklı derişimlerinin anti-bakteriyel özellikleri, *P. aeruginosa* PA01 suşları icin agar difüzyon tekniği kullanılarak test edilmiştir [26].

Biyofilm Testi

Gül uçucu yağı, sitronellol, geraniol ve nerolün anti-biyofilm özellikleri, Zhang vd. [32] tarafından önerilen yöntem kullanılarak değerlendirilmiştir. Bu amaçla, 1 mL LB sıvı besiyeri içeren plastik tüplere 0.5 McFarland bulanıklığa eşdeğer bulanıklıkta ayarlanmış olan gecelik *P. aeruginosa* kültürü eklenmiştir. Ardından Minimum İnhibitör Konsantrasyon (MİK) seviyelerinin altındaki derişimlerde gül yağı %1, %0.5, %0.25 (h/h), sitronellol (2.25mM, 1.125mM, 0.5625 mM), geraniol (1.44mM, 0.72mM, 0.36mM), nerol (0.81mM, 0.405mM, 0.2025mM) ve bu bileşenlerin karışımı CGN ilave edilmiştir. Tüpler iki gün 37°C'de inkübe edilmiştir. İnkübasyondan sonra besiyerleri dökülerek her tüp saf su ile üç kez yıkanmış, kurutulmuştur. Biyofilm tabakası %0.1 kristal viyole ile 30 dakika boyanmıştır. Ardından kristal viyole dökülerek, tüpler üç kez saf su ile yıkanmıştır. Daha sonra, tüplere 1 mL %95 etanol (hacim/hacim) ilave edilmiş ve 15 dakika bekletilmiştir. Süre sonunda absorbans 570 nm'de ölçülmüştür. Her deney üç kez tekrar edilmiştir. % biyofilm inhibisyonu aşağıdaki formül kullanılarak hesaplanmıştır.

$$\%\text{Biyofilm İnhibisyonu} = 100 - \frac{\text{Örnek Absorbans}}{\text{Kontrol Absorbans}} \times 100$$

Kayma Hareketi Testi

Gül uçucu yağı, sitronellol, geraniol ve nerolün *P. aeruginosa* için kayma hareketine etkisi Rashid ve Kornberg'in [33] metoduna göre incelenmiştir. Bunun için %0.5 ve noble agar ve glikoz içeren besiyeri

hazırlanmıştır. Hazırlanan besiyerine sitronellol (2.25mM), geraniol (1.44mM), nerol (0.81mM), CGN ve %1 oranında gül uçucu yağı ilave edilmiştir. Sonrasında gecelik *P. aeruginosa* PA01 kültürü, swarming agar petrilerinin ortasına inoküle edilmiş ve 37°C'de 24 saat inkübe edilmiştir. Kayma hareketi, inokülasyon noktasından etrafa doğru hareket eden bakteri hücrelerinin oluşturduğu çaplar ölçülerek değerlendirilmiştir. Her deney üç kez tekrarlanmıştır.

İstatistiksel Analiz

Tüm deneyler üçer tekrarlı yapılarak ortalamaları alınmış ve standart sapmaları hesaplanmıştır.

BULGULAR ve TARTIŞMA

GC-MS Analizi

GC-MS analiz sonuçlarına göre, *R. damascena* Mill. uçucu yağının %70'ini sitronellol (%35), geraniol (%22), nerol (%13)'ün oluşturduğu belirlenmiştir (Tablo 1). Bu sonuçlara göre sitronellol, geraniol ve nerol sırasıyla %35, %22 ve %13 oranlarında karıştırılıp CGN hazırlanmıştır.

Tablo 1. Gül yağının GC-MS analiz sonuçları
Table 1. GC-MS analysis results of rose essential oil

Bileşen Adı	Gül Yağı (%)
Etanol	1.08
Linalool	0.5
Sitrenellol	35
Nerol	22
Geraniol	13
Fenil etil alkol	1
Nanodekan	13
Metil öjenol	1.33
Öjenol	0.82

Antibakteriyel Etki

Agar difüzyon yöntemi ile sitrenellol, geraniol, nerolün 100 mM- 6.25 mM ve gül uçucu yağının %20-%2.5 (hacim/hacim) derişimlerinde *P. aeruginosa* PAO1 suşu için antibakteriyel etkileri incelenmiştir. Test edilen derişimlerde bu maddelerin *P. aeruginosa* PAO1 suşu için antibakteriyel etki göstermedikleri belirlenmiştir.

Biyofilm Oluşumu

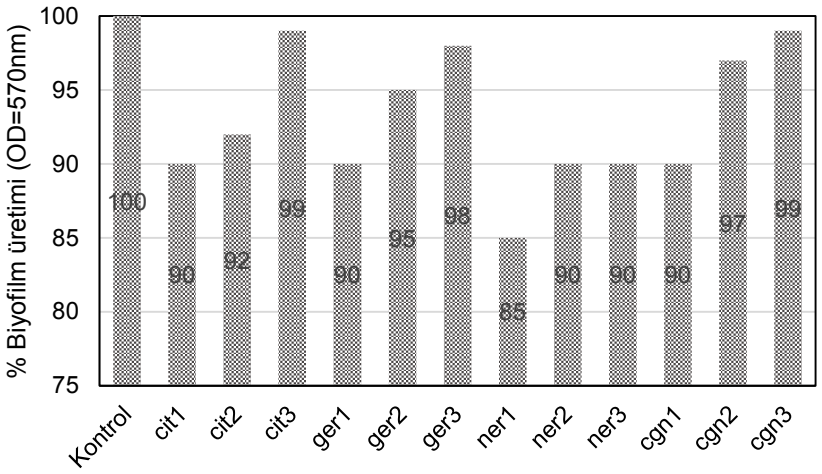
P. aeruginosa'nın biyofilm hücrelerinin, antibiyotiklere ve biyositlere, planktonik hücrelere göre daha dirençli olduğu bildirilmektedir. Bu nedenle bu bakteri ile enfekte olmuş hastaların tedavisi oldukça zor olmaktadır. Son zamanlarda yapılan birçok çalışma, QS iletişim sisteminin inhibisyonunun, virülans faktörlerinin üretimini ve biyofilm oluşumunu doğrudan etkileyebileceğini tutarlı bir şekilde vurgulamaktadır. Bu nedenle, *P. aeruginosa*'da QS iletişim sisteminin baskılanmasının, virülans özellikleri kontrol etmenin potansiyel bir yolu olabileceği ve enfeksiyon hastalıklarının kontrolü ile biyofilm oluşumuna karşı yeni tedavi alternatiflerin keşfedilmesini sağlayabileceği düşünülmektedir [34, 35].

Bu çalışmada sitronellol, geraniol, nerol, CGN ve gül uçucu yağının *P. aeruginosa* PAO1 suşu için biyofilm oluşumuna etkisini değerlendirmek için statik biyofilm testi yapılmıştır. Sitronellol, geraniol, nerol ve CGN *P. aeruginosa* PAO1 için biyofilm oluşumunu önemli seviyede baskılamamıştır. Sitronellol (2.25mM), geraniol (1.44mM), nerol (0.81mM) ve CGN biyofilm oluşumunu sırasıyla %10, %10, %15, %10 oranlarında azaltmıştır (Şekil 1). Bununla birlikte, gül uçucu yağı, biyofilm oluşumunu %1, %0.5 ve %0.25 (hacim/hacim) derişimlerinde sırasıyla %68, %57 ve %54 oranında baskıladığı belirlenmiştir (Şekil 2). Sonuç olarak gül uçucu yağı yüksek antibiyofilm aktivitesine sahipken, onun temel bileşenleri sitronellol, geraniol, nerol ve bunların karışımı olan CGN'nin düşük antibiyofilm aktivitesi gösterdiği belirlenmiştir.

Kayma Hareketi Analizi

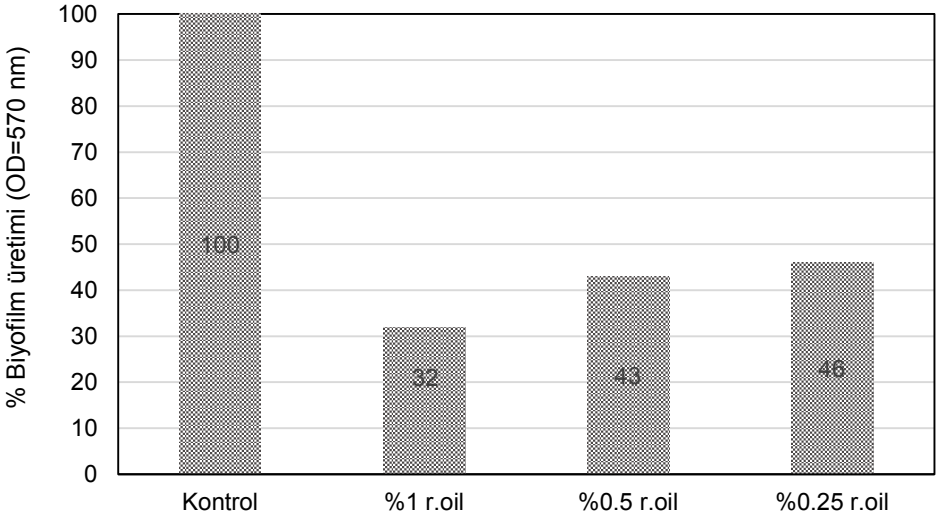
Kayma hareketi (swarming), *P. aeruginosa* ve bazı başka bakterilerde tanımlanan kamçıya bağlı yüzey hareketliliğinin bir şeklidir [36]. Bu hareket, bakterinin bir yüzeye tutunarak orada kolonize olmasını sağlamaktadır [37, 38]. Kayma hareket yeteneği bulunan patojenler, hızla değişen ortamlarda karşılaştıkları çeşitli zorluklara uyum sağlayabilmektedirler. Kayma hareketinin, bakterinin biyofilm oluşumu ve virülansında önemli rol oynadığı iyi bilinmektedir [39, 40]. Ayrıca, bu hareket yeteneğinin antibiyotik direnci ile de ilişkili olduğu bilinmektedir. Bu nedenle, kayma hareketini kontrol etmek, yeni anti-enfektif stratejilerin geliştirilmesi için büyük ilgi görmektedir [41, 42].

Bu çalışmada gül uçucu yağının, sitronellol, geraniol, nerol ve CGN karışımının *P. aeruginosa* PAO1suşu için kayma hareketine etkileri incelenmiştir. Gül uçucu yağı, %1 derişimde kayma hareketini %83 oranında engellemiştir. 2.25 mM sitronellol, 1.44 mM geraniol, 0.81 mM nerol ve CGN ise *P. aeruginosa* PAO1 suşunun kayma hareketini %61 ila %75 oranında inhibe etmiştir [Şekil 3, 4]. Sonuç olarak, gül uçucu yağının ana bileşenlerden daha yüksek oranda kayma hareketini engellediği belirlenmiştir.



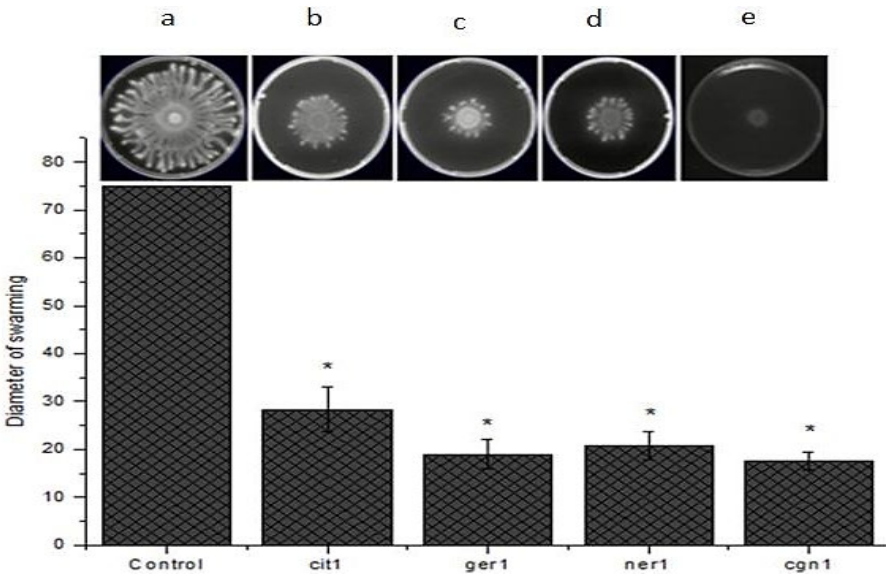
Şekil 1. Farklı derişimlerde sitronellol (cit 1: 2.25mM, cit 2: 1.125mM, cit 3: 0.5625mM), geraniol (ger 1:1.44mM, ger 2: 0.72mM, ger 3: 0.36mM), nerol (ner 1: 0.81mM, ner 2: 0.405mM, ner 3: 0.2025mM) and CGN'nin *P. aeruginosa* PAO1 üzerinde biyofilm oluşumuna etkileri. (Kontrol= Majör bileşen içermeyen *P. aeruginosa* PA01 kültürü)Tüm deneyler en az 3 kez tekrarlanmış ve ortalamaları alınmıştır.

Figure 1. Effects of different concentrations of citronellol (cit 1: 2.25mM, cit 2: 1.125mM, cit 3: 0.5625mM), geraniol (ger 1:1.44mM, ger 2: 0.72mM, ger 3: 0.36mM), nerol (ner 1: 0.81) mM, ner 2: 0.405mM, ner 3: 0.2025mM) and CGN on biofilm formation on P. aeruginosa PAO1. (Control= P. aeruginosa PA01 culture without major component) All experiments were repeated at least 3 times and averaged.



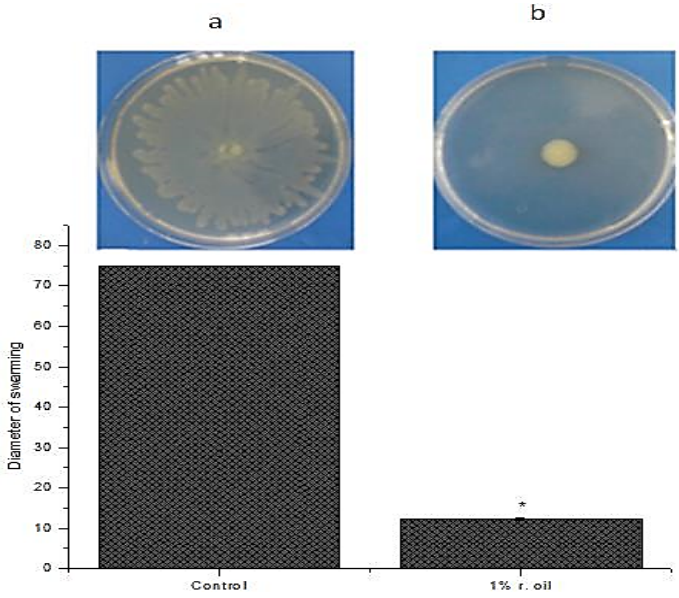
Şekil 2. Farklı derişimlerde *Rosa damascena* Mill. uçucu yağının (%1, %0.5, %0.25) *P. aeruginosa* PAO1 üzerinde biyofilm oluşumuna etkisi. (Kontrol= *Rosa damascena* uçucu yağı içermeyen *P. aeruginosa* PAO1 kültürü) Tüm deneyler en az 3 kez tekrarlanmış ve ortalamaları alınmıştır.

Figure 2. Effects of different concentrations of rose essential oil (1%, 0.5%, 0.25%) on biofilm formation on P. aeruginosa PAO1. (Control= P. aeruginosa PA01 culture without rose essential oil) All experiments were repeated at least 3 times and averaged.



Şekil 3. Kayma hareketi testi. (a) Kontrol *P. aeruginosa* PA01 (Kontrol= Majör bileşen içermeyen *P. aeruginosa* PA01), (b) sitronellol (cit1: 2.25mM), (c) geraniol (ger1: 1.44mM), (d) nerol (ner1: 0.81mM), (e) CGN. Tüm deneyler en az 3 kez tekrarlanmış ve one-way ANOVA kullanılarak istatistiksel olarak değerlendirilmiştir. Asterix, kontrole göre istatistiksel olarak anlamlı verileri göstermektedir (*P<0.05*).

Figure 3. Swarmig motility test. (a) Control P. aeruginosa PA01 (Control P. aeruginosa PA01 culture without major component), (b) citronellol (cit1: 2.25mM), (c) geraniol (ger1: 1.44mM), (d) nerol (ner1: 0.81mM), (e) CGN. All experiments were repeated at least 3 times and their statistical significance was evaluated using one-way ANOVA. Asterix shows statistically significant data compared to control (P<0.05).



Şekil 4. Kayma hareketi testi. (a) Kontrol *P. aeruginosa* PA01 (Kontrol= Etken madde içermeyen *P. aeruginosa* PA01), (b) *Rosa damascena* Mill. uçucu yağı (%1). Tüm deneyler en az 3 kez tekrarlanmış ve one-way ANOVA kullanılarak istatistiksel olarak değerlendirilmiştir. Asterix, kontrole göre istatistiksel olarak anlamlı verileri göstermektedir (*P<0.05*).

Figure 4. Swarmig motility test. (a) Control P. aeruginosa PA01 (Control P. aeruginosa PA01 culture without major component), (b) R. damascena Mill essential oil (1%). All experiments were repeated at least 3 times and their statistical significance was evaluated using one-way ANOVA. Asterix shows statistically significant data compared to control (P<0.05).

QS iletişim sistemi, *P. aeruginosa* dahil birçok Gram-negatif bakterinin çoklu ilaç direnci ve patogenezinde önemli rol oynayan düzenleyici sistem olarak kabul edilmektedir [43]. *P. aeruginosa*, bilinen antibakteriyel tedavileri sonuçsuz bırakan en tehlikeli patojen bakterilerden biridir [44]. Bu yüzden son zamanlarda, *P. aeruginosa*'nın da neden olduğu enfeksiyon hastalıkları ile mücadelede yeni antibakteriyel stratejilerinin geliştirilmesine büyük ilgi duyulmaktadır. Bu stratejilerden, mikroorganizma canlılığını veya büyümesini doğrudan etkilemeyen, dolayısıyla kullanılan ajana direnç geliştirmeyen ama patogenezi engelleyen sonuçlar beklenmektedir. Bu nedenle test edilen ajanların MIK seviyesinin altında kullanılması gerekmektedir [45].

Soković vd. [46] *Thymus vulgaris, Origanum vulgare* gibi bazı uçucu yağların antibakteriyel etkilerini, yağ içerisinde oransal olarak çok bulunan mentol, timol, karvakrol gibi bileşenlerle ilişkilendirmişlerdir. Ancak bazı uçucu yağ örneklerinin antibakteriyel aktiviteleri ile bileşenlerin oranları arasında anlamlı bir ilişki olmadığını belirtmişlerdir. Bu durumun uçucu yağ içerisindeki farklı bileşenlerin sinerjik etkisilerinden ve/veya düşük konsantrasyonlarda bile aktif olabilen diğer bileşenlerin varlığıyla açıklanabileceğini belirtmişlerdir.

Bu çalışma sonucunda gül uçucu yağının hem kayma hareketini hem de biyofilm oluşumunu yağın başlıca bileşenlerinden daha çok baskıladığı belirlenmiştir. Sonuç olarak, *P. aeruginosa* PAO1 suşu için sitronellol, geraniol, nerol ve bu üç molekülün kombinasyonunun belirli seviyede kayma hareketi ve biyofilm oluşumunu azalttığı belirlense de, gül uçucu yağının kayma hareketi ve biyofilm oluşumunu önemli seviyede inhibe ettiği tespit edilmiştir.

SONUÇ

Patojenik bakterilerin biyofilm oluşturma özellikleri, gıda endüstrisi ve insan/hayvan sağlığı için büyük bir sorun olarak kabul edilir. Bakteriyel biyofilm oluşumunu da düzenleyen QS iletişim mekanizmanın engellenmesi ve/veya bozulması, biyofilm oluşumunun önlenmesine ve biyofilm kaynaklı birçok sağlık sorununun çözülmesine yardımcı olabilir.

Bu çalışmada, gül uçucu yağının, saf etken maddelere göre daha etkili şekilde *P. aeruginosa*'da biyofilm oluşumunu ve kayma hareketini engellediği belirlenmiştir. Bu etkinin detaylarının ortaya konabilmesi için, gül uçucu yağ bileşenlerinin sinerji ve antagonizm özelliklerinin detaylı olarak araştırılması gereklidir.

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Review Paper / Derleme Makale

Potential Effects of Bilberry (*Vaccinium myrtillus* L.) on Cancer: A Narrative Review

Gülşen Özduran[1,2], Sevinç Yücecan[3]

[1]Near East University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Nicosia, Northern Cyprus
[2]DESAM Institute, Near East University, Nicosia, Northern Cyprus
[3]Lokman Hekim University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Ankara, Türkiye

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Corresponding author (Yazışmalardan Sorumlu Yazar): glsn_ozdrn@hotmail.com (G. Özduran)
+90 392 223 6464 (3429) +90 392 223 6461

ABSTRACT

Bilberry (*Vaccinium myrtillus* L.) is a fruit with high polyphenolic content and rich in anthocyanins. Due to its strong antioxidant capacity, it has potential effects in improving human health and reducing the risk of diseases. In addition to its antioxidant effect, it also possesses potential anti-inflammatory, anti-carcinogenic, anti-angiogenic, anti-proliferative, anti-atherogenic, anti-microbial, anti-diabetic, anti-lipidemic, neuroprotective, anti-metastatic, anti-radical effects, as well as preventing lipid oxidation, reducing oxidative stress and improving eye health. Bilberry consumption can potentially protect against and reduce the risks of chronic inflammation, dyslipidemia, hyperglycemia, increased oxidative stress, cardiovascular diseases, diabetes, dementia, and other age-related diseases and cancer. This review focuses on the potential mechanisms of action of bilberry in cancer.

Keywords: *Vaccinium myrtillus* L., Billberry, Cancer, Anthocyanins, Functional food

Yabanmersininin (*Vaccinium myrtillus L.*) Kanser Üzerindeki Potansiyel Etkileri: Geleneksel Derleme

ÖZ

Yabanmersini (*Vaccinium myrtillus L.*) polifenolik içeriği yüksek antosiyaninlerden zengin bir meyvedir. Antioksidan kapasitesi çok güçlü olduğu için sağlığı geliştirici ve hastalık risklerini azaltıcı potansiyel etkileri vardır. Antioksidan etkisinin yanı sıra anti-inflamatuar, anti-kanserojenik, anti-anjiyogenik, anti-proliferatif, anti-aterojenik, anti-mikrobiyal, anti-diyabetik, anti-lipidemik, göz sağlığını geliştirici, nöroprotektif, anti-metastatik, anti-radikal, lipit oksidasyonunu önleyici ve oksidatif stresi azaltıcı potansiyel etkileri de bulunmaktadır. Yabanmersini tüketimi kanser dahil olmak üzere kronik inflamasyon, dislipidemi, hiperglisemi, artmış oksidatif stres, kardiyovasküler hastalıklar, diyabet, demans ve yaşa bağlı diğer hastalıkların risklerinin azaltılmasında, önlenmesinde ve tedavisinde koruyucu potansiyel etkilere sahiptir. Bu derlemede antosiyaninlerden zengin olan yabanmersininin kanser üzerindeki potansiyel etki mekanizmaları anlatılmıştır.

Anahtar Kelimeler: *Vaccinium myrtillus* L., Yabanmersini, Kanser, Antosiyaninler, Fonksiyonel gıda

INTRODUCTION

Cancer is a complex disease characterized by uncontrolled cell division, abnormal cell growth, and influenced by genetic makeup and environmental conditions. Cancer ranks second among the leading causes of death worldwide, following cardiovascular diseases. According to the World Health Organization,

cancer accounts for 10 million deaths worldwide each year. Globally, one in every six deaths is attributed to cancer [1-3]. The factors contributing to cancer etiology include excessive consumption of salty, spicy, and smoked foods, inadequate intake of fruits and vegetables rich in antioxidant vitamins and bioactive compounds, insufficient fiber intake, high-carbohydrate and high-fat diets, and the presence of *Helicobacter pylori*. Other factors influencing cancer etiology include low socioeconomic status, age, gender, race, ethnic, reproductive factors, metabolic factors, environmental factors such as tobacco use, alcohol consumption, ultraviolet exposure, inadequate physical activity, immunity, as well as genetic factors [4-8].

Epidemiological studies have provided strong evidence that a diet rich in fruits and vegetables reduces the incidence of cancer. Fruits and vegetables exert their potential anti-carcinogenic actions through phytochemicals, which are functional food components [9-11]. Phytochemicals include carotenoids, phenolics, alkaloids, nitrogen-containing compounds, and organosulfur compounds. Phenolic compounds are further categorized as phenolic acids, flavonoids, stilbenes, tannins, and coumarins. Flavonoids, in turn, are divided into six subclasses including flavonols (e.g., quercetin, kaempferol, myricetin), flavones (e.g., apigenin, luteolin), flavanones (e.g., hesperidin, naringenin), flavanols (e.g., catechin, epicatechin, epigallocatechin, theaflavin), isoflavones (e.g., genistein, daidzein, glycitein), and anthocyanins (e.g., delphinidin, cyanidin, peonidin, malvidin, pelargonidin, petunidin) [12-14].

The potential anti-carcinogenic effects of flavonoids have been demonstrated through experimental evidence both *in vitro* and *in vivo*. Flavonoids have been shown to have positive effect various cancer processes, including proliferation, migration, inflammation, angiogenesis, invasion, and metastasis. They exert these effects by modulating mitogen-activated protein kinase (MAPK), protein kinase C (PKC), phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), and beta-catenin (β-catenin) pathways, by inhibiting the activation of nuclear factor kappa B (NF-kB) and tumor activator protein-1 (AP-1), inducing cell cycle arrest, and promoting apoptosis and autophagy [13, 15 - 17].

Bilberry, cranberry, raspberry, mulberry, elderberry, and strawberry are berries that belong to the berry family, and are rich in nutrients and several bioactive compounds including phenolic acids, tannins and flavonoids, especially anthocyanins [4, 10, 18]. Especially, among these berries, the anthocyanin content of bilberry is higher compared to other Vaccinium species and is one of the richest natural sources [19]. Bilberry has the highest antioxidant capacity owing to its phytochemical content. In addition to its antioxidant capacity, studies have shown that it also has anti-inflammatory, anti-proliferative, anti-invasion, anti-angiogenic, anti-metastatic, anti-migration, apoptotic, anti-adhesion, anti-tumor and chemopreventive effects. It has been emphasized that these effects may have strong anti-carcinogenic effects on organ cancers and blood-related cancers [4, 10, 18-25].

Recently, researchers have provided many and different information about the nutrient components contained in bilberry, the properties of these components and their effects on health. It appears to be an important and promising functional food due to its functional nutrient components. The increase in the number of in vitro and in vivo studies examining the relationship between bilberry and cancer has led to a significant increase in the information on the subject in the literature. However, there are limited number of reviews in the literature describing its general potential effect mechanisms of action on cancer. Therefore, the purpose of this review is to evaluate the potential effects of bilberry on cancer.

Search Strategy

The electronic databases of Medline, Pubmed, Web of Science, Science Direct and TUBITAK ULAKBIM Turkish Medical Directory were searched in order to determine the studies on the potential effects of *Vaccinium myrtillus* L. on cancer. “*Vaccinium myrtillus* L.” AND “Cancer”, “Billberry” AND “Cancer”, “*Vaccinium myrtillus* L.” OR AND “Billberry” AND “Anthocyanins” keywords were used. Databases were searched from inception to 2022 without year limitation. Studies examining the relationship between *Vaccinium myrtillus* L. or billberry and cancer were included in this review. This review was prepared by using the Documentary Source Analysis method, including a total of 76 articles.

Bilberry and its Characteristics

Bilberry is a plant species belonging to the *Ericaceae* family, and grows in grasslands, meadows, and moist coniferous forests in Northern Europe, North America, and Asia. It is a small, blue-colored, seed-bearing, fleshy fruit with a diameter of 5-9 mm [18, 26, 27]. It produces its blue-purple fruits from April to June. In Turkey, it grows in mountains and forests, and is known as "çalı çiçeği" or "çoban üzümü" [28].

In traditional medicine, the fruit of bilberry is used for its anti-diarrheal properties, while the leaves are used as a coagulant and diuretic. The leaves of bilberry contain tannins, flavonoids, and a small amount of arbutin [29]. Blueberries are also a fruit rich in dietary fiber content. It is stated that it can prevent and protect against intestinal diseases such as constipation, hemorrhoids and colon cancer [30]. The fruit of bilberry is rich in phenolic compounds such as flavonols (e.g., quercetin, myricetin), flavanols (e.g., catechin, epicatechin), tannins, ellagitannins, phenolic acids (e.g., gallic, caffeic, ferulic, and chlorogenic acids), and anthocyanins. Among the phenolic compounds, bilberry is particularly abundant in anthocyanins, including delphinidin, cyanidin, peonidin, petunidin, and malvidin [18, 27, 31 - 34]. Table 1 shows the anthocyanin content of bilberry [18].

Table 1. The anthocyanin content of bilberries

Anthocyanins	%
Delphinidin	15.17
Delphinidin-3-O-glucoside	5.81
Delphinidin-3-O-galactoside	5.04
Delphinidin-3-O-arabinoside	4.32
Siyanidin	8.36
Cyanidin-3-O- glucoside	3.42
Cyanidin-3-O- galactoside	2.75
Cyanidin-3-O-arabinoside	2.19
Petunidin	6.64
Petunidin-3-O- glucoside	3.67
Petunidin-3-O- galactoside	1.89
Petunidin-3-O-arabinoside	1.08
Malvidin	5.43
Malvidin-3-O- glucoside	3.35
Malvidin-3-O- galactoside	1.27
Malvidin-3-O-arabinoside	0.81
Peonidin	1.87
Peonidin-3-O- glucoside	1.31
Peonidin-3-O- galactoside	0.34
Peonidin-3-O-arabinoside	0.22

Bilberry contains approximately 40% of anthocyanins. Delphinidin and cyanidin contain about 60% of the total anthocyanin content. The anthocyanin content is ranked as follows: delphinidin (15.17%), cyanidin (8.36%), petunidin (6.64%), malvidin (5.43%), peonidin (1.87%). Depending on environmental conditions and ripeness, approximately 100 grams of bilberry contains about 300-700 mg of anthocyanins. In addition to anthocyanins, 100 grams of bilberry contains approximately 3 mg of quercetin, 20 mg of catechins, 100 mcg of beta-carotene, and 64 mcg of lutein [18, 26, 32, 35]. Table 2 shows the energy and nutrient content of 100 grams of bilberry [35].

Table 2. Energy and nutrient content of bilberries

	Component	Unit	Average amount (100 g)
	Energy	kcal	44
	Water	g	87.76
Macro nutrients	Protein	g	0.46
	Total fat	g	0.34
	Carbohydrate	g	8.49
	Total fiber	g	2.73
	Soluble fiber	g	0.30
	Insoluble fiber	g	2.43
Micro nutrients	Iron	mg	0.55
	Phosphorus	mg	20
	Calcium	mg	13
	Magnesium	mg	7
	Potassium	mg	98
	Sodium	mg	6
	Zinc	mg	0.15
	Vitamin C	mg	21.9
	Thiamin	mg	0.009
	Riboflavin	mg	0.025
	Niacin	mg	0.321
	Vitamin B6	mg	0.077
	Vitamin A	RE	8
	Beta-carotene	mcg	100
	Lutein	mcg	64

Bilberry has the highest antioxidant capacity among berries. It shows this effect with the nutrients and functional food components it contains. Also, its beneficial phytochemical content enables it to have potential effects such as anti-inflammatory, antiseptic, anti-lipidemic and anti-radical, as well as antioxidant effects. It can improve nutritional quality, improve health and reduce the risk of chronic diseases [18, 26, 31, 36, 37].

Anthocyanins

Anthocyanins are water-soluble flavonoids commonly included in fruits and vegetables. Essentially, they are the pigments responsible for the pink, red, blue, and purple colors of many flowers, leaves, vegetables, and fruits [7, 18, 38].

Anthocyanins are glycosylated forms of anthocyanidins (aglycones). These compounds are formed by the flavylium cation backbone hydroxylated at different positions (usually at C3, C5, C6, C7 and C3', C4', C5' carbons). The properties of anthocyanins depend on the degree and pattern of hydroxylation and methoxylation of the skeletal structure [39, 40]. Anthocyanins exhibit color changes based on pH. They appear red in acidic pH (below pH 2), purple at neutral pH, blue at alkaline pH, and colorless at higher pH levels. The anthocyanin pigments responsible for the red color are primarily included in the form of flavylium cations. The flavylium cation formed at low pH allows anthocyanins to be highly soluble in water. Cyanidin appears red at pH < 3, violet at pH 7-8, and blue at pH > 11, while peonidin is cherry red at low pH and dark blue at pH 8. Anthocyanin molecules consist of an anthocyanidin core with sugar (glucose, galactose, xylose, arabinose, or rhamnose) moieties attached at various positions. Anthocyanins vary based on the number and position of hydroxyl and methoxy groups, depending on the structure of the anthocyanidin [18, 38]. Although there are fewer than twenty naturally occurring anthocyanidins, hundreds of different anthocyanins exist. The most common anthocyanins include cyanidin, delphinidin, pelargonidin, peonidin, malvidin, and petunidin. The distribution of these anthocyanidins in fruits and vegetables is approximately 50% cyanidin, 12% delphinidin, 12% pelargonidin, 12% peonidin, 7% malvidin, and 7% petunidin. Cyanidin is responsible for the purplish-red color and is the main pigment in berries. Delphinidin has a similar chemical structure to anthocyanidins and exhibits a bluish-red color. It is responsible for the blue tones in flowers. Pelargonidin differs from most other anthocyanidins and appears as a red-colored pigment in nature. It gives an orange color to some fruits and a red color to certain flowers. Peonidin is a methylated anthocyanidin included in high amounts in fruits, wines, and berries. It has a violet color. Malvidin is another methylated anthocyanidin with a purple appearance. It is responsible for the red color in red wine and appears as a dark red in unripe wines. Petunidin is a methylated anthocyanidin and is a water-soluble dark red or purple pigment [38].

Regarding the bioavailability of anthocyanins, it has been included that their absorption is rapid but relatively low. Unlike other polyphenolic flavonoids, they are absorbed without structural degradation [18]. Anthocyanins are metabolized in the mouth after consumption. In the mouth, glycosidic groups are removed and chalcones are formed under the influence of the oral microbiota. They start from the stomach and passes through the gastrointestinal tract. Despite the acidic pH of the stomach, they do not undergo significant changes and can be absorbed by bilitranslocase or reach the intestinal epithelium. Anthocyanins reach the liver via portal vein circulation. From here they are directed to the systemic circulation and used by target organs and tissues. Unabsorbed metabolites are excreted in urine and feces [41]. Depending on their structure, anthocyanins are absorbed from the stomach and small intestine at a rate of 11-22%. Anthocyanins can be detected in the plasma shortly after oral intake, typically within a few minutes. They reach its maximum plasma concentration between 30-120 minutes and are eliminated within 6 hours. The maximum plasma concentration ranges from 5 to 50 nmol/L [18, 41].

The anthocyanin content of fruits can vary depending on factors such as sunlight exposure, pH, temperature, soil nitrogen and phosphorus levels, oxygen, processing time, storage conditions, harvest time, and fruit ripeness. Anthocyanins are primarily included on the outer surface of fruits. Damage to the fruit's outer surface during harvest can reduce the anthocyanin content. The optimal harvest time is typically in August and early September. As the fruit ripens, the anthocyanin content increases [18, 26].

Anthocyanins have potential health promoting and disease-preventive effects. Their antioxidant, apoptotic, anti-proliferative, anti-angiogenic, anti-carcinogenic, anti-diabetic, anti-obesity, anti-microbial, cardioprotective, eye health- promoting, neuroprotective, and anti-hypertensive effects contribute to their health benefits [18, 38, 41 - 43].

Potential Effects of Bilberry on Cancer and Relevant Studies

Bilberry plays a crucial role in the prevention and treatment of cancer due to its potential antioxidant, anti-radical, anti-inflammatory, anti-proliferative, anti-carcinogenic, anti-angiogenic, anti-metastatic, and apoptotic effects.

Antioxidant and Anti-radical Effects of Bilberry

When the concentrations of heavy metals exceed optimal levels, they can disrupt the normal functioning of the cell by affecting the cellular components and events. They have been associated with increased reactive oxygen species (ROS). This species can interfere with macromolecules, resulting in impairment of cellular functions and metabolism in normal cells. For example, lipid peroxidation and protein inactivation can result in DNA damage. Detoxification is required to minimize the

damage caused by ROS. Detoxification occurs through two separate pathways: enzymatic and non-enzymatic. The enzymatic pathway involves antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR). The non-enzymatic pathway involves tripeptide glutathione, proline, cysteine, ascorbate, and small molecules containing non-protein compounds rich in sulfhydryl (-SH) groups [27, 44].

Bilberry exhibits antioxidant activity by chelating metals such as iron involved in redox reactions, scavenging hydroxyl radicals, hydrogen peroxide radicals, superoxide anion radicals, and ROS. It increases the levels of glutathione, a powerful antioxidant, and antioxidant enzymes (SOD, CAT, GPX, and GR), thereby enhancing antioxidant capacity [3, 18, 28, 45]. Induction of phase I and phase II antioxidant enzymes involved in detoxification protects against ROS while inhibiting the CYP1A1 gene [33, 34]. Figure 1 shows antioxidant and anti-radical effects mechanisms of *Vaccinium myrtillus* L. in cancer cells [3, 18, 28, 45 - 47]..

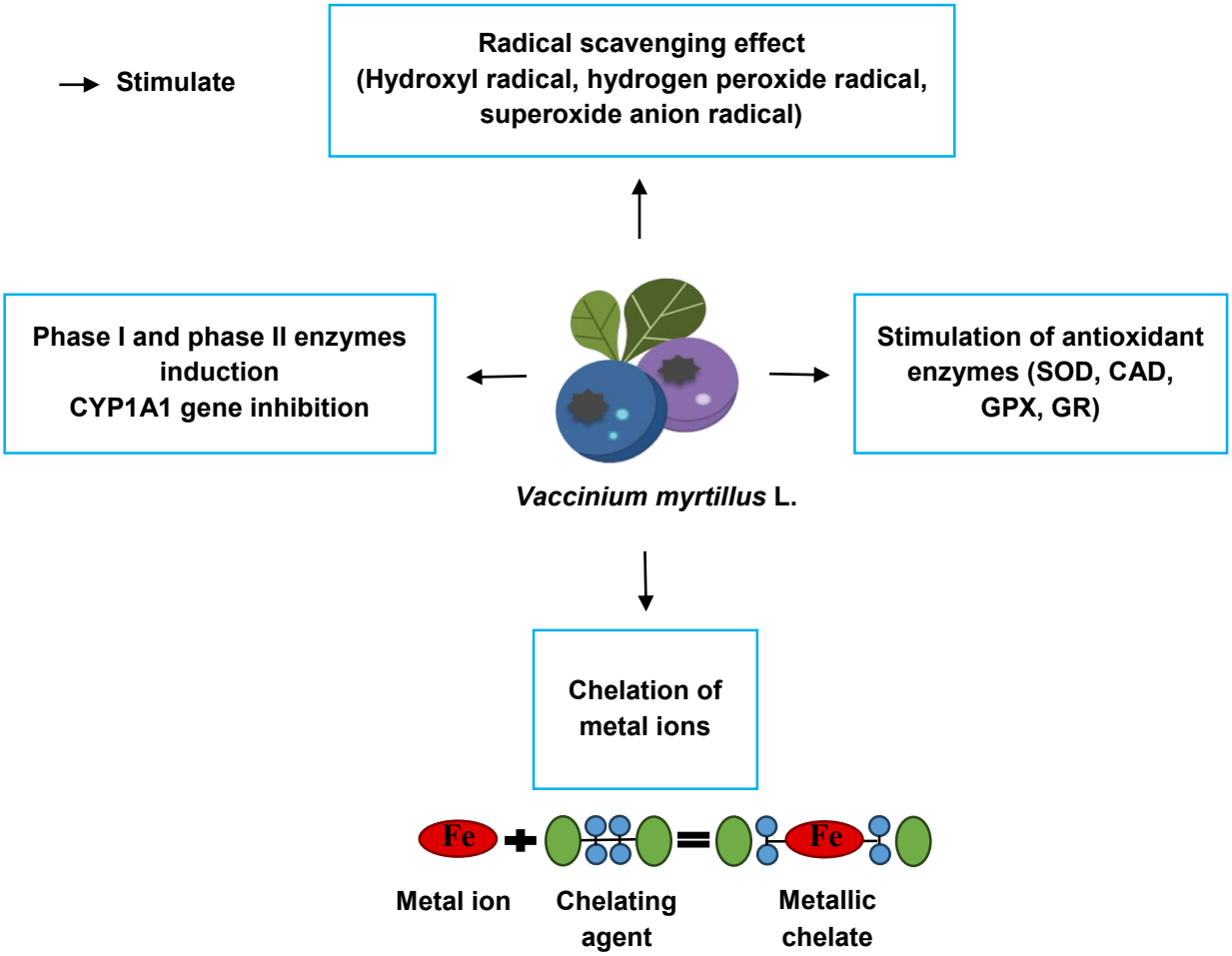

Figure 1. Antioxidant and anti-radical effects mechanisms of *Vaccinium myrtillus* L. in cancer cells [3, 18, 28, 45-47].

In a study by Juadjur et al. [48] on Caco-2 and HT-29 human colon cancer cell lines, 500 mcg/mL of *Vaccinium myrtillus* L. extract significantly reduced ROS levels in Caco-2 cells after 1 hour of incubation. A slight decrease in ROS levels was also observed in HT-29 cells after 24 hours of incubation. Additionally, an increase in total glutathione levels was detected in Caco-2 cells treated with 500 mcg/mL of bilberry extract after 24 hours of incubation. The extract reduced oxidative DNA damage and increased total glutathione levels at high doses. In a study, Šaponjac et al. [49] investigated the antioxidant effects of dried bilberry extract using three different extract fractions. The first extract fraction (Fr1) contained 1.02 mg/100 g of vitamin C, the second extract fraction (Fr2) contained six flavonoids (with quercetin being the most abundant at 243.3 mg/100 g of dried bilberries), and the third extract fraction (Fr3) contained eight phenolic acids, with p-coumaric acid being the most prominent (57.87 mg/100 g of dried bilberry). The most significant effect on the transformation and stabilization of hydroxyl radicals was observed in Fr3 ($EC^{\bullet OH}50$ = 0.117 mg/mL), while the best free radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was observed in Fr2 ($EC^{DPPH\bullet}50$ = 0.025 mg/mL). Fr1 exhibited the lowest free radical scavenging effect on both DPPH and hydroxyl radicals ($EC^{DPPH\bullet}50$ = 0.204 mg/mL, $EC^{\bullet OH}50$ = 1.213 mg/mL). The highest anti-radical effect was observed in Fr2. In addition, Fr2 and Fr3 inhibited the growth of cervical epithelioid carcinoma, breast

adenocarcinoma, and colon adenocarcinoma cell lines. In Bao et al.'s study [50], mice were orally (0.1 mL/10 g of body weight) administered bilberry extract at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg for 5 days. An increase in mitochondrial membrane potential, an increase in sodium/potassium ATPase activity, and a decrease in ROS levels were observed at the dose of 200 mg/kg. In a study by Esselen et al. [51], when HT-29 colon carcinoma cells were incubated with 500 mg/mL of bilberry extract for 72 hours, cell growth was inhibited. It was reported that at a concentration of 25 mg/mL, topoisomerase I activity was strongly inhibited, and at concentrations ≥50 mcg/mL, topoisomerase I activity was completely suppressed. Topoisomerase II activity was reduced at concentrations ≥1 mcg/mL. The study concluded that bilberry extract suppressed the levels of topoisomerase I and II, which covalently bind to DNA and cause damage to the DNA chain, thus preventing DNA damage. Ancillotti et al. [31] examined the antioxidant and anti-radical activities of *Vaccinium myrtillus* L. and *Vaccinium uliginosum* subsp. gaultherioides, and reported that *Vaccinium myrtillus* L. exhibited greater antioxidant and anti-radical activities due to its delphinidin and cyanidin content. In a study by Kandziora-Ciupa et al. [44], the concentrations of heavy metals (cadmium, lead, zinc, iron, and manganese) in the soil and their bioavailability in *Vaccinium myrtillus* L. were investigated. A positive correlation was found between the concentrations of cadmium, manganese, and zinc in *Vaccinium myrtillus* L. leaves and proline. An increase in manganese accumulation was observed to lead to a decrease in antioxidant response, while an increase in non-protein -SH groups and glutathione content resulted in an increase in antioxidant response. It was also found that bilberry has a high capacity for manganese accumulation.

Anti-inflammatory and Anti-proliferative Effects of *Vaccinium myrtillus* L.

Oxidative stress, along with the stimulation of the microenvironment, activates several pathways such as NF-kB, signal transducer and transcription activator 3 (STAT3), hypoxia-inducible factor-1 alpha (HIF-1α), AP-1, and nuclear factor erythroid 2-related factor 2 (Nrf2), leading to increased release of inflammatory cytokines such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-1 beta (IL-1β), interleukin-10 (IL-10) and tumor necrosis factor (TNF)-α. Chronic inflammation, coupled with increased levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS), may result in malignant cell transformation in healthy cells and tissues, thereby increasing the risk of cancer occurrence [3, 33, 46, 52]. Bilberry with its phytochemical content, particularly anthocyanins, exhibits anti-inflammatory and anti-proliferative effects, inhibiting the formation and proliferation of cancer cells. It exerts this effect by scavenging ROS, inhibiting the NF-kB pathway, activating the Nrf2-antioxidant response element (ARE) signaling pathway, inhibiting STAT3, and reducing inflammatory markers [TNF-α, IL-1β, IL-6, IL-10, iNOS, COX-2, prostaglandin E2 (PGE2), phosphoprotein 65 (p-p65)] [47, 53-56]. Figure 2 shows anti-inflammatory and anti-proliferative effects mechanisms of *Vaccinium myrtillus* L. in cancer cells [3, 33, 46, 47, 52-56].

In a study by Schantz et al. [57] using Caco-2 and HT-29 human colon cancer cell lines, *Vaccinium myrtillus* L. exhibited anti-inflammatory effects on HT-29 cells (after 24-hour incubation at 250 mcg/mL) and Caco-2 cell lines (after 1-hour incubation at 50 mcg/mL), and significantly reduced ROS levels. They also observed a significant reduction in DNA damage in Caco-2 cells after 24-hour incubation at 5 mcg/mL. In a randomized controlled study by Karlsen and coworkers [58], the control group (n=31) was given water, while the experimental group (n=31) received 330 mL/day of bilberry juice (diluted with 1 liter of water) for 4 weeks. The study found a significant decrease in plasma concentrations of C-reactive protein (CRP), IL-6, and IL-15 as well as an increase in TNF-α level in the group that was given bilberry juice. The increase in TNF-α stimulates the release of IL-10, an anti-inflammatory cytokine. It was concluded that the polyphenols present in bilberry can modulate inflammatory processes.

Anti-carcinogenic and Apoptotic Effects of *Vaccinium myrtillus* L.

The potential anti-carcinogenic, anti-invasion, anti-adhesion, anti-migration, anti-angiogenic, and anti-metastatic effects of *Vaccinium myrtillus* L. include protection of cells against oxidative damage, suppression of inflammation, regulation of cell cycle, induction of apoptosis leading to inhibition of cell proliferation, inhibition of angiogenesis, prevention of cell migration and adhesion, and prevention and repair of DNA damage [3, 54, 59]. *Vaccinium myrtillus* L. exhibits anti-angiogenic effects by inhibiting the release of vascular endothelial growth factor (VEGF) induced by stress and cytokines [60, 61]. It reduces the release of pro-angiogenic factors such as c-Myc, c-jun, and c-fos. It also exhibits a potential preventive effect against cell adhesion by downregulating the expression of cell adhesion molecules such as β-catenin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), while upregulating the expression of carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) [54]. By suppressing matrix metalloproteinases (MMPs) (MMP-2 and MMP-9) and urokinase-type plasminogen activator (u-PA), *Vaccinium myrtillus* L. can prevent cell invasion [3, 46]. Tumor cells promote angiogenesis by creating new blood vessels from existing ones to access oxygen and nutrients, and they induce metastasis by migrating to other tissues through blood and lymphatic vessels [62]. *Vaccinium myrtillus* L. exhibits anti-metastatic effects by inhibiting AP-1, which accelerates the epithelial-mesenchymal transition of tumor cells (the first step in metastasis) [46]. Figure 3 shows anti-invasion, anti-adhesion, anti-migration, anti-angiogenic and anti-metastatic effects mechanisms of *Vaccinium myrtillus* L. in cancer cells [3, 46, 54, 59 - 61].

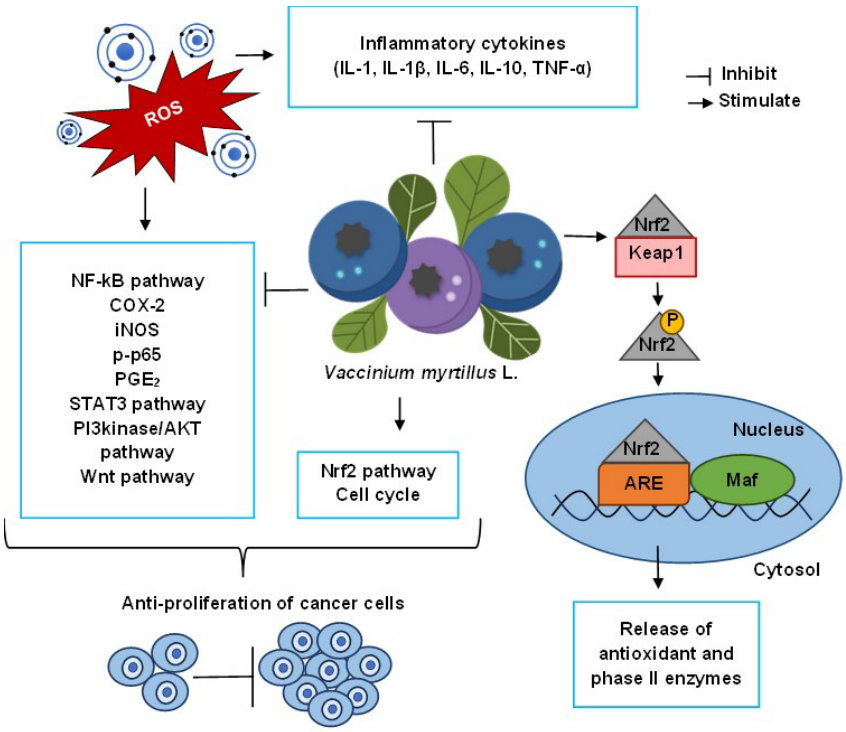

Figure 2. Anti-inflammatory and Anti-proliferative Effects Mechanisms of *Vaccinium myrtillus* L. in Cancer Cells [3, 33, 46, 47, 52-56].

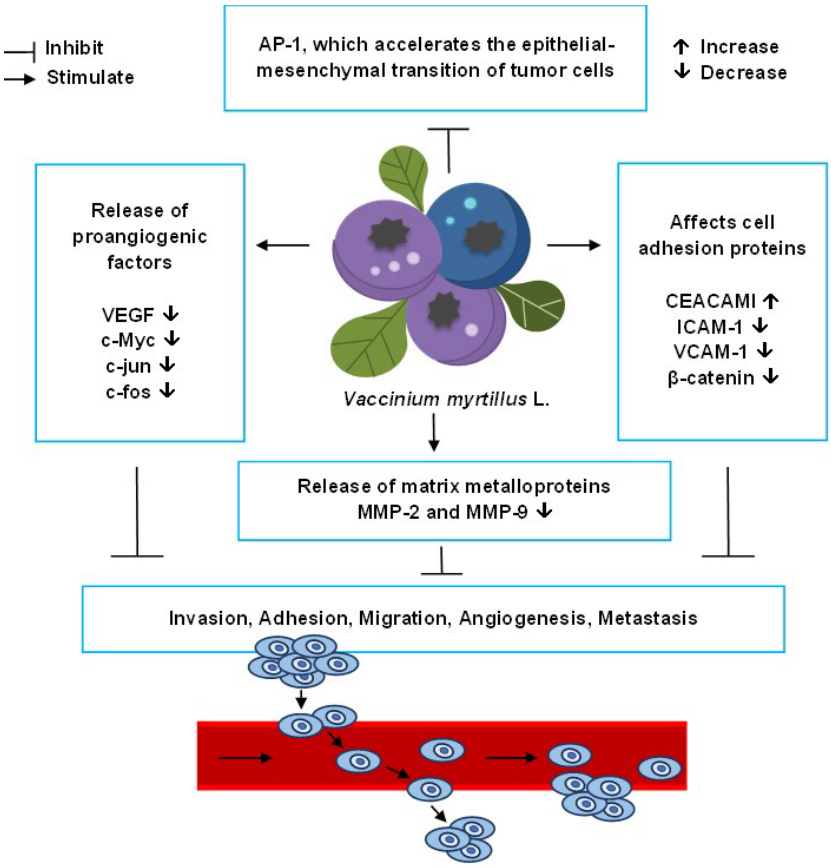

Figure 3. Anti-invasion, anti-adhesion, anti-migration, anti-angiogenic and anti-metastatic effects mechanisms of *Vaccinium myrtillus* L. in cancer cells [3, 46, 54, 59-61].

The cell division cycle is a biochemical phase controlled by cyclin-dependent kinases (CDKs). They are regulated by the synthesis and degradation of cyclins, as well as the phosphorylation and inhibition of CDKs. There are also several transcription factors that control the cell cycle. p53 is a gene that increases the release of CDK inhibitors, thereby halting the cell cycle. Uncontrolled cell division, aberrant signaling pathways, anti-apoptotic effects, metastatic effects, immortality, increased angiogenesis, and mutations in tumor suppressor genes such as proto-oncogenes and p53 are observed in tumor cells [63]. Bilberry exhibits potential anti-proliferative and cell cycle regulatory effects, preventing the uncontrolled growth and proliferation of tumor cells. It reduces the activity of CDK-2, CDK4, cyclin A, cyclin B1, cyclin D1, cyclin E, protein phosphatase 2 (Cdc2), and Cdc25C, while increasing the activity of CDK inhibitors p16, p21, and p27 [3, 54].

Genetic alterations and mutations in cancer cells are associated with signaling pathways that control tumor formation. DNA mutations can lead to overexpression of affected genes or production of mutated proteins with erratic activity. Proteins found in signaling pathways that are widely activated in various physiological responses include growth factor receptor tyrosine kinases (e.g., epidermal growth factor receptor), guanosine triphosphatases (e.g., Ras), serine/threonine kinases (e.g., Raf and Akt), cytoplasmic tyrosine kinases (e.g., Src and Abl), lipid kinases (e.g., Phosphatidylinositol-3-kinase or PI3K), and nucleotide receptors (e.g., estrogen receptor). The components of the signaling pathways such as Wnt, Hedgehog, Hippo, and Notch can also be affected. The most important pathways regulating cell proliferation are PI3K-Akt and Ras-Raf-ERK. Ribosomal S kinase (RSK) and MAPK are phosphorylated by ERK. Akt and RSK lead to activation of the mammalian target of rapamycin (mTOR) pathway [62]. In addition to its anti-inflammatory and anti-proliferative actions, anthocyanin-rich *Vaccinium myrtillus* L. suppresses Ras-Raf-MAPK-ERK, PI3K-Akt-mTOR, and Wnt signaling pathways [54].

p53 is a transcription factor that regulates the cell cycle and controls DNA repair mechanisms and apoptosis. Mutate p53 loses its tumor suppressor function, leading to increased cancer cell formation and proliferation [3, 54]. Through its anthocyanin content, *Vaccinium myrtillus* L., exhibits potential effects in preventing p53 mutation and DNA damage, promoting DNA repair, and inducing phase II enzymes such as quinone reductase. These effects contribute to the inhibition of cell proliferation and induction of apoptosis [18, 47, 64]. Apoptosis is programmed cell death. There are two main pathways for apoptosis: the extrinsic pathway (death receptor pathway) and the intrinsic pathway (mitochondrial pathway). In the extrinsic pathway, TNF is induced by the interaction of extracellular ligands such as the Fas ligand (Fas-L) and TNF-related apoptosis-inducing ligand (TRAIL) with transmembrane receptors (death receptors). Binding of the Fas-associated death domain (FADD) and the TNF receptor-associated death domain (TRADD) results in the formation of the death-inducing signaling complex (DISC). The resulting DISC activates pro-caspase-8, which in turn activates pro-caspase-3, an effector caspase that initiates apoptosis [65, 66]. In the intrinsic pathway, the mitochondria play a crucial role. Cytochrome c (Cyo-c), an electron transport chain protein involved in adenosine triphosphate (ATP) production is located in the inner mitochondrial membrane. tBid is formed when active caspase-8 cleaves the pro-apoptotic protein Bid. The resulting tBid integrates into the mitochondrial membrane and increases its permeability. Increased membrane permeability allows the release of Cyo-c into the cytoplasm through the pores formed in the membrane. The release of Cyo-c from the mitochondria into the cytoplasm indicates that apoptosis is irreversible. Cyo-c, ATP, procaspase-9 and apoptosis protease activating factor 1 (APAF-1) together form a complex known as apoptosome. Procaspase-3 is converted to active caspase-3. The caspase-dependent mechanism of apoptosis is activated [60, 63, 67]. Apoptosis is induced by activation of caspase-3, caspase-8, and caspase-9, induction of Bax and Cyo-c release, suppression of Bcl-2 and poly ADP ribose polymerase (PARP) release, mitochondrial damage, and stimulation of Cyo-c release [46, 47, 54]. Anthocyanins also exert an apoptotic effect by inhibiting the NF-kB pathway and arresting the cell cycle in the G2/M phase [5, 68, 69]. In addition to these effects, they inhibit the expression of polycomb group (PcG) proteins. PcG proteins are epigenetic regulators that downregulate tumor suppressor genes and ensure cancer cell survival [59]. Figure 4 shows anti-angiogenic and apoptotic effects mechanisms of *Vaccinium myrtillus* L. in cancer cells [3, 5, 18, 47, 54, 64, 68-70].

In a study by Misikangas et al. [71] on Min/1 mice to investigate the chemopreventive properties of *Vaccinium myrtillus* L. (rich in anthocyanins), *Vaccinium vitis-idaea* (rich in proanthocyanidins), and *Rubus chamaemorus* (rich in ellagic acid) with different phenolic contents on intestinal tumor formation, four different groups were constructed: *Vaccinium myrtillus* L., *Vaccinium vitis-idaea*, *Rubus chamaemorus*, and a control group. The control group was fed a high-fat diet consisting of 41% fat, 39% carbohydrates, and 19% proteins, while the other groups were fed high-fat diets containing 10% berries for 10 weeks. Berries inhibited the formation of intestinal adenomas by 15-30% and suppressed cell growth. They also reduced β-catenin and showed chemopreventive activity, resulting in a decrease in tumor formation by 60%.

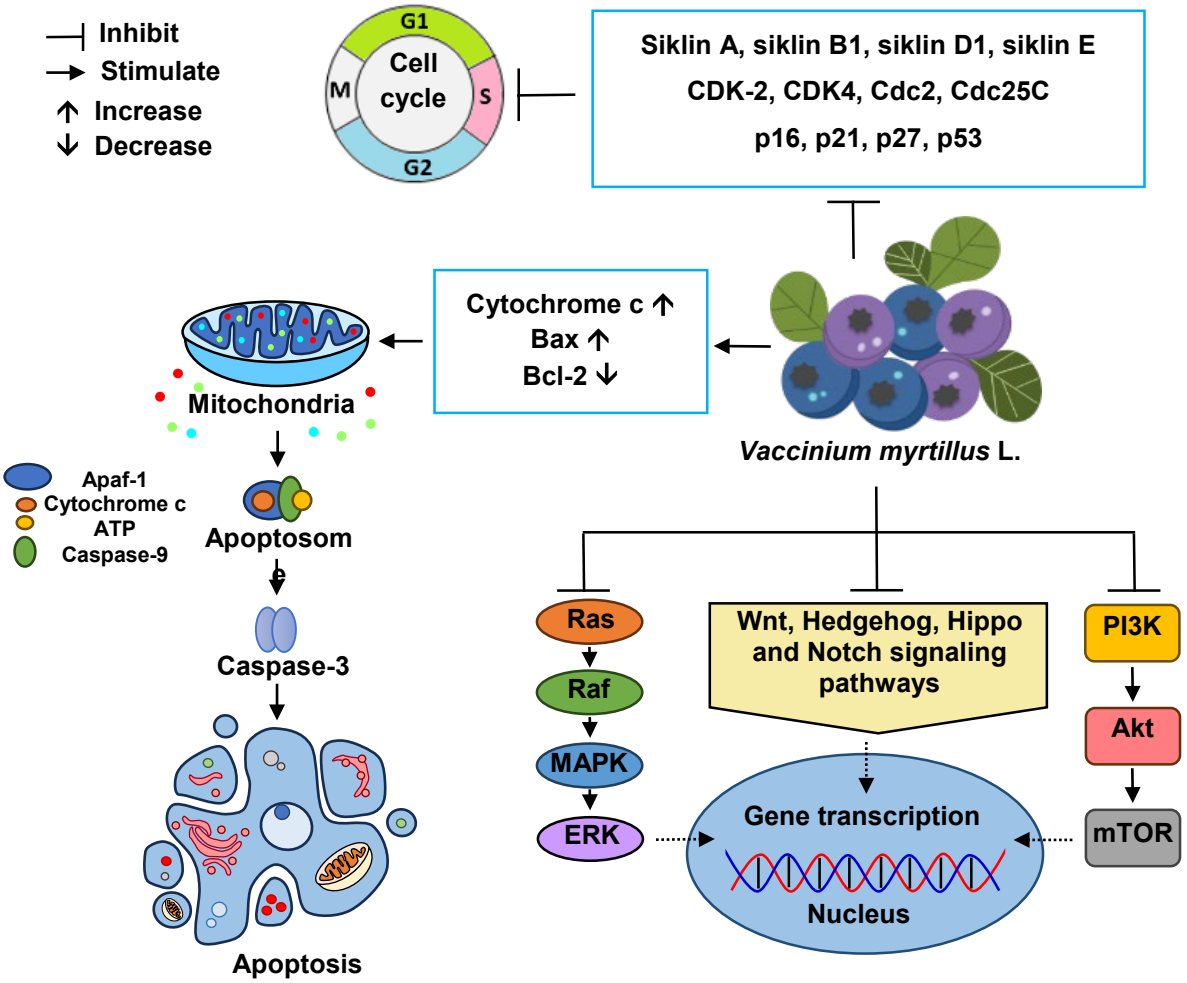

Figure 4. Anti-carcinogenic and apoptotic effects mechanisms of *Vaccinium myrtillus* L. in cancer cells [3, 5, 18, 47, 54, 64, 68 - 70].

Wu et al. [72] reported that 10 mg/mL of bilberry extract reduced cell proliferation by 30% in HT-29 colon cancer cells. They observed that the extract resulted in an increase in Bax expression and a decrease in Bcl-2 expression. In a study, Alhosin et al. [73] investigated the apoptotic effects of a bilberry extract containing 50% anthocyanins (Antho 50) on B-cell chronic lymphocytic leukemia cells. They found that Antho 50 increased ROS generation, caspase-3 activation, and levels of p21 and p73, while decreasing p-Akt, histone deacetylase (HDAC), and DNA methyltransferase 1 (DNMT1). It was observed that ROS generation induced by hydrogen peroxide and Antho 50 was prevented by catalase. The study concluded that Antho 50 inhibited the expression of PcG proteins in Jurkat cells and increased apoptosis by 75% at a concentration of 75 mg/mL. León-González et al. [59] examined the effects of Antho 50 on the expression of PcG proteins in Jurkat cells in which Jurkat cells were treated with different concentrations of Antho 50 (10, 25, 50, 75, and 100 µg/mL) for 24 hours. It was observed that apoptosis began in 50% of the cells treated with 100 µg/mL Antho 50, and there was a loss of mitochondrial membrane potential and a significant increase in intracellular ROS levels. Additionally, there was a 60% decrease in the expression levels of HDAC1, DNMAT1, and UHRF1, which are proteins that act in conjunction with PcG. Also, HDAC2 expression was reduced by 40%. Furthermore, significant increases were observed in the expression levels of the tumor suppressor p73, cell cycle regulator p21, and caspase-3 in cells treated with 75 and 100 µg/mL Antho 50. In an *in vitro* study by Katsube et al. [74] investigating the effects of 10 different berry extracts, including *Vaccinium myrtillus* L., on HL60 human leukemia cells and HCT116 human colon carcinoma cells, it was found that at a concentration of 4-6 mg/mL, bilberry extract reduced the cell viability of HL60 human leukemia cells by 84-88%, and at a concentration of 2-4 mg/mL, it reduced the cell viability of HCT116 human colon carcinoma cells by 66-97%. In the same study, 4 mg/mL bilberry extract was observed to induce apoptosis in HCT116 human colon carcinoma cells, while its apoptotic effect was greater in HL60 human leukemia cells. In another study by Nguyen et al. [75], the effects of *Vaccinium myrtillus* L. on MCF7 human breast cancer cells were assessed. It was reported that, at a concentration of 0.3-0.4 mg/mL, *Vaccinium myrtillus* L. induced apoptosis and reduced cell proliferation by 50%. In a study by Aaby et al. [76], anti-proliferative effects of bilberry extracts obtained at different temperatures (22, 40, 60, 80, and 100°C) for 4, 15, 30, and 45 minutes were investigated in three different colon cancer cell lines (Caco-2, HT-29, and

HCT 116). It was found that, at a concentration of 125 mg/L, the extract obtained at 100 °C inhibited cell proliferation in Caco-2 cells 1.4 and 1.7 times more than the extracts obtained at 60°C and 40°C, respectively. In HT-29 cells, when the extract obtained at 100°C was given at a concentration of 250 mg/L, 2.2 and 2.5 times greater anti-proliferative effect was observed compared to the extracts obtained at 60°C and 40°C, respectively. In HCT 116 cells, at a concentration of 250 mg/L, the extract obtained at 100 °C and exhibited 4.0 and 5.6 times more anti-proliferative activity compared to the extracts obtained at 60°C and 40°C, respectively. These findings indicate that extracts obtained at higher temperatures (80-100°C) have a greater inhibitory effect on colon cancer cell proliferation compared to extracts obtained at lower temperatures. In a pilot study by Thomasset et al. [42] involving 15 patients with colorectal adenocarcinoma and 10 patients with colorectal liver metastasis, 10 patients received 1.4 g of a standardized bilberry extract (containing 36% of anthocyanins), 8 patients received 2.8 g and 7 patients received 5.6 g of the standardized extract three times a day for 7 days. 1.4 g of the standardized extract contained 0.5 g of anthocyanins, equivalent to 370 g of fresh bilberries. A 9% reduction in cancer cell proliferation was observed in the group receiving 1.4 g of the standardized extract, while the reductions in the other groups were nonsignificant. In a study by Zhao et al. [36] on HT-29 colorectal adenocarcinoma cell line and NCM460 colon cell line, *Vaccinium myrtillus* L. extract was reported to inhibit cell proliferation by 7% at 48 and 72 hours.

CONCLUSION

Vaccinium myrtillus L. has an anti-oxidant effect by stimulating the chelation of metal ions, scavenging of reactive oxygen species and releasing of anti-oxidant enzymes. It can prevent oxidative stress and inflammation by activating the Nrf2 pathway and suppressing the NF-kB, STAT3, Wnt, PI3kinase/AKT pathways and inflammatory markers (TNF-α, IL-1β, IL-6, IL-10, IL-1 etc.). Moreover, it has a potential preventive effect against cell adhesion by decreasing cell adhesion molecules such as ICAM-1, VCAM-1, b-catenin and increasing CEACAM1. It has a potential inhibitory effect on angiogenesis and metastasis by reducing VEGF, c-Myc, c-jun, c-fos, MMP-2 and MMP-9. It also prevents the uncontrolled growth and proliferation of tumor cells by showing anti-proliferative and cell cycle regulatory potential. It reduces the activity of CDK-2, CDK-4, cyclin A, cyclin B1, cyclin D1, Cdc2 and Cdc25C, while it increases CDK inhibitors p16, p21 and p27. Additionally, it provides activation of caspase-3, caspase-8 and caspase-9, induction of the release of p53, Bax and Cyo-c, suppression of Bcl-2 and PARP release, formation of mitochondrial damage and stimulation of cytochrome c release and stimulates apoptosis. Thus, it has been shown to have potential anti-oxidative, anti-inflammatory, anti-carcinogenic, anti-proliferative, anti-angiogenic, anti-radical, anti-mutagenic, anti-metastatic and apoptosis-inducing effects on cancer. There are studies examining the effects of *Vaccinium myrtillus* L. on types of cancer on organ cancers such as colorectal, colon and breast, and blood-related cancers such as leukemia and lymphoma. According to the Turkey Nutrition Guide, at least 5 portions (at least 400 g/day) of fruits and vegetables should be consumed per day. At least 2-3 portions of these should be fruit. Consuming fruits of different colors is necessary to absorb different nutrients and bioactive nutritional components. In addition, cancer patients can choose blueberries as one portion of their daily fruit consumption in order to get the nutrients and components found in bilberry. This review is a precursor for future studies that will examine the relationship between *Vaccinium myrtillus* L. and cancer. There is a need for larger prospective, large-scale clinical and human studies describing the relationship between *Vaccinium myrtillus* L. and cancer. New studies should be planned as specified.

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1. Çalışmalar A4 boyutunda hazırlanmalı, üstten 2.45 cm, alttan 2.45 cm, sağ ve soldan 1.75 cm boşluk bırakılmalı ve tek kolon olarak hazırlanmalıdır. Metin çift satır aralıklı yazılmalı, paragraflar arasında tek satır boşluk bırakılmalıdır. Metinde bütün satırlar (sürekli) numaralandırılmalıdır.

2. Çalışma başlığı 14 punto Arial, koyu, küçük harflerle ve ortalanmış olarak yazılmalıdır. Başlıktan sonra bir satır boşluk bırakılmalı (11 punto); yazar isimleri (yalnızca ilk harfler büyük) 10 punto Arial ve ortalanmış olarak verilmelidir. Yazarların adresleri, telefon ve faks bilgileri ile yazışmalardan sorumlu yazarın e-posta adresi hemen alt satırda 9 punto Arial, ilk harfler büyük olacak şekilde ve ortalanmış olarak yazılmalıdır. Yazarların çalıştıkları kuruluşlar (ve/veya adresler) farklı ise her bir yazar isminin sonuna rakamlarla üst indis konulmalıdır.

3. Metin içindeki kısımların başlıkları (ÖZ, ABSTRACT, GİRİŞ vb.) 10 punto Arial ve koyu olarak büyük harflerle yazılmalı, başlıktan sonra bir satır boşluk bırakılarak metine geçilmelidir. Alt başlıklarda ilk harfler büyük, 10 punto Arial ve koyu yazı karakteri kullanılmalıdır. ÖZ'ün altına bir satır boşluk bırakıldıktan sonra en fazla 5 adet Anahtar Kelime konmalıdır. Anahtar Kelimelerden sonra bir satır boşluk bırakılarak İngilizce başlık ve altına ABSTRACT ve Keywords yazılmalıdır. Bir satır boşluk bırakılarak ana metine geçilmelidir.

4. Ana metin 9.5 punto Arial olarak hazırlanmalıdır.

5. Çalışma başlıca şu kısımlardan oluşmalıdır: Başlık, Yazar İsimleri, Adresleri, İletişim Bilgileri, Yazışmalardan Sorumlu Yazarın E-posta adresi, Öz, Abstract, Ana Metin (Giriş, Materyal ve Metot, Bulgular ve Tartışma, Sonuç), Teşekkür (gerekiyorsa), Kısaltmalar (gerekiyorsa), Kaynaklar.

6. Öz ve Abstract 250 kelimeyi geçmemeli, çalışmanın amacını, metodunu ve önemli sonuçlarını içermelidir. Öz tek paragraf olarak yazılmalı ve öz içinde kaynaklara atıf yapılmamalıdır.

7. Çalışma içerisinde geçen mikroorganizma isimleri ile Latince ifade ve isimler italik olarak yazılmalı ve kısaltmalarda uluslararası yazım kuralları göz önünde bulundurulmalıdır.

8. Tablo başlıkları tablonun üstüne, şekil başlıkları ise şeklin altına yazılmalı ve numaralandırılmalıdır. Kullanılan tablo ve şekillere metin içinde mutlaka atıf yapılmalıdır. Metin içinde geçen veriler tablo ve şekillerin tekrarı olmamalıdır. Tablo ve şekillerin başlıkları içerikleriyle uyumlu ve anlaşılabilir olmalıdır. Şekiller ve resimlerin yüksek çözünürlükte olmasına dikkat edilmelidir. Resimler (ve gerekiyorsa Şekiller) *.jpg formatında metin içerisinde yer almalıdır.

9. Metin içerisinde atıflar köşeli parantez içerisinde rakamlarla yapılmalı [1] ve Kaynaklar bölümünde bu numara sırasıyla detayları yazılmalıdır. Kaynakların numaralandırılması MS Word Numaralandırma Kitaplığı kullanılarak yapılmalıdır.

10. Kullanılan matematiksel denklemler numaralandırılmalı ve metin içerisinde bu denklemlere atıf yapılmalıdır.

11. Kaynaklar kısmı APA yazım stili kullanılarak hazırlanmalıdır. Kaynakların yazımında aşağıdaki örnek yazım biçimleri kullanılmalı ve makalelerin yayınlandığı dergi isimleri kısaltma kullanılmadan ve italik olarak yazılmalıdır. Web adreslerine atıf yapılacağında (mümkün olduğunca Resmi web sayfalarına atıf yapılmalıdır) mutlaka ilgili web adresine erişim tarihi verilmelidir.

Makale

[1] Bozkurt, H., İçier, F. (2009). İnegöl köfte üretiminde ohmik pişirmenin uygulanabilirliğinin incelenmesi. *Akademik Gıda,* 9(1), 6-12.

Kitap

[2] Kılıç, S. (2001). Süt Endüstrisinde Laktik Asit Bakterileri. Ege Üniversitesi Ziraat Fakültesi Yayınları, Ege Üniversitesi Matbaası, Bornova, İzmir.

Kitap Bölümü

[3] Gibson, G.R., Saavedra, J.M., MacFarlane, S., MacFarlane, G.T. (1997). Probiotics and Intestinal Infections. In Probiotics 2: Applications and Practical Aspects, Edited by R. Fuller, Chapman & Hall, 2-6 Boundary Row, London SE1 8HN, England, 212p.

Kongre-Sempozyum Bildirisi

[4] Gürsoy, O., Akdemir, O., Hepbaşlı, A., Kınık, Ö. (2004). Recent situation of energy consumption in Turkey dairy industry. *International Dairy Symposium: Recent Developments in Dairy Science and Technology*, May 24-28, 2004, Isparta, Turkey, Book of Proceedings, 10-16p.

12. Hakem görüşleri doğrultusunda düzeltilmek üzere yazar(lar)a gönderilen çalışmaların gerekli düzeltmeleri yapılarak yayın ofisine ulaştırılması gereklidir. Editörler tarafından belirtilen süre zarfında gönderilmeyen çalışmalar "ilk defa gönderilmiş çalışma" olarak değerlendirilecektir.

13. Yukarıdaki kurallara uygun olarak hazırlanmamış çalışmalar değerlendirmeye alınmaz.

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Manuscripts for the Akademik Gıda® (Academic Food Journal) must be sent via the electronic article submission system, which can be located in the official website of the journal, www.academicfoodjournal.com. Manuscripts sent by e-mail are not considered for evaluation. For questions related to the electronic article submission system, contact the editor via e-mail at ogursoy@yahoo.com.

- Authors must specify the type of the manuscript (research articles, review articles, research briefs, mini-review articles, comments and letters to the editor).
- Authors should provide at least 3 potential referees and their contact information (mailing address, e-mail address and phone number).
- Manuscripts to be submitted should be free from any spelling or grammatical error.
- Authors must guarantee that the submitted manuscript is not published anywhere previously and will not be submitted to anywhere before the editorial board makes a final decision on the manuscript.
- The editorial board is authorized to make necessary changes in manuscripts accepted for publication.

Peer review policy

Manuscripts pass through initial screening in the editorial office followed by internal review by Editors. After the first evaluation, manuscripts are double-blind-reviewed by a peer review system involving at least two independent reviewers to ensure high quality of manuscripts accepted for publication. The Editors have the right to decline formal review of a manuscript if it is (i) on a topic outside the scope of the Journal, (ii) lacking technical merit, (iii) fragmentary and providing marginally incremental results or (iv) poorly written.

Publication fee

There is a 300 TL submission fee for Turkish manuscripts submitted after January 15, 2023. This fee may not be waived. No fee is charged for articles submitted to the journal in English.

Ethics Statement

Detailed information about journal publication policy, evaluation of manuscripts and ethical issues can be found in the Ethics Statement section.

Preparation of a manuscript

1. Manuscripts should be prepared in A4 size, and the text must be prepared in a single column format. The text must be double-spaced, and a single space should be left between paragraphs. All lines and pages must be continuously numbered.

2. The title must be 14pt Arial, bold, small letters and centered. A blank line should be left after the title, and the names of authors should be given in 10pt Arial and centered. In addition to each author's contact address, the phone and fax numbers and e-mail address of the corresponding author should be provided. If the institutions of the authors are different, superscript numbers should be used to indicate their addresses.

3. The headings (e.g. Abstract, Introduction, Materials and Methods etc.) must be 10pt Arial, and should be typed in bold capital letters. Each heading should appear on its own separate line. A blank line should be left after each heading. A list of keywords, a maximum of 5, should be provided below the abstract section of the manuscript.

4. The main text should be prepared in 9.5pt Arial.

5. Typical articles mainly consist of the following divisions: Title, Author Names, Addresses, Contact Information, Corresponding author's e-mail address, Abstract, Main text (Introduction, Materials and Methods, Results and Discussion, Conclusions), Acknowledgements (if necessary), Abbreviations (if necessary) and References.

6. The abstract should not exceed 250 words, and the main purpose and method and the most significant result and conclusion should be presented in the abstract. The abstract should be prepared as a single paragraph, and should not include any citation.

7. Latin names in the text should be in italics, and names and abbreviations should follow international rules. If abbreviations that are not standard are unavoidable, they must be defined at their first mention in the text. Consistency of abbreviations throughout the article must be ensured. Internationally accepted rules and conventions must be followed, and the international

system of units (SI) must be used. If other units are mentioned, their equivalents in SI must be provided.

8. Table headings should be on the top of each table and figure captions below each figure. Each table or figure must be numbered consecutively in accordance with their appearance in the text. All figures and tables should be cited in the text. The data presented in the tables and figures should not be repeated in the text. Table headings and figure captions should be self-explanatory. Figures and pictures must be provided in high resolution, and pictures (and, if necessary figures) should be included in the text as *. jpg format.

9. References in the text should be cited in numbers in square brackets [1] and details of the citations must be provided in the Literature or References section with their respective numbers.

10. Mathematical equations should be numbered and cited in the text.

11. References should be given according to the APA manual of style. The following formats should be used for the details of cited references, and the journal names must be typed in italics. References to the Web addresses (if necessary, the official web pages should be preferred) must include full web address and the date of access.

Article

[1] Güzeler, N., Kaçar, A., Say, D. (2011). Effect of milk powder, maltodextrin and polydextrose use on physical and sensory properties of low calorie ice cream during storage. *Akademik Gıda,* 9(2), 6-12.

Book

[2] Kilic, S. (2001). Lactic Acid Bacteria in Dairy Industry. Ege University Faculty of Agriculture Publications, Ege University Press, Bornova, Izmir, Turkey.

Book Chapter

[3] Gibson, G.R., Saavedra, J.M., MacFarlane, S., MacFarlane, G.T. (1997). Probiotics and Intestinal Infections. In Probiotics 2: Applications and Practical Aspects, Edited by R. Fuller, Chapman & Hall, 2-6 Boundary Row, London, England, 212p.

Proceedings of the Congress-Symposium

[4] Gursoy, O., Akdemir, O., Hepbasli, A., Kinik, O. (2004). Recent situation of energy consumption in dairy industry in Turkey. *International Dairy Symposium: Recent Developments in Dairy Science and Technology*, May 24-28, 2004, Isparta, Turkey, Book of Proceedings, 10-16p.

12. A list of the corrections requested by the referees must be provided by the authors, and it must be sent to the editorial office.

13. Studies that are not prepared in accordance with the rules above will not be considered for evaluation.

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Yayın Politikası

Akademik Gıda dergisine gönderilen tüm makaleler Dergi Editörleri için Davranış Kuralları ve En İyi Uygulama Kılavuzları ve Dergi Yayıncıları için Davranış Kurallarında (Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers) belirtilen Genel Kılavuzlara uygun olarak değerlendirilmektedir. Bilimsel yazılar dergiye gönderilmeden önce derginin Yazım Kurallarının okunmasını önemle tavsiye ederiz. Yazarlar aynı zamanda Avrupa Bilim Editörleri Birliği'nin (EASE) (European Association of Science Editors) İngilizce olarak basılacak makaleler için "Bilimsel Makalelerin Yazarları ve Çevirmenleri İçin Rehber"e uymalıdır. Yazarlar, insan veya hayvan verilerini içeren araştırmaları için Uluslararası Tıp Dergisi Editörleri Komitesinin (International Committee of Medical Journal Editors) önerilerini takip etmelidir.

Makalelerin Değerlendirilmesi

Dergiye gönderilen tüm makaleler, bilimsel içeriklerinin özgünlüğü ve kalitesi ölçütlerine göre değerlendirilir.

- Dergiye gönderilen tüm yazılar, ilk olarak yayın ofisindeki (teknik ve genel kalite değerlendirilmesi açısından) eleme işleminden geçer ve ardından teknik ve bilimsel editörler tarafından değerlendirilir.
- İlk değerlendirmeden sonra, editörler (i) dergi kapsamı dışında kalan bir konu hakkında hazırlanmış makaleleri (ii) teknik olarak eksik/yetersiz makaleleri, (iii) kısmi ve marjinal artan sonuçları içeren makaleleri veya (iv) kötü yazılmış makaleleri reddetme hakkına sahiptir.
- İlk inceleme sonucunda makalenin ileri değerlendirme için uygun olduğuna karar verilirse, dergide yayımlanmak üzere kaliteli makalelerin seçimini yapmak amacıyla, makaleler çift-körlü (hakemin ve yazar/yazarların birbirlerini görmedikleri) değerlendirme sistemi ile en az iki bağımsız hakemden oluşan bir değerlendirme sürecinde bilimsel incelemeye alınır.
- Hakemler tarafından talep edilirse, makalenin hakem görüşleri doğrultusunda yazarlar tarafından revize edilmiş versiyonu orijinal hakemler tarafından tekrar değerlendirilir. Değerlendirmelerin ardından editörler hakem önerileri doğrultusunda makale hakkındaki nihai kararlarını verirler. Gerekirse editörler, hakemlerin istedikleri tüm şartların yerine getirilmesi için yazarlardan ilave revizyon isteyebilir.
- Kabul edilen makalelerin son versiyonu, yayın öncesi taslağın (galley proof) hazırlanması için teknik editörlere gönderilir. Yazarlardan, makalelerinin dizgisi hazırlanmış taslaklarını son kontrol için yayın öncesinde incelemeleri istenir.
- Tüm makaleler, nihai formlarında DOI numarası almış ve çevrimiçi olarak pdf dosyaları halinde yayımlanır. İlgili veritabanlarında bu şekilde indekslenir.

Yayın Ücreti

Akademik Gıda dergisinde makalelerin yayınlanması için herhangi bir yayın ücreti talep edilmemektedir.

Gizlilik

Editörler, Akademik Gıda'ya gönderilen tüm makaleleri tam bir gizlilikle ele alır. Editörler, hakemler haricinde, COPE tavsiyelerine uyulmadığı takdirde, üçüncü şahıslara makale ile ilgili hiçbir bilgi vermezler. Yayınlanmak üzere dergiye gönderilen makaleler hakemler için de gizlidir ve bilimsel değerlendirme için aldıkları makalelerin herhangi bir bölümünü üçüncü şahıslarla paylaşmalarına veya dağıtmalarına izin verilmez. Suiistimal şüphesi olduğunda, hakemlerin derhal gizli bir şekilde yayın ofisine başvurmaları önerilir. Hakemler ayrıca, Dergi Editörleri İçin Davranış Kuralları ve En İyi Uygulama Kuralları ile Dergi Yayıncıları için Davranış Kuralları'nı (Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers) takip ederek editöre gizli yorumlarında belirli bir eylem önerebilirler.

Akademik Gıda, çift-kör bir hakem inceleme süreci yürütür, yani çalışmanın eleştirel değerlendirmesini sağlamak için hakemlerin isimleri gizlidir. Hakemlerden, raporlarında adlarını veya irtibat bilgilerini açıklamamaları istenir. Hakem raporları yazarlara gönderilemeden önce bu açıdan kontrol edilir.

Yazarlık

Bir yazar, bir araştırmanın fikrine veya tasarımına, verilerin elde edilmesine, verilerin analizine veya yorumlanmasına büyük ölçüde katkıda bulunan, makalenin hazırlanmasında, yazılmasında veya gözden geçirilmesinde entelektüel içeriğe eleştirel katkı yapan bireydir. Katkıda bulunanlar diğer kişiler makalenin Teşekkür bölümünde belirtilmelidir ve çalışmanın yazarı olarak kabul edilemez. Tüm yazarların doğru ve tam isimleri ile ORCID kimlikleri dergiye gönderilen

makalenin başlık sayfasında yer almalıdır. Yazarların isimlerinin yanında çalıştıkları kurumlar ve yazışmalardan sorumlu yazarın geçerli bir adresi verilmelidir. Yazışmalardan sorumlu yazarın telefon ve faks numaraları ile e-posta adresi makalenin ilk sayfasında belirtilmelidir. Tüm yazarlar, gönderilen makalenin daha önce herhangi bir yerde yayınlanmadığını ve makale hakkında Akademik Gıda dergisi nihai bir karar vermeden önce makaleyi başka bir dergiye göndermeyeceklerini garanti etmelidir.

Destekleyen/Finans Sağlayan Kuruluşlar

Araştırmanın tüm finans kaynaklarına ilişkin detaylar, Teşekkür bölümünde belirtilmelidir. Yazarlar, resmi finansman kurum/larının tam isimlerini ve proje/hibe numaralarını belirtmelidir.

Yazarlarda Değişiklik

Makalenin Akademik Gıda'ya sunulmasından sonra yazar isimlerinde değişiklik ancak revizyon sırasında gerekli olan ek çalışmalar durumunda olabilir. Makalenin yayına kabul edilmesinden sonra herhangi bir değişikliğe izin verilmez. Yazarlıktaki değişiklik, hakem görüşlerine verilen cevaplar sırasında yazışmalarda belirtilmeli ve tüm yazarlar tarafından kabul edilmelidir. Yazışmalardan sorumlu yazar, yazarların sırası da dahil olmak üzere makalenin revize edilmiş versiyonundaki değişikliklerden sorumludur.

Çalışma Verilerinde Düzeltme

Yayınlanan verilerin doğruluğundan tüm yazarlar sorumlu olmalıdır. Verilerin düzeltilmesi için, yazışmalardan sorumlu yazardan yayın öncesi taslağı (galley proof) incelemesi ve makalenin yayınlanmasından 4 gün önce dikkatlice düzeltmesi istenir.

Makalenin Geri Çekilmesi

Bir makalenin geri çekilmesi, gönderim veya yayın hatalarını düzeltmek için kullanılır. Yazarlar makaleyi geri çekebilir ve bu durumda Yayın Etiği Komitesi (COPE) Geri Çekme Kurallarına [(COPE) retraction guidelines] uymalıdır. Tekrarlanan veya benzerlik oranı yüksek bir yayın, verilerin hileli kullanımı, intihal veya etik dışı araştırma yapılması durumunda, makale editör tarafından geri çekilecek ve geri çekilen makale linklerine bağlantı korunacak ancak elektronik veri tabanına (makale sayfasına) bir geri çekme bildirimi eklenecektir.

Etik Hususlar

Çıkar çatışması:

- Yazar/lar başvuru sırasında herhangi bir çıkar çatışması varsa beyan etmelidir. Yazar/ların başvuru sırasında bilimsel değerlendirme için en az üç potansiyel hakem önermeleri istenir. Önerilen hakemler çalışma arkadaşları, ortak çalıştıkları kişiler veya çalıştıkları kurumların üyeleri olamazlar.
- Hakemler makaleyi değerlendirmelerini önleyen herhangi bir çıkar çatışması olması durumunda Editörleri bilgilendirmesi ve bu konuda COPE kurallarına uyması tavsiye edilmektedir.
- Editörler Kurulu üyeleri veya kurul üyelerinin ortak çalıştıkları kişiler tarafından dergiye gönderilen makaleler için, değerlendirme sırasındaki önyargıları en aza indirgemek amacıyla, değerlendirme süreci ilgili kurul üyelerini dışarıda tutacak şekilde değiştirilerek uygulanır.
- Düzeltmeler (revizyonlar) sırasında, editörler Dergi Editörleri İçin Davranış Kuralları ile En İyi Uygulama Kılavuzu ve Dergi Yayıncıları İçin Davranış Kurallarını (Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers) takip ederler.

İnsan denekleri, hayvan veya bitki içeren araştırmalar

- Araştırmanın insan denekleri veya hayvanları içermesi durumunda, yazarların Uluslararası Tıp Dergisi Editörleri Komitesinin (the International Committee of Medical Journal Editors) yönergelerini izlemeleri önerilir.
- İnsan denekleri içeren çalışmalarda, deneklerin çalışmaya katılmak için imzaladıkları onamlar yazarlar tarafından sağlanmalıdır. 18 yaşın altındaki deneklerin çalışmaya katılmaları için ebeveyn veya velileri tarafından izin verilmelidir.
- Test edilen tüm denekler için, makalenin, ilgili kurallara ve/veya uygun izinlere veya lisanslara uyumunu gösteren belgelerin sunulması gerekir.
- Hayvanlar üzerinde yapılacak her türlü araştırma kurumsal, ulusal veya uluslararası kurallara uygun olmalı ve etik kurul tarafından onaylanmalıdır.
- Bitki materyallerinin toplanması dahil, bitkiler üzerinde yapılan deneysel araştırmalar, kurumsal, ulusal veya uluslararası kurallara uygun olmalıdır.
- Saha çalışmaları yerel mevzuata uygun olarak yapılmalı ve uygun izinleri ve/veya lisansları belirten bir açıklama makalede yer almalıdır.

Yayın suistimali

- Akademik Gıda dergisi, Dergi Editörleri İçin Davranış Kuralları ile En İyi Uygulama Kılavuzları ve Dergi Yayıncıları İçin Davranış Kurallarını (Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers) takip eder.
- Makalenin aynı anda birden fazla dergiye gönderilmesi, intihal, yayınlanmış makalenin yeniden yayınlanması, etik kuralların ihlali vb. şüpheli bir suiistimal durumunda, araştırmacılar, hakemler veya okuyucular Yayın Ofisi (ogursoy@yahoo.com) ile iletişime geçmeye teşvik edilir.
- Makaledeki benzerlik oranı tek bir kaynaktan %10'dan fazla olmamak üzere en fazla %25 ile sınırlandırılmıştır. Bu koşula uymayan makaleler reddedilir. Bu şartların ihlal edilmesi durumunda, COPE (COPE recommendations) tavsiyeleri izlenecek ve ilgili tüm taraflara bildirilecektir.

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General Guidelines stated in the Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers are followed by all papers submitted to Academic GIDA. Prior to submission, authors are highly recommended to read the Journal's Instructions to Authors. Authors should also follow the European Association of Science Editors (EASE) Guidelines for Authors and Translators of Scientific Articles to be Published in English. For any research involving human or animal data, the recommendations of the International Committee of Medical Journal Editors should be followed by the authors of the manuscripts.

Peer Review

All contributions are evaluated according to the criteria of originality and quality of their scientific content.

- All manuscripts pass through an initial screening process (technical and overall quality evaluation) in the editorial office followed by an internal review by the technical and scientific editors.
- After the first evaluation, editors have the right to decline formal review of a manuscript if it is (i) on a topic outside the scope of the Journal, (ii) lacking technical merit, (iii) fragmentary and providing marginally incremental results or (iv) poorly written.
- If the manuscript is considered suitable for further evaluation, manuscripts are double-blind-reviewed by a peer review system involving at least two independent reviewers to ensure high quality of manuscripts accepted for publication.
- If requested, the revised version is evaluated by the reviewers, and editors make a decision about final acceptance based on their suggestions. If necessary, further revision can be asked for to fulfil all the requirements of the reviewers.
- The final version is then sent to the technical editor in order to produce a galley proof, and the authors receive this proof for final check before publishing.
- All manuscripts are posted online as pdf files in their final form, indexed in databases with the assigned DOI numbers.

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Akademik GIDA conducts a double-blind peer review process, i.e. the names of the reviewers are confidential to ensure the critical evaluation of the work. Reviewers are asked not to disclose their names or contact details in their comments for authors.

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An author is an individual who substantially contributed to the idea or design of a research, acquisition of data, analysis or interpretation of data, was involved in drafting, writing or revising the manuscript critically for important intellectual content. Other contributors should be mentioned in the Acknowledgements section of the manuscript and cannot be considered as authors of the study. Correct and full names of all authors and their ORCID IDs should be on the title page of the manuscript. Names of authors must be supplemented with their affiliations and a valid address of the corresponding author. The phone and fax numbers and e-mail address of the corresponding author should be stated in the first page of the manuscript. All authors must guarantee that the submitted manuscript is not published anywhere previously and will not be submitted to anywhere before the editorial board makes a final decision on the manuscript.

Funding Sources

Details for all funding sources of the research should be stated in the Acknowledgements. Authors should provide

the full official funding agency name(s) and grant number(s).

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Alteration in authorship after the submission of the manuscript to Akademik GIDA can be justified only by the additional work required during the revision. Any change is not allowed after the acceptance of the manuscript for publication. Alteration in authorship should be indicated in the responses to reviewers, and should be accepted by all authors. The corresponding author is primarily responsible for any alteration in the revised version of the manuscript, including the order of authors.

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All authors should be responsible for the accuracy of the published data. For the correction of data, the corresponding author receives the galley proof of the paper and is asked to correct it carefully within 4 days before publication.

Retraction of an Article

A retraction of an article is used to correct errors in submission or publication. Authors can retract the paper and should follow the Committee on Publication Ethics (COPE) retraction guidelines. In case of a duplicate or overlapping publication, fraudulent use of data, plagiarism or unethical research, the paper will be retracted by the editor, and a retraction notice will be included into the electronic database while all links to the retracted article will be maintained.

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Conflict of interest:

- Authors should declare any conflict of interest in their submission form. Authors are requested to suggest at least three potential reviewers before submission, and these reviewers cannot be their colleagues, collaborators or members of their institutions.
- Reviewers should notify the editors on any conflict of interest which prevents them from reviewing the paper, and they are recommended to follow the COPE guidelines.
- For the manuscripts submitted by the members of the Editorial Board or their collaborators, peer reviewing is modified to exclude them from the entire evaluation process in order to minimize any bias during the evaluation.
- During revision, the editors follow the Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers.

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Fevzipaşa Blv. Çelik İş Merkezi No:162 K:3 D:302 Çankaya / İZMİR
Tel: +90 232 441 60 01 Fax: +90 232 441 61 06 E-mail: sidasmedya@gmail.com

SİDAS MEDYA