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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effect of Different Drying Methods on Some Physical, Chemical and Antioxidant Properties of Elderberry (*Sambucus nigra* L.) Powder Substituted Cookies*

Farklı Kurutma Metotlarının Kara Mürver (*Sambucus nigra* L.) Tozu İkameli Bisküvilerin Bazı Fiziksel, Kimyasal ve Antioksidan Özellikleri Üzerine Etkisi

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Abstract

The development of novel and functional food formulations for improved body immunity has importance. Elderberry fruit is getting a lot of attention with high phenolic content and antioxidant properties concerning functionality. For protecting bioactive compounds, microwave and vacuum drying methods are accepted as good alternatives. This study aims to enhance the nutritional value of a highly consumed food product, wire-cut cookies, with elderberry powders obtained with different drying methods. For this purpose, elderberry fruits were dried by different drying methods (convection, microwave, vacuum), and elderberry powders were substituted to wire-cut cookie formulations at different ratios (0, 5, 10, 15, and 20%). Elderberry powder substitution resulted in a decrease in L^* and b^* values while an increase in a^* values. Better color properties occurred in MDEC samples. MDEC, VDEC samples, and higher substitution ratios had better physical properties. The use of elderberry powders resulted in high fat, ash, and low carbohydrate containing cookies. The highest values of bound and total phenolics were obtained in vacuum dried elderberry powder samples. As the substitution ratio increased, free, bound and total phenolic contents (from 5.35, 18.13, 23.49 mg GAE g^{-1} to 10.17, 27.15, 37.32 mg GAE g^{-1}, respectively) and free and bound antioxidant activities (DPPH (from 1.83, 42.19 mmol TE 100 g^{-1} to 2.14, 55.97 mmol TE 100 g^{-1}, respectively) and FRAP (from 0.11, 3.55 mmol TE 100 g^{-1} to 1.50, 12.48 mmol TE 100 g^{-1}, respectively)) of the products highly increased. Vacuum drying and 10% elderberry powder substituted cookies were the most suitable samples for sensory acceptability. The use of elderberry powders increased the functionality of cookies. Elderberry powder has a good potential to enhance the nutritional quality of food matrices. Using microwave and vacuum drying methods promises to protect the functionality of antioxidant-rich food materials.

Keywords: *Sambucus nigra*, Convection drying, Microwave drying, Vacuum drying, Free phenolics, Bound phenolics

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Öz

Vücut bağışıklığının güçlendirilmesi için yeni ve işlevsel gıda formülasyonlarının geliştirilmesi önem taşımaktadır. Kara mürver meyvesi, fonksiyonellik açısından yüksek fenolik bileşik içeriği ve antioksidan özellikleriyle oldukça ilgi görmektedir. Biyoaktif özellikteki bileşikleri korumak için mikrodalga ve vakum kurutma yöntemleri iyi alternatifler olarak kabul edilmektedir. Bu çalışma, farklı kurutma yöntemleriyle elde edilen kara mürver tozu ikamesiyle, çok tüketilen bir gıda ürünü olan bisküvilerin besin değerini artırmayı amaçlamaktadır. Bu amaçla, kara mürver meyveleri farklı kurutma yöntemleriyle (konveksiyonel, mikrodalga, vakum) kurutulmuş ve elde edilen kara mürver tozları, bisküvi formülasyonlarında farklı oranlarda (%0, 5, 10, 15 ve 20) ikame edilmiştir. Kara mürver tozu ikamesi, örneklerde $L*$ ve $b*$ değerlerinin azalmasına, $a*$ değerlerinde ise artmasına neden olmuştur. MDEC örneklerinde daha iyi renk özellikleri elde edilmiştir. MDEC, VDEC örnekleri ve daha yüksek ikame oranlarına sahip örneklerin daha iyi fiziksel özelliklere sahip olduğu bulunmuştur. Kara mürver tozu ikamesi, yüksek yağ, kül ve düşük karbonhidrat içerikli bisküvilerin eldesi ile sonuçlanmıştır. En yüksek bağlı ve toplam fenolik değerleri vakumla kurutulmuş kara mürver tozu ikameli örneklerde elde edilmiştir. İkame oranı arttıkça ürünlerin serbest, bağlı ve toplam fenolik madde içerikleri (sırasıyla 5.35, 18.13, 23.49 mg GAE g^{-1}'dan 10.17, 27.15, 37.32 mg GAE g^{-1}'a) ile serbest ve bağlı ekstraktların antioksidan aktiviteleri (DPPH için sırasıyla 1.83, 42.19 mmol TE 100 g^{-1}'dan 2.14, 55.97 mmol TE 100 g^{-1}'a ve FRAP için ise sırasıyla 0.11, 3.55 mmol TE 100 g^{-1}'dan 1.50, 12.48 mmol TE 100 g^{-1}'a) büyük oranda artış göstermiştir. Vakumla kurutulmuş tozların ikame edildiği ve %10 kara mürver tozu ikameli bisküvi örnekleri, duyusal kabul edilebilirlik açısından en uygun örnekler olmuştur. Kara mürver tozlarının kullanımı bisküvilerin fonksiyonelliğini artırmıştır. Kara mürver tozu, gıda matrislerinin besin kalitesini artırmada iyi bir potansiyele sahiptir. Mikrodalga ve vakumlu kurutma yöntemlerinin kullanılması ise antioksidan açısından zengin gıda maddelerinin fonksiyonelliğini korumayı vaat etmektedir.

Anahtar Kelimeler: *Sambucus nigra*, Konveksiyonel kurutma, Mikrodalga kurutma, Vakumlu kurutma, Serbest fenolik, Bağlı fenolik

1. Introduction

Elderberry (*Sambucus nigra* L.) is a plant species that belongs to the Adoxaceae family and is native to Europe, Asia, North Africa, and the USA (Charlebois et al., 2010). Nine Sambucus species have been defined: *S. ebulus, S. adnata, S. wightiana, S. gaudichaudiana, S. javanica, S. australasica, S. australis, S. racemosa* and *S. nigra*. Sambucus plant also has wild, semi-wild and uncultivated species (Mikulic-Petkovsek et al., 2015). Elderberry fruits are in the form of a bunch, like the flowers of the plant. Each bunch has 150 to 200 fruits. The elderberry is a round-shaped fruit with a diameter of 3 to 5 mm, which turns black or dark purple as it matures, has a fleshy structure, and contains between 3 and 5 seeds. Elderberry fruits are cultivated from mid-summer (August) to the end of summer (September) in the tropical and subtropical regions of the world (Costică et al., 2019). Elderberry fruits are rarely consumed fresh but generally as jam, marmalade, juice, vinegar, tea, wine, liquor and fried form (Mikulic-Petkovsek et al., 2015). Such fruit has been widely used as food and medicine in Europe since ancient times traditionally (Agalar, 2019). Elderberry fruit is a rich source of C, A and B vitamins, flavonoids, carotenoids, calcium and iron. Particularly, elderberry fruits are exhibited multiple bioactivities, such as antioxidant, antiviral, immunomodulatory, anti-inflammatory, antimicrobial, anticonvulsant and antidepressant (Agalar, 2019). It is known that elderberry has been used as a natural agent against hypertension, obesity and diabetes. These aforementioned effects of elderberry can be attributed to its chemical ingredients, including anthocyanins, quercetin derivatives, proanthocyanidins, monoterpene glycosides, phytosterols and sesquiterpene contents.

Freshly harvested elderberry is susceptible to deterioration due to containing approximately 80% of water (USDA, 2018). Thus, such fruits require appropriate drying to maintain quality, prolong shelf-life, and reduce nutrient loss. Drying is a widely used technology in food processing and preservation. Drying foods reduces the cost of packaging, storage and transportation by reducing the volume and weight of the product. Also, a variety of artificial drying methods, have been applied to fresh herbs, plants, and vegetables to prepare dehydrated products for industrial production (Yan et al., 2019). Drying is generally performed by evaporating the water in the food, and in modern industries, new drying methods are sought to obtain higher quality products in a reliable and efficient way, while saving energy (Çelen et al., 2016). Contrary to the new drying methods, convectional drying (CD) methods such as hot air drying have some disadvantages including high energy consumption, long drying time, low efficiency, high cost, and inferior quality of dried products (Yan et al., 2019). Microwave drying (MD) which is another food drying method prevents the decrease in product quality while saving energy by reducing the drying time (Li et al., 2010). The vacuum drying (VD) method is performed at low temperatures and in an oxygen-free environment. Therefore, it has more efficient than conventional drying methods and preserves the volatile compounds and flavor of the dry product (Sun et al., 2019). In this respect, the drying processes play an important role in the development of new, high-quality nutrient food ingredients from dry elderberries. The biscuit and cookie industries have an enormous contribution to the food production industry and the global economic value is approximately 7.4 billion USD. For Türkiye, the economic value of the biscuit and cookie industry consists of 3-4% of the worldwide. Thus it is important to maintain healthy product production in this industry to obtain economic value added (Erenler et al., 2022).

Elderberry fruit has a good potential for utilization in food, medicine, and health care due to its bioactive contents such as phenolics and antioxidants. Consequently, enhancing an efficient drying method is necessary to remove the moisture for decreasing the deterioration of fruit, and improve product quality. To the best of our knowledge, this is the first study aiming at investigating the impact of the use of microwave and vacuum-dried elderberry powders on the physical (color, texture, diameter, thickness and spread ratio), chemical (moisture, crude oil, crude protein, ash, carbohydrate and energy, phenolic contents (free, bound and total)), and antioxidant capacity (using DPPH and FRAP methods) of cookies. Therefore, the aims of this study were to compare the effects of three different drying methods (CD, MD and VD) on the color, proximate composition and antioxidant properties of elderberry powder, to develop cookies with dried elderberry powders by CD, MD and VD methods at 0, 5, 10, 15 and 20% ratios, and to determine physical, textural, chemical characteristics and antioxidant quality of cookies containing elderberry powders. The results of this research will contribute to the selection of an appropriate drying method for elderberries and expand the potential for applying dried elderberries to functional foods.

2. Materials and Methods

2.1. Materials

The fresh elderberry (*Sambucus nigra* L.) fruits harvested in 2019 were obtained from the Yalova Atatürk Horticultural Central Research Institute. Fruits were kept in cooling-controlled containers during transportation to the laboratory and stored in the deep freeze at -18 °C until their use. Commercial wheat (*Triticum aestivum*) flour (Hekimoğlu, Konya, Turkey; contains 0.54% ash and 10.29% protein), shortening, powdered sugar, fructose syrup, milk powder, salt and sodium bicarbonate were purchased from local markets in Konya, Turkey.

2.2. Preparation of elderberry powders

Elderberry fruits were separated from their stems and pureed with a blender (Korkmaz A446, Turkey). Then, elderberry fruit purees were spread as a thin layer on drying papers and dried with three different methods. Drying conditions were determined based on the preliminary tests which were carried out by changing drying parameters (eg. temperature and time for convection and vacuum drying, W and time for microwave drying). In the CD method, elderberry puree was dried in a tray dryer (Eksis, Turkey) at 60 °C for 3 h. Secondly, elderberry puree was dried in a microwave oven (LG Solar DOM, Korea), and drying conditions of 360 watts and 20 min were used. Drying was not applied continuously in the microwave and a 30 second pause was required every 5 minutes. In the third method, elderberry puree was dried in a vacuum dryer (JSVO-60T, Korea) at 60 mmHg absolute pressure, 60 °C for 3 h. Dried elderberry fruits were ground with a laboratory-type grinder (Alveo, Turkey). Elderberry powders were stored in polyethylene packages at +4 °C until used.

2.3. Preparation of cookie samples

Modified AACC Standard No: 10-54 production method was used in the preparation of cookies (AACC, 1990). Control cookie without substitution was prepared with 100 g wheat flour, 42 g powdered sugar, 40 g shortening, 1.5 g fructose syrup, 1.5 g sodium bicarbonate, 1.25 g salt, 1 g milk powder, and 20 ml milk. All ingredients were kneaded in a laboratory-type mixer (Kenwood KMX, UK) for 8 min until obtaining homogeneous dough. At the end of the time, the dough was given a round shape with a thickness of 5.0 mm and a diameter of 55.0 mm. Cookie samples were baked at 200 °C for 26 min in an oven (Vestel SF8401, Turkey). Substituted cookies were prepared by replacing wheat flour with elderberry powders at 5, 10, 15 and 20% ratios. Utilization ratios of elderberry powders were determined by preliminary tests that investigated the lower and higher ratios according to consumer acceptability.

2.4. Physical analyses

Color parameters (L^* [(0) black - (100) white], a^* [(+) red - (-) green] and b^* [(+) yellow - (-) blue]) of the raw materials and cookie samples were measured with Minolta CR 400 (Konica Minolta Inc., Japan). The color measurements were replicated five times and made at three different points for each sample.

Diameter (mm) and thickness (mm) values of cookie samples were measured using a digital caliper (0.001 mm Mitutoyo, Minoto-Ku, Tokyo, Japan). Spreading ratio values were determined by dividing the diameter values by the thickness values (ACCC, 1990).

Hardness and fracturability values were measured with a texture analyzer (Stable Micro Systems TA-XT Plus, Surrey, UK) after 24 h. A three-point bend rig, a distance of 5.0 mm and a speed of 3.0 mm sec^{-1} were used in the texture measurements.

2.5. Chemical analyses

Moisture (AACC Method 44-19.01), ash (AACC Method 08-01.01), crude protein (AACC Method 46-12.01) and crude fat (AACC Method 30-25.01) content of the raw materials (wheat flour and elderberry powders) and cookie samples were determined using standard methods (AACC, 1990). The carbohydrate and energy values of the samples were calculated according to Schakel et al. (1997).

2.6. Phenolic and antioxidant analyses

Free (soluble) and bound (hydrolyzable, insoluble) polyphenol extractions were carried out according to Naczk and Shahidi (2004) and Vitali et al. (2009) with some modifications. Powdered samples (1 g) were extracted for 2 h with 10 ml solvent (methanol:HCl:distilled water, 8:1:1, v/v/v) at 20 °C to obtain free phenolic extracts. The extracts were separated by centrifuge at 3500 rpm (4 °C) for 10 min. The residues of the samples were extracted in 40 ml of solvent (methanol:H_2SO_4, 10:1, v/v) at 85 °C for 20 h to obtain bound phenolic extracts. At the end of the period, the extracts were separated by centrifuge at 3500 rpm (4 °C) for 10 min. Obtained supernatants were used for the determination of phenolics using Folin–Ciocalteu spectrophotometric method. The calibration curve was made using gallic acid and therefore obtained amounts of free and bound phenolics were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight.

Antioxidant activity was measured using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Brand-Williams et al., 1995). The supernatants of free and bound extracts (50 µl) were reacted with freshly prepared 60 µM DPPH solution (950 µl). DPPH solution is prepared in the concentration of 60 µM with methanol. The absorbances were measured at 517 nm by a UV/Visible spectrophotometer model Libra S22 (Biochrom, Cambridge, UK). DPPH antioxidant activity was expressed as millimoles of Trolox equivalents (TE) per 100 grams of dry weight.

In order to determine the ferric reducing antioxidant power (FRAP) of samples, the analysis was carried out according to Benzie and Strain (1996). The supernatants of free and bound extracts (75 µl) were reacted with FRAP reagent (2.25 ml) and distilled water (225 µl). After 30 min of incubation at dark, the absorbances were measured at 593 nm by a UV/Visible spectrophotometer model Libra S22 (Biochrom, Cambridge, UK). FRAP values were expressed as millimoles of TE per 100 grams of dry weight.

2.7. Sensory analysis

The sensory evaluation of cookies was carried out by 10 panelists aged from 22 to 48 years, healthy and nonsmokers. Cookies were presented to the panelists with random numbers in porcelain dishes at ambient temperature (25±2 °C). Sensory evaluation was performed in a bright environment. Samples were assessed for color, taste, odor, appearance, crispness and overall acceptability parameters evaluated using a 5-point scale (1: dislike very much, 5: like very much).

2.8. Statistical analysis

This study was carried out according to the (2 × 5) × 2 factorial design. JMP (SAS Institute Inc., Cary, NC, USA) statistical program version 13.2.0 was used for statistical data analysis. Raw material and cookie data were compared by one-way and two-way analysis of variance (ANOVA), respectively. Drying method and substitution ratio are the main variance sources. Tukey's HSD test was used to determine significant differences. The values of $p < 0.05$ were accepted as significantly different.

3. Results and Discussion

3.1. Color, proximate composition and antioxidant properties of raw materials

Color values of raw materials are demonstrated in *Table 1*. *L*, a** and *b** values of MDE were found higher than CDE and VDE. Similarly, Contreras et al. (2008) reported that the *a** value of microwave-dried strawberry was found higher than traditional dried ones. The MD method protects the nutritional value, color and flavor of food products, and provides higher quality. Besides the protection of texture and color properties, MD provides shorten drying time. Cyanidin-3-glycoside and cyanidin-3-sambubioside anthocyanin compounds are responsible for the color of the elderberry fruit (Schmitzer et al., 2012). Anthocyanins are affected by external factors such as oxygen, temperature and light (Samotchia et al., 2016). The shortening of the drying process in the MD method allowed for achieving better results of color preservation.

Crude fat content of raw materials ranged between 1.45% and 15.43% (*Table 1*). Crude fat contents of elderberry powders were found higher than wheat flour. This finding may be because elderberry fruit, especially its seeds, had high fat content (Dominguez et al., 2020). The crude protein content of elderberry powders was found lower than wheat flour (*Table 1*). The highest ash content was found in CDE (4.85%), while the lowest was found in wheat flour (0.54%) (*Table 1*). When CD was used to dry fresh elderberry fruits, the solubility of the minerals could be affected by the energy and wave strength of the convective drying method, further resulting in higher ash content. A similar finding was also observed in a previous study for drying rosemary samples (Özcan and Arslan, 2008). The carbohydrate contents of elderberry powders were found lower than wheat flour, while the energy values were found higher (*Table 1*). The differences in carbohydrate and energy values may be because of the results of the differences in the chemical composition of samples. Phenolic compounds exist in free (soluble) and bound (insoluble) forms with respect to structurally and depending on their relationship with the food matrix. In this study, free, bound and total phenolic contents of raw materials were summarized in Table 1. Free phenolic contents of raw materials ranged between 6.50 and 96.44 mg GAE g^{-1}. The drying treatments had no significant influence on the free phenolic contents of the dried elderberry samples ($p > 0.05$).

Table 1. Some properties of raw materials[1]

			Wheat Flour	CDE[2]	MDE[3]	VDE[4]
Color properties		*L**	94.04 ± 0.01[a]	18.83 ± 0.45[c]	20.11 ± 0.18[b]	19.91 ± 0.15[bc]
		*a**	-0.54 ± 0.01[d]	5.43 ± 0.08[b]	6.26 ± 0.06[a]	4.33 ± 0.05[c]
		*b**	10.07 ± 0.01[a]	-0.62 ± 0.01[c]	-0.28 ± 0.01[b]	-0.55 ± 0.01[c]
Chemical contents (%)		Moisture	9.79 ± 0.01[a]	11.86 ± 1.03[a]	9.50 ± 0.53[a]	12.44 ± 0.28[a]
		Crude fat	1.45 ± 0.68[b]	15.12 ± 0.64[a]	15.43 ± 0.47[a]	14.96 ± 0.38[a]
		Crude protein	10.29 ± 0.68[a]	8.66 ± 0.12[ab]	7.96 ± 0.62[b]	7.35 ± 0.00[b]
		Ash	0.54 ± 0.03[c]	4.85 ± 0.09[a]	3.76 ± 0.02[b]	3.86 ± 0.03[b]
		Carbohydrate	77.92 ± 0.02[a]	59.51 ± 0.59[b]	63.35 ± 1.59[b]	61.40 ± 0.63[b]
		Energy value (kcal 100 g^{-1})	366.00 ± 3.33[b]	408.76 ± 7.66[a]	424.12 ± 0.35[a]	409.58 ± 0.87[a]
Phenolic contents (mg GAE g^{-1})		Free phenolic	6.50 ± 0.02[b]	96.44 ± 5.44[a]	95.75 ± 2.14[a]	89.30 ± 1.17[a]
		Bound phenolic	25.12 ± 0.49[c]	100.58 ± 1.16[b]	96.26 ± 0.87[b]	122.92 ± 2.03[a]
		Total phenolic	31.62 ± 0.47[c]	197.01 ± 4.29[b]	192.02 ± 3.01[b]	212.21 ± 0.86[a]
Antioxidant properties (mmol TE 100 g^{-1})	DPPH values	Free extract	1.82 ± 0.03[b]	2.98 ± 0.25[a]	2.87 ± 0.09[a]	2.90 ± 0.02[a]
		Bound extract	45.58 ± 0.25[b]	165.65 ± 7.16[a]	151.34 ± 10.07[a]	183.40 ± 20.14[a]
	FRAP values	Free extract	0.04 ± 0.01[b]	16.78 ± 1.49[a]	15.63 ± 2.27[a]	18.84 ± 0.01[a]
		Bound extract	5.27 ± 0.01[b]	24.05 ± 3.09[a]	22.49 ± 0.22[a]	24.26 ± 0.35[a]

1 Means followed by the same letter within a row are not significantly different ($p < 0.05$). Values are the average of duplicate measurements on the duplicate samples, and are based on dry matter. 2 Convectional dried elderberry 3 Microwave dried elderberry 4 Vacuum dried elderberry

On the contrary, the different drying methods significantly influenced the bound and total phenolic contents ($p < 0.05$). The lowest bound phenolic content was found in wheat flour (25.12 mg GAE g^{-1}), while the highest was found in VDE (122.92 mg GAE g^{-1}), whereas CDE and MDE had no statistically significant differences. Issis et al. (2019) reported that the degradation of phenolic compounds in maqui berries subjected to VD was lower than convective dried ones at the same temperature. Moreover, according to these findings, it was suggested that VD could be more convenient than convective drying to maintain phenolic compounds, possibly due to the lack of oxygen and a shorter drying time. Similar to bound phenolic content, the highest total phenolic content was determined in VDE as 212.21 mg GAE g^{-1}. Similarly, Quispe-Fuentes et al. (2018) found that the total phenolic content of vacuum-dried maqui berries, another berry fruit, was higher than conventional dried ones. Also, Yokuş and Akpınar Borazan (2014) reported that the total phenolic content of vacuum-dried apples was higher than microwave-dried ones. *Table 1* shows that a considerable amount of total phenolics consists of bound forms. Similarly, Sosulski et al. (1982) reported that 69.2% of the total phenolic content of wheat consists of bound phenolic compounds, and their amount is 2 to 5 times higher than free phenolic compounds. It must be noted that the phenolic content of agricultural samples could change considering species and growing conditions. Also, the extraction ratio could affect the determined phenolic contents (Baumgartner et al., 2018). Moreover, previous studies described that the drying process facilitates the extraction of phenolic compounds, which results in a positive effect on bound and total phenolic contents (Delgado-Nieblas et al., 2017).

The antioxidant activities of the samples were shown in *Table 1*. The DPPH antioxidant activities of the raw materials ranged between 1.82 and 2.98 mmol TE 100 g^{-1} for the free extract, and 45.58 and 183.40 mmol TE 100 g^{-1} for the bound extract. Similar to phenolic contents, the lowest DPPH scavenging activity values were found in the free and bound extracts of wheat flour (1.82 and 45.58 mmol TE 100 g^{-1}, respectively). For both free and bound extracts, DPPH scavenging activity of elderberry powders dried by different methods has numerical differences, while had not any statistically significant ($p > 0.05$) differences (*Table 1*). Similar to our findings, previous studies also observed that DPPH values of bound extracts of raspberry, blackcurrant, blackberry, cranberry, black chokeberry, blueberry (Kim, 2018), other berry fruits, were higher than free extracts. Similar to DPPH values, FRAP values of wheat flour were lower than those of elderberry powders and different drying methods had no statistically significant effects on the antioxidant power of elderberries ($p > 0.05$) (*Table 1*). Effects of different drying methods on FRAP value were consistent with the previous studies which investigated the antioxidant properties of other berry fruits (Samotchia et al., 2016). Lou et al. (2020) reported that the antioxidant activities of free extracts of Crataegus pinnatifida was found lower than bound extracts. These results are also consistent with our findings.

3.2. Color values of cookie samples

The color values and physical properties of the cookie samples are summarized in *Table 2*. Color in bakery products is an important quality factor for consumers. In addition, factors such as texture, taste and color in cookies affect the acceptability of the end product. Among tested factors, both drying methods and substitution ratios showed significant effects on the color properties of cookie samples (*Table 2*). The most positive effect on the L^* value has been seen in MDEC samples. a^* values of CDEC and MDEC samples were found significantly higher than VDEC samples. For drying methods, the highest b^* values were found in CDEC samples, while the lowest were found in VDEC samples. Rozylo et al. (2019) stated that the stability of pigments depends on a variety of factors such as the structure and concentration of pigments, pH, temperature, enzymes, oxygen, ascorbic acid and sugar. Therefore, such factors could be the reason for the higher L^*, b^*, and lower a^* values in CDEC samples.

For substitution ratios, as the substitution ratio increased, both L^* and b^* values decreased. On the contrary, it was seen an increase in the a^* values. Similarly, Rozylo et al. (2019) reported that freeze-dried elderberry and chokeberry addition resulted in an increase in a^* values and a decrease in L^* and b^* values in gluten-free wafer sheets. Also, Da Silva et al. (2019) reported similar changes in color values in elderberry juice added croissants samples. Similar variations of color values have also been reported for cookie samples substituted with black currant and jostaberry (Molnar et al., 2015), blackcurrant powder (Mofasser Hossain et al., 2017) and raspberry, red currants, and strawberry pomace flour (Tarasevicіene et al., 2020). Topka et al. (2023) reported a decreased L^* and increased a^* and b^* values in gingerbread cookies covered in chocolate enriched with elderflower dry extract and elderberry juice concentrate. It was predicted the most important factor in the color changes of samples was the own color of substituted elderberry. Also, similar color changes in the biscuit samples were explained by occurs Maillard reaction during the cooking process of samples (Molnar et al., 2015).

3.3. Physical measurements of cookie samples

The size which is one of the basic quality criteria of the cookies is determined by the diameter and thickness values. The spread ratio affects biscuit size in terms of both quality and product packaging.

Table 2. Color values, physical and textural properties of cookie samples[1]

Factor	L*	a*	b*	Diameter (mm)	Thickness (mm)	Spread Ratio	Hardness (g)	Fracturability (mm)
Drying Method								
CDEC[2]	44.50 ± 0.09[c]	8.87 ± 0.08[a]	8.46 ± 0.12[a]	55.48 ± 0.25[c]	8.00 ± 0.08[a]	6.96 ± 0.05[c]	4600.46 ± 226.11[b]	38.97 ± 0.11[a]
MDEC[3]	47.31 ± 0.24[a]	8.78 ± 0.10[a]	8.18 ± 0.19[b]	55.81 ± 0.21[b]	7.94 ± 0.11[a]	7.06 ± 0.08[b]	4877.19 ± 568.46[ab]	38.70 ± 0.12[b]
VDEC[4]	45.06 ± 0.14[b]	8.50 ± 0.18[b]	6.21 ± 0.14[c]	56.41 ± 0.30[a]	7.92 ± 0.08[a]	7.15 ± 0.06[a]	5211.38 ± 221.55[a]	38.70 ± 0.12[b]
Substitution Ratio (%)								
0	75.12 ± 0.08[a]	2.44 ± 0.13[e]	25.57 ± 0.49[a]	54.50 ± 0.42[e]	8.70 ± 0.14[a]	6.26 ± 0.05[e]	3649.36 ± 339.18[d]	39.44 ± 0.05[a]
5	44.74 ± 0.18[b]	8.81 ± 0.10[d]	4.49 ± 0.04[b]	54.92 ± 0.26[d]	8.15 ± 0.07[b]	6.74 ± 0.03[d]	4392.82 ± 195.22[c]	39.14 ± 0.32[a]
10	40.64 ± 0.24[c]	9.86 ± 0.18[c]	3.37 ± 0.07[c]	56.00 ± 0.19[c]	7.87 ± 0.09[c]	7.12 ± 0.09[c]	5018.00 ± 513.52[bc]	38.70 ± 0.15[b]
15	36.48 ± 0.14[d]	10.76 ± 0.05[b]	2.57 ± 0.05[d]	56.57 ± 0.24[b]	7.68 ± 0.07[d]	7.37 ± 0.06[b]	5304.37 ± 318.23[b]	38.43 ± 0.05[bc]
20	31.14 ± 0.16[e]	11.70 ± 0.13[a]	2.10 ± 0.09[e]	57.52 ± 0.16[a]	7.37 ± 0.09[e]	7.80 ± 0.10[a]	6117.17 ± 327.39[a]	38.24 ± 0.02[c]

1 Means followed by the same letter within a column are not significantly different ($p < 0.05$). Values are the average of duplicate measurements on the duplicate samples, and are based on dry matter. 2 Convectional dried elderberry powder substituted wire-cut cookies 3 Microwave dried elderberry powder substituted wire-cut cookies 4 Vacuum dried elderberry powder substituted wire-cut cookies

Among the drying methods, the highest diameter and spread ratio values were found in VDEC samples, while the lowest values were found in CDEC samples (*Table 2*). Drying methods had no statistically significant effects on the thickness values of cookies ($p < 0.05$). It was found that there was a parallel relationship between diameter, spread ratio values and substitution ratio, on the contrary thickness value. An increase in the substitution ratio resulted in an increase in diameter and spread ratio values. Hadinezhad and Butler (2009) reported that the amount of dough fermentation and gravity are always considered constant, so only dough viscosity affects the spread ratio of cookie samples. On the other hand, it was stated that the higher lipid content resulted in an increase in diameter and spread ratio in cookies. Thus, it could be mentioned that the obtained results related to the cookie dough viscosity which is decreased with elderberry powder substitution and/or the lipid content of cookie dough. Low thickness, wide diameter and high spread ratio is desired in a high-quality cookie. Therefore, it could be concluded that the elderberry powder substitution led to an increase in the physical quality of cookies.

3.4. Texture measurements of cookie samples

The texture is an important parameter in evaluating the quality of bakery products, and it contributes positively to the quality of the end cookie product (Mofasser Hossain et al., 2017). Hardness and fracturability which are the main textural characteristics of cookies are defined as the strength of the product to resist deformation. Among the drying methods, the highest hardness was found in VDEC, while the highest fracturability was in CDEC (*Table 2*). For fracturability values, MDEC and VDEC samples had no significant differences ($p < 0.05$). The substitution ratios and hardness values had a parallel relationship. On the contrary, an increase in substitution ratios led to a decrease in fracturability values. In addition, fracturability of cookie samples which has 0 and 5% substitution ratios had no significant differences ($p < 0.05$). A similar trend in textural properties of cookies depending on substitution ratios was also reported for other berry fruits (Molnar et al., 2015; Mofasser Hossain et al., 2017; Taraseviciene et al., 2020). The change in hardness values could be related to the interaction between protein and starch molecules in the product, in brief, hydrogen bonds (Mofasser Hossain et al., 2017). Similarly, Molnar et al. (2015) determined an increase in hardness values with increased berry substitution ratios related to the starch composition and starch-protein interactions. Also, Jukic et al. (2019) stated that the increase in diameter and decrease in thickness of the cookies could cause an increase in the hardness values.

3.5. Chemical contents of cookie samples

The chemical composition of cookie samples showed in *Table 3*. Similarly, Borczak et al. (2022) found that elderberry cookies contained 94.91, 0.71, 9,57, 20.39, and 62.55 g 100 g^{-1} of dry matter, ash, fat, protein, and carbohydrates, respectively. The moisture content of cookies should not exceed 14% and the best moisture content for cookies is 5% (Uchoa et al., 2009). Therefore, it could be concluded that the moisture contents are in the desired range. Among tested factors, only substitution ratios showed a significant effect on the crude fat content of the cookies. The crude fat content of elderberry powder substituted cookies were found higher than the control sample. This result could be attributed to the high fat content of elderberry fruit, especially its seeds (Dominguez et al., 2020). Similar results on crude fat content changes were also reported for different berry powder substituted cookies (Tarasevicíene et al., 2020). Similar trends were observed for crude protein and ash content of cookie samples according to the drying methods used (*Table 3*). Crude protein and ash contents of VDEC samples were found lower than other samples, while ash content of MDEC and VDEC had no significant differences ($p < 0.05$). The protein content of samples had an inverse relationship with the substitution ratio contrary to ash content. An increase in substitution ratio led to an increasing trend in ash contents. But 15 and 20% substitution ratios had no significant effect on the ash content of samples. These findings are consistent with previous studies (Özcan and Arslan, 2008; Molnar et al., 2015; Taraseiciene et al., 2020). The differences between crude protein and ash results were expected due to elderberry containing more ash (related to high mineral content) and less protein than wheat flour (Divis et al., 2015).

Table 3. Chemical composition of cookie samples[1]

Factor	Moisture (%)	Crude Fat (%)	Crude Protein (%)	Ash (%)	Carbohydrate (%)	Energy (kcal 100 g^{-1})
Drying Method						
CDEC[2]	7.03 ± 0.39^{a}	17.33 ± 0.56^{a}	7.33 ± 0.10^{a}	1.73 ± 0.02^{a}	66.57 ± 0.60^{a}	451.61 ± 4.29^{a}
MDEC[3]	6.97 ± 0.33^{a}	17.53 ± 1.06^{a}	7.22 ± 0.12^{a}	1.63 ± 0.02^{b}	66.64 ± 1.29^{a}	453.27 ± 4.27^{a}
VDEC[4]	7.04 ± 0.12^{a}	17.31 ± 0.89^{a}	6.49 ± 0.12^{b}	1.62 ± 0.01^{b}	67.08 ± 0.86^{a}	451.94 ± 4.47^{a}
Substitution Ratio (%)						
0	6.79 ± 0.14^{a}	16.44 ± 1.45^{b}	7.57 ± 0.18^{a}	1.41 ± 0.02^{d}	67.79 ± 1.15^{a}	449.42 ± 7.70^{b}
5	6.94 ± 0.24^{a}	16.64 ± 1.12^{ab}	7.27 ± 0.11^{b}	1.56 ± 0.01^{c}	67.58 ± 1.13^{ab}	449.20 ± 5.64^{b}
10	7.07 ± 0.15^{a}	17.64 ± 0.46^{ab}	7.17 ± 0.08^{bc}	1.65 ± 0.02^{b}	66.47 ± 0.69^{ab}	453.33 ± 1.90^{ab}
15	7.12 ± 0.57^{a}	17.77 ± 0.53^{ab}	7.03 ± 0.08^{c}	1.82 ± 0.02^{a}	66.25 ± 0.88^{ab}	453.04 ± 3.45^{ab}
20	7.15 ± 0.31^{a}	18.48 ± 0.63^{a}	6.79 ± 0.12^{d}	1.86 ± 0.01^{a}	65.71 ± 0.74^{b}	456.38 ± 3.03^{a}

1 Means followed by the same letter within a column are not significantly different ($p < 0.05$). Values are the average of duplicate measurements on the duplicate samples, and are based on dry matter. 2 Convectional dried elderberry powder substituted wire-cut cookies 3 Microwave dried elderberry powder substituted wire-cut cookies 4 Vacuum dried elderberry powder substituted wire-cut cookies

Preference for high fiber and low carbohydrate content foods is gradually increased. In addition, researchers stated that low-carbohydrate and high-fiber foods both have a protective effect on human health and help digestion (Grewal, 2018). Drying methods had no significant effects on both carbohydrate contents and energy values (*Table 3*). Similar to reported studies about other berry supplemented cookies, carbohydrate contents of elderberry substituted cookies were found higher than the control sample (Grewal, 2018; Da Silva et al., 2019). Plain biscuits were rich in carbohydrates but lacked components such as dietary fiber, minerals and vitamins. Tarasevicíene et al. (2020) reported that the substitution of berry fruits in cookie flour reduces the glycemic index and is an important source of phytochemicals in terms of enriching cookies. Therefore, the dietary fiber composition of substituted elderberry powder could be the reason for the increasing carbohydrate content in cookies. Carbohydrate, protein and fat components contribute to the energy value in foods. Also, fat content is known as a good source of energy (Grewal, 2018). For substitution ratios, as the substitution ratio increased, the energy values of cookies increased (*Table 3*). These changes could be explained by a hypothesis that high fat content of substituted elderberry powder affected the energy values of cookie samples. There has been an increasing trend toward foods that are low in carbohydrates and rich in dietary fiber for health (Grewal, 2018). Thus, it could be remarked that elderberry powder substituted cookies can be considered as an alternative product for these desired requests.

3.6. Phenolic and antioxidant properties of cookie samples

Phenolic compounds are the most active antioxidants in our diet and consuming foods which rich in phenolic compounds causes an increase in antioxidants in the human body (Vitali et al., 2009). Phenolic compounds exist in fruits in two forms, free and bound. Free phenolic compounds are rapidly absorbed and spread in the human body. On the other way, phenolic compounds in bound form cannot be digested by the stomach and small intestine, but reach the colon after converting to the free form and performing their bioactivity (Li et al., 2019). Elderberry fruit has therapeutic properties against many diseases due to its rich phenolic content (Pliszka, 2017). Free phenolic contents of cookie samples ranged between 5.35 and 10.17 mg GAE g^{-1} (*Table 4*). No significant effect was determined in terms of drying methods on free phenolic contents ($p > 0.05$). But increased substitution ratio had an enhancing effect on free phenolic contents ($p < 0.05$). For drying methods, both bound and total phenolic contents were found significantly higher in VDEC samples ($p < 0.05$). Also, bound and total phenolic contents of CDEC and MDEC were found to be statistically similar.

Table 4. Functional properties of cookie samples[1]

		Drying Method			*Substitution Ratio (%)*				
		CDEC[2]	**MDEC[3]**	**VDEC[4]**	**0**	**5**	**10**	**15**	**20**
Phenolic Content (mg GAE g^{-1})	**Free**	7.95 ± 0.19[a]	7.68 ± 0.45[a]	7.87 ± 0.37[a]	5.35 ± 0.51[e]	6.89 ± 0.43[d]	7.88 ± 0.29[c]	8.86 ± 0.07[b]	10.17 ± 0.39[a]
	Bound	22.85 ± 0.55[b]	22.84 ± 0.22[b]	23.80 ± 0.89[a]	18.13 ± 0.52[d]	20.04 ± 1.23[c]	24.79 ± 0.39[b]	25.67 ± 0.29[ab]	27.15 ± 0.34[a]
	Total	30.78 ± 0.70[b]	30.53 ± 0.57[b]	31.67 ± 1.07[a]	23.49 ± 1.03[e]	26.93 ± 1.26[d]	32.67 ± 0.61[c]	34.53 ± 0.26[b]	37.32 ± 0.73[a]
DPPH values (mmol TE 100 g^{-1})	**Free**	1.95 ± 0.03[a]	1.96 ± 0.02[a]	1.98 ± 0.04[a]	1.83 ± 0.01[c]	1.87 ± 0.03[c]	1.96 ± 0.05[b]	2.02 ± 0.01[b]	2.14 ± 0.04[a]
	Bound	48.47 ± 1.51[a]	50.63 ± 1.21[a]	52.06 ± 6.50[a]	42.19 ± 0.50[b]	48.58 ± 2.81[ab]	51.51 ± 4.78[a]	53.68 ± 3.06[a]	55.97 ± 4.20[a]
FRAP values (mmol TE 100 g^{-1})	**Free**	0.78 ± 0.01[a]	0.77 ± 0.06[a]	0.82 ± 0.07[a]	0.11 ± 0.01[e]	0.48 ± 0.05[d]	0.78 ± 0.05[c]	1.05 ± 0.10[b]	1.50 ± 0.04[a]
	Bound	8.05 ± 0.23[a]	7.76 ± 0.32[a]	7.44 ± 0.50[a]	3.55 ± 0.88[e]	5.98 ± 0.06[d]	7.16 ± 0.24[c]	9.59 ± 0.51[b]	12.48 ± 0.86[a]

1 Means followed by the same letter within a column are not significantly different ($p < 0.05$). Values are the average of duplicate measurements on the duplicate samples, and are based on dry matter. 2 Convectional dried elderberry powder substituted wire-cut cookies 3 Microwave dried elderberry powder substituted wire-cut cookies 4 Vacuum dried elderberry powder substituted wire-cut cookies

Borczak et al. (2022) found the total phenolic content of elderberry cookies to be 144.69 mg 100 g^{-1}. They also reported that ABTS and FRAP antioxidant activity of elderberry cookie samples were 9.42 µmol g^{-1} and 11.37 µmol g^{-1}, respectively. Topka et al. (2023) found the free and bound phenolic contents of gingerbread cookies covered in chocolate enriched with elderflower dry extract as 277.44 and 67.77 µg CE g^{-1}, while 256.16 and 68.79 µg CE g^{-1} for gingerbread cookies covered in chocolate with elderberry juice concentrate, respectively. The differences between results could be attributed to the differences in cookie recipes, elderberry samples used, and used analysis method.

For all those samples, there was a parallel relationship between substitution ratio and bound and total phenolic contents (*Table 4*). This parallel relationship between substitution ratio and phenolic contents is consistent with previous studies about other berry fruits (Mofasser Hossain et al., 2017; Grewal, 2018). Heat treatments cause different effects on phenolic compounds in foods, and this is due to the differences in the composition and types of phenolic compounds. Higher bound and total phenolic contents of VDEC samples could be attributed to the low oxygen level of the environment during VD and the preservation of the amount of phenolic substance in the product. Thus, it is thought that the amount of bound and total phenolic substances comes to the forefront compared to other techniques (Quispe-Fuentes et al., 2018). Abdel-Aal and Rabalski (2013) reported that after the baking process of lutein-enriched cookies, the bound phenolic content in the control and lutein-substituted samples increased by 11% and 12%, respectively. These findings could be related to the changes in phenolic contents occurring during the production of samples depending on heat processes in both the drying and cooking.

Additionally, in the literature, it was reported that the use of elderberry in dessert recipes resulted in anthocyanin loss for jam as 60%, for muffin as 35%, for crumble 35%, and for mousse as 10%. It was stated elderberry anthocyanins are more prone to water extraction and degradation by heat (Cordeiro et al., 2021). Therefore, heatless or low-heated drying methods seem promising to preserve the anthocyanins of elderberry.

Oxidation in foods are reactions that cause the formation of undesirable substances and flavors that can harm human health. Various antioxidants are used in the food industry to prevent or delay this process (Brand-Williams et al., 1995). Antioxidants are naturally present in fruits and vegetables. The antioxidant activities of fruits and vegetables depend on the type and amount of antioxidant substances responsible for the product. The antioxidant properties of cookie samples were demonstrated in *Table 4*. No significant differences according to the drying methods occurred in the DPPH scavenging activity and FRAP values of the cookie samples ($p > 0.05$). On the contrary, it was found a parallel relationship between substitution ratios and antioxidant properties. The increased substitution ratio led to a statistically significant increase in both DPPH and FRAP antioxidant properties (p <

0.05). For the DPPH scavenging activity, samples with 0 and 5% substitution ratios and with 10 and 15% substitution ratios were found to be statistically similar for free extracts. Also, for the DPPH values of bound extracts, samples with 10, 15 and 20% were found to be statistically similar ($p > 0.05$). All substitution ratios caused an increase in both free and bound extracts in FRAP values, significantly ($p < 0.05$). Similar changes in antioxidant properties in cookie samples were attributed to the high antioxidant properties of berry fruit powders substituted against wheat flour by reported studies (Mofasser Hossain et al., 2017). It was also stated that the Maillard reaction that occurred during the cooking process of the samples could be another reason for the increase in new compounds that have antioxidant properties (Ning et al., 2020). Therefore, in light of the aforementioned studies, it could be concluded that the increase in antioxidant properties of cookie samples is a result of the high antioxidant properties of substituted elderberry powder (Pliszka, 2017; Dominguez et al., 2020).

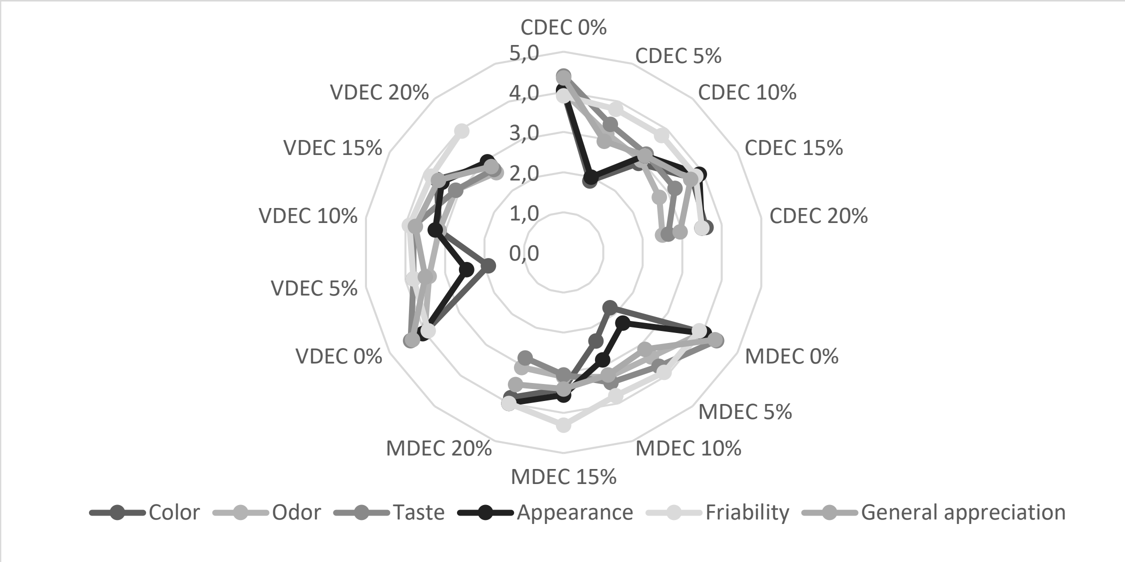

Figure 1. Sensory properties of cookie samples[1]

1 CDEC 0%: 100% wheat flour; CDEC 5%: 95% wheat flour + 5% CDE; CDEC 10%: 90% wheat flour + 10% CDE; CDEC 15%: 85% wheat flour + 15% CDE; CDEC 20%: 80% wheat flour + 20% CDE; MDEC 5%: 95% wheat flour + 5% MDE; MDEC 10%: 90% wheat flour + 10% MDE; MDEC 15%: 85% wheat flour + 15% MDE; MDEC 20%: 80% wheat flour + 20% MDE; VDEC 5%: 95% wheat flour + 5% VDE; VDEC 10%: 90% wheat flour + 10% VDE; VDEC 15%: 85% wheat flour + 15% VDE; VDEC 20%: 80% wheat flour + 20% VDE.

3.7. Sensory evaluation of cookie samples

The sensory properties of cookie samples are presented in Figure 1. Control samples had the highest color, odor, taste, appearance and general appreciation scores. However, it can be said that elderberry powder substituted samples also had acceptable levels of sensory appreciation. Color values of 5% and 10% elderberry powder substituted samples were lower than 15% and 20% substituted ones. Contrarily to color values, increased substitution ratios resulted in lower odor values. Besides the control, the best odor values were found in samples with 10% ratios for all samples regarding the drying method factor. The taste scores were found acceptable up to 15% of the substitution ratio. Similar to odor, the highest scores were found in samples that had 10% substitution among the elderberry powder used samples. The most admirable sample was the VDEC according to the drying method factor. For appearance, samples that had 15% elderberry powder for CDEC and VDEC and samples that had 20% elderberry powder for MDEC had the highest points, besides the control. Friability scores were not significantly different for overall samples. For general appreciation, the most admirable samples that had elderberry powder were as follows: sample containing 15% elderberry for CDEC, sample containing 20% elderberry for MDEC and sample containing 10% elderberry for VDEC. Briefly, among all sensory parameters, MDEC and VDEC samples were found more desirable.

4. Conclusions

Overall findings indicated that the use of elderberry powders increased the nutritional quality of cookies. The increasing amount of elderberry powder in cookie formulations decreased $L*$ and $b*$ values and increased $a*$ value.

Better color properties occurred in MDEC samples. MD and VD methods and higher substitution ratios provided better physical characteristics. The high substitution ratios affected the textural quality of products but can be evaluated to be within acceptable limits. CDEC samples had higher protein and ash contents. As the elderberry is a good source of functional components, phenolic contents and antioxidant capacity of cookie samples improved with elderberry powder substitution. Bound and total phenolic contents of VDEC samples were higher than other samples. In further studies, it is recommended to use elderberry powder in the production of other food matrices such as bread, cakes and pasta to benefit from its superior phenolic content and antioxidant capacity. Also, extending the usage of VD and MD methods in the industrial drying process is recommended due to the protective effects.

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Ethical Statement

Conflicts of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Authorship Contribution Statement

Concept: Demir, M. K.; Design: Demir, M. K.; Data Collection or Processing: Alıç, B., Olcay, N.; Statistical Analyses: Alıç, B., Olcay, N.; Literature Search: Alıç, B., Olcay, N., Demir, M. K.; Writing, Review and Editing: Olcay, N., Demir, M. K.

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