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The Production of Pestil (Fruit leather) from Different Hawthorn (*Crataegus* spp.) Fruits

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ABSTRACT

Pestil is one of the important foods traditionally produced in Türkiye, with high energy, vitamin and mineral content. It is obtained by naturally drying the pulp obtained from different kinds of fruits after mixing it with ingredients such as sugar and flour. In regions where the fruiting period depends on the season, fruit pulp is produced in order to benefit from it in winter. In addition, bioactive components originating from the fruit increase the nutritional value. In this study, the physicochemical and antioxidative properties of the pestil obtained from three different Hawthorn fruits (*Crataegus meyeri*, *Crataegus turkestenica* and *Crataegus orientalis*) known to have positive effects on health were investigated. The results revealed that, depending on the fruit type, pestil samples have high antioxidative properties (IC_{50}: 50.11±0.16-52.1±2.14 mg/mL) due to the high phenol content (39.8±0.16-52.95±1.21 mg GA/g DW). In this context, in the present study, it has been seen that the pestil prepared with the traditional method from three different Hawthorn fruits should be industrially produced as a product for people to access healthy and various foods today.

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Introduction

Since fruits can spoil when fresh, they are processed into products such as marmalade, jam, pulp and molasses in order to extend their shelf life and preserve their nutritional value longer. One of these products, fruit pulp, is a popularly consumed traditional fruit product characterized by the addition of sucrose and starch to the pulp obtained from different fruits (eg, grape, apricot, plum, mulberry) and the drying of this pulp (Suna and Özkan-Karabacak, 2019). This product is very popular in Türkiye called "pestil" also referred to by names such as fruit leather, lavashak, qamar al deen and bastegh in the different countries and regions of world (Tontul and Topuz, 2018).

Türkiye is home to many medicinal and aromatic plants due to its rich flora. In the regions where the plants grow naturally, they are used by the people directly or in the form of tea, food and spices for the treatment of both human and animal diseases. The consumption of leaves, sprouts, fruits and roots of Hawthorn species, which is one of the plants used for this purpose, is common among the people (Özderin et al., 2016). As a matter of fact, this plant is mainly used in the treatment of high blood pressure, hemorrhoids and cough, as well as heart pain, tonsillitis, kidney diseases and liver pain. It is known to be used in traditional Chinese medicine for similar purposes (Ercisli et al., 2015). Hawthorn is in the deciduous trees class of the genus *Crataegus* L. belonging to the Rosaceae family, which are usually thorny. Although it is reported worldwide Hawthorn genus of about 200 species it has been reported to be the recipient of 26 taxa in Türkiye (Dönmez, 2004). Despite this information, the fact that the fruit harvesting period is limited to the fall season restricts access to the Hawthorn fruits. In this context, it is understood that access to this fruit, which has pharmacological effects as previously mentioned, should be throughout the year. This can only be possible by transforming the fruit into a durable product (marmalade, jam, pulp, etc.).

Nowadays, consumers' interest in natural products increases gradually in terms of nutrition and health properties. This trend promotes the production of traditional products and different types of food in the food industry (Yildiz and Boyraci, 2020). In this context, pestil production potential from three different Hawthorn fruits harvested from eastern region of Türkiye was investigated. For this purpose, some physicochemical, phenolic content and antioxidant capacity of the Hawthorn fruits used as raw materials, as well as the physicochemical, antioxidant, phenolic and flavonoid contents and sensory properties of Hawthorn fruit leather (pestil) were examined.

Material and Methods

Materials

Mature Hawthorn fruits (*Crataegus* spp.) were hand harvested from eastern Türkiye (latitude 39°38′N and longitude 40°55′E; fruit ripening season September-October) (Figure 1). Approximately 1 kg fully matured fruits were randomly selected from those that do not contain any defects, transferred to the laboratory and held at 1±1°C, 90±5% relative humidity until use.

Methods

Pomological Characteristics of Hawthorn Fruit

For fruit mass, dimensions, flesh/seed ratio and color measurements, a total 20 fruits (3 replicates) were used. Height and width of Hawthorn fruits were measured with a micrometer. Total weight and seed weight of Hawthorn fruits were measured with an electronic balance of 0.01 g sensitivity. In order to determine the flesh/seed ratio, the fruits were first cut and the flesh separated from the seeds. After weighing both parts separately, the flesh/seed ratio was calculated with the following formula:

Flesh/seed ratio (%) = (Fruit weight – seed weight)/ fruit weight × 100

Dry matter content of Hawthorn fruits was analyzed according to AOAC 925.09 method (AOAC, 2005). Soluble solids content (SSC) (% Brix) analysis in slurried fruits was performed using a digital refractometer. Levels of pH were determined using standard methodology (AOAC, 2005) with a pH meter (GLP 21, Crison Instruments, SA, Barcelona, Spain). For titratable acidity, fruit samples of 10 g were exactly weighed and homogenized in 25 mL of distilled water and then titrated by 0.1 mol L^{-1} sodium hydroxide using phenolphthalein as indicator. Total acidity was expressed as malic acid equivalent (g kg^{-1}).

Production of Pestil from Hawthorn Fruit

Before production, Hawthorn fruits were thoroughly washed several times with distilled water. Then Hawthorn fruits were mixed with distilled water in a ratio of 1:1 (w/v) and crushed thoroughly with the help of a wooden spoon. It was then stirred for about 1 hour at a constant temperature of 60°C in a stainless-steel container. The mixture was then filtered through cotton cloth. The filtrate obtained was mixed with distilled water at a ratio of 1:1 (w/v) and heated at 60°C for about 30 min. This mixture was combined with the initial filtrate after filtration. The obtained filtrate was poured on cotton cloths as a thin layer, while on the other hand, they were spread by smoothing them with a trowel. These procedures were carried out for all three types of Hawthorn fruits. In the spreading process, it was paid attention to spread all three fruits in equal volumes and in equal volume areas using a stainless-steel container (530*325*150). The pulp, which was dried for 2 days at 40℃ in an oven (UL 55, Memmert 854 Schwabach, Germany) with relative humidity of 50%, was peeled off the cloth and turned into a roll and kept at +4℃ until analyzed.

Physicochemical characteristics of Hawthorn fruits and Pestil samples

Dry matter and ash content of Hawthorn fruits and pestil samples were analyzed according to AOAC 925.09 and AOAC 923.03 method, respectively. Levels of pH were determined using standard methodology (Official 2005) with a pH meter (GLP 21, Crison Instruments, SA, Barcelona, Spain). For titratable acidity, fruits and pestil samples of 10 g were exactly weighed and homogenized in 25 mL of distilled water using an Ultra-Turrax homogenizer (T25, Ika Works Inc., USA) and then titrated by 0.1 mol L^{-1} sodium hydroxide using phenolphthalein as indicator. Total acidity was expressed as % malic acid equivalent.

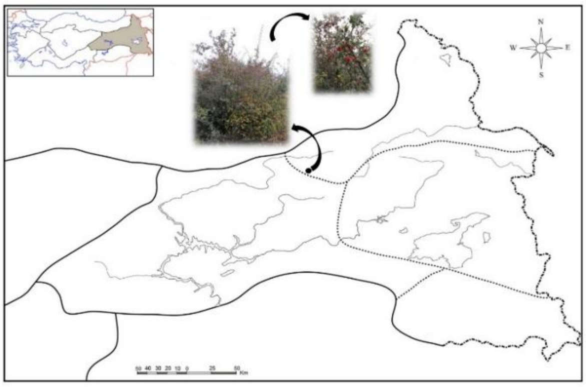

Figure 1. Study area where mature fruits were harvested from in September, 2018

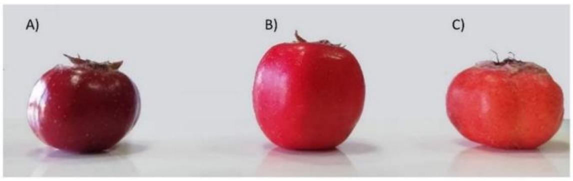

Figure 2. The Hawthorn fruits used in the study
A: *Crataegus meyeri*; B: *Crataegus turkestenica*; C: *Crataegus orientalis*

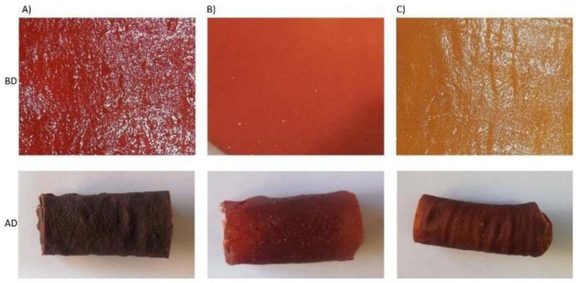

Figure 3. Hawthorn pestils before and after drying
A: Pulp and Pestil of *C. meyeri*; B: Pulp and Pestil of *C. turkestenica*; C: Pulp and Pestil of *C. orientalis*; BD: Before drying; AD: After drying

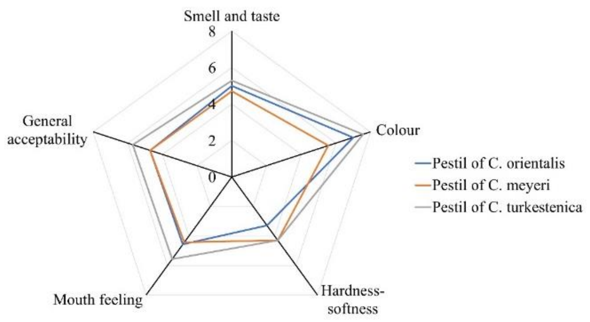

Figure 4. Radar chart representing mean scores of the sensory analyses for the freshly prepared Hawthorn fruit pestil samples

Color Analysis

Colors of Hawthorn fruit pestil were measured as L, a, and b by using a colorimeter (AR10, China) computed as means of at least three measurements from different positions and expressed as L; darkness (0, black) / lightness (100, white); –a, greenness; +a, redness; –b, blueness; and +b, yellowness.

Sensorial Analysis

The sensory properties of the pestil samples produced in this study were evaluated according to the method reported by Yildiz (2013). The panel consisted of 10 non-smoking women and 10 men aged 22 to 35 years old. Pestil samples obtained from three different Hawthorn fruits were served under white light and at room temperature in plastic plates randomly labeled with different codes. Between evaluations, water was offered to the panelists to clean the palate. The evaluation results were scored on a 9-point (1: strong dislike, 5: neither dislike nor dislike, 9: intense liking) in traditional hedonic scale. In this context, it was requested to evaluate in terms of 5 different criteria (Smell and taste; Color; Hardness-softness; Mouth feeling; General acceptability).

Phenolic Content and Antioxidant Capacity Analysis

Determination of Total Phenolic Content

For obtaining the water extracts, a 25 g pestil sample was homogenized into 500 ml boiling water by Ultra-Turrax homogenizer (T25, Ika Works Inc., USA), and filtered through Whatman No. 1 paper. The amount of total phenolic components in water extracts obtained from pestils was determined according to the Folin-Ciocalteau method. Briefly; after 0.5 mL of sample was added to 1 mL of Folin-Ciocalteau reagent medium and mixed, 0.5 mL of 1% sodium carbonate was added to the reaction medium and the medium was neutralized. It was then incubated at room temperature for 2 hours and will be read against a blank of distilled water at 760 nm. Phenolic contents were calculated as mg^{-} gallic acid equivalents for the water extracts.

DPPH$^{\bullet}$ Free Radical Scavenging Capacity

The DPPH$^{\bullet}$ free radical scavenging capacity of water extract samples from pestils was performed according to a modification of the Blois method (Blois, 1958). 1 mM DPPH$^{\bullet}$ solution was used as free radical. Samples with different concentrations of water extracts (15, 30 and 60 µg/mL) were transferred to the test tubes, respectively, and their total volumes were made up to 3 mL with ethanol. Then, 1 mL of DPPH$^{\bullet}$ solution was added to each sample and after incubation for 30 minutes at room temperature in the dark, the absorbance changes at 517 nm were measured against the blank consisting of ethanol. As a control sample, 3 mL of ethanol and 1 mL of DPPH$^{\bullet}$ solution were used. The decreased absorbance indicates the remaining amount of DPPH$^{\bullet}$ solution, that is, the free radical scavenging capacity (Celik, Kucukoglu, et al., 2014; Celik, Nadaroglu, et al., 2014). Calculations for DPPH$^{\bullet}$ free radical was made according to the following equation:

$$\text{DPPH}^{\bullet}\ \text{radical scavenging capacity (\%)} = \left(1-\frac{A_{Sample}}{A_{Control}}\right)\times 100$$

In this equation, A_{Sample} represents the absorbance value found after adding the sample to the DPPH$^{\bullet}$ radical solution, and $A_{Control}$ represents the absorbance value of the control sample containing only DPPH$^{\bullet}$ radical solution. BHT was used as positive controls.

Superoxide Anion Radical Scavenging Activity

Superoxide radicals were produced with a minor modification of the method described by Zhishen et al. (1999). All solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The reaction mixtures containing Hawthorn aqueous extracts (15-60 µg mL-1) prepared at different concentrations were incubated under a fluorescent lamp (20 W) for 40 minutes at 25 ºC. Thus, photo-induced reactions were initiated. Absorbance changes were measured at 560 nm and BHT was used as a standard.

$$\text{Superoxide anion radical scavenging activity (\%)} = \left(1-\frac{A_{Sample}}{A_{Control}}\right)\times 100$$

Statistical Analysis

Statistical analysis of the data obtained in the study was done using SPSS (SPSS Inc., Chicago, IL, USA) program. Descriptive statistics including mean and standard deviation were calculated. Homogeneity test was applied to all data and one-way analysis of variance (ANOVA) with post hoc Tukey honestly significant difference (HSD) test was applied for those with normal distribution, and Dunnett T3 test was applied for those with non-normal distribution. All analyses were done in technical and experimental triplicate.

Results and Discussion

Three different Hawthorn fruits harvested from eastern Türkiye were identified as *Crataegus meyeri* (*C. meyeri*), *Crataegus turkestenica* (*C. turkestenica*) and *Crataegus orientalis* (*C. orientalis*) according to morphological characteristics (skin color, thorn shape, aril color, aril thickness etc.) (Figure 2). In this context, some pomological and chemical characteristics were examined for all three species. As seen in Table 1, there were statistically significant differences between the species in terms of some morphological and chemical properties.

Fruit size and weight are accepted as an important quality criterion in Hawthorn as it affects consumer preferences. Weight (g), width (mm) and height (mm) of all Hawthorn types tested in this study ranged from 1.62 to 3.47 g, 14.22 to 20.22 mm and 12.09 to 19.15 mm, respectively. Previously, Gundogdu et al. (2014) reported Hawthorn fruit weight, width and height from Erzincan province, Türkiye ranged from 0.58 to 3.48 g, 1.44 to 17.68 mm and 1.29 to 15.72 mm, respectively. Mean values of fruit weight, width and length in different genotypes of Hawthorn fruit from Malatya province, Türkiye were ranged from 0.65 to 4.19 g, 9.88 to 20.39 mm and 10.06 to 18.07 mm, respectively, while the respective values were 0.98 to 6.76 g, 10.27 to 24.96 mm, and 8.27 to 19.56 mm for the Hawthorn fruits grown in Akçadağ-Hekimhan district of the same province (Bektaş et al., 2017). As seen in the Table 1, a statistical difference was found between the types of hawthorn fruits used in this study, in terms of weight and height, except for width. The data of fruit flesh ratio, another important quality criterion in Hawthorn fruits, are given in Table 1. The fruit flesh / seed ratio for three different Hawthorn fruits ranged from 56.00 to 81.56%. Ağlar et al. (2020) reported that Hawthorn genotypes were between 72.53 and 86.31% for flesh / seed ratio. In another study, in Hakkari (Şemdinli) region, Yavıc et al. (2016) reported that fruit flesh / seed ratio is between 77.84 and 85.99%. One of the most important fruit characteristics desired in Hawthorn breeding programs is high fruit weight and meat ratio (Ercisli, 2004).

Biochemical properties that affect fruit quality such as SSC, pH and acidity can also affect consumer preferences. SSC and acidity levels, which are used as criteria in determining the maturity level of fruits, are ideal for consumption during the maturity period (Ağlar et al., 2020). In terms of SSC, *C. turkestenica* had the lowest SSC content (20.62%) while *C. orientalis* and *C. meyeri* had the highest amount (22.26 and 22.28%, respectively) (Table 1). Again, acidity levels were similar in *C. orientalis* and *C. meyeri* (0.74 and 0.84%, respectively). In this study, the lowest pH value was determined in *C. turkestenica* and the highest pH value in *C. meyeri*. The average pH value determined for all three species was found to be statistically different from each other. The acidity value of the Hawthorn fruits was between 0.7 and 1.15%. Results obtained from studies conducted in different regions of Türkiye reveal that values such as SSC, acidity and pH may be variable. Indeed, Ercisli et al. (2015) reported the SSC ratio between 6.71 and 14.85% and the pH value between 2.88 and 3.65 in the Hawthorn fruits from the Malatya region, Türkiye. In another study, Gundogdu et al. (2014) stated that the SSC ratio was between 3.05 and 20.00%, the pH was between 4.22 and 5.99, and the acidity was between 0.22 and 2.40% in Hawthorn fruits from Erzincan region, Türkiye. Ağlar et al. (2020) reported that SSC ratio, acidity and pH of Hawthorn in growing naturally in Suşehri, Türkiye was between 14.80-21.8%, 0.38-1.58%, and 4.10-5.22, respectively.

As known, moisture content is very important in determining the physicochemical properties of Hawthorn fruit such as fruit density, bulk density, pulp mass, porosity, static and dynamic friction coefficient. In the dry matter analysis carried out in this context, it was determined that the dry matter content in Hawthorn fruits was between 23.02 and 41.96%. On the other hand, the highest dry matter content was determined in *C. meyeri* (38.77%). Yalçın Dokumacı et al. (2021) reported the average moisture content of *C. monogyna* type Hawthorn fruits as 68.98 ± 2.20, consistent with our study. It is observed that the physical and chemical qualities of Hawthorn fruits used in the current study differ from the results obtained in different studies. Studies have shown that such properties of fruits are affected by climate, soil structure, harvest time and genetic factors (Ercisli et al., 2015; Gundogdu et al., 2014). Considering all these results, the different results determined can be attributed to the variability of the Hawthorn species used in the studies and other environmental factors. Also, results demonstrated that origin of species had significant effects regarding some physicochemical characteristics (pH, titratable acidity, SSC) of Hawthorn fruits. Considering all the results, the physicochemical properties of Hawthorn fruits changed depending on the species.

Table 1. Pomological characteristics and soluble solid content (SSC), pH, acidity and dry weight of Hawthorn species

Parameter	*C. meyeri*	*C. turkestenica*	*C. orientalis*
Fruit weight (g)	1.75±0.1^{a}	3.28±0.16^{b}	2.49±0.16^{c}
Fruit width (mm)	15.28±0.77^{a}	18.4±0.74^{b}	18.24±0.66^{b}
Fruit height (mm)	12.3±0.57^{a}	17.6±0.63^{b}	16.13±1.58^{c}
Seed weight (g)	0.34±0.09^{a}	0.64±0.14^{b}	0.61±0.34^{b}
Seed number	1.6±0.55^{a}	2.8±0.84^{b}	4.8±0.45^{c}
Fruit flesh ratio	68.2±9.53^{ab}	76.4±3.15^{a}	69.71±1.83^{b}
Fruit SSC (%)	22.26±0.25^{a}	20.62±0.23^{b}	22.28±0.09^{a}
Fruit pH	4.11±0.02^{a}	3.9±0^{b}	4.08±0.03^{c}
Fruit acidity (% malic acid)	0.74±0.05^{a}	1.07±0.08^{b}	0.84±0.03^{a}
Fruit dry weight (%)	38.77±2.79^{a}	31.49±0.69^{b}	25.82±2.55^{c}

*Each value is expressed as means±standard deviation (n:3) (P<0.05). Means within the same row (a, b, c) with different letters are significantly different (P<0.05).

Table 2. Some physicochemical properties of Hawthorn fruit pestil

Parameter	Pestil of *C. orientalis*	Pestil of *C. meyeri*	Pestil of *C. turkestenica*
Moisture (g. 100 g^{-1})	7.54±2.27a	3.39±0.61b	4.75±1.87ab
Ash (%)	2.23±0.15a	1.57±0.04b	0.99±0.02c
pH	3.43±0.12	3.34±0.15	3.38±0.04
Acidity (% malic acid)	1.08±0.02a	1.33±0.02b	1.29±0.06b

*Each value is expressed as means±standard deviation (n:3) (P<0.05). Means within the same row (a, b, c) with different letters are significantly different (P<0.05).

Table 3. CIE-*L*a*b** color parameters of Hawthorn fruit pestil samples

Samples	*L**	*a**	*b**
Pestil of *C. orientalis*	22.33±1.58[a]	83.20±4.73[a]	12.59±2.52[a]
Pestil of *C. meyeri*	20.58±0.86[a]	77.41±6.61[a]	8.15±0.50[a]
Pestil of *C. turkestenica*	48.00±3.21[b]	19.87±12.05[b]	38.95±5.16[b]

*Each value is expressed as means±standard deviation (n:3) (P<0.05). Means within the same column (a, b, c) with different letters are significantly different (P<0.05).

Table 4. Total phenolic content, antioxidant activity and antioxidant capacity of Hawthorn fruit pestil samples

Samples	Total phenolic content (mg GA/g DW)	DPPH scavenging activities (mg/mL) (IC_{50})	SARS (mg/mL) (IC_{50})
C. orientalis	48.3±1.15a	52.95±1.21a	45.30±1.21a
C. meyeri	39.8±0.16b	50.11±0.16ab	42.35±1.50a
C. turkestenica	52.1±2.14c	51.87±1.16ab	52.35±2.32b
BHT	-	48.95±0.14b	21.15±1.33c

*Each value is expressed as means±standard deviation (n:3) (P<0.05). SARS: Superoxide anion radical scavenging activity

In this study, the usability, acceptability and some physicochemical and biochemical properties of Hawthorn fruit as a raw material in the production of pestil which is traditionally produced widely in Türkiye and is very popular were investigated. By applying the traditional production procedure, pestil was produced from the pulp of the Hawthorn fruits without adding any additives (Figure 3). The problem of seasonally limited consumption of Hawthorn fruit, which has been shown to have many important positive effects on health in previous studies, can be overcome by transforming it into longer-lasting products such as fruit leather. In traditional fruit pestil production, products such as flour and starch are added as hydrocolloid substances to thicken the pestil obtained from different fruits (Tontul and Topuz, 2018). However, interestingly, in this study, it was determined that Hawthorn fruit pestil spontaneously polymerized and gained consistency without the need for such materials. The reason for this is that the Hawthorn fruit contains procyanidins (Cui et al., 2006) and pectin (Lozano-Grande et al., 2016), which have a high degree of polymerization, as previously reported. As is known, ingredients such as flour and sugar appear to be important driving force of metabolic dysfunction, overweight and inflammation in individuals. It is also thought to have a profound effect on cardiovascular disease or may even be initiators (Rubio-Ruiz et al., 2015). In our current study, it is seen that a healthier fruit leather is produced that does not contain these nutrients.

As can be seen in Table 2, some physicochemical properties of pestil obtained from different Hawthorn fruits are given. The moisture content of Hawthorn fruit pestil was determined between 2.70 and 9.62%. The moisture content for different pestil and fruit leathers reported between 2.83 and 21.99% (Anju et al., 2014; Cagindi and Otles, 2005; Huang and Hsieh, 2005; Offia-Olua and Ekwunife, 2015; Tontul and Topuz, 2018). Moisture content in pestil obtained from three different types of Hawthorn fruit varies relatively from each other. However, all Hawthorn fruit pestil samples had low-moisture food system owing to the relative low water content. The moisture content of all pestil samples was lower than 15 g/100 g, which is known the critical limit for microbial growth and other deteriorative reactions such as lipid oxidation, non-enzymatic browning, and crystallization (Suna, Tamer et al. 2014). The ash content of Hawthorn fruit pestil was determined between 0.97 and 2.40%. The ash content for different pestil reported between 0.66 (Yildiz, 2013) and 2.32 (Şengül et al., 2020). Also, for Hawthorn fruit reported between 2.28% (Özcan et al., 2005) and 2.41% (Lou et al., 2020). As can be seen in Table 2, ash content of pestil samples obtained from three different Hawthorn fruits was statistically significant ($p <0.05$). The observed pH difference in the initial raw material was not detected in the pestil samples obtained from Hawthorn fruits. In addition, the pH of the pestil samples has decreased in comparison with the pH of the raw materials. Also, as expected, the pH and the acidity level changed inversely. The total titratable acidity showed that all the samples were significantly different ($p<0.05$) from one another with sample of *C. orientalis* pestil. Considering all the results, there is variability in the content of these physicochemical properties analyzed among the pestil samples. This situation can be explained by the difference in the content of the Hawthorn fruits used.

Since it gives information about the production conditions (especially heat treatment) and the content of the food, the color in the appearance of the food is a very important parameter. The boiling process performed during fruit leather production causes browning reactions. This has a major effect on the increase in water-soluble diglycosides and thus on the color formation of the pestil (Maskan et al., 2002). As seen in Table 3, it shows that the pestil obtained from other Hawthorn fruits, except for the samples of *C. turkestenica* pestil, are statistically in the same group (b) in terms of all color values ($p <0.05$). While the darkest colored products with the lowest L value at 20.58 are *C. meyeri* pestil, the lightest colored products are *C. turkestenica* pestil with a higher L value of 48.00. The "a value" indicative of redness was positive for all samples. This value was measured as 11.06 in *C. turkestenica* pestil. Considering the consumer taste, it is stated that since it is thought to be related to the scope of the heat treatment applied in the production of pestil and the caramelization that occurs as a result, it is not desired that the a* color value is too high and that the pulp with a lower a value is preferred (Maskan et al., 2002; Yildiz, 2013). Again, positive b values between 7.57 and 42.74 indicating the presence of yellowish color were measured. This value was measured as 38.95 in *C. turkestenica* pestil.

Sensory properties are among the most important properties of food products. Sensory qualities can be demonstrated by determining the structural, mechanical and surface properties of foods using vision, hearing, touch and kinesthetic observation tools (Szczesniak, 2002). As can be seen in Figure 4, significant differences were found in pestil obtained from different Hawthorn fruits in this study. When all the sensory parameters were examined, it was found that the highest score was clearly on *C. turkestenica* pestil. On the other hand, although the panelists were asked to score out of 10, the general acceptability of the products remained below 5. However, it has been reported that this value is between 6.25-7.66 in samples of pestil produced traditionally from different fruits (Özkan Karabacak, 2019; Suna and Özkan-Karabacak, 2019; Yildiz, 2013). In this study, it is thought that moral absolutism has an important role as an obstacle in the acceptance of the pestil obtained from a different fruit. Questioning the habits of individuals regarding their food consumption is seen as an important barrier for the production of new products.

It is known that reactive oxygen species (ROS) (superoxide anion (O^{2-}) radicals, hydroxyl radicals (OH•) and hydrogen peroxide (H_2O_2)) produced in the organism can cause different diseases such as cancer, atherosclerosis, diabetes) if they exceed the level that the metabolism can tolerate. Many phytochemicals have been determined to inactivate excessively produced ROS, which are called antioxidants. Studies reveal the free radical scavenging potential and antioxidant properties of polyphenols found in fruits and vegetables. These substances that contribute to color and sensory properties such as bitterness and astringency in plants, it has been assumed that it is an important nutritional component known to have health-promoting effects. In this context, it is clear that these components, which are indispensable for human life, should be taken regularly from fruits and vegetables. In this study, the phenolic content and its antioxidant capacity have been investigated. Table 4 shows the phenolic contents and antioxidative properties of fruit pestil samples.

It was determined that DPPH and superoxide anion radical scavenging activities of Hawthorn fruit pestil showed stronger activity compared to BHT. It is widely used to determine the radical scavenging capacity of compounds whose activity is determined by DPPH radical scavenging activity assay (Nadaroglu et al. 2007; Nadaroglu et al. 2009). For pestil of *C. orientalis*, *C. meyeri* and *C. turkestenica*, the required sample concentration to reduce the initial concentration of DPPH by 50% (IC_{50}) was determined. A lower value of IC_{50} indicates a higher rate of antioxidant capacity. Among the samples, the best free radical scavenging activity was achieved in the order *C. orientalis* > *C. turkestenica* > *C. meyeri* > BHT. From the results obtained, it was determined that pestil of *C. orientalis*, with a rate of IC_{50}: 52.95±1.21 mg/mL, showed stronger DPPH radical scavenging activity with a higher inhibition percentage *in vitro*. Superoxide anions are converted to oxygen and hydrogen peroxide by superoxide dismutase or react with nitric oxide to form peroxynitrite. In this context, the superoxide scavenging capacity in the metabolism is very important as the first stage of protection against oxidative stress. Superoxide scavenging activity was performed in all Hawthorn pestil samples and the results are given in Table 4. *C. turkestenica* > *C. orientalis* > *C. meyeri* > BHT sequence showed the best superoxide scavenging activity. The ranking obtained from the color development resulting from the reaction of superoxide in the reaction medium with NBT was IC_{50}: 52.35±2.32%> 45.3±1.21%> 42.35±1.50%> 21.15±1.33 mg/mL. It was determined that the pestil samples showed higher superoxide anion radical scavenging activities compared to the standard control BHT. Alirezalu et al. (2020) reported that the highest antioxidant capacity in the fruits of *C. pentagyna* (1.84 ± 0.21 mmol Fe^{++}/g DW). On the other hand, Çaliskan et al. (2012) detected that the *C. monogyna* subsp. *azarella* had the highest antioxidant capacity (81.9%). The reported results were relatively higher than the values we found. It was thought that this was due to the heat treatment applied during the production of the pestil. It was reported that the total phenolic compounds are the main responsible compounds for the antioxidant capacity of Hawthorn fruits (Alirezalu et al., 2020).

Accordingly, the phenolic contents of the pestil samples obtained from *C. orientalis*, *C. meyeri* and *C. turkestenica* were found to be 48.3±1.15 mg GA g^{-1}, 39.8±0.16 mg GA g^{-1} ve 52.1±2.14 mg GA g^{-1}, respectively. Alirezalu et al. (2020) reported that 40.04 mg GA g^{-1}, 58.17 mg GA g^{-1} and 21.19 mg GA g^{-1}, respectively, for the same spices of Hawthorn fruits. On the other hand, Yılmaz et al. (2017) also reported the total phenolic content in the pomegranate pestil was 7.8–8.6 g GAE/kg. Özkan Karabacak (2019) determined the content of total phenolics was 150–152 mg GAE/100 g db in blackthorn pestil.

In a study, it was suggested that the most detected phenolic components in Hawthorn fruits (*C. monogyna* subsp. *monogyna* Jocq, *C. atrosanguinea* Pojark, *C. orientalis* var. *orientalis* Pallasex. Bieb and *C. meyeri* Pojark.) were rutin, catechin and caffeic acid (Muradoğlu et al., 2019). When the studies on the total phenolic content, phenolic components and antioxidant capacity of the same type of fruit are examined, it has seen that different results are recorded depending on many factors such as genetic variations between species, season in which the fruit is harvested, ripening, and climatic conditions in which it is grown. As a matter of fact, it has been demonstrated by many studies (Chang et al., 2013; Liu et al., 2011; Mraihi et al., 2015) that different hawthorn fruits may have different levels of bioactive components depending on localization.

Conclusion and Recommendations

The current study shows that pestil obtained from different Hawthorn fruits are extremely rich food sources, especially in terms of phenolics. The positive effects of Hawthorn fruits on the mentioned health have brought a different perspective to the traditionally produced pestil. It is recommended as an alternative to supplementary snack foods especially for children and adults with circulatory system disorders. This kind of pestil, which we have produced as a result of our research, can also be used as food sources and complementary food, as it contains functional compounds. However, from a sensory point of view, it has been observed that this product, which consumers have never tasted of a pestil produced from different Hawthorn fruits, is an important barrier towards the acceptability. Therefore, researching the elements that can contribute more to the acceptance of the product is among the important outputs of this study. The results obtained in this study can be used in the development of traditional foods.

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