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Kozalak ekstraktı pekmezlerinin bazı fizikokimyasal ve biyoaktif özelliklerinin gıda güvenliği ve kalite açısından değerlendirilmesi

Evaluation of some physiochemical and bioactive properties of the cone extract molasses in terms of food safety and quality

Emine İNCEMEHMETOĞLU[1], Müge HENDEK ERTOP[2]*

[1]Kastamonu Universitiy, Graduate School of Natural and Applied Sciences, Department of Sustainable Agriculture and Natural Resources, Kastamonu, Turkey.
incemehmetoglu@tarimorman.gov.tr
[2]Kastamonu Universitiy, Faculty of Engineering and Architecture, Department of Food Engineering, Kastamonu, Turkey.
mugeertop@kastamonu.edu.tr

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Öz

Bu çalışmada, Karaçam (Pinus nigra), Göknar (Abies nordmanniana) ve Sarıçam (Pinus sylvestris) ağaçlarından elde edilen kozalak pekmezinin bazı fizikokimyasal ve biyoaktif özelliklerinin belirlenmesi, kalite standardizasyonu ve gıda güvenliği açısından değerlendirilmesi amaçlanmıştır. Örneklerin pH değerleri 3.47 ile 4.75 arasında, toplam asitlik değerleri ise %1.28 ile %2.56 arasında değişim göstermiştir. Viskozite 150.42 ile 11164.67 cP arasında, °Brix değerleri %64.63 ile %78.68 arasında ve elektriksel iletkenlik sırasıyla 376.67 ile 944.67 (µS/cm) arasında bulunmuştur. Kozalak pekmezlerindeki kül içeriği %0.24 ile %0.96 arasındadır. Tüm pekmez örnekleri mineral açısından özellikle K (208.0-4146.9 mg/kg) ve Ca (27.5-1580.0 mg/kg), başta olmak üzere P, Mg ve Na açısından zengin iken Zn, Fe ve Mn düzeyleri ise en düşük seviyede bulunmuştur. Renk değerleri L için 0.41 ile 29.,04, a* için 6.54 ile 44.11, b* için 3.16 ile 36.45 arasında belirlenmiştir. Pekmez örneklerinin şeker profilleri oldukça değişken bulunmuştur. Fruktoz içeriği %5.92-30.23, glukoz içeriği %12.03-36.48, invert şeker miktarı %17.95-66.71, sakaroz içeriği %4.67-45.32, toplam şeker miktarı %49.31-75.80 olarak tespit edilmiştir. Toplam fenolik içerikleri 875.00 ile 2750.00 µg GAE/g arasında, antioksidan aktiviteleri %44.06 ile 89.77 arasında, Hidroksimetilfurfural (HMF) içerikleri ise 4.54-1101.58 mg/kg aralığında olup geniş bir aralıkta değişmiştir. Elde edilen sonuçlar bu ürünün biyoaktif niteliklerine ilişkin fonksiyonelliğini değerli kılsa da, kozalak pekmezi üretiminde özellikle geleneksel yöntemlere bağlı yüksek sıcaklık, uzun işlem süresi ve şeker içeriği açısından standardizasyonun eksik olması temel kalite parametrelerinde önemli değişikliklere neden olmuştur. Pekmez olarak isimlendirilmekle birlikte, yönetmelikler açısından belirli bir ürün grubuna ait olmadığından kalite limitlerine göre karşılaştırılamamaktadır. Ancak bu çalışma kapsamında elde edilen sonuçlar mevcut regülasyonlara göre değerlendirilmiştir. Bağımsız reçetelerle ve konvansiyonel yöntemlerle üretilen kozalak melasının üretim tekniklerinin geliştirilmesi ve kalite kriterleri açısından sürdürülebilir standardizasyonun sağlanması gerekmektedir.*

Anahtar kelimeler: Kozalak pekmezi; Kozalak ekstraktı; Çam kozalağı; Kozalak şurubu; Gıda Güvenliği

Abstract

This study aimed to determine some physicochemical and bioactive properties of the cone molasses, which was origin Black Pine (Pinus nigra), Fir (Abies nordmanniana) and Scotch Pine (Pinus sylvestris) trees, to evaluate them in terms of quality standardization and food safety. pH values varied from 3.47 to 4.75. Total acidity changed between 1.28 and 2.56%. The viscosity varied from 150.42 to 11164.67 cP, °Brix values varied from 64.63 to 78.68%, and electrical conductivity varied from 376.67 to 944.67 (µS/cm), respectively.The ash contents of cone molasses ranged from 0.24 to 0.96%. Regarding the minerals, all the molasses samples was rich in especially K (208.0-4146.9 mg/kg) and Ca (27.5-1580.0 mg/kg) and also P, Mg, and Na, while Zn, Fe, and Mn levels were the lowest.. The color values were found between 0.41 and 29.04 for L, 6.54 and 44.11 for a*, 3.16 and 36.45 for b*. The sugar contents of cone molasses samples ranged remarkably. The fructose level was between 5.92-30.23%, glucose level was 12.03-36.48%, saccharose content was 4.67-45.32%, total sugar amount was 49.31-75.80%, invert sugar amount was 17.95-66.71%. Total phenolic contents varied from 875.00 to 2750.00 at µg GAE/g level, antioxidant activities from 44.06 to 89.77%, and the hydroxymethylfurfural (HMF) contents in the wide range of 4.54-1101.58 mg/kg. Although the results obtained makes this product's functionality related to the qualities of bioactive valuable, the fact that lack of the standardization, especially in terms of high temperature, long process time, and sugar content in the production of cone molasses caused significant changes in the quality parameters. Considering that it does not belong to a specific product group regarding regulations, it is not compared according to the quality limits. However, the results obtained within the scope of this study were evaluated according to current regulations.To develop the production techniques of cone molasses produced with independent recipes and conventional methods and to provide sustainable standardization in terms of quality propeties, it is necessary.*

Keywords: Cone molasses; Cone extract; Pine cone; Cone syrup; Food safety

1 Introduction

The nutrition concept is developing day by day, food products are not only consumed for their nutritional values but also provide positive effects on health thanks to the different functional compounds. A functional food must demonstrate its effects in amounts that can typically be expected to be consumed in the diet as it is part of the regular food pattern (1).

The use of fractions such as pine crust, pine needle, and pinecones to develop functional food, food supplement, or natural food additives is also an exciting subject and there are limited study on it. The data acquired as a result of the studies

*Corresponding author/Yazışılan Yazar

done about particular pine types (2)-(3)-(4)-(5)-(6)-(7) shows that the waste assessment in this field can obtain new value-added foods to remove chronic food deficiency which affects more than two billion people across the world (8).

There are few studies on using these fractions, such as extract, powder, and capsules, as a food additive and supplement (9). The leaves, pine cones, needles and resins of the plants that belong to the *Pineacea* family have healing properties, and these are used in the treatment of various illnesses such as stomach disorders, asthma, and cough (10). The various parts of the pines are commonly consumed in Eastern Asian countries as food and food supplements to assist health (11). The herbology books compiling information related to the healing properties of the local food gives place to the pine cones of other types of pine, primarily *P. koraiensis* (6). Pine cones are not estimated as toxic in medicine. They are used to moisturize the lungs, prevent cough, to reduce fever. In addition to this, extended studies show that the pine cone obtained from *P. koraiensis* has various bioactive matters such as phenolic compounds, polysaccharides, and flavonoids having anti-tumor, anti-inflammatory, anti-bacterial, and antioxidant activities (5)-(12). It is reported that the extracts obtained from pine cones have a sweeping effect on the reactive oxygen and reduce serum lipids (13).

It is also known that pine cone is used in different forms especially extracts as traditional medicine and a food supplement in the Aegean and Black Sea region, particularly in Kastamonu, where pine forests are dense. It was reported that the cone extract obtained by decoction in water was generally used and more effective (14)-(15). "Pine cone syrup" was defined as young pine cones (*Pinus sylvestris*), which are boiled down with drinking water, sugar, and optionally citric acid, as well as the molasses produced by the cooking of the pine cones, by European Parliament and of the Council (2015). It falls under the category of "The food produced from plants or their parts". Although the term 'molasses' is also used for 'cone molasses' in Anatolia, it is different from the other fruit molasses in terms of the use of pine cones as raw material instead of fruits such as grape, mulberry, and the production stages. Though the pine cone and its shoots used in the production of cone molasses are not fruits directly consumed, it has been reported that the cone fraction of some trees (such as *Cupressus sempervirens* (cypress), *Juniperus communis* (common juniper) *and Juniperus sabina* (black juniper)) has been accepted as "fruits" of them, according to the 'Turkish Food Codex Usable Herbs and Herbal Preparate in Foods Communique' (16). Therefore, the cones and shoots of trees such as Black Pine and Fir Trees could also be accepted as their fruits. Unlike the production of fruit molasses, some process steps are not implemented for cone molasses, such as neutralization of acidity and clarification with molasses soil. Even though the sweetness and sugar content in other fruit molasses comes from the fruit, sugar must be added to give sweetness and consistency to cone molasses. Because of this, it is different from other molasses concerning content and product qualities. There is no regulation about the quality standards of the product.

This study aims to review some physicochemical and bioactive qualities of cone molasses, commonly produced domestically and on a micro-scale, used as a functional food and food supplementary, concerning quality standards and food security. In order to serve as a safe food for this product whose sustainability, protection, and promotion were applied for geographical indication, the determination of ultimate quality parameters was focused. The data from this study are of considerable value for the creation of the communiqué of the product. There was no regulation to evaluate the data obtained from the study. The studies conducted on the cone and its extracts in literature and the regulations about fruit molasses and scientific data were used for the interpretation of the analysis results (17)-(18)-(19)-(20).

2 Material and methods

2.1. Material

The sugar (sucrose, glucose and fructose) and HMF (≥ 99%) standard materials were supplied from AFG Bioscience LLC (Northbrook, USA), Merck (Darmstadt, Germany) and Sigma Aldrich (Saint-Louis, MO, USA). Acetonitrile (HPLC grade), methanol and all the other reagents (analytical purity) were supplied from Sigma Aldrich and Merck. The types of cone molasses samples used in the study and their supply locations were given in Table 1. These locations were preferred due to where the cone molasses production was high. The samples were produced from the cones of the conifers found in the forest flora in the period of May-June 2020. The samples were taken directly from the manufacturer.

Table 1. Types of cone molasses and their supply locations.

Sample	Type	Location (district/village-quarter)
S1	Black pine cone molasses	Azdavay/ Bakırcı village
S2	Black pine cone molasses	Azdavay/ Ahat village
S3	Black pine cone molasses	Azdavay/ Evlek village
S4	Black pine cone molasses	Azdavay/Çocukören village
S5	Black pine cone molasses	Azdavay/ Yumacık village
S6	Black pine cone molasses	Azdavay/ Başören village
S7	Black pine cone molasses	Azdavay/ Tas village
S8	Black pine cone molasses	Azdavay/Sabuncular village
S9	Fir cone molasses	Azdavay/Sabuncular village
S10	Black pine cone molasses	Azdavay/ Center
S11	Fir cone molasses	Azdavay/ Sökü village
S12	Black pine cone molasses	Merkez/ Kırışoğlu village
S13	Scotch cone pine molasses	Şenpazar/Kahraman quarter
S14	Black pine cone molasses	Daday/ Çölmekciler village
S15	Black pine cone molasses	Pınarbaşı/Center

The traditional production methods of cone molasses involve quite similar stages despite the individual modality of the different manufacturers. The cone molasses samples in this research were produced according to the main production stages reported by Incemehmetoğlu (21). To produce cone molasses, fresh cones are cleaned, washed, and separated into 2–3 pieces if necessary and boiled under open heating conditions (30 min) (1st boiling). Generally, 1 part water is used to 1 part cone mass. The first boiling water is discarded, and the cones are separated. Then the 2nd stage water is added to cones and passed to the main cooking stage (2-3 h). When the cones gain a soft texture, and the extract reaches to red color, the extract is filtered and then re-boil. Because the extract has a very intense sour and robust taste in this stage, sugar is generally added in this stage (at the rate of 2/3 of molasses mass) to provide a consumable taste and consistency. After adding sugar, the molasses continued to boil to become concentrated. When the desired molasses consistency is achieved, it is taken from the heating device, filled into glass jars, and packaged.

2.2. Methods

The analyzes performed in the study were given below, the analyzes were performed in at least two parallel and two replications and the results were given as mean±standard deviation. All analyzes were carried out at Kastamonu University Central Research Laboratory.

2.2.1. Physicochemical properties

°Brix values of molasses samples were determined by Abbe refractometer at 20°C, and the results were given as °Brix. For titration acidity, 10 grams of sample were taken, mixed with 90 mL of water and homogenized, and titrated with 0.1 N NaOH in the presence of phenolphthalein indicator. pH values were measured with a digital pH meter (Heidolph, Germany). The electrical conductivity of molasses samples was determined using a 20% solution of each sample and a conductivity measuring device (22). Deionized ultrapure water with a conductivity of 0.05 µS/cm was used for dilution and the results of the measurements made at 20°C were given as µS/cm (23). The colors of the molasses samples were measured colorimetrically and the values were given as L* (lightness), a* (redness), and b* (yellowness). The viscosities of the samples were determined by a rotational viscometer (Fungilab, Spain) and reading at 50 rpm using Spindle No 6 (23).

2.2.2. Ash content

The ash content of the samples was determined by burning 1 g sample in an ash furnace for 8 hours and 600°C overnight. They were kept in the desiccator for about 1 hour until they reached constant weight, weighed and the ash percentages were determined according to the initial weight.

2.2.3. Protein content

To determine the total protein content of the samples; Kjeldahl method was used (24). The digestion was performed by adding catalyst tablets and H_2SO_4 (98%). Then the neutralization of the digested solution with NaOH and distillation steps were carried out. The distillation vapor was trapped in H_3BO_4. At the final stage of the titration was carried out with 0.1 M of HCl until pH 4.6. To calculate the crude protein content, the nitrogen-to-protein conversion factor (6.25) was used.

2.2.4. Hydroxy Methyl Furfural (HMF) content

A High-Performance Liquid Chromatography (HPLC) device (Shimadzu, LC-20A Prominence, Japan) equipped with a Diode Array Detector was used for the HMF analyses. The analysis conditions for HPLC were presented in Table 2.

Table 2. Characterization of sugar profile. Types of cone molasses and their supply locations.

	HMF Analysis	Sugar composition analysis
Deaerator	DGU/20/A/5R Prominence	DGU/20/A/5R Prominence
Pump	LC/20 AT Prominence	LC/20 AT Prominence
Control unit	CBM-20A Prominence	CBM-20A Prominence
Auto-sampler	SIL- 20AC HT	SIL- 20AC HT
Colon oven	CTO- 10AS VP	CTO- 10AS VP
Colon	Inertsil/ ODS-3 Reverse Phase (5 µm-125*4.6 mm)	CarboSep/CHO/682
Flow rate	1.3 mL/ min	0.4 mL/min
Mobile Phase A	(90:10) / (Water: Methanol)	Ultrapure water
Solvent	Ultrapure water	Ultrapure water

The standard HMF solutions in the ranges of 0.8, 4.0, 8.0, 12.0, 20.0, 40.0, and 80.0 mg/kg were used to construct a calibration curve. Also, the sugar concentrations were calculated through the calibration graphs by the chromatograms obtained from the HPLC analysis.

The etraction of the samples to chromatographic analyses: A homogenized sample of 2.5 g was dissolved with 25 mL of water. The flask content was diluted to 50 mL with water. Then. it filtered through a 0.45 µm membrane filter.

The quantities of sugars were determined using a HPLC device equipped with Refractive Index (RI) detector. The analysis conditions were given in Table 2. The sugar concentrations were calculated through the calibration graphs by the chromatograms obtained as a result of the HPLC analysis. The standard solutions of sugars (purity ≥ %99.0) in 15-80% ranges were prepared for the calibration curve. All samples filtered through a 0.45 µm filter were fed to the device.

2.2.6. Mineral content

The mineral content was measured using the microwave (Millestone MLS 1200, Italy) nitric acid digestion procedure, followed by induction-coupled plasma optical emission spectrometry (Spectro Blue ICP-OES). The samples 1±0.1g were mixed with HNO_3 (67% v/v) ve H_2O_2 and subjected to the following digestion program: pre-digestion for 15 min at room temperature, rise to 1200W at 150°C, hold steady for 10 min 1200W at 150°C, cooling 250W (30 min). After the digested samples were cooled to room temperature, the mineral contents analyses were performed at the ICP-OES system.

2.2.7. Total phenolic content (TPC)

80% methanol solution (50 mL) and the sample of 5 g were mixed at 37°C for four hours and then filtered for the extraction. The level of phenolic compounds in the samples was determined by the Folin–Ciocalteu colorimetric method (25). 0.200 mL of Folin-Ciocalteu reagent was added to 40 µL extract. The mixture was allowed to equilibrate for 2 min and then mixed with 2400 µL of pure water and 600 µL of $NaCO_3$ solution. It was kept in the dark at room temperature for 2 hours. After the incubation, the absorbance value was measured at 765 nm by UV-VIS spectrophotometer (Agilent, UV-Visible, USA). The results represented as gallic acid equivalent (GAE) for samples dry basis.

2.2.8. Antioxidant activity

The antioxidant activities of the samples were determined using the DPPH method based on free radical scavenging. For preparing the radical solution, (0.1 mM) DPPH (2,2-diphenyl-1-picrylhydrazyl) and 80% methanol were used. 2000 µL of DPPH radical solution was added to 100 µL of each sample extract in the test tube and mixed using a vortex. The tube content was kept dark at room temperature (30 min). Then, the absorbances (A sample) were measured at 517 nm through a UV-VIS spectrophotometer (Agilent, UV-Visible, USA). DPPH radical scavenging activity was calculated as inhibition% (26) by using the formula (1);

$$\text{İ}nhibition\ \% = [\,1 - \left(\frac{Asample}{Acontrol}\right) * 100 \qquad (1)$$

2.2.9. Statistical analysis

The experiments of analyses were performed in two replicates, the color measurement was measured in five replicates. The results were given as mean±standard deviation values. Analyses of variance (ANOVA) (SPSS 17.0.1, Chicago, IL) was

used for the comparison ($p<0.05$) of the results. Pearson Correlation Coefficients were calculated to explain the relationships between some attributes and were evaluated at the statistical significance level ($p<0.05$).

3 Results and discussion

3.1 Physicochemical properties of cone molasses samples

The results of some physicochemical properties of 15 cone molasses samples (S1-S15) were given in Table 3. The electrical conductivity value of cone molasses samples varied on a wide scale. (376.67-944.67 µS/cm). Türkben et al. (27) found that the electrical conductivity value of the molasses samples obtained from different types of grapes was between 1.96 mS/cm and 4.51 µS/cm. Electrical conductivity can vary according to various factors, particularly fruit origin. Electricity in food is carried with ions, unlike metals that are carried with electrons, and the electrical conductivity of food is closely related to pH and physicochemical features such as °Brix value, protein, phenolic content, organic acid, and mineral content (28). The conductivity value and mineral content are closely linked. It was reported that tomato juice had the highest mineral content and also had the highest conductivity value in a study conducted on tomato, orange, and apple juice (29). Whereas in a study in which the link between physicochemical features of various fruit wines and electrical conductivity was observed, weak-positive-tendentious relation was found between protein, pH, and acidity and electrical conductivity; a strong-positive-tendentious relation was found between electrical conductivity and phenolic content, K, P, Mg and Ca minerals (28). There wasn't any statistically significant correlation between electrical conductivity and phenolic content. However, high (r^2:0.82) and significant ($p<0.05$) correlation was determined between the results of electrical conductivity and K contents of the samples in this study. While electrical conductivity increases in concentrations up to 30°Brix, as consistency thickens in higher concentrations, ion activity decreases; thus electrical conductivity declines. Electrical conductivity increases as temperature goes up and foods having low pH value and high solid material content convey electricity better (30). As the primary raw material of molasses samples was the cone fraction, it was thought that they showed lower electrical conductivity than fruit-based molasses. According to the Grape Molasses Communique (17), molasses whose pH value is between 5.0-6.0 is grouped as 'sweet' and whose pH value is between 3.5-5.0 is grouped as "sour". According to the regulations, the pH value of mulberry molasses is supposed to be between 5.0 and 6.0 (20), pH value of carob molasses is supposed to be between 4.5 and 6.0 (19). In this study, the pH values of cone molasses were between 3.47 and 4.79. According to the current regulations, all samples can be regarded in the "sour molasses" group. Total acidity values of molasses samples ranged from 1.28% to 2.90%. The total acidity amount of andiz (*Juniperus drupacea*) molasses samples was found to be between 0.28% and 1.16% (31) in a study and also found as 0.97% (32) in another study. Titration acidity of mulberry molasses was found between 0.48% and 0.96% in different studies (33)-(34)-(35). The acidity values of cone molasses samples examined in this study were higher than those stated in the literature.

The degree Brix (°Bx) is the most common scale for measuring dissolved or soluble solids. Most of these compounds for molasses are sugar, and some other compounds affect the °Brix value, such as pectin, mineral, and vitamin. During the clarification and filtration stages of molasses production, using of molasses soil and another clarification agent and most importantly the time of the boiling process implemented for evaporation has a significant impact on the °Brix value of molasses (31). °Brix values of cone molasses were found between 64.63% and 78.68%. According to the Grape Molasses Communique (17), °Brix is supposed to be at least 68% in liquid grape molasses. The water of all molasses examined in this study was evaporated by boiling in an open cauldron cooker with conventional production ways, and 'fixing the turbidity' or 'adjusting the acidity' processes was not implemented. Implementing different cooking times, different amounts of sugar used by producers, the factors such as visual control of the fluidity of the end product, and deciding the cooking level caused variable °Brix values. °Brix values of different fruit molasses are variable in literature as well. It was detected in a study conducted by Tüzün et al. (36) that the highest dry matter amount was in mulberry molasses (71.42 °Brix) and Mardin grape molasses (69.77 °Brix), carob molasses (68.42 °Brix) and Batman grape molasses (67.78 °Brix) respectively. The molasses of pomegranate (64.28 °Brix) and black mulberry (64.12 °Brix) were detected to have the lowest values. Karataş (37) reported that the values in different mulberry molasses were between 71.25% and 82.17%. The studies conducted in the literature shown that the molasses dry matter levels changed according to the fruit used and conventional production methods.

It was found that the viscosity values of cone molasses samples were in a wide range (150.42-11164.67 cP). It was thought that the difference in viscosity values results from the dry matter amount affected by the temperature and time implemented during the production period. It was observed that viscosity values were relatively high in samples whose dry matter content (°Brix) was high. When viscosity and °Brix values of samples were evaluated with Pearson correlation, the result was $r^2=0.861$ ($p=0.000$), which means a high and statistically significant correlation ($p<0.01$) (Table 4). The color values of cone molasses samples were evaluated as colorimetric L* (0, black; 100, white), a* (+red, -green), b* (+yellow, -blue) and showed changes in a wide range. L* values between 0.41 and 29.04, a* values between 6.54 and 44.11, b* values between 3.16 and 36.45 were found ($p<0.05$). The non-enzymatic browning, depending on heat treatment in molasses production and dry matter of molasses and soluble dry matter content are the causes determining the color (37)-(38). Color browning occurs when a certain amount of sugar contained in molasses caramelizes. The high a* color values, commonly caused by the caramelization of sugars, is not desired. Therefore, the decrease in redness a* and the increase in L* value show that these products are more qualitative and preferable. The research shows that the lightness value (L*) of molasses in the evaporation of unfermented grape juice decreases in parallel with the increase of dry matter amount. It was explained in another study that more viscous and darker color molasses are obtained by keeping the boiling time long and applying high temperatures (37). The samples' correlation parameter between °Brix and a* values obtained in the study was detected as $r^2=0.439$ ($p:0.015$);

Table 3. Some physicochemical properties of cone extract molasses samples.

Sample	Electrical conductivity (µS·cm)	pH	Total titration acidity (%)	°Briks	Viscosity (cP)	Color		
						*L**	*a**	*b**
S1	473.00 ± 0.00^{g*}	3.71 ± 0.064^{ef}	2.82 ± 0.20^{bcd}	78.63 ± 0.13^{a}	10878.62 ± 19.91^{b}	4.14 ± 0.36^{de}	13.67 ± 5.93^{bc}	4.57 ±0.50^{de}
S2	574.33 ± 0.47^{d}	3.87 ± 0.099^{de}	2.50 ± 0.05^{ef}	68.00 ± 0.00^{f}	1234.20 ± 45.28^{i}	0.41 ± 0.04^{g}	20.55 ± 0.29^{b}	5.67± 0.09^{cde}
S3	520.00 ± 1.41^{e}	3.86 ± 0.101^{de}	2.56 ± 0.50^{a}	75.40 ± 0.10^{c}	6328.98 ± 39.84^{c}	0.76 ± 0.00^{g}	14.77 ± 0.01^{bc}	4.52± 0.09^{e}
S4	376.67 ± 6.85^{i}	3.47 ± 0.005^{f}	2.90 ± 0.80^{b}	77.50 ± 0.00^{b}	3512.65 ± 10.65^{g}	29.04 ± 0.03^{a}	13.37 ± 0.03^{bc}	19.97±0.04^{b}
S5	596.67 ± 0.47^{c}	4.47 ± 0.036^{abc}	1.58 ± 0.50^{def}	77.13 ± 0.13^{b}	5768.53 ± 17.24^{d}	1.92 ± 0.14^{fg}	40.44 ± 0.59^{a}	19.31 ±0.09^{b}
S6	607.00 ± 0.00^{c}	4.01 ± 0.037^{de}	1.74 ± 0.40^{cde}	71.65 ± 0.05^{e}	2000.46 ± 16.77^{h}	3.45 ± 0.14^{ef}	13.79 ± 1.00^{bc}	3.73± 0.21^{e}
S7	564.00 ± 0.00^{d}	3.93 ± 0.033^{de}	1.95 ± 0.50^{bc}	78.50 ± 0.00^{a}	11164.67 ± 65.80^{a}	5.41 ± 0.35^{d}	19.11 ± 1.55^{b}	5.24 ±0.46^{cde}
S8	416.67 ± 0.47^{h}	4.79 ± 0.019^{a}	1.28 ± 0.20^{f}	68.00 ± 0.00^{f}	174.30 ± 1.99^{l}	16.97 ± 0.33^{b}	16.21 ± 0.22^{bc}	36.45± 0.60^{a}
S9	502.33 ± 0.47^{f}	4.16 ± 0.070^{cd}	1.77 ± 0.10^{cde}	68.18 ± 0.08^{f}	561.29 ± 7.76^{j}	3.63 ± 0.04^{ef}	14.26 ± 0.43^{bc}	3.16 ±0.09^{e}
S10	944.67 ± 2.05^{a}	4.45 ± 0.061^{bc}	1.79 ± 0.50^{cde}	66.25 ± 0.00^{g}	150.42 ± 4.08^{l}	3.45 ± 0.21^{ef}	8.75 ± 0.90^{c}	7.90± 0.04^{cd}
S11	506.33 ± 0.47^{f}	4.62 ± 0.005^{ab}	1.69 ± 0.04^{cde}	68.10 ± 0.00^{f}	395.93 ± 5.15^{k}	4.61 ± 0.37^{de}	14.84 ± 1.20^{bc}	3.85 ±0.30^{e}
S12	527.33 ± 0.47^{e}	4.43 ± 0.061^{bc}	1.73 ± 0.09^{cde}	75.60 ± 0.00^{c}	3872.02 ± 8.88^{f}	4.32 ± 0.02^{de}	8.91 ± 0.21^{c}	3.20± 0.00^{e}
S13	421.67 ± 0.47^{h}	4.75 ± 0.068^{ab}	1.31 ± 0.20^{f}	78.68 ± 0.08^{a}	5831.01 ± 13.75^{d}	14.68 ± 0.91^{c}	44.11 ± 1.99^{a}	35.21 ±2.07^{a}
S14	849.67 ± 0.47^{b}	4.56 ± 0.008^{ab}	1.80 ± 0.10^{bcde}	73.00 ± 0.00^{d}	5001.28 ± 11.10^{e}	3.29 ± 0.12^{ef}	6.54 ±0.30^{c}	3.18 ±0.07^{e}
S15	936.33 ± 1.70^{a}	4.65 ± 0.069^{ab}	1.51 ± 0.20^{ef}	64.63 ± 0.13^{h}	279.98 ± 22.99^{kl}	3.51 ± 0.08^{ef}	8.32 ±0.62^{c}	7.99 ±0.18^{c}

*: Different letters in the same column indicate a statistically significant difference between the datas (p<0.05).

Table 4. Some nutritional contents, sugar profile and HMF values of cone extract molasses samples.

	HMF (mg/kg)	Ash (%)	Protein (%)	Inhibition (%)	Total phenolic content** (µg GA·g)	Fructose (g/100 g)	Glucose (g/100 g)	Sucrose (/100 g)	Fructose/ Glucose	Total sugar (g/100 g)	Invert sugar (g/100 g)
S1	160.29±0.5^{d*}	0.55±0.067^{cdef}	0.088±0.00^{c}	85.81±0.17^{ab}	1739.15± 58.45^{f}	22.16±0.2^{d}	28.90±1.0^{b}	24.74±0.9^{cd}	0.766	75.80±0.7^{a}	51.06±0.6^{b*}
S2	141.75±0.9^{e}	0.65±0.04^{bcd}	0.178±0.01^{b}	83.33±0.50^{ab}	2073.53±12.48^{e}	23.36±0.0^{c}	28.30±0.7^{b}	6.93±0.0^{gh}	0.825	58.59±0.2^{fg}	51.66±0.4^{b}
S3	1101.58±1.4^{a}	0.59±0.06^{cde}	0.089±0.00^{c}	86.84±0.04^{ab}	2359,09±81.96^{d}	30.23±0.5^{a}	36.49±0.5^{a}	4.67±0.1^{h}	0.828	71.38±1.9^{bcd}	66.71±0.5^{a}
S4	236.40±1.1^{c}	0.34±0.00^{efg}	0.089±0.00^{c}	44.06±3.05^{g}	1129,03±68.43^{h}	30.16±0.9^{b}	35.72±0.2^{a}	6.79±0.1^{gh}	0.844	72.68±0.4^{abc}	65.89±0.6^{a}
S5	51.74±0.5^{h}	0.48±0.03^{defg}	0.174±0.02^{b}	65.02±3.05^{ef}	1178,20±2.38^{gh}	11.43±0.9^{l}	17.90±0.0^{f}	43.80±1.1^{a}	0.638	73.13±0.7^{ab}	29.34±0.5^{g}
S6	80.40±0.8^{g}	0.42±0.07^{defg}	0.087±0.00^{c}	86.84±0.21^{ab}	2102,23±66.71^{de}	19.34±0.6^{g}	23.91±0.5^{c}	25.99±0.8^{bc}	0.808	69.25±0.6^{cd}	43.25±0.6^{cd}
S7	9.64±0.8^{i}	0.53±0.07^{defg}	0.088±0.00^{c}	89.77±0.08^{a}	3503,18±49.54^{b}	19.01±0.1^{h}	24.14±0.3^{c}	30.25±0.2^{b}	0.787	73.39±0.2^{ab}	43.15±0.2^{d}
S8	4.54±0.3^{k}	0.27±0.04^{fg}	0.086±0.00^{c}	74.04±0.83^{cd}	1433,82±57.19^{g}	5.92±0.0^{n}	12.03±0.0^{g}	45.33±1.2^{a}	0.492	63.28±0.4^{e}	17.95±0.0^{h}
S9	11.04±0.7^{j}	0.47±0.07^{defg}	0.087±0.00^{c}	79.84±1.98^{bc}	3971,10±25.93^{a}	20.91±0.5^{f}	24.66±0.5^{c}	16.96±0.8^{f}	0.847	62.52±0.6^{e}	45.57±0.5^{c}
S10	162.33±0.6^{d}	0.91±0.07^{ab}	0.085±0.00^{c}	64.86±0.91^{ef}	3366,03±85.39^{b}	13.75±0.6^{k}	18.39±0.1^{ef}	25.56±0.7^{cd}	0.747	57.70±0.5^{fg}	32.14±0.4^{f}
S11	4.91±0.5^{k}	0.24±0.03^{g}	0.121±0.01^{b}	64.86±1.24^{ef}	3836,27±57.11^{a}	15.63±0.2^{i}	22.67±0.0^{cd}	19.18±0.2^{ef}	0.690	57.48±0.1^{fg}	38.30±0.1^{e}
S12	370.68±1.2^{b}	0.40±0.04^{defg}	0.166±0.01^{b}	65.80±0.58^{ef}	2344,57±70.15^{d}	21.22±0.4^{e}	29.08±0.4^{b}	10.64±0.1^{g}	0.730	60.94±0.3^{ef}	50.30±0.4^{b}
S13	27.96±0.9^{i}	0.25±0.08^{g}	0.057±0.01^{c}	62.43±0.66^{f}	1884,21±125.82^{ef}	14.75±0.0^{j}	23.61±0.9^{cd}	30.25±1.6^{b}	0.625	68.60±0.8^{d}	38.35±0.5^{e}
S14	42.43±0.6^{i}	0.84±0.03^{abc}	0.499±0.02^{a}	71.76±0.41^{de}	3085,61±67.80^{c}	15.05±0.2^{i}	20.94±0.2^{de}	18.81±0.9^{ef}	0.719	54.79±0.4^{g}	35.98±0.2^{e}
S15	120.48±0.4^{f}	0.96±0.01^{a}	0.701±0.01^{a}	64.55±1.65^{ef}	3434,93±33.92^{b}	10.66±0.0^{m}	17.23±0.0^{f}	21.42±0.5^{de}	0.619	49.31±0.1^{h}	27.89±0.0^{g}

*: Different letters in the same column indicate a statistically significant difference between the datas.

**: The results were given on dry sample bases.

the relationship between them was found of weak but statistically significant correlation (p<0.05). The difference in L* values of molasses samples in this study shows that factors such as the intensity of the heating source applied during the production of molasses and cooking time and production parameters are not standard. The color, which is the most important physical quality parameter of different molasses in literature, was detected as the primary criterion and it was reported to change in a wide range in the studies conducted about the quality aspects of local molasses types (35). This case indicates that the process parameters individually vary in conventional production methods. However, it was reported that the color of the samples selected from industrial trademarks was shown to change in a limited range (39). The raw material types used in molasses production; such as mulberry, grape, and carob, are one of the significant factors influencing the product color parameters. Moreover, fruit types and the genus of the fruits (such as red, black, or white grapes) have even impacted the color parameters (40).

According to the conventional production methods of molasses using in this study, the cone extracts obtained in the first stage were boiled until their color turned red. The boiling was continued by adding sugar to the extracts. As a result, chemical browning reactions were taken place both during the boiling of the extract and after the addition of sugar. a* values of the samples of cone molasses in the study were higher than the other molasses types' a* values in literature. This case indicated the formation of chemical browning reactions and increased HMF content.

3.2 The sugar composition of cone molasses samples

According to the regulations, it is reported that grape molasses cannot be produced by dilution and multiplication with commercial glucose, fructose, and similar sugar types (17). One of the most common adulterations in fruit-based molasses is raising the end-product amount by adding sugar or sugar syrups. For that reason, there are quality limits related to sugar profiles in molasses types. The sugar content of molasses types which are subjects of product communique and standards, results from the raw materials used. It is necessary to flavor the cone extract with sugar added to be able to consume it, which has an intensely sour taste. Therefore, cone molasses stays out of the sugar profile limits of the other molasses. For instance,

according to the Grape Molasses Communique (17), grape molasses' Fructose/ Glucose amount is supposed to be between 0.9 and 1.1, saccharose amount is supposed to be 1% at most. It was found that the Fructose/Glucose amount of the cone molasses in this study was between 0.49 and 0.85, and saccharose content was between 4.67% and 45.33% (Table 4). Sugar compositions of molasses samples exhibited variance in a wide range. It was found that fructose content was between 5.92%-30.23%, glucose amount 12.03%-36.48%, and saccharose amount 4.67%-45.32%. Also, the Fructose/Glucose ratio was in the range of 0.49-0.84, the total sugar amount was 49.31%-75.80%, and the invert sugar amount was 17.95%-66.71%. In molasses obtained from other sources except for the grape, the addition of sugar is necessary due to the production technology and these products were allowed to have higher sucrose levels in the related product communique and standards.

For example, the saccharose level of carob molasses must be between 20% and 40% (19), for Type 1 mulberry molasses contents must be max. 14%, and for Type 2 mulberry molasses must be max. 17% (20). The total sugar amount of cone molasses in this study was between 49.3% and 75.8% and the saccharose, glucose, fructose, and its related invert sugar contents varied considerably. The primary purpose of adding sugar to cone molasses is to gain a drinkable taste to the sour cone extract, increase the dry matter amount and get consistency. So; in low-capacity or household productions, from diabetic and dietetic reasons and sensory preferences, the addition of sugar depends on the initiative of manufacturers. On the other hand; it was thought that the difference in saccharose and invert sugar profile results from the inversion of saccharose added to cone molasses extracts in the last boiling stage. One of the most essential aspects of sugar inversion is the medium's acidity level. For this purpose, Pearson Correlation Parameters between pH and Total Titration Acidity values (TTA) and invert sugar contents of the molasses sample were determined (Table 5). Whereas the negative correlation r^2:-0.827 (p:0.00) between invert sugar and pH values was found statistically significant ($p<0.01$), the positive correlation r^2=0.832 (p:0.00) between invert sugar and TTA values was found statistically significant ($p<0.01$). This result indicated that the different acidity levels of the molasses affected the inversion of added saccharose and the invert sugar level.

Besides the domestic production at a small scale, the total sugar amount of the products must be supposed to be limited to produce these products commercially. Total sugar content provides standardization of the end-products °Brix value, flavor, and color properties. Thus, the positive correlation (r^2=0.812) between °Brix and the total Sugar contents of samples in this study was found to be statistically significant ($p<0.01$).

3.3 HMF contents of cone molasses samples

HMF values of cone molasses samples widely ranged from 4.54 mg/kg to 1101.58 (Table 4). According to regulations, the highest HMF limit value in grape and mulberry molasses is 75 mg/kg (17)-(20), the highest in carob molasses is 40 mg/kg (19). Eight cone molasses in this study were found to have over 75 mg/kg limit values for HMF. It is a compound that does not naturally exist in fruits but is formed in the presence of monosaccharides with the help of temperature and acid effect, and its amount is limited by regulations to prevent and control extreme temperature application in many products. HMF is a quality factor that shows excessive heat treatment (temperature and time) applied in foods (41). HMF indicates whether the heating process is applied correctly in molasses and sugary products exposed to all boiling processes and also indicates the suitability of healthy food. Molasses is a highly nutritious food in terms of its trace elements and minerals. Additionally, it was remarked that the cations such as Ca^{+2}, Fe^{+2}, Cu^{+2}, and Mg^{+2} and minerals such as Na^{+}, K^{+}, and Zn^{+2} stimulate HMF formation in many studies (42)-(43). Because the HMF is the intermediary step product of the Maillard reaction resulting from heat treatment of the foods containing reducing sugar and amino groups, it closely relates to the compounds of the food. The positive correlation (r^2=0.635) between HMF and reducing sugar contents of the samples in this study was found statistically significant ($p<0.05$). Although the product's composition is decisive in forming HMF, the type of heat treatment applied, temperature, and time parameters also have considerable effects.

The fact that the samples in this study were traditionally cooked in open boilers with sensorial visual control and non-standard times caused the samples to have high HMF content. Similarly, over-limit values of HMF were detected in some studies conducted to determine the quality properties of different molasses in various regions (39).

Table 5. Correlation matrix for the quality properties of the samples

R-Pearson values**	1	2	3	4	5	6	7	8	9	10	11	12
1 °Brix	1											
2 Viscosity	**0.861****	1										
3 Sucrose	0.035	0.09	1									
4 Invert sugar	0.454	0.339	**-0.808****	1								
5 Total sugar	**0.812****	**0.705****	0.178	0.435	1							
6 pH	-0.395	-0.408	**0.525***	**-0.827****	**-0.578***	1						
7 TTA	0.343	0.381	-0.632*	**0.832****	0.424	**-0.910****	1					
8 Protein	-0.377	-0.201	-0.083	-0.329	**-0.675****	0.348	0.220	1				
9 HMF	0.201	0.177	**-0.556***	**0.635***	0.210	-0.361	-0.467	-0.110	1			
10 Total phenolic	-0.337	-0.058	-0.200	-0.083	-0.445	0.183	-0.220	0.231	-0.108	1		
11 Inhibition %	0.012	0.348	0.040	0.093	0.216	-0.260	0.125	-0.172	0.169	0.267	1	
12 Ash	-0.39	-0.071	-0.178	-0.134	-0.496	0.016	0.091	**0.667****	0.118	0.335	0.126	1

*: Correlation is significant at the 0.05 level (2-tailed).
**: Correlation is significant at the 0.01 level (2-tailed).

3.4 Some nutritional properties of cone molasses samples

The limit values of ash (%) content indicated for different molasses types according to the codex and standards vary. The maximum limit values are 2.5% for grape molasses, 4.0% for type 1 mulberry molasses, and 3.0% for carob molasses. In this study, the ash contents of molasses samples were determined between 0.24% (S11) and 0.96% (S15). Ash% contents were variable in the studies conducted with different fruit molasses. Ash (%) values were reported as 3.79% for traditional andiz (*Juniperus drupacea*) molasses (32), 2.02% for mulberry molasses, and 4.42% for sweet sorghum molasses (44). Ash, which expresses the total mineral content, differs in each fruit and is present in small amounts. Most of the minerals in fruits, generally in the form of salt, are soluble in organic and inorganic acids and water. Therefore, a significant part of the fruit mass passes into the fruit juice during the processing of the fruit. In addition to the mineral content of the fruit, the cleaning applied in the processing of the raw material, whether the clarification process is done well, the amount and composition of the molasses soil used, the cooking method of the molasses (32) and the storage period can also affect the ash (%) content of molasses. In the study, the cause of low ash (%) content compared to other molasses types could be that the cone does not have high mineral content as many as that of fruits and the first boiling water's spilling and not adding to the molasses content. In addition; due to their hard texture, cones do not thoroughly pass into the extract by softening like other fruits during the extraction stage. The mineral contents of the final molasses are composed of substances that pass from the cone to the second boiling water.

In addition, molasses soil, added to reduce acidity in producing other molasses, is not used in cone molasses affects the total ash amount. Since the primary protein source of molasses is fruit, the type of fruit and even its origin used in making molasses is the most determining factor in the protein amount of the final product. Proteins provide suspension of substances that cause turbidity in the most, such as pectin and polyphenols and in order not to adversely affect the quality of the final product, tannin-gelatin is added to the deacidified must and they are removed from the must by clarification and filtration (45). Therefore, the protein content of molasses is lower than the protein content of the fruit used as a raw material. For example, in a study, the protein content of andiz fruit was 2.45%; andiz molasses was found to be 0.72% (46). There is no regulation about molasses' protein content in codex and standards. In this study, the cone molasses samples' protein contents were detected between 0.057% and 0.701% ($p<0.05$). In a study conducted by Erbil (23), protein content was found that 0.19-1.20% for traditional grape molasses, 0.35-1.81% for carob molasses, 0.14-2.27% for mulberry molasses, and 0.14-0.27% for andiz molasses. Although there is no clarification process for turbidity in the production of cone molasses, the main reason for the low protein content is thought to be the low protein content of the cones used as raw material. The antioxidant capacity of molasses samples in this study ranged from 44.06% (S4) to 89.77% (S7) ($p<0.05$). The antioxidant activities of molasses obtained from different raw materials were also variable in the literature. It was reported that the antioxidant activity of mulberry molasses was between 10.65-35.17% (37), while the antioxidant activity of grape molasses was between 86.44% and 92.83% (26). In another study, the average antioxidant activity of grape molasses was determined as 64.13% (47). The processes applied to foods leads to some changes in both phenolic compound content and antioxidant activity (48). While one of the most critical factors affecting the phenolic content of foods is the cooking time, other factors such as cooking degree, piece sizes of the food, food-boiling water ratio, whether the cooking vessel is open or closed, the choice of the cooking vessel and the type of food also affect it (49). There was reported an increase in antioxidant activity during the boiling process in the production of grape molasses; while the antioxidant activity was 12.17% in the initial must stage, it was 93.40% in the final product (40). In the production of molasses from red, white, and black grape varieties with the traditional method, it was reported that the antioxidant activity of molasses samples at the initial stage (37.37%; 50.42%; 63.56%, respectively) increased in the final product molasses (93.40%; 92.83%, 86.44% respectively) (26).

In this study, it was impossible to determine the antioxidant activity of cones used as raw material in the production since cone molasses were collected from different regions. However, in a study conducted by Şahin and Üner (50), it was determined that the antioxidant activity of black pine cones was 11.59% and that of Scotch pine cones was 7.56%. In this study, the antioxidant activities of cone molasses were found to be between 44.06-89.77%. The removal of water from the cone extracts by boiling and the increased concentration of components with antioxidative activity in the product also caused the molasses samples to have high antioxidant activity. It is also known that products such as melanoidin, which are formed as a result of the Maillard reaction, have antioxidative effects (51), which should be considered in the increase in the antioxidant activity of the end product. Indeed, Piva et al. (52) determined that the total antioxidant activity of the "grape must" increased depending on the increase in temperature and concentration, but the antioxidant activity of the phenolic fraction decreased.

In this study, the phenolic content of pine molasses samples was found to be between 1129.03 µg GAE/g (S4) and 3971.10 µg GAE/g (S9) ($p<0.05$). The phenolic content of cones used as raw materials in this study was not analyzed; however, Şahin and Üner (50) have stated in a conducted study that the total phenolic matter content of black pine was 330 mg-kg GAE, and that of scotch pine was 300 mg-kg. The total phenolic contents of molasses samples in this study are higher than the total phenolic content of the cones in the literature. This case may be due to the excellent transition of phenolic compounds from the cone into the water during the extraction by boiling and the condensation of the phenolic compounds in the final product as a result of the boiling and evaporation of the water. In a study carried out by Tüzün et al. (36), carob molasses was 7487.4 µg GAE/100 g, mulberry molasses 2198.5 µg GAE/100 g, grape molasses 1661.4 µg GAE/100 g total phenolic substance values were determined. Studies show that the total phenolic content of molasses mainly varies according to the raw material. In the molasses samples examined in this study, it was determined that, unlike other fruit molasses, cones were used instead of a consumable fruit, but they had phenolic contents comparable to other molasses types in the literature. Phenolic compounds can be found in plants' fruits, seeds, flowers, leaves, branches, and stems. The flavonoids, an essential group of phenolic compounds, are polyphenolic antioxidants found naturally in herbal teas, fruits, and vegetables. Polyphenols as food components are important in terms of their effects on the formation of aroma and flavor (such as bitterness-sourness),

their participation in color formation (such as yellow, yellow-brown, and red-blue), their antimicrobial and antioxidative effects, their enzyme inhibition, and their being purity criteria in some foods (53). It was reported that polyphenols are beneficial compounds for health because they decrease cholesterol, regulate eicosanoid synthesis, inhibit low-density lipoprotein oxidation, prevent hypertension and cardiovascular diseases, and show antimutagenic and anticarcinogenic effects (54)-(55). However, they cause different problems, such as enzymatic browning in processing fruits and vegetables in the final product.

3.5 Mineral contents of cone molasses samples

The total mineral contents of molasses samples ranged between 497.06 (S13) and 6595.30 mg/kg (S15) (Table 6). The change in mineral matter contents of samples except for selenium (Se) was found ($p<0.05$) statistically significant. It is thought that due to the production of the samples in different non-standard conditions and with conventional production methods, there may be a decrease in some minerals and even an increase in the amounts of some minerals such as iron (Fe) and copper (Cu) because of the use of metal cooking tools (32). It can be said that the ripeness level of cones used in molasses production, tree type, production parameters of molasses, and extraction conditions affect this differentiation. Whereas Se concentration was detected to be under detection limits for S1 and S11-15 samples, it ranged from 2.57 to 3.07 in S2-S10 samples, but the difference was not found statistically significant ($p>0.05$). The molasses samples contained the most potassium (K) and Calcium (Ca) elements on the general average. Magnesium (Mg), Phosphorus (P), Sodium (Na), Zinc (Zn), Iron (Fe), and Manganese (Mn) elements followed them, respectively. According to the Grape Molasses Communique (17), the Zn element must be 5 mg/kg at most. Ten of the 15 molasses samples in this study were found above the specified limit. In a study, Velioğlu and Artık (56) examined whether some molasses samples were proper to Grape Molasses Standards (18) and found that the Fe and Zn contents of twenty grape molasses samples varied between 17-125 mg/kg and between 3-55 mg/kg, respectively. Moreover, data from the study presented a crucial standardization problem in molasses.

4 Conclusion

The cone molasses samples in the study were the samples whose water was removed by boiling in open boilers with the conventional production method and unlike the traditional fruit molasses, the clarification process was not applied. Although cone molasses has a traditional production method, the data obtained in this study have varied widely due to the variability of application parameters and the effects of individual techniques. As a product for which a Geographical Indication Registration application has been made, the quality limits should be kept narrow to serve a standard product to the consumer. Although it is consumed in the daily diet and a valuable product in traditional folk medicine due to its different functional qualities, no regulation can be compared in terms of quality and food safety criteria.

Unlike other fruit molasses, the cone molasses samples have differed from other molasses types in the literature and regulations regarding qualities such as acidity, pH and ash because its raw material was the cones. Since necessary to add a source of sugar in production, the sugar content and composition of the molasses samples have also differed considerably compared to other molasses types.

Table 6. Mineral contents of cone extract molasses samples (mg/kg)

	Selenium (Se)	Sodium (Na)	Magnesium (Mg)	Phosphorus (P)	Potassium (K)	Calcium (Ca)	Manganese (Mn)	Iron (Fe)	Zinc (Zn)
S1	nd	54.8±0.00^{cd}	49.3±8.38^{h}	58.8± 8.20^{ef}	616.5± 0.05^{i}	444.6± 0.03^{f}	2.9± 0.32^{h}	8.3± 1.31^{cde}	33.97 ± 4.44^{ab}
S 2	2.89 ±0.60^{a*}	64.2±0.01^{b}	67.4±4.22^{fg}	104.6 ± 1.00^{c}	1029.9± 0.03^{e}	410.0± 0.05^{h}	8.5± 0.09^{d}	14.3± 3.83^{bc}	30.38 ±1.16^{b}
S 3	3.07 ±0.58^{a}	56.8±0.01^{c}	68.0±1.60^{fg}	80.5± 3.80^{cde}	803.9± 0.02^{h}	852.4± 0.03^{d}	3.5± 0.28^{gh}	18.2± 1.24^{b}	36.25 ±1.36^{ab}
S 4	2.81 ±1.08^{a}	48.1±0.01^{fg}	24.4±1.41^{ij}	17.1± 2.36^{h}	208.0± 0.08^{k}	441.0± 0.01^{fg}	4.2± 0.20^{g}	8.9± 0.25^{cde}	30.88 ±0.60^{b}
S 5	3.07 ±0.23^{a}	49.8±0.01^{ef}	72.6±2.50^{ef}	93.7± 2.84^{c}	786.8± 0.07^{h}	417.7± 0.03^{gh}	6.0± 0.15^{f}	13.4± 0.50^{bc}	33.50 ±0.74^{ab}
S 6	2.93 ±1.29^{a}	54.6±0.00^{d}	86.5±3.53^{de}	86.4± 0.99^{cd}	959.1± 0.07^{f}	458.5± 0.07^{f}	7.9± 0.44^{de}	12.3± 0.24^{bcd}	39.45 ±2.55^{a}
S 7	2.57 ±0.92^{a}	66.2 ±0.00^{b}	54.3±1.09^{gh}	86.0± 3.31^{cd}	1069.0± 0.04^{d}	253.7± 0.03^{i}	7.3± 0.13^{e}	8.1± 0.54^{cde}	7.82 ±0.24^{def}
S 8	2.61 ±0.61^{a}	34.4±0.01^{i}	23.3±0.54^{ij}	21.7± 1.51^{h}	234.0± 0.03^{k}	500.9± 0.01^{e}	0.8± 0.04^{i}	9.1± 0.94^{cde}	10.54 ±0.15^{cd}
S 9	2.65 ±0.42^{a}	42.8±0.01^{h}	56.5±0.66^{fgh}	62.9± 1.19^{def}	912.4± 0.08^{g}	139.9± 0.01^{k}	10.9± 0.46^{a}	6.0± 0.42^{de}	9.20± 0.16^{cde}
S 10	2.67 ±0.57^{a}	47.1±0.01^{g}	155.3±2.16^{c}	288.9± 12.56^{a}	2181.4± 0.02^{c}	1100.2± 0.03^{b}	12.2± 0.35^{c}	13.4± 0.20^{bc}	16.53 ± 0.23^{c}
S 11	nd	106.7± 1.10^{a}	32.1± 0.50^{i}	3.7± 0.36^{h}	1091.6± 8.60^{d}	27.5± 0.30^{l}	5.5± 0.05^{f}	3.8± 0.28^{e}	0.84 ± 0.03^{f}
S 12	nd	34.1± 0.30^{i}	95.7±0.90^{d}	49.1± 0.20^{f}	1063.1± 11.60^{de}	191.3± 2.80^{j}	4.0± 0.03^{gh}	3.6± 0.12^{e}	1.00 ± 0.01^{f}
S 13	nd	5.2± 0.30^{j}	9.8± 0.03^{j}	11.0± 0.05^{h}	343.2± 3.40^{j}	120.3± 1.80^{k}	1.5± 0.00^{i}	5.5± 0.00^{e}	0.56 ± 0.00^{f}
S 14	nd	34.8± 0.80^{i}	304.9± 1.10^{b}	202.2± 0.66^{b}	3357.8±14.90^{b}	927.8± 11.10^{c}	27.4± 0.05^{b}	16.3± 1.12^{b}	2.77 ± 0.00^{def}
S 15	nd	51.5± 0.30^{e}	424.3± 3.10^{a}	292.0± 1.41^{a}	4146.9± 14.40^{a}	1580.0± 12.30^{a}	12.5± 0.09^{c}	85.9 ± 0.10^{a}	2.23 ± 0.01^{ef}

*: Different letters in the same column indicate a statistically significant difference between the datas ($p<0.05$).

**: The results were given on dry sample bases.

Therefore, to be produced commercially and served to consumers except for household consumption, it needs a communique including quality limits in terms of °Brix, sugar profile, and acidity like other molasses. Unlike other molasses, it must be sweetened before it can be consumed, so natural sweetener sources and limits that can be used in its production must be specified in the regulation. It is detected that antioxidant activity and total phenolic matter contents of cone molasses are pretty high. Although it is promising as a functional food in terms of its effective bioactive properties, it must be standardized in basic quality properties such as HMF, pH, Brix and total sugar or sucrose content.

The main stages of cone molasses preparation are constant, but the use of personal techniques in household production and the variations in process conditions such as boiling time and heating have caused the HMF contents to be very variable. Whereas the acceptable limit for molasses types is 75 mg/kg at most, the samples examined in this study have reached a maximum of 1101.58 mg/kg. Consumption of this product, used as a traditional folk medicine with a high level of HMF content, is not found to be safe according to food security. There are several studies carried out with extracts obtained from the cones, shoots, needles (leaves) and bark parts of plants in the Pinaceae family and there are even commercialized products as food supplements. However, it is thought that the relationship between raw materials and ingredients regarding bioactive components should be examined in future studies. Besides being used locally as a food supplement, cone molasses is used as a protective and curative in asthma, bronchitis and the common cold in traditional folk medicine and it is thought that more advanced examinations should be conducted so that these qualities could be pharmacologically.

5 Teşekkür

6 Yazar katkı beyanı

The authors declare that they have contributed equally to the article.

7 Contribution Rate Statement Summary of Researchers

There is no need to obtain ethics committee permission for the article. The authors of the article declare that there is no conflict of interest between them.

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