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Food Supplement Production from Propolis, Honey, and Mulberry Molasses and Its Optimization

Propolis, Bal ve Dut Pekmezli Takviye Edici Gıda Üretimi ve Optimizasyonu

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Abstract

In this study, twelve different mixtures were formed by different ratios of honey (15%-78.5%) and propolis (1.5%-5%) to mulberry molasses to increase its antioxidant, phenolic compound, and mineral values, and the physical and chemical properties of these mixtures and pure products were investigated. In the analysis conducted in the study, it was determined that among the twelve different mixtures, the highest antioxidant activity was determined to be in sample A1, with a value of 400.4 ± 1.0 mg/100 g $FeSO_4$, whereas the lowest activity was found in sample B4, with a value of 203.1 ± 0.4 mg/100 g $FeSO_4$. Regarding the total phenolic content, the highest activity was identified in sample A1, with the highest value of 184.3 ± 0.9 mg GAE/100 g, while the lowest was noted in sample B4, with a value of 110.3 ± 0.05 mg GAE/100 g. Furthermore, in the determination of sugar components, the Fructose/Glucose ratio was observed to be highest in sample C4, at 1.16 ± 0.03, and lowest in sample B1, at 0.95 ± 0.003. In this study, it was determined that an increase in the percentage of propolis in the mixtures led to an increase

in the antioxidant and phenolic compound levels, thereby imparting antioxidant properties to the product. Additionally, it was observed that the nutritional content was enriched because of the increase in glucose and fructose amounts as the percentage of honey increased.

Keywords: Mulberry Molasses, Propolis, Honey, Health, Food Supplement.

Özet

Bu çalışmada, dut pekmezine; antioksidan, fenolik madde ve mineral değerlerinin artırılması amacıyla farklı oranlarda bal (%15-78,5) ve propolis (%1,5-5) eklenerek 12 ayrı karışım oluşturulmuş ve bazı fiziksel ve kimyasal özellikler araştırılmıştır. Çalışma kapsamında yapılan analizlerde, 12 farklı karışım arasında en yüksek antioksidan aktivitesinin A1 örneğinde 400,4 ± 1,0 mg/100 g $FeSO_4$ değeriyle, en düşük aktivitesinin ise B4 örneğinde 203,1 ± 0,4 mg/100 g $FeSO_4$ değeriyle gözlemlendiği tespit edilmiştir. Toplam fenolik madde içeriği açısından en yüksek aktivite A1 örneğinde en yüksek 184,3 ± 0,9 mg GAE /100 g değeriyle, en düşük ise B4 örneğinde 110,3 ± 0,05 mg GAE /100 g değeriyle belirlenmiştir. Ayrıca, şeker bileşenlerinin belirlenmesinde Fruktoz / Glukoz oranının en yüksek olarak C4 örneğinde 1,16 ± 0,03, en düşük olarak ise B1 örneğinde 0,95 ± 0,003 olduğu gözlenmiştir. Bu çalışmada, karışımlarda propolis yüzdesinin artmasıyla antioksidan ve fenolik madde miktarının arttığı tespit edilmiş ve böylece ürüne antioksidan özellikler kazandırılmıştır. Ayrıca, bal yüzdesinin artmasıyla glukoz ve fruktoz miktarlarındaki artış sonucunda besinsel içeriğin zenginleştiği görülmüştür.

Anahtar Kelimeler: Dut Pekmezi, Propolis, Bal, Sağlık, Takviye Edici Gıda.

Abbreviations: HPLC, High performance liquid chromatography; TPTZ, Tripyridyl Triazine; GAE, Gallic acid equivalent; ICP-OES, inductively coupled plasma optical emission spectrometer.

1. INTRODUCTION

Rapid shifts in living and working conditions have led to changes in dietary habits, causing malnutrition and unbalanced nutrition. The fast pace of life has increased reliance on dietary supplements to meet nutritional needs not adequately met by daily diets (Coşkun & Velioğlu, 2020). Diet plays a significant role in health (Işık, 2014), and food supplements help meet nutritional needs by adapting to changing eating habits (Karataş & Şengül, 2018). Adequate nutrition involves consuming a variety of nutrients from both animal and plant foods and their proper utilization by the body (Zohoori, 2020). Consuming antioxidant-rich foods is emphasized, especially in developed countries, to support health (Güngör, 2007).

Molasses, a sweet, dense liquid obtained from fresh or dried fruits using traditional or industrial methods (Figure 1), is characterized by high sugar content, predominantly glucose and fructose (Batu, 1993; Karaca, 2009). It provides quick energy and is used traditionally for various diseases due to its high iron content (Yılmaz, 2012; Bayrak & Aygün, 2018).

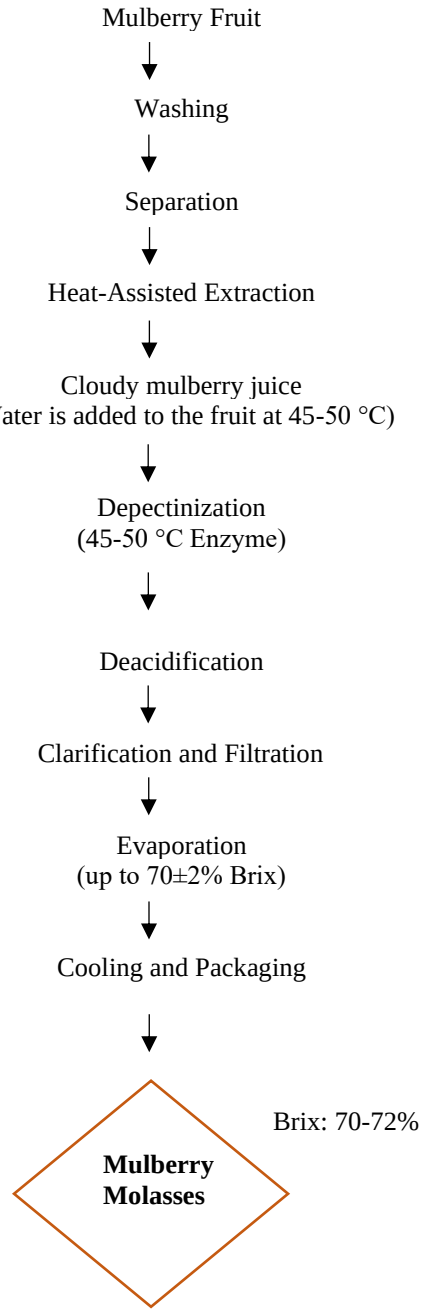

Figure 1. Mulberry Molasses Production Flow Diagram

Mulberry fruits, known for their low-calorie content and health benefits, are abundant in polyphenols, minerals, and vitamins, supporting overall health (Özbalcı et al., 2023). Mulberries are rich in essential minerals like potassium (K), calcium (Ca), phosphorus (P), magnesium (Mg), sulfur (S), and iron (Fe) (Akbulut et al., 2007). They also contain essential fatty acids—linolenic, linoleic, and arachidonic acids—vital for cellular membrane integrity, optimal brain and nervous system function, and the synthesis of eicosanoids, which regulate blood pressure, viscosity, and immunity (Pawlovski et al., 1996; Simopoulos and Salem, 1996). Thus, incorporating mulberries into the diet ensures the provision of these critical fatty acids essential for bodily well-being.

Propolis is a natural product produced and utilized by honeybees (Apis mellifera) in the construction, adaptation, and protection of the hive (Cibanal & Sulaeman et al., 2019). Its composition varies depending on the source plant, containing approximately 45-55% resin, 23-35% waxes and fatty acids, 10% essential oils, 5% pollen, and 5% other organic substances and minerals (Burdock, 1998; Ertürk et al., 2013). Propolis contains over 300 compounds, including phenolics, terpenoids, lignans, stilbenoids, alcohols, benzaldehyde derivatives, and various minerals and amino acids, with fatty acids being the most abundant lipids (Huang et al., 2014; Crane & Walker, 1987). It has a wide range of biological activities, including antibacterial, antitumor, anti-inflammatory, antifungal, cytotoxic, immunomodulatory, and antioxidant properties (Krol et al., 2013; Kalsum et al., 2017).

Honey, another product of honeybees, is a functional food with protective and therapeutic properties against many diseases due to its various vitamins, minerals, organic acids, and enzymes (Alkın & Özmen, 2006; Dashora et al., 2011; Can, 2014; Molan, 2000). This rich content enables honey to have various positive effects on health (Sajtos et al., 2019 & Solayman et al., 2016). The mineral content of honey varies between 0.02% and 1%. The main minerals include potassium (K), calcium (Ca), phosphorus (P), and magnesium (Mg). Additionally, it contains trace elements such as iron (Fe), copper (Cu), zinc (Zn), selenium (Se), fluorine (F), and chlorine (Cl) (Can, 2014).Honey aids in the treatment of numerous diseases, including ulcers, stomach diseases, heart failures, palpitations, bone diseases, cough, allergies, bronchitis, anemia, throat pain, skin problems, and nervous system disorders. It also provides solutions to constipation, improves blood circulation, strengthens the heart, facilitates fat digestion, and heals wounds and burns (Molan, 2000). Thus, honey is a versatile natural product with a wide range of health benefits.

Natural products like propolis, honey, and molasses are rich in antioxidants and phenolic substances, protecting the body against oxidative stress and damage caused by free radicals (Karataş & Şengül, 2018).

Phenolic compounds, found in various plant sources, have numerous health benefits, providing protection against several diseases (Çağlar & Demirci, 2017; Khalil et al., 2020; Kolaç et al., 2017).

The principal objective of this scientific investigation was to comprehensively explore the physicochemical attributes of twelve distinct mixtures, each comprising varying proportions of honey (ranging from 15% to 78.5%), propolis (constituting 1.5% to 5%), and mulberry molasses to create a novel new product with desired properties. The study was meticulously designed to augment the inherent antioxidant capacity, phenolic compound content, and mineral profiles of these composite formulations. Through rigorous analysis of the samples, the researchers aimed to elucidate the intricate relationships between compositional variations and their multifaceted biological effects. Specifically, they probed the impact of altered ratios on antioxidant potential, and mineral composition. Furthermore, the investigation delved into the nutritional enrichment arising from fluctuations in glucose and fructose levels as the proportion of honey increased within the mixture.

2. MATERIALS and METHODS

2.1. Materials

In this research, dried mulberries procured directly from local producers in the Manisa region in July 2023 were employed for experimentation. These dried mulberries were utilized in the industrial production of mulberry molasses by SEM-AS Food Industry Trade Ltd. Co. The resulting mulberry molasses, identified as batch number 07S17, were transported to the laboratory in glass jars from the manufacturing facility. The acquired molasses samples were stored under controlled conditions in a dark environment at a constant temperature of 20 ± 2 ºC. All experiments were conducted in triplicate.

Propolis, in its raw form, was directly introduced into the mixtures, and a detailed analysis of the propolis samples was conducted using a pure water extraction method. The use of water in propolis extraction was preferred to comply with halal certification standards. 1 g of propolis sample was extracted with 100 mL of pure water to obtain a homogeneous mixture. The resulting mixture was dissolved in a stirrer overnight.

The flower honey utilized in this research, denoted as batch number 07S11, was produced by Semas Food Industry Trade Ltd. Co. and subsequently brought to the laboratory in glass jars. Furthermore, all analyses were conducted using deionized ultrapure water obtained from the Ultra-Pure Water System (Millipore, Synergy, Germany).

2.2. Method

The investigation involved the formulation of twelve different mixtures by introducing varying proportions of propolis (1.5-5%) and honey (15-78.5%) into mulberry molasses (as outlined in Table 1). These mixtures were prepared concurrently, and the outcomes were subsequently presented using the mean values derived from these formulations.

Table 1. Composition of mulberry molasses mixtures

MIXTURE	PROPOLİS (%)	HONEY (%)	MULBERRY MOLASSES (%)
Propolis	100 ± 0.01	0	0
Honey	0	100 ± 0.03	0
Mulberry Molasses	0	0	100 ± 0.01
A1 Mixture	5 ± 0.02	15 ± 0.03	80 ± 0.2
A2 Mixture	5 ± 0.01	35 ± 0.1	60 ± 0.01
A3 Mixture	5 ± 0.1	55 ± 0.3	40 ± 0.04
A4 Mixture	5 ± 0.003	75 ± 0.05	20 ± 0.02
B1 Mixture	2.5 ± 0.1	17.5 ± 0.03	80 ± 0.4
B2 Mixture	2.5 ± 0.02	37.5 ± 0.2	60 ± 0.01
B3 Mixture	2.5 ± 0.04	57.5 ± 0.07	40 ± 0.03
B4 Mixture	2.5 ± 0.03	77.5 ± 0.06	20 ± 0.004
C1 Mixture	1.5 ± 0.1	18.5 ± 0.07	80 ± 0.3
C2 Mixture	1.5 ± 0.03	38.5 ± 0.02	60 ± 0.01
C3 Mixture	1.5 ± 0.02	58.5 ± 0.1	40 ± 0.02
C4 Mixture	1.5 ± 0.01	78.5 ± 0.4	20 ± 0.006

2.2.1. pH Value

The pH assessment of the mixtures adhered to the TS 1728 ISO 1842 standard, employing the pH meter (Hanna, HI 2020, US) for the measurements (TS 1728 ISO 1842, 2001). The measurements were systematically executed with three replicates at a controlled temperature of 20 ºC.

2.2.2. Electrical Conductivity

The electrical conductivity of the mixtures was assessed using conductivity measurement instrument (Ohaus, St300c, US). Adhering to the TS 13366 Honey-Electrical Conductivity Determination standard, a 20% aqueous solution was prepared for each sample, and measurements were executed at 20 ºC. To

prevent the conductivity results from being influenced by the water used for dilution, deionized ultra-pure water (Millipore, Synergy, Germany) with a conductivity of 0.0006 mS/cm was utilized.

2.2.3. Determination of Soluble Solids Content (Brix°)

The analysis of soluble solids content, expressed in degrees Brix (º), employed a Refrakto Abbe (Ertick, Abbe-2, Germany) tabletop manual refractometer. Measurements were conducted at 20 ºC, and the results were expressed as a Brix° percentage.

2.2.4. Ash Determination

The determination of ash content was conducted using the gravimetric method, as detailed by Cemeroğlu (2010). Precision scale (RADWAG, AS220.R2, Poland) was utilized for sample analysis. Approximately 2 g of sample from each mixture, with an accuracy of 0.01 mg, was taken into porcelain crucibles brought to a constant weight, first burned with a bunsen burner flame, then heated in a muffle furnace at 550 ºC until it turned light gray-white, burned, and then weighed and the amount of ash was determined as a percentage with each mixture sample undergoing incineration, followed by weighing to ascertain the percentage of ash.

2.2.5. Hydroxymethylfurfural (HMF) Determination

The quantification of HMF involved weighing a 5 g mixture sample with a precision 0.01 mg and dissolving it in 100 mL ultrapure water. Following that, 2 mL of Carrez I —15 g potassium ferrocyanide (Merck, Darmstadt, Germany) was dissolved in pure water and water was added up to make 100 mL solution— and Carrez II — 30 g zinc acetate (Merck, Darmstadt, Germany) was dissolved in pure water and water was added to make 100 mL solution — reagents were added to the sample and the resulting solution was filtered through a 0.45 µm filter. After this, 2 mL of the sample solution were transferred to two separate test tubes and 5 mL of p-toluidine (Sigma-Aldrich, Munich, Germany) solution were added to each tube. Afterwards, 1 mL of barbituric acid (Sigma-Aldrich, Munich, Germany) was added to one tube (sample) and 1 mL of pure water was added to the other (blank), the tubes were thoroughly mixed. The absorbance values at 550 nm were measured using a spectrophotometer (SHIMADZU, UV-1900I, Japan). The obtained results were multiplied by the correction factor of 192 to calculate HMF (hydroxymethylfurfural) quantities in mg/kg (Güngör, 2007). The calculation formula for HMF content (Equation 1) is provided below.

$$HMF = A \times 192$$ (Equation 1)

2.2.6. Water Activity Determination

The water activity (a_w) of the mixture samples was measured using water activity determination device (Novasina Labmaster, 1119971, Switzerland) at room temperature.

2.2.7. Hunter Color Analysis (L, a, b)

The Hunter color values (L, a, b) of the homogenized mixtures were determined using color measurement device (Konica Minolta, CR-410, Japan). The values L (100: white, 0: black), a (+red, -green), and b (+yellow, -blue) were recorded.

2.2.8. Sugar Profile Analyses

The quantification of glucose, fructose, sucrose, maltose, and lactose in the mixture samples was performed using High-Performance Liquid Chromatography (HPLC) based on the DIN 10758 method. This method includes honey, jams, marmalades, molasses, confectionery, and fruit juices. 10000, 15000 and 20000 ppm standards of glucose, fructose and sucrose were prepared and injected into the HPLC device (SHIMADZU, Reservoir Tray, Japan), and the calibration curve was drawn. Then, 5 g of sample was weighed with a precision of 0.01 mg and dissolved in 40 mL of water. Following that, sample and 25 mL of methanol was taken into a volumetric flask and completed to 100 mL using water and the mixture were filtered through a 45 µm filter. Chromatographic conditions are given in Table 2.

Table 2. Chromatographic conditions for HPLC

Device	HPLC SHİMADZU, Reservoir Tray model
Mobile Phase	Water/Acetonitrile solution (20/80)
Detector	Agilent RID Detector, wavelength 284 nm
Column	Agilent Zorbax NH2 analytical column (4.6x250 mm, 5 µm)
Flow Rate	1.8 mL/min
Column Temperature	30 ºC
Injection Volume	20 µL

2.2.9. Antioxidant Assessment

The quantification of antioxidants in the samples was conducted employing the Ferric Reducing Antioxidant Power (FRAP) assay. The FRAP method relies on the reduction of the Fe(III)-TPTZ —2,4,6-tris(2-pyridly)-S-triazin— (Sigma-Aldrich, Munich, Germany) complex in the presence of antioxidants, forming the blue Fe(II)-TPTZ complex. The complex formed exhibits maximum absorbance in a spectrophotometer (SHIMADZU, UV-1900I, Japan) at 593 nm (Benzie IFF and Strain, 1996). A calibration curve was prepared using varying concentrations of $FeSO_4.7H2O$ (Sigma-Aldrich, Munich, Germany) (31.25-62.5-125-250-500-1000 µM) for calibration (Figure 3). A mixture of 3 mL of FRAP reagent—300 mM pH 3.6 acetate buffer (Merck, Darmstadt, Germany), a 10 mM TPTZ, and 20 mM $FeCl_3$ (Sigma-Aldrich, Munich, Germany) mixture in a ratio of 10:1:1— was combined with 100 µL of the sample. The results were compared against a standard $FeSO_4.7H_2O$, tested under the same conditions, and expressed as the µM $FeSO_4.7H_2O$ equivalent antioxidant power. Pipetting was performed as described in Table 3 (Can, 2014).

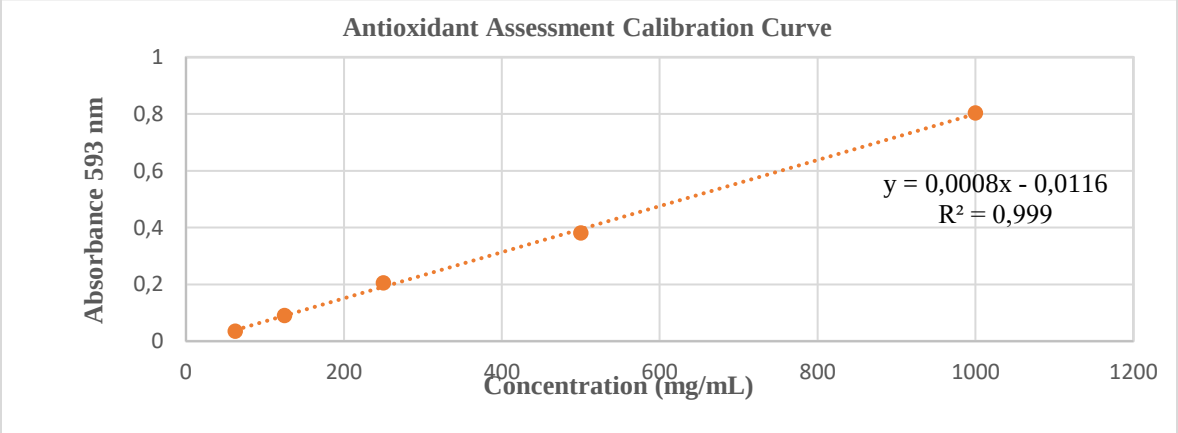

Figure 2. Antioxidant assessment calibration curve

Table 3. Pipetting procedure in FRAP determination.

	Blank MeOH	**Test (Sample)**	**Color Blank MeOH**	**$FeSO_4.7H_2O$**
FRAP Reagent	3 mL	3 mL	-	3 mL
Sample	-	100 µL	100 µL	-
$FeSO_4.7H_2O$(Variable Conc.)	-	-	-	100 µL
Methanol	100 µL	-	3 mL	-
In the 4th minute, absorbance is read at 593 nm.				

Color Blank MeOH: Color blank for the sample dissolved in methanol.

2.2.10. Total Polyphenol Analysis

The basis of the determination of total phenolic content relies on the redox reaction where phenolic compounds reduce the Folin-Ciocalteu (Sigma-Aldrich, Munich, Germany) reagent, an oxidative compound in a basic medium, converting them into their oxidized form. Following the reaction, the total amount of phenolic compounds in the sample is calculated by measuring the absorbance of the reduced reagent's resulting purple-blue color in a spectrophotometer (SHIMADZU, UV-1900I, Japan) at 760 nm. In the preparation of the standard curve, various concentrations of gallic acid (1; 0.5; 0.25; 0.125; 0.0625; 0.03125; and 0.015625 mg/mL) were utilized (Figure 3). The total polyphenol content was determined in terms of gallic acid (Sigma-Aldrich, Munich, Germany) equivalents (Slinkard, 1977; Singleton, 1999). The detailed procedures for the determination of total polyphenols are explained in Table 4.

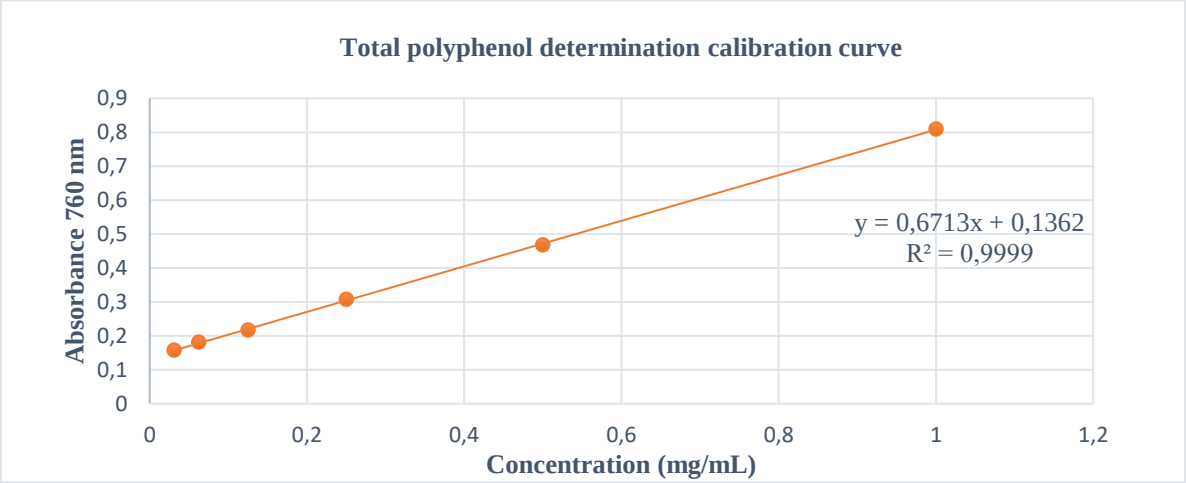

Figure 3. Total polyphenol determination calibration curve

Table 4. Pipetting procedure for total polyphenolic determination

	Blank	Standard	Sample
Distilled Water	700 µL	680 µL	680 µL
Standard (Various Conc.)	-	20 µL	-
Mixture Samples	-	-	20 µL
0,2 N Folin Reagent	400 µL	400 µL	400 µL
The tubes were mixed by vortex and after 3 minutes following chemical was added.			
%10 Na_2CO_3	400 µL	400 µL	400 µL
The absorbance was read against the blank at 760 nm.			

2.2.11. Aflatoxin Quantification (B1, B2, G1, G2)

To determine aflatoxin B1, B2, G1, and G2 in the mixture samples, samples were homogenized and prepared according to the AOAC 999.07 method (AOAC, 2007). For this purpose of 50 g of the sample, 5 g NaCl (Sigma-Aldrich, Munich, Germany), 100 mL deionized water, and 125 mL 70% methanol (Merck, Darmstadt, Germany), was stirred for 30 minutes at room temperature using a shaking extraction technique. Subsequently, the mixture was filtered first through filter paper and then through Whatman No. 4 paper before being analyzed using HPLC (SHIMADZU, Reservoir Tray, Japan). The chromatographic conditions
are shown in Table 5.

Table 5. Chromatographic HPLC conditions

Device	HPLC SHİMADZU brand, Reservoir Tray model
Mobile Phase	Water/Methanol/Acetonitrile (550/300/200)
Detector	Fluorescence Detector (360 nm- 440 nm)
Column Length	ODS-2 (C18 -250 mm-5µm- 4.6 mm)
Flow Rate	1 mL/min
Column Temperature	25 ºC
Injection Volume	100

2.2.12. Determination of Mineral Content

In the study, approximately 0.5 g of a homogeneous mixture was taken into a Teflon crucible, and 6 mL of pure HNO_3 (Sigma-Aldrich, Munich, Germany) and 3 mL of H_2O_2 (Sigma-Aldrich, Munich, Germany) were added. The samples were burned in a Milestone microwave oven, and the resulting ashes were diluted with distilled water to a volume of 25 mL. The mineral elements in the samples, including calcium (Ca), sodium (Na), phosphorus (P), potassium (K), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn), were determined using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Thermo ICAP, 7400, Japan) (Yıldız et al., 2009).

2.2.13. Determination of Titratable Acidity

The titratable acidity of samples were determined according to TS 1125 ISO 750.For this, 25 mL of the mixture samples were taken and diluted to 250 mL. A 50 mL aliquot of this solution was titrated with a

standardized 0.1 N NaOH (Merck, Darmstadt, Germany) solution using phenolphthalein as an indicator (Anonymous, 2002).

3. RESULTS AND DISCUSSION

Different ratios of honey (15%-78.5%) and propolis (1.5%-5%) were added to the mulberry molasses, creating 12 separate mixtures. Some physical and chemical properties related to propolis, honey, and mulberry molasses, along with the analysis results of these mixtures, are presented in Tables 6 and 7. The mineral analysis results for propolis, honey, mulberry molasses, and the 12 mixture samples are provided in Table 8.

Table 6. Analysis Results of Propolis, Honey and Mulberry Molasses

Composition Element	Honey	Propolis	Mulberry Molasses
pH Value	4.98 ± 0.2	6.61 ± 0.02	5.06 ± 0.03
Electrical Conductivity (mS /cm)	0.381 ± 0.006	1.079 ± 0.007	2.860 ± 0.01
Water Soluble Dy Matter (% Brix°)	78.7 ± 0.3	0.4 ± 0.003	71.8 ± 0.2
Ash (%)	0.3 ± 0.006	4.14 ± 0.01	1.99 ± 0.1
HMF Analysis (mg / kg)	18.4 ± 0.08	-	25.1 ± 0.5
Determination of Water Activity (a_w)	0.604 ± 0.006	-	0.703 ± 0.009
Hunter Color Analysis	L:18.8 ± 0.1 a:2.59 ± 0.03 b:3.6 ± 0.05	L:17.94 ± 0.05 a:1.91 ± 0.03 b:4.68 ± 0.06	L:16.4 ± 0.2 a:0.01 ± 0.001 b:0.75 ± 0.07
Glucose (%)	31.59 ± 0.07	-	30.85 ±0.05
Fructose (%)	36.12 ± 0.08	-	29.97 ± 0.04
Fructose / Glucose	1.14 ± 0.02	-	0.97 ± 0.03
Antioxidant (mg/ 100 g $FeSO_4$)	120.6 ± 1.2	180.5 ± 1.3	389.5 ± 1.7
Total Phenolic Substance (mg GAE /100g)	121.5 ± 1.4	97.83 ± 0.1	675.95 ± 1.9
Determination of Aflatoxin (B1.B2.G1.G2) (µg/kg)	0	0	0
Titratable Acidity (Citric acid equivalent) (g /100 mL)	0.5 ±0.03	-	0.308 ± 0.007

Table 7. Mineral Analysis Results of Propolis, Honey and Mulberry Molasses and 12 Mixture Samples

Mineral (mg/kg)	Honey	Propolis	Mulberry Molasses	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4
Ca	20 ± 1.2	38 ± 0.3	95.6 ± 0.03	93 ± 0.5	81.2 ± 0.5	64.1 ± 0.02	51 ± 0.4	82,2 ± 0.05	71.5 ± 0.1	68.1 ± 0.2	61.4 ± 0.03	74.2 ± 0.02	67.3 ± 0.03	62.1 ± 0.4	58.4 ± 0.07
Na	9.1 ± 0.7	6.2 ± 0.02	58.4 ± 0.02	56.8 ± 0.02	52.1 ± 0.06	48.4 ± 0.01	42.3 ± 0.7	48.7 ± 0.03	41 ± 0.4	38.2 ± 0.1	30.8 ± 0.02	45.7 ± 0.04	43.3 ± 0.05	38.4 ± 0.02	35.1 ± 0.3
K	4.1 ± 0.03	55.4 ± 0.04	432.9 ± 0.09	428.8 ± 0.7	422.2 ± 0.8	410.6 ± 0.07	402.8 ± 0.3	398.7 ± 0.5	381.3 ± 0.06	374.2 ± 0.6	355.4 ± 0.3	354.2 ± 0.06	336.1 ± 0.4	325.8 ± 0.5	312.5 ± 0.6
Mg	1.2 ± 0.02	1.5 ± 0.02	68.1 ± 0.1	67.3 ± 0.3	65.1 ± 0.3	61.8 ± 0.05	58.1 ± 0.07	65.7 ± 0.03	62.4 ± 0.2	59.4 ± 0.06	57.1 ± 0.4	58.1 ± 0.07	54.5 ± 0.2	48.3 ± 0.03	47.2 ± 0.05
Cu	0.25 ± 0.001	0.4 ± 0.03	4.32 ± 0.02	4.07 ± 0.04	3.45 ± 0.05	2.85 ± 0.3	2.02 ± 0.04	3.56 ± 0.04	3.21 ± 0.05	2.14 ± 0.01	1.87 ± 0.01	3.12 ± 0.003	3.01 ± 0.06	2.77 ± 0.01	2.64 ± 0.04
Fe	0.47 ± 0.004	0.65 ± 0.006	1.25 ± 0.09	1.02 ± 0.08	1 ± 0.08	1.01 ± 0.04	0.94 ± 0.2	1.01 ± 0.2	0.98 ± 0.08	0.95 ± 0.02	0.81 ± 0.03	0.94 ± 0.007	0.92 ± 0.02	0.81 ± 0.03	0.77 ± 0.08
Zn	0.14 ± 0.01	1.62 ± 0.007	1.2 ± 0.04	1.15 ± 0.04	1.07 ± 0.03	0.95 ± 0.08	0.56 ± 0.04	1.1 ± 0.3	0.87 ± 0.05	0.77 ± 0.01	0.69 ± 0.01	0.84 ± 0.02	0.71 ± 0.01	0.66 ± 0.05	0.54 ± 0.06
Mn	0.05 ± 0.008	0.57 ± 0.002	0.4 ± 0.01	0.4 ± 0.02	0.35 ± 0.04	0.21 ± 0.05	0	0.45 ± 0.07	0.43 ± 0.2	0.37 ± 0.01	0.34 ± 0.05	0.41 ± 0.04	0.38 ± 0.02	0.27 ± 0.07	0.22 ± 0.4
P	0.8 ± 0.01	7.1 ± 0.07	55 ± 0.6	42.2 ± 0.6	41.9 ± 0.06	41.3 ± 0.1	40.3 ± 0.3	41.7 ± 0.01	41.5 ± 0.3	40.5 ± 0.3	40.1 ± 0.02	41.4 ± 0.08	40.7 ± 0.1	40.2 ± 0.2	39.8 ± 0.1

3.1. pH Values of Mixture Samples

The pH values of the mixture samples examined in the study have a significant impact on the flavor and robustness properties of molasses. The samples of propolis, honey, and mulberry molasses, as well as the 12 different mixtures that were examined, had pH values ranging from 4.98 ± 0.2 to 6.61 ± 0.02. Based on the obtained data, the pH value of propolis was found to be 6.61 ± 0.02, while honey to be 4.98 ± 0.2, and mulberry molasses to be 5.06 ± 0.03 (Table 6). The product A1, whose pH value is 5.56 ± 0.04, was found to have the highest value among the prepared mixtures. In contrast, the mixture with the lowest value, C4, the 12th product, has a pH value of 5.33 ± 0.01 (Table 8).

Table 9. Average Analysis Results of Mixtures A (A1, A2, A3, A4), Mixtures B (B1, B2, B3, B4) and Mixtures C (C1, C2, C3, C4)

Composition Element	**A1**	**A2**	**A3**	**A4**	**B1**	**B2**	**B3**	**B4**	**C1**	**C2**	**C3**	**C4**
pH Value	5.56 ± 0.04	5.52 ± 0.02	5.46 ± 0.04	5.41 ± 0.1	5.53 ± 0.02	5.5 ± 0.03	5.44 ± 0.07	5.36 ± 0.06	5.54 ± 0.02	5.49 ± 0.03	5.4 ± 0.02	5.33 ± 0.01
Electrical Conductivity(mS /cm)	2.470 ± 0.02	9.26 ± 0.01	1.046 ± 0.01	1.163 ± 0.01	2.280 ± 0.003	0.596 ± 0.004	1.047 ± 0.3	2.276 ± 0.002	2.180 ± 0.003	1.162 ± 0.003	0.875 ± 0.004	0.469 ± 0.006
Water Soluble Dry Matter(% Brix^{o})	73 ± 0.3	74.5± 0.4	71.1 ± 0.6	77.5 ± 0.6	72.9 ± 0.3	74.5 ± 0.2	75.9 ± 0.1	77.4 ± 0.05	73 ± 0.4	74.6 ± 0.04	76 ± 0.6	77.8 ± 0.07
Ash (%)	1.68 ± 0.02	1.26 ± 0.01	0.84 ± 0.005	0.71 ± 0.001	1.57 ± 0.01	1.18 ± 0.003	0.76 ± 0.008	0.62 ± 0.03	1.69 ± 0.04	1.27 ± 0.06	0.85 ± 0.04	0.65 ± 0.03
HMF Analysis (mg / kg)	19.3 ± 0.2	20.7 ± 0.1	22.3 ± 0.1	24.2 ± 0.06	22.9 ± 0.03	23.8 ± 0.2	25.2 ± 0.3	27.9 ± 0.01	26.8 ± 0.05	27.1 ± 0.3	27.4 ± 0.05	28.5 ± 0.06
Determination of Water Activity (a_W)	0.693 ±0.005	0.675 ±0.006	0.657 ± 0.001	0.631 ± 0.004	0.694 ± 0.004	0.671 ± 0.006	0.654 ± 0.005	0.63 ± 0.09	0.691 ± 0.002	0.674 ± 0.005	0.651 ± 0.004	0.629 ± 0.007
Hunter Color Analysis L a b	L:16.53 ± 0.06 a:0.18 ± 0.04 b: 0.91 ± 0.07	L:16.8 ± 0.03 a:0.49 ± 0.07 b:1.21 ± 0.05	L:17.34 ± 0.09 a:0.8 ± 0.04 b:1.61 ± 0.03	L:18.1 ± 0.07 a:1.38 ± 0.02 b:2.13 ± 0.03	L:17.68 ± 0.04 a:0.23 ± 0.01 b:0.88 ± 0.03	L:17.63 ± 0.07 a:0.24 ± 0.02 b:0.79 ± 0.01	L:17.0 ± 0.04 a:0.29 ± 0.03 b:1.16 ± 0.06	L:17.7 ± 0.03 a:1.24 ± 0.2 b:2.02 ± 0.09	L:16.57 ± 0.01 a:0.09 ± 0.02 b:0.8 ± 0.07	L:16.59 ± 0.03 a:0.1 ± 0.02 b:0.86 ± 0.05	L:16.61 ± 0.02 a:0.01 ± 0.04 b:0.91 ± 0.02	L:17.33 ± 0.01 a:0.46 ± 0.03 b:1.62 ± 0.06
Glucose (%)	30.72 ± 0.07	30.86 ± 0.03	31.56 ± 0.06	31.83 ± 0.02	31.14 ± 0.08	31.46 ± 0.05	31.78 ± 0.02	31.82 ± 0.04	30.47 ± 0.006	30.57 ± 0.001	30.65 ± 0.05	30.79 ± 0.03
Fructose (%)	29.54 ± 0.04	30.26 ± 0.2	32.99 ± 0.2	34.46 ± 0.01	29.44 ± 0.05	31.24 ± 0.01	32.15 ± 0.04	33.53 ± 0.06	30.54 ± 0.005	32.87 ± 0.002	33.90 ± 0.002	35.57 ± 0.004
Fructose/Glucose	0.96 ± 0.04	0.98 ± 0.03	1.04 ± 0.02	1.08 ± 0.03	0.95 ± 0.003	0.99 ± 0.004	1.01 ± 0.05	1.05 ± 0.04	1.00 ± 0.2	1.08 ± 0.03	1.11 ± 0.02	1.16 ± 0.03
Antioxidant (mg/ 100 g FeSO4)	400.4 ± 1.0	392.6 ± 0.8	298.6 ± 0.02	264.2 ± 0.4	361.4 ± 0.1	303.5 ± 0.8	282.9 ± 0.7	203.1 ± 0.4	345.3 ± 0.04	275.1 ± 0.06	235.7 ± 0.2	222.0 ± 0.04
Total Phenolic Substance(mg GAE /100g)	184.3 ± 0.9	108.3 ± 0.07	128.7 ± 0.002	118.7 ± 0.08	156.9 ± 0.06	140.7 ± 0.06	127.1 ± 0.3	110.3	133.1 ± 0.02	131.1 ± 0.08	125.5 ± 0.03	111.1 ± 0.07
Aflatoxin (B1.B2.G1.G2) (µg/kg)	0	0	0	0	0	0	0	0	0	0	0	0
Titratable Acidity (Citricacid equivalent) (g /100 mL)	0.732 ± 0.005	0.738 ± 0.001	0.748 ± 0.007	0.752 ± 0.004	0.756 ± 0.004	0.762 ± 0.003	0.766 ± 0.002	0.775 ± 0.02	0.824 ± 0.003	0.835 ± 0.002	0.842 ±0.01	0.859 ± 0.005

The pH values ranged from 4.98 ± 0.2 to 6.61 ± 0.02 across the samples, indicating a slight acidic to neutral pH environment. Propolis exhibited the highest pH value, likely due to its weakly acidic nature, while honey and mulberry molasses displayed lower pH values, indicative of their acidic properties. These variations in pH values could influence the sensory attributes and stability of the final product.

According to TS 12001 Mulberry Molasses Standard, it is known that the pH value determined for mulberry molasses should be between 5.0 and 5.5 (Anonymous, 1996). In particular, the use of natural ingredients such as propolis and honey in the molasses production process may cause changes in the chemical composition of molasses. Therefore, it is thought that the pH fluctuations in the results are caused by natural ingredients such as propolis and honey in the product. These variations in pH values could influence the sensory attributes and stability of the final product.

3.2. Electrical Conductivity

Contrary to metals, electricity in food is carried by ions, not electrons, and a food's conductivity is directly correlated with its physicochemical characteristics, including pH, Brix value, protein, phenolic substance, organic acid, and mineral content (Lee et al., 2013).

The electrical conductivity measurement results for propolis, honey, and mulberry molasses samples, and 12 different mixtures based on analysis range from 0.38 ± 0.006 to 9.26 ± 0.01 mS / cm. These values are directly associated with food's physicochemical characteristics. The analysis results show that, out of 12 different mixtures, the A2 sample has the highest electrical conductivity (conductivity value: 9.26 ± 0.01 mS / cm), and the C4 product, has the lowest electrical conductivity (conductivity value: 0.496 ± 0.006 mS / cm) (Table 8).

The measurements ranged from 0.38 ± 0.006 to 9.26 0.01 mS/cm, indicating diverse conductivity levels across the samples. Product A2 exhibited the highest electrical conductivity, possibly due to its higher mineral content or ion concentration, while product C4 displayed the lowest conductivity. These differences in conductivity could be attributed to variations in the composition and concentration of the ingredients, affecting the overall quality and stability of the product.

3.3. Amount of Water-Soluble Dry Matter (Brix^{o} Values)

Within the scope of the research, samples of propolis, honey, and mulberry molasses as well as the 12 mixtures had water-soluble dry matter (WSS) ranging from 0.4 ± 0.003 to 78.7 ± 0.3 Brix^{o}. Based on the obtained data, the C4 product had the highest water-soluble dry matter quantity (77.8 ± 0.07

Brixº) out of 12 different mixtures, while the A3 product had the lowest water-soluble dry matter amount (71.1 ± 0.6 Brixº) (Table 8).

The range of Brixº values observed (0.4 ± 0.003 to 78.7 ± 0.3) suggests significant variability in the concentration of soluble solids across the samples. Product C4 exhibited the highest Brixº value, likely due to its higher honey concentration, which contributes to increased sweetness and viscosity. In contrast, product A3 displayed the lowest Brixº value, indicating lower soluble solid content. These differences in Brixº values could influence the taste, texture, and nutritional content of the final product.

3.4. Ash Content Determination

The total mineral components that constitute ash are present in varying and minute amounts in every fruit. Most of the mineral components present in the fruit have formed water-soluble salts with organic and inorganic acids. Consequently, many of them pass into fruit juice during processing (Cemeroğlu, 1982). Within the scope of research, it was found that samples of propolis, honey, and mulberry molasses and 12 different mixtures of these samples contained ash contents at rates varying between 0.3 ± 0.006 % and 4.14 ± 0.01 % (Table 6 and Table 8). Furthermore, Table 8 revealed that the product with the highest ash amount, C1, had 1.69 ± 0.04 %, while the product with the lowest ash amount, B4, had 0.62 ± 0.03%. The observed ash content ranged from 0.3±0.006% to 4.14 ± 0.01%, indicating variations in mineral content across the samples. Product C1 exhibited the highest ash content, possibly due to its higher concentration of mineral-rich ingredients, while product B4 displayed the lowest ash content. It is thought that this increase in the amount of ash is due to mulberry molasses.

3.5. HMF (5-Hydroxymethylfurfural) Analysis

It isstated that heat treatment or long-term storage under inappropriate conditions generally causes an increase in the amount of HMF (5-hydroxymethylfurfural). In addition, it is known that high amounts of HMF exposure is cytotoxic and has irritation effect, and there are many studies supporting that HMF is genotoxic and has mutagenic and carcinogenic effects (Capuano and Fogliano, 2011).

Within the scope of this study, HMF results of the mixtures show values ranging between 18.4 ± 0.08 and 28.5 ± 0.06 mg/kg. According to the results obtained, the C4 product had the greatest HMF value, measuring 28.5 ± 0.06, while the A1 product had the lowest value, measuring 19.3 ± 0.2 (Table 8).

The observed HMF values ranged from 18.4 ± 0.08 to 28.5 ± 0.06 mg/kg, indicating varying degrees of heat exposure and potential risks associated with HMF formation. Product C4 exhibited the highest HMF value, suggesting prolonged heat exposure or inadequate storage conditions, while product A1 displayed the lowest HMF value. However, in this study, no evaluation was made on how storage conditions and duration affect the HMF content.

The study by Bozkurt and his team (1998) examined how HMF amounts changed when molasses samples prepared at different concentrations and pH levels were exposed to heat. This study revealed that the occurrence time of the browning reaction varies depending on various factors, and as condensation increases and the pH level decreases, the adaptation period decreases. Therefore, it was concluded that the HMF formation rate increased. It was determined that the browning reactions in molasses production played a critical role in the formation of color and taste, but it was emphasized that the formation of intermediate products such as HMF should be kept under control. Therefore, it is important to consider the impact of storage conditions on HMF formation and manage it appropriately.

3.6. Determination of Water Activity (Aw)

The amount of free water available for chemical reactions and microbiological development inside the food matrix is measured and defined as water activity. Water is the primary necessity for microorganisms to sustain their activity. In general, bacteria function at greater a_w values than molds and yeasts. Water activity is therefore a crucial factor to consider when predicting the microbiological and chemical deterioration of food during preparation and storage (Jay, Loessner and Golden, 2008). Generally, it is known that yeasts and molds operate at lower water activity values (range 0.61-0.88) than bacteria (>0.90) (Özbey et al., 2013).

Water activity values ranged from 0.604 ± 0.006 to 0.703 ± 0.009 among 12 different mixtures and samples of propolis, honey, and mulberry molasses, according to the research (Tables 6 and 8). B1 had the highest water activity among the mixtures, with 0.694 ± 0.004, while C4 had the lowest water activity, with 0.629 ± 0.007.

The observed water activity values ranged from 0.604 ± 0.006 to 0.703 ± 0.009, indicating differences in moisture content and potential for microbial growth across the samples. Product B1 exhibited the highest water activity, suggesting a higher risk of microbial spoilage, while product C4 displayed the lowest water activity, indicating better stability and shelf-life.

In addition, in the study conducted by Salik and his team (2021), it was determined that water activity values in mulberry molasses samples varied between 0.59 and 0.75. The values are compatible in the research.

3.7. Color Analysis (Hunter) Test

Color is an important parameter in foods. In the Hunter Lab color model, L represents lightness with values ranging from 0 to 100, a denotes the green-red axis with negative values indicating green and positive values indicating red, and b represents the blue-yellow axis with negative values indicating blue and positive values indicating yellow, collectively providing a comprehensive description of color appearance for precise measurement and analysis (Dobrzansk and Rybcyzynsk, 2002).

In this research, the color analysis of 12 different mixtures and samples of propolis, honey, and mulberry molasses were performed to determine their color parameters (L, a, and b). L values range from 18.8 ± 0.1 to 16.4 ± 0.2, a value from 2.59 ± 0.03 to 0.01, and b value from 4.68 ± 0.06 to 0.75 ± 0.07 (Tables 6 and 8). It was concluded that there was no discernible color change when the honey content increased. This demonstrates that switching to a specific honey ratio has no significant impact on color change.

3.8. Determination of Sugar Content

When the sugar profiles of these products are examined, it is seen that the fructose/glucose ratio is close to 1.0. Within the scope of this study, it was found that the Fructose / Glucose ratio was highest in C4 (1.16 ± 0.03) and lowest in B1 (0.95 ± 0.003) among 12 different mixtures, propolis, honey, and mulberry molasses (Table 8).

Product C4 had the highest sugar content among the 12 different mixtures examined. This feature can be considered as a suitable food alternative for consumption in case of fast and high energy requirements (Kolayli et.,2013; Ischayek and Kern,2006).

3.9. Determination of Antioxidants

Fruits, particularly berries and vegetables are rich sources of antioxidants, which are phenolic compounds with antimutagenic and anticarcinogenic qualities (Güngör, 2007). Tables 6 and 8 show that the mixture samples tested have antioxidant activity ranging from 120.6 ± 1.2 to 400.4 ± 1.0 mg / 100 g $FeSO_4$. It was found that, with 400.4 ± 1.0 mg/100 g $FeSO_4$, A1 had the highest antioxidant content, while B4 had the lowest, with 203.1 ± 0.4 mg/100 g $FeSO_4$ (Table 8). An increase in the

amount of antioxidants has been observed with the increase in propolis in Mulberry Molasses (Zab, 2021; Szajdek,2008). It can be considered that these products can be used as food additives.

3.10. Total Phenolic Substance

It was discovered that the total amount of phenolic compounds varied between 108.3 ± 0.07 and 184.3 ± 0.9 mg GAE /100 g among 12 different mixtures and the samples of propolis, honey, and mulberry molasses. Out of 12 distinct mixtures, product A1 had the highest amount of phenolic compounds (184.3 ± 0.9 mg GAE /100 g), while A2 had the lowest amount (108.3 ± 0.07 mg GAE /100 g) (Table 6 and Table 8). The mixture samples contain significant amounts of phenolic substances.

It was observed that phenolic substance increased with the increase in the amount of propolis, but decreased with the increase in honey (Saroğlu, Bayram and Özçelik.,2023).Among 12 different mixtures, the presence of 184.3 ± 0.9 mg GAE /100 g phenolic substance in product A1 shows that this product can be used as a food supplement.

3.11. Mineral Analysis

Minerals are essential food components that the body needs to consume on a regular basis. They serve a variety of purposes in the body, including structural support, influencing, and balancing physiological processes, and supporting the neurological and muscular systems. Sodium, potassium, calcium, and magnesium are known as macro minerals, and copper, iron, zinc, and manganese are known as micro minerals (Güngör, 2007).

Table 7 lists the mineral contents of 12 distinct mixtures and the samples of propolis, honey, and mulberry molasses. The results of the analysis show that mulberry molasses have a higher mineral content than other samples, with product A1 having the highest value out of 12 distinct mixtures.
It contains elements such as potassium, calcium, sodium, iron, phosphate, copper, and zinc, which are the richest minerals in the A1 product. In case of deficiency of these minerals, people may be advised to choose this food.

3.12. Determination of Total Acidity Content

Fruit varieties and types can have a different flavor, which is influenced by the acidity/sugar content of their structural makeup (Güngör, 2007). In the study, propolis, honey, mulberry molasses samples, and 12 distinct mixtures had total acidity levels ranging from 0.308 ± 0.007 to 0.859 ± 0.005 g /100 mL (Table 6 and Table 8). Based on the obtained data, product C4 had the highest total acidity (0.859

± 0.005 g/100 mL), while product A1 had the lowest total acidity (0.732 ± 0.005 g/100 mL) (Table 8). It is thought that there is no relationship between acidity value and pH in propolis, honey and mulberry molasses mixtures and the acidity in the products arises from the natural acidity coming from the mulberry fruit (Koyuncu,2004; Bozhüyük,2015; Krishna et al.,2020).

3.13. Aflatoxin Analysis

Aflatoxins, harmful toxins produced by certain fungi, were analyzed in the mixture samples. In the study, 12 different mixtures and samples of propolis, honey, and mulberry molasses were examined; no aflatoxin residue was discovered (Tables 6 and 8). These results assure the safety and quality of the mixtures, indicating compliance with regulatory standards and consumer safety requirements (Official gazette,2011).

4. CONCLUSION

In this research, mulberry molasses -which has a high nutritional value and carbohydratecontent- was combined with natural items that have rich vitamin and mineral content, like propolisand honey, to create a new food additive. Through their synergistic effects, these additives increasethe nutritional value while also contributing to the provision of bioavailability and bioactive substances. When propolis was added to mulberry molasses, there was a noticeable increase in its antioxidant capacity. Furthermore, an essential substitute for the recommended daily intake of potassium is provided by the observed rise in potassium (K) content of mixtures. It is believed thatthese mixture samples might be advised to be consumed, particularly when there is an increased requirement for minerals. Mulberry molasses have been given a more palatable taste and flavor profile with the addition of varying amounts of honey. Moreover, the product gains antibacterial, antioxidant, anti-inflammatory, and immune system-supporting qualities from the addition of propolis. It is advised to conduct more research on the different additive alternatives in light of these findings. For sensory analysis testing, products also need to be assessed by sensory analysts.Finally, it is believed that the products of mixing propolis and honey with mulberry molasses can be utilized as useful food additives or natural antioxidant.

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