GIDA
THE JOURNAL OF FOOD
E-ISSN 1309-6273, ISSN 1300-3070

Research / Araştırma
GIDA (2024) 49 (6) 1038-1061
doi: 10.15237/gida.GD24071

BIOCHEMICAL COMPOSITION AND DETERMINATION OF ADULTERATION OF BLACK MULBERRY EXTRACTS

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Received/Geliş: 18.07.2024; *Accepted*/Kabul: 17.10.2024; *Published online*/Online baskı: 21.10.2024

Şirin, Y., Erdem, B., Cengiz, S., Gürkan, S., Gürkan, P. (2024) Biochemical composition and determination of adulteration of black mulberry extracts. GIDA (2024) 49 (6) 1038-1061 doi: 10.15237/ gida.GD24071

Şirin, Y., Erdem, B., Cengiz, S., Gürkan, S., Gürkan, P. (2024) Karadut özlerinin biyokimyasal bileşimi ve tağşişin belirlenmesi. GIDA (2024) 49 (6) 1038-1061 doi: 10.15237/ gida.GD24071

ABSTRACT

Black mulberry is crucial for its high content of bioactive substances, such as phenolic compounds and vitamin C, as well as nutritional values, such as carbohydrates, fats, fibre and minerals. This study aimed to determine the physical, biochemical and nutritional properties of 35 different brands of black mulberry extracts sold in our country. Consequently of the studies, it was observed that some of the extracts sold in our country had a very high HMF value; most of them contained no protein and additional maltose and derivatives. In addition, all of these black mulberry extracts were low in minerals and contained additional sweeteners, preservatives and synthetic colourings. Synthetic colourings were found in many of these black mulberry extracts and carbon 13 levels were not appropriate. When BME-34 and 35 brands of black mulberry extract were analysed, all of the above values were appropriate, and consistent with previous studies in the literature.

Keywords: Black mulberry, fruit extract, food

KARADUT ÖZLERİNİN BİYOKİMYASAL BİLEŞİMİ VE TAĞŞİŞİN BELİRLENMESİ

ÖZ

Karadut, içeriğindeki yüksek miktarda fenolik bileşikler ve C vitamini gibi biyoaktif maddelerin yanı sıra karbohidrat, yağ, lif, mineral gibi besleyici değerlerden dolayı önemlidir. Bu çalışmada ülkemizde satışı yapılan 35 farklı marka karadut özlerinin fiziksel, biyokimyasal ve besinsel özelliklerinin belirlemesi amaçlanmıştır. Yapılan çalışmalar sonucunda ülkemizde satışı yapılan karadut özlerinin bazılarında HMF değerinin çok yüksek olduğu, birçoğunun protein içermediği ve ilave maltoz ve türevlerini içerdiği görülmüştür. Ayrıca bu karadut özlerinin tamamının mineralce fakir olduğu, ilave tatlandırıcı, koruyucu ve sentetik boya içerdiği tespit edilmiştir. Bu karadut özlerinin birçoğunda sentetik boyaya rastlanmış ve karbon 13 değerlerinin de uygun olmadığı görülmüştür. BME-34 ve 35

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marka karadut özü incelendiğinde ise yukarıda bahsedilen tüm değerlerin uygun olduğu ve literatürle de benzer sonuçlar verdiği görülmüştür. Sonuç olarak ülkemizde karadut özü olarak satılan ürünlerin hileli bir ürün olduğu tespit edilmiştir.
Anahtar kelimeler: Karadut, meyve özü, gıda

INTRODUCTION

Recently, there has been a marked increase in the demand for fresh fruits. This surge can be attributed to a combination of factors, including changes in production methodologies, innovative marketing approaches, and the effective utilization of social media platforms to emphasize the rich presence of bioactive compounds in fruits (Skrovankova et al. 2015). Fruits, a significant source of nutrients, play a pivotal role in human health and are integral to a balanced diet. They are primary provitamin A and vitamin C sources and are abundant in phytochemicals and bioactive constituents. These components have demonstrated potential therapeutic effects against cardiovascular diseases and may also mitigate cancer risks (Serçe et al. 2010). Furthermore, fruits contribute dietary fiber and essential minerals to an adult diet while offering a diverse range of flavors and colors. The presence of these attributes and the concentration of bioactive compounds in fruits are significantly influenced by their genotypes and the environmental conditions under which they are grown (Skrovankova et al. 2022).

Specifically, berries have garnered substantial attention in nutritional science, earning the title 'super-food' due to their positive impact on human health (Kalt et al. 2017). This is primarily due to their high content of anthocyanins and other phenolic compounds. Berry fruits are typically consumed in their fresh state but are also available in frozen and processed forms (Okatan and Çolak 2019).

The black mulberry (*Morus nigra*), a member of the Moraceae family within the *Morus genus*, is cultivated for its fruit, leaves, and wood. Widely distributed across Asia, Europe, and America (Özgen et al. 2019), it shares prominence with two other well-researched mulberry species: white mulberry (*Morus alba* L.) and red mulberry (*Morus rubra* L.). Black mulberry fruits are flavorful and rich in nutritional value. Their high content of vitamins, minerals, and antioxidants makes them desirable for human consumption (Dalmagro et al. 2019; Shekarabi et al. 2020).

In regions, such as India, China, and Japan, black mulberry is primarily grown for its leaves, which play a crucial role in silkworm breeding. Silkworms (*Bombyx mori*) exclusively feed on mulberry leaves, which provide essential nutrients for their growth and silk production. The genetic basis for this feeding preference involves the GR66 gene, which influences silkworms' affinity for mulberry leaves (Zhang et al. 2018; Vijayan et al. 1997). In contrast, European countries like Türkiye and Greece focus on cultivating black mulberry for its fruit (Gerasopoulos and Stavroulakis 1997; Ercisli 2005). Notably, Anatolia is renowned for high-quality black mulberry cultivation (Erarslan et al. 2021), with Türkiye leading in production, especially in the Middle Eastern Anatolia and Black Sea regions (Toğrul and Hayoğlu 2020).

Black mulberry fruits, which are approximately 2.5 cm in length, mature from a reddish hue to a deep black color, resembling raspberries. Harvested during July and August (3), these fruits are versatile in consumption, being enjoyed fresh or processed into dried fruit, molasses, and juice (Kutlu et al. 2011). Their palatable taste has led to their extensive use in jams, liqueurs, and natural dyes, and they are also valued in the cosmetic industry due to their beneficial properties (Memete et al. 2023; Nguyen et al. 2022).

Among fruits and vegetables, black mulberries are recognized as one of the richest sources of antioxidants (Zhang et al. 2018). Specifically, they contain high concentrations of phenolic compounds, including anthocyanins, flavonoids, and other polyphenols, contributing to their strong antioxidant properties (Liu and Zhang 2024). Anthocyanins, glycosides formed by the attachment of sugar groups to anthocyanidins, are categorized into two subgroups: sugar-free

anthocyanidin aglycones and sugar-containing anthocyanin glycosides. Over 500 distinct anthocyanins have been identified, with the six most prevalent anthocyanidins in fruits and vegetables being cyanidin (50%), delphinidin (12%), pelargonidin (12%), peonidin (12%), petunidin (7%), and malvidin (7%). The 3-glycoside, 3,5- and 3,7-diglycoside derivatives of anthocyanidins are more abundant, and 3-glycoside derivatives are 2.5 times more abundant than 3,5-diglycosides. The most common anthocyanin in nature is cyanidin-3-glycoside (Castañeda-Ovando et al. 2009).

Anthocyanins, responsible for the vibrant colors observed in many fruits and vegetables, play a significant role in preventing or treating health conditions associated with inflammation and oxidative stress. Anthocyanins have been recognized for their potential therapeutic applications against various diseases, including oral and dental diseases, hypertension, diabetes, anemia, and cancer (Suh et al. 2003; Mahesh et al., 2017; Naseri et al. 2018; Ahmed et al. 2023).

These compounds exhibit a fascinating color variability that depends on their concentration, pH levels, and the presence or absence of co-pigments (Sendri and Bhandari 2024; Guo et al., 2023). In acidic environments, anthocyanins appear red; in neutral environments, they turn purple; and in basic environments, they range from blue-green to violet (Espín et al. 2000; Ananga et al., 2013). These pigments are used as natural colorants in food products due to their stability and health benefits (Katsube et al. 2006; Palonen and Weber 2019; Svanberg et al. 2019).

The antioxidant content of black mulberries is influenced by several factors, including soil composition, geographical location, and growing conditions (Kostić et al. 2019; Pruteanu et al. 2023). For instance, soil rich in humic substances can enhance the transport of nutrients and water, redox reactions, chelate formation, and the secretion of various compounds (i.e., organic acids, sugars, phenols, and amino acids) by plant roots (Rodrigues de Queiroz et al., 2023). Black mulberries contain significant amounts of anthocyanins, such as cyanidin-3-glycoside, cyanidin-3-rutinoside, pelargonidin-3-glycoside, and pelargonidin-3-rutinoside, which have shown various health benefits, including inhibitory effects on cancer cells (Chen et al. 2006). Cyanidin, a type of anthocyanin, has been suggested to form a complex with DNA, providing a protective effect. The formation of this complex is believed to shield both cyanidin and DNA from oxidative damage, particularly from the attack of hydroxyl radicals (Sarma et al. 1999; Mattioli et al. 2020). This protective mechanism underscores the potential of anthocyanins in mitigating oxidative stress-related damage.

Black mulberries are packed with essential nutrients, including vitamins C and K, iron, potassium, and dietary fiber, supporting overall health, including boosting immunity, aiding digestion, and promoting proper blood clotting. They have shown potential in reducing cholesterol, blood sugar levels, and cancer risk due to their phenolic compounds, organic acids, and sugar content (Naeem 2020; Gundogdu et al. 2011).

In Türkiye, a significant portion of black mulberries is used to produce molasses (70%) (Aybastier 2021). Recently, the industrial production of black mulberry juice has increased, offering more opportunities for its use in various products (Polat 2004). However, the commercial use of anthocyanins is limited by their low extraction yields and susceptibility to degradation by heat, light, oxygen, and pH changes during processing and storage (Cavalcanti et al. 2011).

Modern developments in the food industry, such as the cold-press method, have enhanced the preservation of nutrients in fruit products. This method, which does not use chemicals or heat, retains the nutritional content of the fruit (Rabrenović et al. 2014). Cold-pressed fruit musts, which have high nutritional value, are increasingly popular due to their sensory properties and health benefits (Kaplan 2022).

The rising demand for black mulberry products has also led to increased incidences of adulteration. Common adulterants include preservatives like sorbic acid and benzoic acid, synthetic dyes, and artificial sweeteners, such as Acesulfame potassium, Saccharin, Aspartame, and Sucralose. These substances can significantly alter the nutritional profile and safety of black mulberry products, raising concerns about their health implications.

Given the rising demand for black mulberry products, the incidence of adulteration has increased, with many products containing synthetic additives. This study aims to analyze the physical-chemical, nutritional, and antioxidant properties of black mulberry extracts from various companies and to identify any adulteration. By examining 35 different brands and analyzing each sample in triplicate, we seek to provide insights and raise public awareness about the quality and authenticity of black mulberry extracts, ensuring consumers have access to genuine and beneficial products. This study also includes an evaluation of BME-34 Black Mulberry Extract and BME-35 Black Mulberry Mix, products by Sem-As Food, to ensure a comprehensive market understanding. The companies producing the extracts are coded and discussed in accordance with ethical guidelines.

MATERIALS and METHODS

Materials

This study undertook the examination of 35 distinct brands of black mulberry extracts available in the Turkish market, with the objective of assessing their physical, biochemical, and nutritional properties to determine their naturalness. To facilitate this, three samples from each of the 35 brands were procured from the market between March 1, 2024, and March 31, 2024, and preserved under standardized conditions pending analysis. For each brand of black mulberry extract, three samples were taken, and each sample was tested in triplicate. Each example was examined with three repetitions. Furthermore, the physical, biochemical, and nutritional characteristics of the black mulberry extracts produced by SEM-AS Gıda Sanayi Ticaret Ltd. Şti. were ascertained for comparative purposes with the other extracts. The black mulberry extracts from all brands were assigned codes to maintain ethical standards. All figures and tables included in the text are provided in the appendix.

Methods

pH Value

The pH values of each sample were determined using a pH meter (Hanna, HI 2020, US) in accordance with the TS 1728 ISO 1842 standard. Measurements were conducted at a controlled temperature of 20°C to ensure accuracy and consistency. Each sample was measured in triplicate to ensure the reliability and reproducibility of the results.

Electrical Conductivity

The electrical conductivity of each sample was measured using a conductivity meter (Ohaus, St300c, US). Measurements were performed at a constant temperature of 20°C. To ensure the precision of the readings, samples were diluted with deionized ultra-pure water (Millipore, Synergy, Germany), which has a conductivity of 0.0006 Ms/cm. Each sample was measured in triplicate to confirm the consistency and accuracy of the data.

Determination of Soluble Solids Content (Brix°)

The soluble solid content of the samples was measured using a tabletop manual refractometer (Refrakto Abbe, Ertich, Abbe-2, Germany). The results were expressed in degrees Brix (°), with the final values reported as percentages of Brix°. Each measurement was conducted in triplicate to ensure the dependability and precision of the results.

Determination of viscosity

The viscosity of the black mulberry extracts was measured using a First Plus Viscometer. The measurements were conducted with an R-4 spindle at a speed of 200 rpm for 60 seconds.

Ash Determination

The determination of ash content was conducted using the gravimetric method, as described by

Czaja (2020). A precision scale (RADWAG, AS220.R2, Poland) was employed for the analysis. Approximately 2 g of each sample, measured with an accuracy of 0.01 mg, were placed in porcelain crucibles that had been brought to a constant weight. The samples were first incinerated using a Bunsen burner flame and then heated in a muffle furnace at 550ºC until they turned light gray-white. The samples were then weighed, and the ash content was determined as a percentage. Each sample was analyzed in triplicate to ensure the accuracy and reproducibility of the results.

Determination of Density

In this study, the densities of the black mulberry extracts were determined using a pycnometer, a standard instrument for such measurements.

Hydroxymethylfurfural (HMF) Determination

The quantification of HMF was performed by weighing a 5 g sample from each sample with a precision of 0.01 mg and dissolving it in 100 mL of ultrapure water. Following this, 2 mL of Carrez I solution (15 g potassium ferrocyanide dissolved in pure water and diluted to 100 mL) and Carrez II solution (30 g zinc acetate dissolved in pure water and diluted to 100 mL) were added to the sample. The resulting solution was filtered through a 0.45 μm filter. Subsequently, 2 mL of the filtrate was transferred to two separate test tubes. To each tube, 5 mL of p-toluidine solution (Sigma-Aldrich, Munich, Germany) was added. Then, 1 mL of barbituric acid solution (Sigma-Aldrich, Munich, Germany) was added to one tube (sample) and 1 mL of pure water to the other (blank). The tubes were thoroughly mixed, and the absorbance values at 550 nm were measured using a spectrophotometer (SHIMADZU, UV-1900I, Japan). The results were multiplied by a correction factor of 192 to calculate HMF content in mg/kg (Güngör 2007). Each sample was measured in triplicate to ensure reliability.

$$HMF = A \times 192 \quad \text{(Equation 1)}$$

Water Activity Determination

The water activity (a_w) of the samples was measured using a water activity meter (Novasina Labmaster, 1119971, Switzerland) at room temperature. Each sample was measured in triplicate to guarantee the accuracy and consistency of the results.

Determination of Protein

The protein content in black mulberry was determined using the Kjeldahl method by measuring nitrogenous compounds and multiplying by a factor of 6.25, as recommended by Bremler (1965). The results were expressed as a percentage (Koyuncu et al. 2014).

Hunter Color Analysis (L, a*, b*)*

The Hunter color values (L*, a*, b*) of the samples were determined using a color measurement device (Konica Minolta, CR-410, Japan). The L* value represents lightness (100: white, 0: black), the a* value represents the red-green spectrum (+a*: red, -a*: green), and the b* value represents the yellow-blue spectrum (+b*: yellow, -b*: blue). Each measurement was conducted in triplicate to ensure precision and reproducibility.

Determination of Titratable Acidity

The titratable acidity of the samples was assessed following the guidelines outlined in TS 1125 ISO 750. Initially, a 25 mL aliquot of each sample was appropriately diluted to a final volume of 250 mL. Subsequently, a 50 mL portion of this diluted solution underwent titration using a standardized 0,1 N sodium hydroxide (NaOH) solution (Merck, Darmstadt, Germany) with phenolphthalein employed as the indicator (Işık and Çelik 2023).

Sugar Profile Analyses

Quantitative analysis of glucose, fructose, sucrose, maltose, and lactose within the samples was performed using high-performance liquid chromatography (HPLC) in accordance with the DIN 10758 methodology. This methodology is universally applicable across diverse food matrices, such as honey, jams, marmalades, molasses, confectionery, and fruit juices. Calibration standards encompassing glucose, fructose, and sucrose at concentrations of 10000, 15000, and 20000 ppm were meticulously prepared and injected into the HPLC system (SHIMADZU, Reservoir Tray, Japan) to

construct a robust calibration curve. Following precise weighing (to 0.01 mg) of 5 g of each sample, dissolution in 40 mL of water ensued. This solution, along with 25 mL of methanol, were quantitatively transferred to a volumetric flask and made up to a final volume of 100 mL with water. Before HPLC analysis, the resultant mixture underwent filtration through a 0.45 μm filter. Detailed chromatographic parameters are delineated in Table 1.

Table 1. Chromatographic conditions for HPLC

Device	HPLC SHIMADZU, 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

Determination of Sorbic acid and Benzoic acid

This investigation employed high-performance liquid chromatography (HPLC) to perform quantitative analysis of benzoic acid and sorbic acid. The sample preparation adhered to the protocol outlined by the Nordic Committee on Food Analysis, specifically the method titled "Liquid Chromatographic Determination of Benzoic Acid, Sorbic Acid, and p-Hydroxybenzoic Acid Esters in Foods" (Karataşlı et al. 2026).

The chromatographic separation of benzoic acid and sorbic acid was facilitated using a C18 column (Agilent, 250 mm length, 4.6 mm outer diameter, inclusive of 5 μm silica particles). The mobile phase was a mixture of an acetate buffer (pH=4.74) and methanol in a 65:35 ratio. The column was conditioned before the analysis by passing the mobile phase through it at a flow rate of 1 ml/min for approximately 40 minutes. The flow rate of the mobile phase was consistently maintained at 1 ml/min throughout the chromatographic separation process. The detection of benzoic acid and sorbic acid was performed at a wavelength of 250 nm.

Determination of Synthetic paint

In this study, the presence of synthetic colorants, which are frequently incorporated into food substances during the manufacturing process, was verified in blackberry extracts. This verification was based on the NMKL-130 method and utilized an HPLC device for the analysis.

Antioxidant Assessment

The antioxidant capacity of the samples was quantified utilizing the Ferric Reducing Antioxidant Power (FRAP) assay. The FRAP methodology is predicated on the reduction of the Fe(III)-TPTZ complex-2,4,6-tris(2-pyridyl)-S-triazine- (Sigma-Aldrich, Munich, Germany) in the presence of antioxidants, leading to the formation of the blue Fe(II)-TPTZ complex. This complex exhibits peak absorbance at 593 nm when measured using a spectrophotometer (SHIMADZU, UV-1900I, Japan) (Benzie and Strain 1996).

A calibration curve was established using a range of concentrations of $FeSO_4.7H_2O$ (Sigma-Aldrich, Munich, Germany) (31,25/62,5/125/250/500/1000 μM) (Figure 1). The FRAP reagent, a mixture of 300 mM pH 3.6 acetate buffer (Merck, Darmstadt, Germany), 10 mM TPTZ, and 20 mM $FeCl_3$ (Sigma-Aldrich, Munich, Germany) in a 10:1:1 ratio, was prepared. A volume of 3 mL of this reagent was combined with 100 μL of the sample.

The results were benchmarked against a standard $FeSO_4.7H_2O$, tested under identical conditions and expressed as the μM $FeSO_4.7H_2O$ equivalent antioxidant power. The pipetting procedure was executed as delineated in Table 2 (Can et al. 2015).

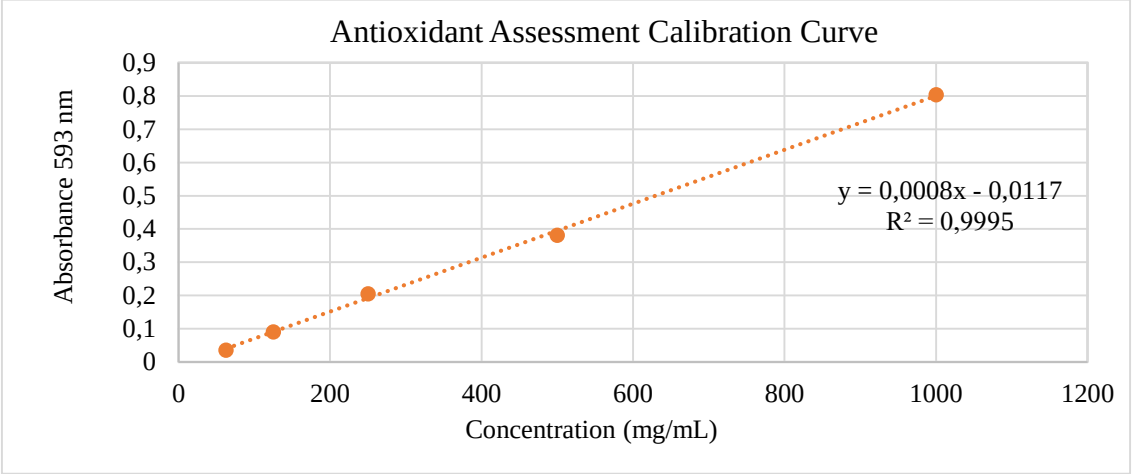

Figure 1. Antioxidant assessment calibration curve

Table 2. 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.

Total Polyphenol Analysis

The quantification of total phenolic content is predicated on a redox reaction, wherein phenolic compounds reduce the Folin-Ciocalteu reagent (Sigma-Aldrich, Munich, Germany), an oxidative compound in a basic medium, thereby transforming them into their oxidized forms. Subsequent to this reaction, the total quantity of phenolic compounds present in the sample is ascertained by measuring the absorbance of the resultant purple-blue color of the reduced reagent using a spectrophotometer (SHIMADZU, UV-1900I, Japan) at 760 nm.

For the construction of the standard curve, a range of concentrations of gallic acid (1, 0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.015625 mg/mL) were employed (Figure 2). The total polyphenol content was quantified in terms of gallic acid (Sigma-Aldrich, Munich, Germany) equivalents (Slinkard and Singleton 1977). The comprehensive procedures for the quantification of total polyphenols are delineated in Table 3.

Table 3. 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 three minutes, the following chemical was added.			
%10 Na_2CO_3	400 µL	400 µL	400 µL

The absorbance was read against the blank at 760 nm.

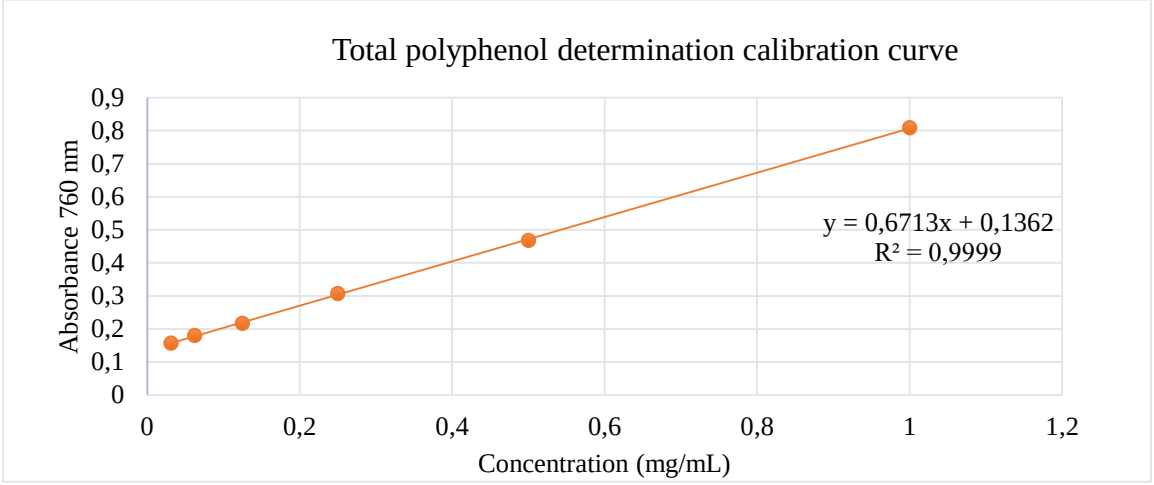

Figure 2. Total polyphenol determination calibration curve

DPPH• Radical Scavenging Activity Analysis

The DPPH• (2,2-diphenyl-1-picrylhydrazyl) radical, a synthetic free radical commercially available, is utilized as a model for quantifying antioxidant activity. The radical form, which exhibits an absorbance at 517 nm, undergoes a color change upon reduction in the presence of an antioxidant agent (Table 4). The efficacy of DPPH radical scavenging was quantified in terms of SC_{50}, a parameter defined as the concentration of the antioxidant agent required to reduce (scavenge) 50% of the radical (Cuendet et al. 1997). A graphical representation was constructed with concentration plotted against absorbance, facilitating the calculation of the SC_{50} value in mg/ml.

Table 4. Pipetting procedure in DPPH determination.

	Blank	Color Blank	Sample
Sample (Black Mulberry Extract)	-	750 µL	750 µL
Methanol	750 µL	750 µL	-
DPPH•	750 µL	-	750 µL
In the 40 minutes, absorbance is read at 517 nm.			

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

Total Flavonoid Substance Analysis

Flavonoids, representing the most extensive subclass within the polyphenol family, are notable bioactive constituents of honey. The total flavonoid content in honey was quantified employing the spectrophotometric method, specifically the aluminum chloride method (Fukumoto and Mazza 2000). The absorbance of the colored complex, formed due to the redox reaction between the flavonoids present in honey and aluminum (III), was measured at 415 nm. A standard working graph or calibration graph was established using a Quercetin standard (1 mg/ml). Working solutions of varying concentrations (0.500/ 0.250/ 0.125/ 0.062/ 0.031 and 0.015 mg/ml) were prepared through serial dilution, and their respective absorbance values were recorded in accordance with the method (Table 5). A standard calibration graph was subsequently generated by plotting the absorbance values against the Quercetin concentration. The results were expressed as mg Quercetin equivalent (mg QE/100 g).

Table 5. Pipetting procedure in Flavonoid determination.

	Blank	Color Blank	Standard	Sample
Sample	-	250 µL	-	250 µL
Standard (Quercetine)	-	-	250 µL	-
Methanol	2.4 mL	2.25 mL	2.15 mL	2.15 mL
10% $Al(NO_3)_3$	50 µL		50 µL	50 µL
1M NH_4CH_3COO	50 µL	-	50 µL	50 µL

In the 4th minute, absorbance is read at 415 nm.

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

Determination of Mineral Content

In this research, a homogeneous sample weighing approximately 0.5 g was placed into a Teflon crucible, to which 6 mL of pure HNO_3 and 3 mL of H_2O_2 (both sourced from Sigma-Aldrich, Munich, Germany) were added. The samples were incinerated in a Milestone microwave oven, and the resultant ashes were diluted with distilled water to achieve a total volume of 25 mL. The mineral constituents within the samples, encompassing calcium (Ca), sodium (Na), phosphorus (P), and potassium (K), were ascertained utilizing an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Thermo ICAP, 7400, Japan) (Yıldız et al. 2009).

Carbon isotope analysis (C13)

The most prevalent method for detecting sugar additives in fruit extracts today is 13C analysis, a technique first implemented in 1978 (Padovan et al. 2007). This method relies on the carbon isotope difference ($^{13}C/^{12}C$) between the fruit protein fraction, serving as a qualitative and quantitative indicator of the purity of the fruit extract. The 13C analysis is an isotopic technique used to detect fruit extracts fed with sugar; it is based on the differences in isotope ratios between C3 and C4 naturally present in plants due to photosynthesis.

The ^{13}C analysis, an isotopic technique used to detect sugar-fed fruit extracts, is based on the differences in isotope ratios between C3 and C4 naturally present in plants due to photosynthesis. Typically, in C4 plants, this ratio is between -22% and -35% compared to nectar-bearing C3 plants. This analysis method, which detects adulteration in fruit extracts produced or fed with externally added C4 sugar, involves the complete combustion of the protein solution obtained from the fruit extract. The resulting CO_2 gas contains a C atom with a $^{13}C/^{12}C$ ratio, which is determined by mass spectrometry, and the % C4 sugar ratio is calculated from this value.

RESULTS and DISCUSSION

The results of the physical, chemical, and nutritional properties of black mulberry extracts sold by various companies in Türkiye, as well as BME-34 brand black mulberry extract and BME-35 black mulberry mix products, are presented in Tables 6-14.

The results of the analysis indicated that the brix values of black mulberry extracts exhibited considerable variability, ranging from 41.9 to 81.8, with an overall mean of approximately 75 brix. The pH values of black mulberry extracts showed a similarly wide range, spanning from 1.76 to 4.9. The literature review revealed that the pH value of black mulberry fruit varies from 2.60 to 4.00. The pH value of the BME-34 and 35 brand black mulberry extract was 3.5. Upon examination of the density values of all black mulberry extracts, it was found that they fall within the range of 1.36 to 1.43 g/cm³ (Table 6).

When the colour analysis was examined, it was determined that the redness value of blackberry extracts containing synthetic dyes was higher, although there was no obvious difference (Table 6). When the water activity of black mulberry extracts was analysed, it was found to vary from 0.555 to 0.934. Considering the viscosity results, it was found that they varied from 795 to 56263 cP (Table 7).

Table 6. Analysis Results of Black Mulberry Extract (Karadut Özü)

Product code	% Brix°	pH	Density (g/cm³)	Hunter color parameters		
				L*	a*	b*
BME-1	76.0±1.1	2.53±0.03	1.39±0.01	18.14±0.2	1.86±0.03	0.7±0.01
BME-2	75.6±1.2	1.86±0.01	1.42±0.01	18.36±0.4	2.31±0.01	1.01±0.04
BME-3	76.3±0.9	1.85±0.01	1.39±0.00	18.25±0.7	1.64±0.06	0.95±0.02
BME-4	75.8±1.4	2.44±0.01	1.41±0.01	18.13±0.1	1.73±0.07	0.73±0.04
BME-5	77±1.1	2.48±0.02	1.38±0.03	17.99±0.8	0.24±0.08	0.44±0.06
BME-6	75.7±1.3	2.03±0.03	1.37±0.01	18.29±0.5	0.18±0.01	0.52±0.02
BME-7	76±0.7	2.58±0.04	1.39±0.02	18.01±0.4	0.2±0.07	0.44±0.05
BME-8	79±1.6	2.08±0.01	1.41±0.01	18.36±0.6	1.95±0.05	0.88±0.01
BME-9	75.3±1.8	1.76±0.02	1.37±0.00	19.47±0.9	1.7±0.09	0.73±0.01
BME-10	41.9±0.5	2.33±0.02	1.18±0.01	18.44±0.3	0.37±0.01	0.46±0.02
BME-11	80.2±0.6	1.93±0.03	1.43±0.04	18.18±0.5	0.54±0.03	0.69±0.04
BME-12	73.1±1.4	3.61±0.01	1.38±0.02	18.63±0.3	-0.18	1.07±0.04
BME-13	77.6±0.4	1.95±0.02	1.43±0.01	18.19±0.8	0.32±0.01	0.57±0.07
BME-14	78.7±0.7	2.01±0.03	1.39±0.02	18.81±0.2	3.11±0.03	1.74±0.02
BME-15	77.4±0.7	2.04±0.04	1.41±0.02	19.72±0.4	0.45±0.01	0.4±0.01
BME-16	75.5±0.9	2.05±0.03	1.37±0.03	18.58±0.6	0.79±0.02	0.68±0.05
BME-17	82.1±1.4	4.39±0.02	1.4±0.02	18.31±0.1	-0.14	0.84±0.06
BME-18	81.7±0.9	4.46±0.03	1.43±0.01	18.79±0.5	-0.32	1.41±0.09
BME-19	80.8±1.4	4.37±0.02	1.4±0.01	18.44±0.7	-0.05	0.94±0.01
BME-20	79.6±1.1	2.28±0.01	1.39±0.00	18.06±0.3	0.78±0.02	0.48±0.01
BME-21	76.7±1.7	2.24±0.02	1.39±0.00	17.91±0.7	0.22±0.03	0.25±0.03
BME-22	75.3±1.4	2.05±0.01	1.43±0.01	18.02±0.3	0.35±0.03	0.41±0.02
BME-23	81±0.7	4.9±0.01	1.42±0.02	18.21±0.4	0.33±0.03	0.44±0.04
BME-24	80.6±1.2	4.62±0.02	1.39±0.04	19.4±0.6	-0.12	2.33±0.07
BME-25	80.2±1.4	4.61±0.01	1.4±0.03	18.13±0.3	0.35±0.01	0.47±0.08
BME-26	75.1±1.4	2.06±0.02	1.36±0.01	17.99±0.4	0.38±0.01	0.45±0.06
BME-27	75.8±1.2	2.01±0.01	1.37±0.01	18.08±0.7	0.31±0.01	0.45±0.07
BME-28	33.5±0.3	2.14±0.04	1.38±0.01	18.35±0.5	1.55±0.02	0.83±0.07
BME-29	79.7±1.2	2.32±0.02	1.39±0.02	18.47±0.2	1.21±0.07	0.91±0.05
BME-30	75.5±1.1	2.07±0.03	1.43±0.02	18.18±0.2	1.89±0.05	0.65±0.01
BME-31	73.6±0.8	2.54±0.03	1.37±0.03	18.39±0.3	2.13±0.06	1.01±0.03
BME-32	77.3±1.2	2.1±0.03	1.38±0.01	18.11±0.6	0.98±0.03	0.88±0.02
BME-33	75±0.8	2.2±0.02	1.39±0.03	18.37±0.9	1.84±0.04	0.76±0.05
BME-34	81.8±1.6	3.5±0.02	1.4±0.02	18.05±0.1	0.39±0.08	0.41±0.02
BME-35	60±1.3	2.7±0.01	1.36±0.03	18.01±0.2	0.42±0.09	0.45±0.07

Table 7. Analysis Results of Black Mulberry Extract (Karadut Özü)

Product code	Water Activity (a_w)	% Ash	Viscosity (cP)	HMF (mg/kg)
BME-1	0.761±0.13	0.09±0.01	6698±62	14±1
BME-2	0.731±0.10	0.06±0.01	6721±47	92±2
BME-3	0.727±0.12	0.05±0.01	6624±101	275±5
BME-4	0.782±0.87	0.06±0.01	6333±42	12±1
BME-5	0.721±0.17	0.05±0.00	6952±121	137±8
BME-6	0.727±0.15	0.15±0.02	4404±53	980±12
BME-7	0.777±0.13	0.07±0.01	6922±81	40±2
BME-8	0.681±0.21	0.035±0.00	11524±113	100±3
BME-9	0.72±0.18	0.005±0.00	3753±78	176±5
BME-10	0.934±0.11	0.14±0.01	795±71	77±3
BME-11	0.742±0.19	0.38±0.03	53207±167	376±8
BME-12	0.745±0.27	0.35±0.03	17834±101	272±15
BME-13	0.784±0.32	0.08±0.01	15141±121	72±6
BME-14	0.685±0.10	0.17±0.02	12349±47	114±11
BME-15	0.71±0.31	0.32±0.03	17650±203	870±25
BME-16	0.804±0.22	0.19±0.02	10800±59	154±12
BME-17	0.555±0.19	1.37±0.01	51496±236	55±3
BME-18	0.558±0.25	1.53±0.02	56263±245	64±7
BME-19	0.568±0.21	1.46±0.03	32575±282	52±11
BME-20	0.785±0.12	0.14±0.01	14953±168	132±7
BME-21	0.801±0.14	0.055±0.01	20545±153	49±3
BME-22	0.814±0.33	0.13±0.01	13864±109	135±11
BME-23	0.569±0.17	0.38±0.01	28822±80	15±3
BME-24	0.572±0.17	0.78±0.01	20603±73	66±7
BME-25	0.581±0.24	0.84±0.04	28263±82	244±18
BME-26	0.749±0.26	0.03±0.00	3933±221	119±21
BME-27	0.809±0.17	0.085±0.00	6589±78	21±3
BME-28	0.798±0.11	0.41±0.01	12895±98	453±31
BME-29	0.813±0.24	0.02±0.00	13684±105	339±18
BME-30	0.804±0.17	0.32±0.00	32134±155	165±11
BME-31	0.752±0.11	0.11±0.01	22890±86	532±15
BME-32	0.791±0.12	0.2±0.01	10903±59	105±9
BME-33	0.777±0.18	0.09±0.01	16534±167	229±17
BME-34	0.562±0.19	1.96±0.01	38906±137	48±21
BME-35	0.792±0.32	1.78±0.02	2459 ±42	35±3

Upon analysis of the ash content of black mulberry extracts, it was observed that the ash values were generally low, indicating that the products were low in minerals. While the lowest ash content was 0.005%, the highest ash content was 1.53%. The ash content of the BME-34 brand black mulberry extract was 1.96%, while that of the BME-35 black mulberry mix was 1.78%. This indicates that the latter is rich in minerals (Table 7).

Taking a look into the analysis of the HMF (5-Hydroxymethyl Furfural) values of black mulberry extracts, it was observed that they exhibited a considerable range, spanning from 12 to 980. The HMF value, known to be harmful to human health, especially in products subjected to high temperatures, was elevated in most products. The HMF value was 48 in the BME-34 brand black mulberry extract and 35 in the BME-35 black mulberry mix (Table 7).

The acidity values of black mulberry extracts ranged from 0.29 to 3.26 (citric acid equivalent) g/100 mL, while the conductivity values were observed to vary from 475 to 2970 µS/cm. The electrical conductivity of the BME-34 brand blackberry extract was 4100 µS/cm (Table 8).

Upon examination of the protein content of black mulberry extracts, it was observed that they demonstrated a very low protein content. The protein content of black mulberry extracts exhibited a range of values between 0.078 and 0.4%. The protein content of the BME-34 brand black mulberry extracts was calculated to be 1,5%, consistent with the literature and 1.2% in the BME-35 black mulberry mix (Table 8).

When the sugar profile of black mulberry fruit was examined, it was observed that the fructose/glucose ratio should be close to 1, there should be no sucrose in it, and the maltose maximum should be around 2%. It was also confirmed with the literature. It was determined that the fructose/glucose ratio of many extracts did not match the expected value and contained additional maltose. Some black mulberry extracts were even found to contain sucrose. It was deduced that the fructose content of black mulberry extracts showed a considerable range, varying from 1.48 to 28.37%. Similarly, the glucose content exhibited a wide range, varying from 2.3 to 38.26%. In most products, the maltose content exceeded 20%, while in some instances, sucrose was present at a concentration of up to 11%. In the case of the BME-34 brand black mulberry extract and BME-35 black mulberry mix, the fructose-to-glucose ratio did not contain sucrose in a concentration close to 1. Furthermore, it was noted that no additional maltose was added (Table 9).

The preservative content of black mulberry extracts was analysed, and it was found that many extracts contained sorbic acid and benzoic acid. Sorbic acid was added between 37 and 446 mg/kg, and benzoic acid was added between 38 and 844 mg/kg in black mulberry extracts. The BME-34 brand blackberry extract and BME-35 mix were found to contain no preservatives (Table 10). Dyes were utilized in many of the extracts to impart coloration. No evidence of the use of dyes was found in the BME-34 brand black mulberry extract and BME-35 black mulberry mix (Table 10).

To ascertain the presence of sweeteners in black mulberry extracts, an investigation was conducted to determine the presence of various sweeteners. The most commonly used sweeteners, namely Acesulfame-K, Saccharine, Aspartame and Sucralose were analysed for this purpose. The results of the analyses indicated that some black mulberry extracts contained Acesulfame-K (30-204 mg/kg), saccharine (34-134 mg/kg), aspartame (2.6-25 mg/kg) and sucralose (0.78-12.8 mg/kg). The BME-34 brand black mulberry extract and BME-35 black mulberry mix were devoid of any sweetener (Table 11).

The results of the analyses conducted to determine the mineral content of black mulberry extracts revealed that the mineral values of the extracts were relatively low. In particular, the amounts of calcium, potassium, sodium and phosphorus, which were most abundant in black mulberry, were analysed. The results

demonstrated that the mineral contents of all extracts were low. The mineral content of the BME-34 brand black mulberry extract and BME-35 black mulberry mix was high (Table 12).

Table 8. Analysis Results of Black Mulberry Extract (Karadut Özü)

Product code	Acidity (Citric acid eq.) (g /100 mL)	Electrical Conductivity (µS/cm)	% Protein
BME-1	0.94±0.05	492±15	0.1±0.01
BME-2	1.86±0.07	483±22	0.2±0.01
BME-3	1.67±0.09	475±17	0.2±0.01
BME-4	1.24±0.06	622±11	0.05±0.01
BME-5	1.18±0.06	626±13	n.d.
BME-6	0.9±0.02	772±27	0.03±0.01
BME-7	1.02±0.07	575±12	0.07±0.01
BME-8	0.86±0.07	570±16	0.1±0.01
BME-9	2.18±0.09	989±19	n.d.
BME-10	1.63±0.07	884±18	n.d.
BME-11	1.35±0.03	765±22	0.09±0.02
BME-12	0.81±0.03	885±23	0.06±0.00
BME-13	3.26±0.17	1235±47	0.3±0.01
BME-14	0.97±0.05	596±15	0.04±0.02
BME-15	0.79±0.03	615±10	0.1±0.02
BME-16	2.31±0.08	856±24	0.03±0.02
BME-17	0.54±0.07	2450±57	0.3±0.01
BME-18	0.43±0.06	2970±59	n.d.
BME-19	0.5±0.08	2955±62	0.16±0.03
BME-20	3.25±0.19	1119±39	0.23±0.05
BME-21	1.42±0.04	803±22	0.4±0.03
BME-22	2.41±0.09	879±14	0.11±0.01
BME-23	0.29±0.06	2653±47	0.3±0.04
BME-24	0.33±0.01	1380±33	n.d.
BME-25	0.4±0.05	367±17	0.3±0.01
BME-26	1.95±0.32	922±18	n.d.
BME-27	2.23±0.23	1115±26	0.36±0.02
BME-28	1.34±0.02	882±18	0.17±0.01
BME-29	1.56±0.08	757±28	n.d.
BME-30	1.28±0.05	1064±43	n.d.
BME-31	1.17±0.09	736±11	0.078±0.00
BME-32	1.24±0.08	670±25	0.13±0.03
BME-33	1.33±0.16	1145±33	0.28±0.04
BME-34	0.8±0.01	4100±86	1.5±0.01
BME-35	0.97±0.02	1875±78	1.2±0.02

Table 9. Sugar Analysis Results of Black Mulberry Extract (Karadut Özü)

Product code	% Fructose	% Glucose	% Saccharose	% Maltose	F/G Ratio
BME-1	4.57±0.4	13.02±0.01	n.d.	30.18±0.06	0.351
BME-2	16.8±0.2	20.31±0.01	n.d.	20.05±0.02	0.827
BME-3	13.74±0.5	24.13±0.01	n.d.	11.41±0.1	0.569
BME-4	3.38±0.6	9.47±0.01	0.42±0.03	28.58±0.9	0.357
BME-5	12.62±0.8	21.56±0.02	n.d.	20.81±0.3	0.585
BME-6	16.16±0.5	21.01±0.01	n.d.	16.23±0.07	0.769
BME-7	4.24±0.9	11.75±0.03	n.d.	31.79±0.4	0.361
BME-8	16.34±0.1	23.05±0.02	n.d.	19.21±0.07	0.709
BME-9	16.69±0.1	19.67±0.02	n.d.	18.09±0.03	0.849
BME-10	7.08±0.2	9.32±0.01	n.d.	10.83±0.9	0.760
BME-11	1.48±0.2	4.38±0.01	0.3±0.01	35.05±0.05	0.338
BME-12	17.81±0.3	21.55±0.00	n.d.	14.57±0.08	0.826
BME-13	0.073±0.0	1.87±0.00	0.3±0.03	37.78±0.6	0.039
BME-14	17.49±0.1	20.12±0.01	n.d.	20.12±0.5	0.869
BME-15	14.73±0.2	20.03±0.01	0.3±0.01	18.53±0.09	0.735
BME-16	0.19±0.0	2.94±0.00	0.29±0.01	36.38±0.00	0.065
BME-17	20.89±0.1	34.14±0.03	12.06±0.04	1.22±0.01	0.612
BME-18	24.25±0.2	38.26±0.01	11.1±0.02	0.75±0.6	0.634
BME-19	24.94±0.1	37.36±0.02	0.33±0.05	0.31±0.08	0.668
BME-20	0.34±0.2	5.56±0.01	n.d.	37.87±0.8	0.061
BME-21	0.076±0.0	2.38±0.04	0.3±0.02	40.83±0.05	0.032
BME-22	0.25±0.0	1.86±0.05	n.d.	37.56±0.3	0.134
BME-23	20.44±0.5	30.68±0.01	0.92±0.01	19.46±0.5	0.666
BME-24	20.42±0.9	30.77±0.00	0.43±0.03	21.32±0.7	0.664
BME-25	28.37±0.2	29.74±0.02	1.14±0.04	14.99±0.3	0.954
BME-26	8.81±0.2	17.71±0.02	n.d.	21.76±0.9	0.497
BME-27	0.08±0.1	2.3±0.01	n.d.	37.8±0.2	0.035
BME-28	12.63±0.5	12.2±0.01	n.d.	0.2±0.00	1.035
BME-29	11.8±0.1	17.17±0.02	n.d.	23.11±0.04	0.687
BME-30	0.36±0.05	2.7±0.4	0.86±0.04	39.45±0.06	0.133
BME-31	13.67±0.6	17.45±0.45	1.1±0.32	22.54±0.4	0.783
BME-32	16.82±0.3	23.5±0.06	n.d.	18.54±0.9	0.716
BME-33	6.21±0.6	10.45±0.08	n.d.	34.72±1.7	0.594
BME-34	35.83±0.4	36.75±0.07	n.d.	1.49±0.05	0.975
BME-35	28.54±0.06	28.89±0.12	n.d.	1.05±0.07	0.988

Table 10. Protector and Paint Analysis Results of Black Mulberry Extract (Karadut Özü)

Product code	Sorbic Acid (mg/kg)	Benzoic Acid (mg/kg)	Synthetic paint
BME-1	224.5±8.3	351.6±14.76	n.d.
BME-2	81.98±3.03	n.d.	Positive
BME-3	229.6±10.1	n.d.	Positive
BME-4	189.5±5.2	288.9±11.5	Positive
BME-5	n.d.	303.34±15.9	Positive
BME-6	176.9±6-8	n.d.	Positive
BME-7	103.5±11.4	207.22±10.8	Positive
BME-8	n.d.	n.d.	n.d.
BME-9	165.49±12.7	189.4±9.6	n.d.
BME-10	445.94±16.5	843.78±35.4	Positive
BME-11	n.d.	n.d.	Positive
BME-12	102.6±3.8	n.d.	n.d.
BME-13	37.23±56	48.8±2.6	Positive
BME-14	n.d.	57.3±6.1	Positive
BME-15	15.08±0.56	38±0.56	Positive
BME-16	80.9±3	182.4±7.7	n.d.
BME-17	124.75±7.8	n.d.	n.d.
BME-18	305.77±8.9	413.9±12.8	Positive
BME-19	219.44±12.8	276.90±17.9	n.d.
BME-20	n.d.	n.d.	n.d.
BME-21	n.d.	146.14±6.14	Positive
BME-22	n.d.	56.88±10.9	Positive
BME-23	175.8±7.91	n.d.	n.d.
BME-24	116.93±13.5	n.d.	n.d.
BME-25	74.76±2.8	135.55±23.4	n.d.
BME-26	n.d.	n.d.	Positive
BME-27	49.2±1.9	96.9±2.26	Positive
BME-28	n.d.	241.98±13.2	n.d.
BME-29	n.d.	n.d.	Positive
BME-30	102.55±6.1	108.89±4.7	Positive
BME-31	89.45±8.4	95.34±2.9	Positive
BME-32	181.5±5.7	278.9±12.54	n.d.
BME-33	171.8±7.1	n.d.	Positive
BME-34	n.d.	n.d.	n.d.
BME-35	n.d.	n.d.	n.d.

Table 11. Sweetener Analysis Results of Black Mulberry Extract (Karadut Özü)

Product code	Acesulfame-K (mg/kg)	Saccharine (mg/kg)	Aspartam (mg/kg)	Sucralose (mg/kg)
BME-1	n.d.	n.d.	n.d.	n.d.
BME-2	n.d.	n.d.	n.d.	n.d.
BME-3	56.73±3.5	52.9±1.8	18.54±2.2	7.15±1.2
BME-4	n.d.	103.92±4.2	n.d.	n.d.
BME-5	101.63±7.8	n.d.	3.7±0.9	n.d.
BME-6	n.d.	n.d.	25.32±4.3	12.8±2.3
BME-7	143.62±8.4	65.7±9.5	n.d.	n.d.
BME-8	n.d.	n.d.	5.8±0.75	n.d.
BME-9	76.30±11.7	19.4±2.8	n.d.	5.2±0.76
BME-10	168.93±7.1	104.4±4.7	21.03±2.11	n.d.
BME-11	n.d.	n.d.	n.d.	n.d.
BME-12	n.d.	n.d.	13.11±1.12	n.d.
BME-13	n.d.	n.d.	n.d.	n.d.
BME-14	203.77±13.4	89.5±4.2	n.d.	2.8±0.3
BME-15	n.d.	n.d.	n.d.	n.d.
BME-16	178.86±9.5	133.96±6.03	n.d.	n.d.
BME-17	n.d.	n.d.	2.6±0.7	0.78±0.13
BME-18	n.d.	42.6±11.9	n.d.	n.d.
BME-19	55.23±8.2	n.d.	n.d.	n.d.
BME-20	104.57±3.8	34.5±3.0	13.76±3.11	n.d.
BME-21	n.d.	n.d.	n.d.	n.d.
BME-22	108.65±4.2	64.8±5.5	n.d.	n.d.
BME-23	116.7±6.5	n.d.	n.d.	n.d.
BME-24	n.d.	n.d.	n.d.	n.d.
BME-25	n.d.	n.d.	3.8±0.55	n.d.
BME-26	83.7±5.7	43.35±3.8	n.d.	n.d.
BME-27	61.3±7.2	n.d.	n.d.	n.d.
BME-28	n.d.	69.32±1.9	4.2±0.3	n.d.
BME-29	29.5±7.2	n.d.	18.9±2.4	n.d.
BME-30	n.d.	101.56±5.2	n.d.	n.d.
BME-31	n.d.	n.d.	2.9±0.11	n.d.
BME-32	n.d.	n.d.	n.d.	n.d.
BME-33	78.34±6.4	n.d.	8.31±1.05	n.d.
BME-34	n.d.	n.d.	n.d.	n.d.
BME-35	n.d.	n.d.	n.d.	n.d.

Table 12. Mineral Analysis Results of Black Mulberry Extract (Karadut Özü)

Product code	Calcium (Ca)	Potassium (K)	Sodium (Na)	Phosphorus (P)
BME-1	35.1±7.4	216±45.4	108.4±22.8	50.4±10.6
BME-2	30±6.3	36.9±7.7	64.4±13.5	16.3±3.4
BME-3	43.6±6.3	103.4±9.5	69.3±10.8	12.8±3.3
BME-4	57.21±12.8	52.5±12.7	57.2±8.45	19.34±3.8
BME-5	27.83±7.2	67.5±6.8	48.7±4.5	24.5±6.2
BME-6	98.44±13.42	98.7±7.7	77.8±12.6	27.3±5.1
BME-7	41.6±10.8	119.8±11.8	101.3±7.9	35.3±9.2
BME-8	43.7±9.2	65.5±13.8	30.4±6.4	21.6±4.5
BME-9	20.05±5.3	142.4±6.6	309.2±13.6	41.6±8.3
BME-10	57.3±12	314±65.9	554.4±116.4	9±1.9
BME-11	16±3.4	n.d.	75.8±15.9	1.6±0.3
BME-12	146.5±30.8	1445.2±303.5	353.5±74.2	194.4±40.8
BME-13	65.9±11.6	308.5±4.9	n.d.	7.4±1.1
BME-14	14.9±2.2	78.14±8.4	109.4±10.5	54.2±9.3
BME-15	27.4±5.8	82.8±17.4	174±36.5	25.5±5.4
BME-16	18.6±3.9	54.3±11.4	356.1±74.8	17±3.6
BME-17	75.44±15.4	61.7±4.0	206.4±7.5	n.d.
BME-18	36.9±8.5	72.1±9.3	401.7±6.6	n.d.
BME-19	48.13±10.5	66.4±8.3	59.3±8.2	19.3±2.6
BME-20	52.9±3.3	243.0±5.8	209.8±12.7	32.6±4.0
BME-21	141.5±29.7	24.9±5.2	365±76.7	275±57.8
BME-22	77.34±13.6	118.6±7.3	56.7±6.8	22.1±52.7
BME-23	18.56±2.5	71.8±12.9	259.1±8.9	56.3±7.6
BME-24	28.22±3.8	n.d.	n.d.	101.5±7.8
BME-25	61.7±9.4	67.9±11.7	207.9±16.3	98.6±5.5
BME-26	101.44±15.3	307.6±34.3	51.8±2.7	62.7±6.3
BME-27	40.6±8.6	22.7±1.9	n.d.	29.4±6.9
BME-28	44.2±7.4	36.8±2.9	48.9±3.1	57.3±6.9
BME-29	65.92±14.5	417.8±33.5	101.7±15.9	32.9±6.3
BME-30	20.8±3.3	86.4±3.9	74.5±9.4	48.9±4.4
BME-31	16.7±2.4	276.7±6.3	78.3±16.9	38.6±7.0
BME-32	39.5±4.9	72.7±11.9	59.13±14.8	108.7±34.1
BME-33	81.56±4.6	138.8±41.3	88.9±3.0	129.22.8
BME-34	1098.2±230.6	8224.6±1727.2	2190.6±460.1	586.5±123.2
BME-35	787.5±34.73	6543.3±57.4	1879.9±101.3	357.3±43.7

When the total phenolic contents of black mulberry extracts were investigated, it was found that the phenolic content could not be calculated in all but one or two of them. Similarly, FRAP and DPPH could not be calculated in most cases. In the BME-34 brand black mulberry extract and BME-35 black mulberry mix, the calculated phenolic contents ranged from 1547 to 1728 mg GAE/100 g. The antioxidant contents were similarly calculated, with FRAP values ranging from 1850 to 2245 mg/100 g $FeSO_4$ and DPPH (SC_{50}) values ranging from 10.9 to 12.6 mg/mL. The total flavonoid contents of the BME-34 brand black mulberry extract and BME-35 black mulberry mix ranged from 350 to 526 mg QE/100 g (Table 13).

Table 13. Antioxidant Analysis Results of Black Mulberry Extract (Karadut Özü)

Product code	Antioxidant - FRAP (mg/ 100 g FeSO4)	Antioxidant - DPPH SC50 (mg/mL)	Total phenolic (mg GAE/100 g)	Total flavonoid (mg QE/100 g)
BME-1	25.7±3.2	n.d.	n.d.	n.d.
BME-2	n.d.	n.d.	n.d.	n.d.
BME-3	n.d.	n.d.	n.d.	n.d.
BME-4	n.d.	n.d.	n.d.	n.d.
BME-5	n.d.	n.d.	n.d.	n.d.
BME-6	n.d.	n.d.	n.d.	n.d.
BME-7	n.d.	n.d.	n.d.	n.d.
BME-8	n.d.	n.d.	n.d.	n.d.
BME-9	n.d.	n.d.	n.d.	n.d.
BME-10	n.d.	n.d.	n.d.	n.d.
BME-11	n.d.	n.d.	n.d.	n.d.
BME-12	86.3±7.2	n.d.	25.9±1.1	2.6±0.3
BME-13	n.d.	n.d.	n.d.	n.d.
BME-14	n.d.	n.d.	n.d.	n.d.
BME-15	n.d.	n.d.	n.d.	n.d.
BME-16	n.d.	n.d.	n.d.	n.d.
BME-17	n.d.	n.d.	n.d.	n.d.
BME-18	52.7±9.1	n.d.	21.3±0.5	n.d.
BME-19	n.d.	n.d.	n.d.	n.d.
BME-20	n.d.	n.d.	n.d.	n.d.
BME-21	n.d.	n.d.	n.d.	n.d.
BME-22	n.d.	n.d.	n.d.	n.d.
BME-23	n.d.	n.d.	n.d.	n.d.
BME-24	n.d.	n.d.	n.d.	n.d.
BME-25	n.d.	n.d.	n.d.	n.d.
BME-26	n.d.	n.d.	n.d.	n.d.
BME-27	n.d.	n.d.	n.d.	n.d.
BME-28	n.d.	n.d.	n.d.	n.d.
BME-29	n.d.	n.d.	n.d.	n.d.
BME-30	n.d.	n.d.	n.d.	n.d.
BME-31	n.d.	n.d.	n.d.	n.d.
BME-32	n.d.	n.d.	n.d.	n.d.
BME-33	n.d.	n.d.	n.d.	n.d.
BME-34	2245.4±38.3	10.9±0.4	1728.3±28.6	525.8±11.8
BME-35	1852.7±19.8	12.6±0.7	1547.4±33.7	349.5±8.4

Upon examination of the carbon 13 isotope analyses of the black mulberry extracts, it was observed that all of them were not suitable. While the carbon 13 isotope value in fruits should be -23 and more negative, it was observed that the value varied from -12 to -15 in all extracts. In the case of the BME-34 brand black mulberry extract and BME-35 black mulberry mix, the calculated value was approximately -25 (Table 14).

Table 14. Carbon 13 Analysis Results of Black Mulberry Extract (Karadut Özü)

Product code	Delta C13 (δ13C)	Product code	Delta C13 (δ13C)
BME-1	-12.36±0.31	BME-19	-12.18±0.31
BME-2	-12.26±0.31	BME-20	-12.69±0.31
BME-3	-12.42±0.32	BME-21	-12.40±0.31
BME-4	-12.56±0.32	BME-22	-12.26±0.34
BME-5	-12.28±0.31	BME-23	-13.45±0.32
BME-6	-12.54±0.32	BME-24	-12.57±0.34
BME-7	-12.29±0.33	BME-25	-12.48±0.31
BME-8	-12.38±0.31	BME-26	-12.40±0.32
BME-9	-12.58±0.32	BME-27	-12.26±0.32
BME-10	-12.66±0.32	BME-28	-14.72±0.33
BME-11	-12.54±0.31	BME-29	-12.83±0.31
BME-12	-14.03±0.35	BME-30	-12.61±0.32
BME-13	-12.39±0.33	BME-31	-12.74±0.32
BME-14	-12.41±0.31	BME-32	-12.52±0.31
BME-15	-12.86±0.32	BME-33	-14.57±0.32
BME-16	-12.84±0.32	BME-34	-25.19±0.63
BME-17	-12.21±0.31	BME-35	-25.18±0.63
BME-18	-14.57±0.33		

To compare the results of this study, various studies with low Brix content were analyzed, and their Brix values were adjusted to 75°Brix for a consistent assessment of the findings. It was found that the total acidity results of all samples were consistent with those in other studies, while the total phenolic contents were generally lower, except for samples BME-34 and BME-35, which were within the expected range (Wang et al., 2022). Regarding total flavonoid content, only BME-34 and BME-35 had results comparable to other studies; however, the values were slightly lower. This discrepancy could be attributed to the fact that other studies used fresh fruit juices, whereas this study involved heat-treated and evaporated fruit extracts (Wang et al., 2022; Ercisli and Orhan, 2007).

Moreover, the mineral content of black mulberry fruit was evaluated post-Brix adjustment. The calcium, potassium, and phosphorus contents of black mulberry fruit were similar to those of samples BME-34 and BME-35, while the other samples contained lower mineral levels. However, the sodium content was slightly higher than the adjusted fruit mineral values (Ercisli and Orhan, 2007; Paunović, Mašković and Milinković, 2020).

In the study by Paunović et al., the protein content and sugar composition of the fruit extracts were also analyzed. The results for samples BME-34 and BME-35 were similar to their findings, except for the sucrose content, which was not found in samples BME-34 and BME-35. However, the remaining samples did not exhibit similar sugar compositions or protein

content (Paunović, Mašković and Milinković, 2020).

CONCLUSION and RECOMMENDATIONS

The increasing demand for black mulberry is driven by its recognized nutritional value, characterized by many antioxidants, vitamins, and minerals. Renowned for its richness in potassium, calcium, and vitamins C and K, black mulberry extract stands as a significant nutritional source, contributing to the enhancement of the human immune system.

This study aims to enhance consumer awareness regarding the black mulberry extracts available in the Turkish market by conducting comprehensive analyses of these products. Notably, many extracts of black mulberry sold within the country contain added sugars, sweeteners, and preservatives.

Evaluation of this study outcomes reveals that while the pH values of several examined black mulberry extracts meet the standards for human consumption, certain extracts exhibit elevated pH levels, posing health risks. Furthermore, many tested extracts show excessive levels of Hydroxymethylfurfural (HMF), a known carcinogenic substance, thus rendering the consumption of these products hazardous.

Except for the BME-34 and 35 brands, the protein content in the analyzed products is notably absent, suggesting a low fruit content or the absence of fruit entirely. Similarly, the sugar profile analysis indicates the absence of fruit sugars, with the presence of maltose and its derivatives, implying the products' lack of complete naturalness. Moreover, varying levels of sweeteners were detected, posing risks, especially for diabetic patients and potentially causing digestive system complications. Hence, product labels should transparently disclose the presence of sweeteners.

Many black mulberry extracts contain sorbic and benzoic acids as preservatives, yet their usage is often undisclosed on product labels. Such preservatives may elicit adverse health effects ranging from skin rash and itching to digestive complications and allergic reactions. Therefore, the inclusion of preservatives on product labels is imperative. Additionally, some extracts contained added colorants.

The mineral analysis of black mulberry extracts indicates lower-than-optimal mineral content. Similarly, the Carbon 13 analysis reveals inadequate fruit content in these extracts. The analysis reveals insufficient phenolic compounds, total flavonoids, and antioxidants in several products despite labels claiming suitability for infants and diabetic patients. However, the findings obtained in the analyses suggest these products are unsuitable for such populations.

Conversely, examination of BME-34 and 35 brand products reveals mineral richness, high antioxidant values, and comparable nutritional profiles to black mulberry fruit as documented in the literature. Notably, these products lack added sugars, preservatives, colorants, and sweeteners and exhibit appropriate Carbon 13 values.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest concerning this research, authorship, and publication of this article.

AUTHOR CONTRIBUTIONS

Yakup Şirin: Investigation, Laboratory Analysis, Methodology, Conceptualization, Funding acquisition, Supervision, Writing - review & editing; Büşra Erdem: Laboratory Analysis, Methodology; Sertan Cengiz: Laboratory Analysis, Writing, Investigation; Semih Gürkan: Investigation, Funding acquisition; Perihan Gürkan: Investigation, Funding acquisition, Supervision, Writing - review & editing.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to everyone who contributed to the successful completion of this study. I would also like to thank SEM-AS Tourism Food Industry Trade Limited Company General Manager Reşat

GÜRKAN for his valuable contributions and Aslı GÜRKAN for his support.

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