ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

MSc THESIS

Sugra Altaf QAZI

THE EFFECT OF ULTRASOUND ON THE QUALITY OF PEELED AND UNPEELED WALNUT KERNEL BEVERAGE

DEPARTMENT OF FOOD ENGINEERING

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ABSTRACT

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THE EFFECT OF ULTRASOUND ON THE QUALITY OF PEELED AND UNPEELED WALNUT KERNEL BEVERAGE

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In this study, the functional features, physicochemical properties, quality attributes, flavor characteristics, and storage stability of peeled and unpeeled walnut beverages were studied after being treated with ultrasound (25 kHz at 100 W for 10 minutes). The results showed that gallic acid, ellagic acid, sugars, particle size, viscosity, L*value, a*value, whiteness index, and E* (color difference) all increased after storage (4°C, 180 days), while total phenolic content, DPPH, FRAP, b*value, C* values reduced significantly. The total phenolic content of unpeeled walnut beverages was notably higher (3 folds) than peeled walnut beverages. The highest FRAP values were determined in unpeeled ultrasonicated (UPUS) samples. The peeled ultrasonicated (PUS) samples exhibited the lowest $D_{4,3}$ value (96 μm), whereas peeled control (PC) samples showed the highest $D_{4,3}$ value (198.67 μm). The PC samples had the highest concentration of linoleic acid (60.71 - 64.65%), whereas UPUS had the lowest. Ellagic and gallic acid was the most abundant phenolic compounds in all the walnut beverage samples. Sucrose, followed by fructose and glucose sugars, increased with storage, and sucrose was shown to be the most prevalent basic sugar component of walnut beverages. Among the analyzed samples, UPC had the greatest magnesium (Mg) level at 95.92 mg/L, while PUS had the lowest at 70.80 mg/L. The PC samples were more appreciated, and the most liked.

Key Words: Walnut beverage, ultrasound treatment, fatty acid composition, particle size, antioxidant activity

ÖZ

YÜKSEK LİSANS TEZİ

ULTRASONUN SOYULMUŞ VE SOYULMAMIŞ İÇ CEVİZ İÇECEKLERİNİN KALİTE ÖZELLİKLERİNE ETKİSİ

Sugra Altaf QAZI

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Bu çalışmada, soyulmuş ve soyulmamış ceviz içeceklerinin ultrases (25 kHz, 100 W, 10 dakika) işleminden geçirildikten sonra fonksiyonel özellikleri, fizikokimyasal özellikleri, kalite özellikleri, lezzet özellikleri ve depolama stabiliteleri incelenmiştir. Sonuçlar, depolamadan sonra gallik asit, ellagik asit, şekerler, partikül boyutu, viskozite, L*değeri, a*değeri, beyazlık indeksi, E* (renk farkı) ve b*değerinin arttığı (4°C, 180 gün), toplam fenolik içerik, DPPH ve FRAP değerlerinin önemli ölçüde azaldığını göstermiştir.En yüksek FRAP değerleri soyulmamış ultrasonik (UPUS) örneklerde belirlenmiştir. Soyulmuş ultrasonik (PUS) örnekler en düşük $D_{4,3}$ değerini (96 μm) sergilerken, soyulmuş kontrol (PC) örnekleri en yüksek $D_{4,3}$ değerini (198,67 μm) göstermiştir. PC numuneleri en yüksek linoleik asit konsantrasyonuna (%60,71 - 64,65) sahipken, UPUS en düşük konsantrasyona sahipti. Ellagik ve gallik asit tüm cevizli içecek örneklerinde en fazla bulunan fenolik bileşik olmuştur. Sakaroz, ardından fruktoz ve glikoz şekerleri depolama ile artmış ve sakarozun ceviz içeceklerinin en yaygın temel şeker bileşeni olduğu gösterildi. Analiz edilen örnekler arasında, UPC 95,92 mg/L ile en yüksek magnezyum (Mg) seviyesine sahipken, PUS 70,80 mg/L ile en düşük seviyeye sahipti. PC örnekleri daha çok beğenildi ve en çok beğenilen örnek olmuştur.

Anahtar Kelimeler: Ceviz içeceği, ultrases işlemi, yağ asidi bileşimi, partikül boyutu, antioksidan aktivite

EXTENDED ABSTRACT

Nuts are nutritionally-dense foods that have been a component of the human diet since prehistoric times. They are an excellent way to provide bioactive substances that promote health. As a result, they are vital beneficial snack products, as well as ingredients in many traditional and innovative gourmet recipes around the world. It is strongly advised to consume nuts or dried fruits on a regular basis in order to properly derive the benefit of the nutrients, bioactive chemicals, and antioxidants included in walnuts and other nuts.

Walnuts have contributed immensely to human nutrition all around the world. *Juglans regia* L. (Juglandaceae) kernels are necessary for human nutrition due to their high protein and oil content. As a result, walnut has been identified as a critical nut for human nutrition and is included in the Food and Agriculture Organization's (FAO) priority plant list.

Several previous studies have demonstrated the utility of ultrasound as an emulsification method. Recent research has employed ultrasound to create walnut protein-stabilized emulsions. Furthermore, it has been observed that ultrasound is useful in combating contaminating microorganisms in liquid foods. Existing work mainly focuses on the characterization of walnut protein/oil emulsion systems. Limited studies have looked into walnut beverages, an actual food system. Furthermore, little research has been conducted to determine the impact of ultrasound on the properties of walnut foods. Therefore, this study investigated the physicochemical properties and differences between peeled and unpeeled walnut beverages treated with ultrasound for 10 min at an ultrasonic frequency of 25 kHz.

The dried walnuts were deshelled manually. The walnut kernels were divided into two parts; one was kept with the pellicle (unpeeled: UP), and another was peeled (P). For peeling, the walnut kernels were weighed and dipped into a beaker containing 12% NaOH solution on a heater for 3 minutes, maintaining a temperature of 90°C. The kernels were removed from the solution and washed

under a jet stream of tap water. An abrasive brush was used to clean the kernels from any remaining pellicles.

Further, the peeled kernels were soaked in distilled water in a jar overnight to neutralize the effect of NaOH. For both the walnut kernels (peeled and unpeeled), 4.5 times their weight of distilled water (50°C) was added. Then the walnut kernels were ground in a blender (Warning, USA) for 2 min. Next, the obtained slurry was homogenized using an Ultra Turrax homogenizer (IKA T 18 Digital Ultra-Turrax, Germany). Finally, the walnut beverage sample (approximately 450 mL) was poured into the double-jacketed 500 mL beaker. Furthermore, the sonotrode was immersed 1 cm upper than the bottom of the beaker containing walnut beverage. The samples were treated with ultrasound for 10 min at 25 kHz. After ultra-sonification, the walnut beverage was poured into bottles and autoclaved at 121°C for 15 minutes. Finally, the bottles were stored at 4°C, kept in storage for six months, and analyzed at 45 days intervals.

According to the findings, changes in total dry matter values (12.80-17.17%) were found to be statistically significant before storage, and an increase occurred during storage (12.97-17.18%)., The ash values of the samples varied between 0.14% and 0.30% at the beginning of storage and between 0.19% and 0.30 % at the end of storage. Differences between samples were considered significant ($p<0.05$). The ellagic and gallic acid content in walnut beverages increased during storage.

Nutritional analysis of walnut beverages revealed that unpeeled ultrasonicated (UPUS) samples contained 2.93% and 12.63 % crude protein and fat, higher than control walnut beverages. Total solids and ash content were highest in unpeeled control (UPC) walnut beverage samples. Mineral content (Mg, Fe) was higher in UPC walnut beverages, and Ca, K, and Zn were found to be highest in UPUS samples. In contrast, sodium and copper were found to be highest in PC and PUS samples, respectively.

When the color changes in the samples were examined during storage, L*, a* whiteness index, and ΔE* values increased. In contrast, hue, b*, and C* values decreased. Changes during storage were found to be statistically significant ($p<0.05$), with the lowest L* value on the 0 days in the UPC sample (34.1), the lowest a* value on day 45 in peeled ultrasonicated (PUS) (0.2) samples, the lowest b* value in the day 180 in PC sample (8.6), and the lowest C* value in the day 180 in peeled control (PC) samples (8.6). The increase observed with storage indicates that the redness in the color of the product increases with storage. When the color values of the ultrasonicated samples were examined at the end of storage, the L* and a* values in the samples were higher compared to the control samples.

During storage, a gradual decreasing tendency was determined in the total phenolic content of PC and PUS samples which decreased by 18.34% and 22.78%, respectively, at the end of the storage. However, the total phenolic content of UPC and UPUS samples showed the highest increase by 9.17% and 34.95%, respectively, at 45 days of storage and later decreased at the end of storage.

Antioxidant activity in terms of FRAP values was highest in UPUS samples, and as storage progressed, it decreased. The antioxidant activity of unpeeled walnut beverage samples was 72.2 folds higher than peeled walnut beverage samples. DPPH showed the same trend as FRAP, with the highest value in the UPUS samples.

Particle size shows a gradual increase in $D_{3,2}$ in control samples, whereas, in ultrasonicated samples, a non-uniform pattern was observed. The PC samples' $D_{4,3}$ value (198.67 μm) was the highest, while the PUS samples showed the lowest $D_{4,3}$ value (96 μm). Also, the highest $D_{3,2}$ value (15.53 μm) was determined in UPC samples, while the lowest value (2 μm) was determined in PUS samples. Volume-weighted mean diameter $D_{4,3}$ values showed similar results with average particle size values $D_{0.5}$.

The most common phenolic components identified in walnut beverages are gallic acid and ellagic acid. There was an increase in the phenolic compounds at

the beginning of storage. The contents of gallic and ellagic acid were the lowest in PC samples at the beginning and end of storage. It was observed that the use of ultrasound increased the gallic and ellagic acid content of the walnut beverage.

The most abundant basic sugar component of walnut beverages was sucrose, followed by fructose and glucose sugars which showed an increasing and decreasing behavior during storage. Also, the ultrasound showed an increasing effect on the sugars.

The sensory evaluation of the walnut beverages was carried out at the beginning of the storage with 11 panelists. The PC samples were more appreciated, and the most liked sample, with a score of 7.77.

GENİŞLETİLMİŞ ÖZET

Kuruyemişler, besin açısından yoğun gıdalardır ve tarih öncesi çağlardan beri insan diyetinin düzenli bir bileşeni olmuşlardır. İnsan sağlığını geliştiren biyoaktif bileşikler sağlamak için mükemmel bir kaynaklardır. Bu nedenle, Dünya çapında birçok geleneksel ve yeni gastronomi tarifinin bir parçası olmanın yanı sıra, temel sağlıklı atıştırmalıklardır. Kuruyemişler içerdiği besinlerden, biyoaktif bileşiklerden ve antioksidanlardan tam olarak yararlanmak için sık sık tüketimi şiddetle tavsiye edilmektedir.

Ceviz tüm dünyada insan beslenmesinde tüketilmektedir. *Juglans regia* L. (Juglandaceae) çekirdeklerinin yüksek protein ve yağ içeriği, bu meyveyi insan beslenmesi için vazgeçilmez kılmaktadır. Sonuç olarak ceviz, insan beslenmesi için hayati bir bitki olarak belirlenmiş ve FAO'nun öncelikli bitkiler listesinde yer almaktadır.

Önceki birkaç çalışma, bir emülsifikasyon yöntemi olarak ultrasesin faydasını göstermiştir. Son araştırmalar, ceviz proteini ile stabilize edilmiş emülsiyonlar oluşturmak için ultrases kullanmıştır. Ayrıca ultrasesin sıvı gıdalardaki kontamine edici mikroorganizmalarla mücadelede faydalı olduğu gözlemlenmiştir. Mevcut çalışmalar temel olarak ceviz proteini/yağ emülsiyon sistemlerinin karakterizasyonuna odaklanmaktadır. Gerçek bir gıda sistemi olan ceviz sütünü inceleyen sınırlı sayıda çalışma vardır. Ayrıca, ultrasesin cevizli gıdaların özellikleri üzerindeki etkisini belirlemek için çok az araştırma yapılmıştır. Bu nedenle, bu çalışmada, 25 kHz ultrasonik frekansta 10 dakika ultrases ile muamele edilmiş soyulmuş ve soyulmamış cevizden elde edilen sütler arasındaki fizikokimyasal özellikler ve farklılıklar incelenmiştir. Kurutulmuş cevizlerin kabukları elle soyulmuştur. Ceviz taneleri ikiye ayrıldı; biri zar ile tutuldu (soyulmamış, UP) ve diğeri soyuldu (P). Ceviz tanelerinin soyulması için taneler tartıldı ve %12 NaOH solüsyonu içeren bir behere daldırılarak bir ısıtıcı üzerinde

90°C'de tutuldu. Taneler çözeltiden çıkarıldı ve bir musluk suyu jeti altında yıkandı. Çekirdekleri kalan zarlardan temizlemek için aşındırıcı bir fırça kullanıldı.

Ayrıca, soyulmuş taneler, NaOH'nin etkisini nötralize etmek için gece boyunca damıtılmış suya batırılmış bir kavanozda tutuldu. Her iki ceviz içi (soyulmuş ve soyulmamış) için ağırlıklarının 4.5 katı saf su (50°C) ilave edildi. Daha sonra ceviz taneleri bir karıştırıcıda (Uyarı, ABD) 2 dakika öğütüldü. Elde edilen bulamaç, Ultra Turrax homojenleştirici (IKA T 18 Digital Ultra-Turrax, Almanya) kullanılarak homojenleştirildi. Ceviz içecek numuneleri (yaklaşık 450 mL), çift cidarlı 500 mL'lik behere döküldü. Ayrıca, ultrasonik prob ceviz içeceği içeren beherin tabanından 1 cm yukarıya daldırılmıştır. Numuneler, 25 kHz'de 10 dakika boyunca ultrases ile muamele edildi. Ultrasonifikasyondan sonra cevizli içecek şişelere döküldü ve 121°C'de 15 dakika otoklavlandı. Son olarak, şişeler 4°C'de saklandı, altı ay süreyle saklandı ve 45 günlük aralıklarla analiz edildi.

Elde edilen bulgulara göre toplam kuru madde değerlerindeki değişim (%12,80-17,17) depolama öncesi istatistiksel olarak anlamlı bulunmuş, depolama sırasında artış (%12,97-17,18) meydana gelmiştir., Numunelerin kül değerleri %0,14 arasında değişmiştir. ve depolamanın başında %0,30 ve depolamanın sonunda %0,19 ile %0,30 arasındadır. Örnekler arasındaki farklar anlamlı kabul edildi (p<0.05). Cevizli içeceklerde ellagik ve gallik asit içeriği depolama süresince artmıştır.

Cevizli içeceklerin beslenme analizi, soyulmamış ultrases uygulanan (UPUS) numunelerin %2,93 ve %12,63 ham protein ve yağ içerdiğini, kontrol cevizli içeceklerden daha yüksek olduğunu ortaya çıkardı. Toplam katı madde ve kül içeriği, soyulmamış kontrol (UPC) cevizli içecek numunelerinde en yüksekti. Mineral içeriği (Mg, Fe) UPC cevizli içeceklerde, Ca, K ve Zn ise UPUS örneklerinde daha yüksek bulundu. Buna karşılık, sodyum ve bakır sırasıyla PC ve PUS örneklerinde en yüksek bulunmuştur.

Depolama süresince örneklerdeki renk değişimleri incelendiğinde L*, a* beyazlık indeksi ve ΔE* değerleri artmıştır. Buna karşılık hue, b* ve C* değerleri azaldı. Depolama sırasındaki değişikliklerin istatistiksel olarak anlamlı olduğu bulundu (p<0.05), en düşük L* değeri 0. günde UPC örneğinde (34.1), en düşük a* değeri 45. günde soyulmuş ultrases uygulanan (PUS) (0.2) örneklerde, en düşük b* değeri 180. günde PC örneğinde (8.6), en düşük C* değeri 180. günde soyulmuş kontrol (PC) örneklerinde (8.6). Depolama ile gözlenen artış, ürünün rengindeki kırmızılığın depolama ile arttığını göstermektedir. Ultrasonikasyon uygulanan numunelerin depolama sonunda renk değerleri incelendiğinde numunelerdeki L* ve a* değerleri kontrol numunelerine göre biraz daha yüksek çıkmıştır.

Depolama süresince PC ve PUS örneklerinin toplam fenolik içeriğinde depolama sonunda sırasıyla %18,34 ve %22,78 oranında kademeli bir azalma eğilimi belirlenmiştir. Ancak UPC ve UPUS örneklerinin toplam fenolik içeriği sırasıyla %9,17 ve %34,95 ile en yüksek artışı 45 günlük depolamada göstermiş ve daha sonra depolama sonunda azalmıştır.

FRAP değerleri açısından antioksidan aktivite UPUS örneklerinde en yüksek olup, depolama ilerledikçe azalmıştır. Soyulmamış cevizli içecek örneklerinin antioksidan aktivitesi, soyulmuş cevizli içecek örneklerinden 72,2 kat daha yüksekti. DPPH, UPUS örneklerinde en yüksek değerle FRAP ile aynı eğilimi gösterdi.

Parçacık boyutu, kontrol numunelerinde $D_{3,2}$'de kademeli bir artış gösterirken, ultrasonik numunelerde üniform olmayan bir model gözlemlendi. PC örneklerinin $D_{4,3}$ değeri (198,67 μm) en yüksek, PUS örnekleri ise en düşük $D_{4,3}$ değerini (96 μm) gösterdi. Ayrıca en yüksek $D_{3,2}$ değeri (15,53 μm) UPC örneklerinde, en düşük değer (2 μm) ise PUS örneklerinde belirlenmiştir. Hacim ağırlıklı ortalama çap $D_{4,3}$ değerleri, ortalama parçacık boyutu değerleri $D_{0.5}$ ile benzer sonuçlar gösterdi.

Cevizli içeceklerde tespit edilen en yaygın fenolik bileşenler gallik asit ve ellajik asittir. Depolama başlangıcında fenolik bileşiklerde artış olmuştur. Gallik ve

ellajik asit içerikleri depolamanın başında ve sonunda PC numunelerinde en düşük seviyedeydi. Ultrases kullanımının ceviz içeceğinin gallik ve ellajik asit içeriğini arttırdığı gözlenmiştir.

Cevizli içeceklerde en fazla bulunan temel şeker bileşeni sükroz olup, onu depolama süresince artma ve azalma davranışı gösteren fruktoz ve glikoz şekerleri izlemiştir. Ayrıca, ultrases şekerler üzerinde artırıcı etki göstermiştir.

Cevizli içeceklerin duyusal değerlendirmesi depolama başlangıcında 11 panelist ile gerçekleştirilmiştir. PC örnekleri 7,77 puanla daha çok beğenildi ve en çok beğenilen örnek olmuştur.

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TABLE OF CONTENTS **PAGE**

LIST OF TABLES **PAGE**

LIST OF FIGURES **PAGE**

ABBREVIATIONS

CMPA	: Cow's Milk Protein Allergy
PC	: Peeled Control
PUS	: Peeled Ultrasound
UPC	: Unpeeled Control
UPUS	: Unpeeled Ultrasound
WHO	: World Health Organization
FAO	: Food And Agricultural Organization
AOAC	: Association Of Official Agricultural Chemists
HPLC	: High-Performance Liquid Chromatography
TPC	: Total Phenolic Compounds
TFC	: Total Flavonoid Content
DPPH	: 2,2-Diphenyl-1-Picrylhydrazyl
FRAP	: Ferric Reducing Antioxidant Power
UPLC/MS	: Ultra Performance Liquid Chromatography Mass Spectrometry
$NaHCO_3$	: Sodium Bicarbonate
IU	: International Unit
ALA	: Alpha-Linolenic Acid
EPA	: Eicosapentaenoic Acid
DHA	: Docosahexaenoic Acid
mcg	: Microgram
mg	: Milligram
mL	: Milliliter
Oz.	: Ounce

1. INTRODUCTION

Recently people around the world have switched towards a plant-based diet that includes different food groups like fruits and vegetables, legumes, pulses, and nuts due to various reasons such as an aversion to animal cruelty, environmental awareness, healthy lifestyle, and avoidance of health problems caused by animal foods (Stuckler and Basu, 2011) like cow's milk protein allergy (CMPA), lactose intolerance. Therefore, new product development research is currently conducted not only for nutritional goals but also for health promotion and illness prevention. As a result, the emphasis is on designing products that promote both mental and physical health. Furthermore, because of the growing market need for dairy-free products and derivatives, such as plant-based milk, creating new products is critical for enterprises; such new products are closely tied to population consumption and need patterns. (Anne Muñoz-Furlong, 2003). Therefore, the current system needs an immediate change to cope with the rapidly growing demands for plant-based products. Innovation can be made to pave the way and meet the needs of consumers, which can be accomplished through beverages, plant-based milk, or plant-milk alternatives like soy, rice, corn, and nuts, among others.

Plant-based milk alternatives' health advantages have been examined for both positive and negative consequences. They have a positive impact by lowering the risk of cardiovascular illnesses, diabetes, and atherosclerosis owing to their significant antioxidant activity and fatty acid content (Zujko and Witkowska, 2014). However, compared to bovine milk, most of these alternatives lack nutritional balance. They do, however, include functionally active components that have health-promoting qualities, such as hypocholesterolemic and anticancer capabilities (Sethi et al., 2016).

According to Mintel’s research, plant-based milk consumption surged by 19% in three months in 2018. Furthermore, each category of plant-based milk

alternatives has grown year after year. For example, between 2017 and 2018, sales of oat milk substitutes increased by 71%, almond milk by 10%, and coconut milk by 16%. They can be sold plain or with some added ingredients. They are classified as aromatic, with or without sugar, low fat, and enriched (Wood, 2019).

However, there is still an immense area to explore in plant-based alternatives from legumes (soybeans, chickpea), cereals (rice, oats), seeds (sesame, sunflower), pseudo cereals, (quinoa), and nuts (Cashew nut, Brazil nut, almonds, hazelnuts) to prepare beverages or drinks. In the past, significant focus has been placed on soy milk owing to its numerous health benefits. However, interest has recently been switched to investigating the use of various cereals, nuts, and oilseeds for new food production based on their functional qualities, which reveal the physical characteristics of food components and their interactions (Sethi et al., 2016).

Plant-based beverages are suspensions and emulsions derived from a variety of sources, including soy, almond, rice, oat, and peanut, in which the nut oil is disseminated in an aqueous phase, and the ingredients play varied roles in the product's stability (Bernat et al., 2015a). Previous studies have reported the development of functional drinks from sea buckthorn syrup, soymilk (Maftei et al., 2012), carrot and soya milk (Banigo, 2015), high protein and fiber-enriched soy and mushroom drink (Farzana et al., 2017). After homogenization with subsequent heating, a stable mixed beverage made of soy and walnut milk (Chen et al., 2014). Cashew milk (Manzoor, 2017), soy-walnut milk drinks (Bolarinwa et al., 2018), sesame milk (Ahmadian-Kouchaksaraei et al., 2014), and almond milk (Briviba et al., 2016). Plant-based beverages do, however, confront several difficulties, and one such barrier is their poor dispersion stability. The cause of the poor dispersion stability has not yet been determined. They have complex dispersion properties that require these products to be submitted to a homogenization step to obtain a stable product during their commercial shelf life (Valencia-Flores et al., 2013)

Currently, novel processing techniques are used to improve the stability and quality of plant-based beverages. In many studies, the ultrasound (US) technique is used to improve the stability of dispersions and emulsions (Abismaïl et al., 1999; Canselier et al., 2010; Chemat et al., 2011). For example, ultrasound has been shown to increase the physical stability of almond 'milk' using 5 minutes at 300 W, coconut milk' using 13 minutes at 55 W/cm2, and peanut 'milk' using three minutes at 400 W, which can be related to alterations in particle size (proteins and lipids) and rheology (Rojas et al., 2022).

Ultrasonic processing is commonly used to expand the shelf life of juices and also boost yield and extraction, and improve the rheological characteristics and turbidity of the same. However, ultrasound alone is insufficient for microbial and enzymatic inactivation; it must be combined with other inactivation methods, such as temperature, irradiation, pressure, and so on, to achieve desired results. The term thermosonication refers to the use of ultrasound at moderate to high temperatures (Irkilmez et al., 2017).

The impact of ultrasound treatments on the quality properties of walnut beverages has not yet been researched. Therefore, this study used ultrasonic treatment at different densities and durations to stabilize the walnut beverages.

This study aimed to analyze the effect of ultrasound treatment on walnut beverages' physical properties in order to define the processing conditions that ensure product quality and stability; also, it aimed to analyze the phenolic and fatty acid composition, chemical composition, basic quality properties like total soluble solids, ash, pH, viscosity, and microstructure of the walnut beverages in terms of storage.

2. LITERATURE REVIEW

Since earlier times, humans have been consuming nuts on a regular basis because of their richness in nutrients. In addition, they are an excellent source of bioactive substances that are good for human health. Therefore, they are a crucial component of many traditional and modern gastronomy recipes worldwide, as well as healthy snack foods. Walnut, with the scientific name *Juglans regia*, is a member of the *Juglandaceae* family. It is one of the nuts that is particularly rich in nutrients and has a great deal of therapeutic potential. Walnut kernels are highly nutritious due to their high protein and oil content. As a result, the Food and Agriculture Organization (FAO) has listed the walnut on its list of priority plants and identified it as a plant that is essential for human nutrition. To reap the full nutritional, bioactive, and antioxidant benefits of walnuts, it is strongly advised to consume nuts or dried fruits on a daily basis. All around the world, walnut has been included as a vital portion of human nutrition (Mohammadi et al., 2011; Mohammadi Delaviz et al., 2012; Mirzaei et al., 2012).

Recently walnut has gained immense popularity due to their health-promoting capabilities, which have been linked to a reduction in the risk of diseases such as hypercholesterolemia, arteriosclerosis, hypertriglyceridemia, diabetes mellitus, and cardiovascular disease (Arós et al., 2013; Pan et al., 2013; Ros et al., 2004). The health characteristics of walnut are generally attributed to ω-3 fatty acids (Ros et al., 2004) and, to a small degree, to the presence of vitamin E (Maguire et al., 2004).

Walnuts were considered to be unhealthy because of their high-fat content. However, with time they became popular due to their high contents of proteins, fats, minerals, and vitamins (Thakur and Singh, 2013). In addition, they also contain a high amount of other bioactive compounds, like sterols, flavonoids, phenolic acids, related polyphenols, and pectic substances, that may positively affect health (Vinson and Cai, 2012).

The total amount of polyphenols in walnut was found to be remarkably more significant as compared to other nuts (peanuts, almonds, and hazelnuts, among others) (Abe et al., 2010). According to reports, among the polyphenols present in walnut, ellagitannins are the most abundant and significant phenolic compounds (Fukuda et al., 2003; Ito et al., 2007).

One serving of walnut, about 28 g, contains 185 calories and 3.9 g of carbohydrates, 1.9 g of fiber, 18.5 g of fat, and 4.3 g of protein. The fat and calories in walnuts are high, while the saturated fat content is minimal. The high fiber content helps promote digestive health (Thakur and Singh, 2013). In addition, walnuts are a whole food with the most excellent alpha-linoleic acid (ALA) content of any edible plant (Ros, 2010).

2.1. Origin and Habitat

The mountainous regions of Central Asia are the natural habitat of the *Juglans regia* tree. This includes the Xinjiang province of western China, parts of Kazakhstan, southern Kirghizia, and Uzbekistan, as well as the lower mountain ranges of Nepal, Bhutan, northern India, Tibet, Pakistan, and Sri Lanka. It also includes portions of Afghanistan, Iran, and Turkmenistan, as well as parts of Armenia, Azerbaijan, eastern Turkey, and Georgia. These countries have significant genetic diversity, particularly ancestral forms with lateral fruiting. While on its migrating journey to western Europe, the common walnut lost this characteristic and evolved into a big tree that bears fruit only in terminal fruiting style. However, a small population of these *Juglans regia* trees in Southern Europe managed to survive the most recent glacial episode. Despite this, most of the wild germplasm found on the Balkan peninsula and throughout much of Turkey was almost certainly brought there from eastern Turkey several thousand years ago through trade and settlement (Shah et al., 2014).

China is the world's leading walnut producer, with Turkey ranking fourth. Turkey produces 217334 metric tons of walnut annually (FAO, 2019). For fruit

production, the most commonly grown walnut species in Turkey is *Juglans regia*, named 'Ceviz' or 'Koz' in Turkish. Walnut has an important place in Turkish culture. Walnut is known as the protein tree in Turkey, with its fruits known to be an aphrodisiac and source of energy (Akca and Yilmaz, 2017).

Table 2.1. Walnut production, cultivation area, and yield (FAOSTAT, 2021).

Country	**Production Quantity (ton)**	**Cultivation Area (ha)**	**Yield (ton/ha)**
China	2521504	631330	3,99
USA	592390	147710	4.01
Iran	321074	44780	7.17
Turkey	225000	124553	1.81
Mexico	171368	102068	1.68
Ukraine	125850	13900	9.05
Chile	122950	40801	3.01
Uzbekistan	50660	4857	10.43
Other countries	367646	195350	

2.2. Walnut nutritional composition

Walnut (*Juglans regia*, L.), a hard-shelled fruit, has a special place among fruit species due to the high nutritional value of its fruit as well as its timber being a valuable raw material for the furniture industry. As a result, it takes second place after hazelnut in terms of production among hard-shelled fruits in Turkey (Selek, 2011). The different walnut parts are shown in Figure. 2.1.

The components of walnut fruit are the skin, kernel, green husk, and shell. The fruit of walnuts produces two important byproducts: the shell and husk. The walnut kernel is consumed as fresh or as an ingredient in various bakery products, tarts, candies, pies, and confectionaries as part of a well-balanced and health-promoting diet. It has a unique flavor and astringency taste. Syrups, Pickles, walnut

chutney, and marmalades are known products made from immature fruits (Hussain et al., 2021).

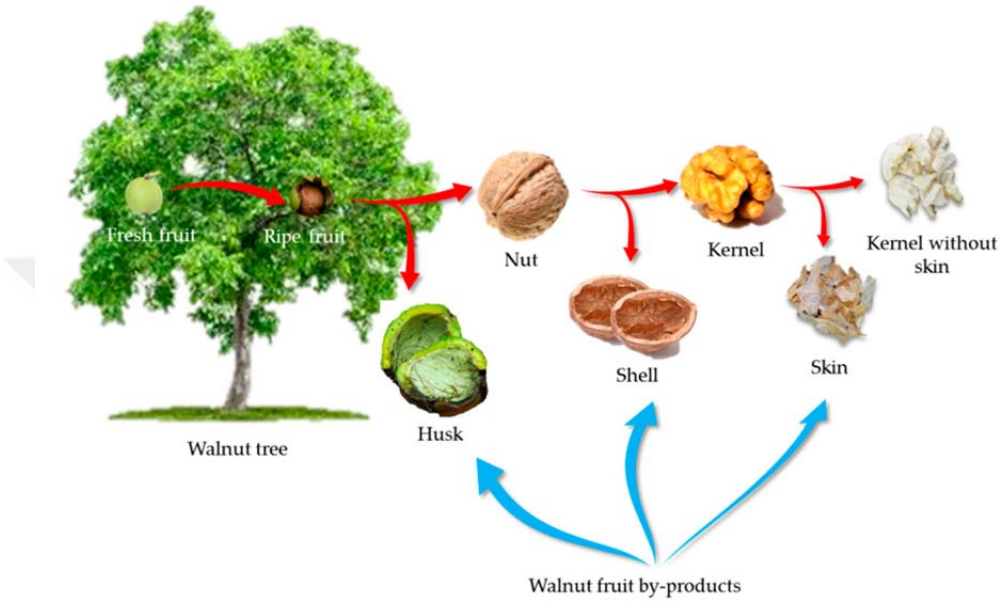

Figure 2.1. Different parts of walnut fruit (Jahanban-Esfahlan et al., 2019)

2.2.1. Fatty acid

In walnuts, most energy comes from fats, about 65% by weight; thus, they are a high-energy and high-calorie food. The major components of walnut oil are free fatty acids, triacylglycerols, monoacylglycerols, diacylglycerols, sterol esters, and phosphatides, which are present in small quantities. The primary fatty acids found in walnut are linolenic acid (18:3), linoleic acid (18:2), and oleic acid (18:1). Linoleic acid (omega-6 fatty acid) is the most abundant fatty acid. They also contain a high percentage of alpha-linolenic acid (ALA), a healthy omega-3 fatty acid (Shah et al., 2014).

Walnuts are considered one of the primary sources of alpha-linolenic acid (ALA). They are the only nuts with high ALA levels, which is good for cardiovascular health. It also aids in the improvement of blood fat composition and

the reduction of inflammation. It also serves as a precursor for the long-chain omega-3 fatty acids EPA and DHA, both of which have several health benefits (Thakur and Singh, 2013) (Table 2.2).

Table 2.2. Fatty acid composition of walnut (Ni et al., 2022).

Nutritional ingredients	Content
Fat (g)	64.5 g/100 g nut meat
Total PUFAs (g)	72.96 g/100 g lipid
Total MUFAs g)	15.28 g/100 g lipid
Linoleic acid (C18:2n6) (PUFA) (g)	59.79/100 g lipid
Alpha-linolenic acid (C18:3n3) (PUFA) (g)	13.17/100 g lipid
Total polyphenols (Gallic acid equivalents) (mg)	1556(mg GAE/100mg)

(PUFA: Polyunsaturated fatty acids, MUFA: Monounsaturated fatty acids, GEA: Gallic acid equivalents)

2.2.2. Tocopherols (Vitamin E)

Gamma-tocopherol, a type of vitamin E, is found in high concentrations in walnuts. Isomers of vitamin E possess a high level of antioxidant activity. They are essential contributors to the oxidization of unsaturated fatty acids in lipid membranes. To this day, there is insufficient documentation regarding the measurement of these isomers in walnut oil. Lavedrine et al. (1999) have provided information regarding the vitamin E content of walnuts farmed in the United States of America and France. Tocopherol was found in fresh walnuts and walnuts that had been stored; however, considerable amounts of the compound were lost during storage at 4 degrees Celsius for three months. In addition, α tocopherol was identified as the main tocopherol in walnut oil (Savage et al., 1999).

2.2.3. Amino Acids

Walnuts have 13.6 to 18.1 g of crude protein per 100 g of dry matter, making them a prime source of protein (Savage and Mcneil, 2001). Additionally, walnuts have significantly high levels of arginine (Table 2.3) and a comparatively minimal amount of lysine (Ruggeri et al., 2004). Walnuts' high arginine levels have been regarded as a good attribute because arginine may be turned into nitric oxide, and this potent vasodilator can decrease platelet adhesion and aggregation (Sabate et al., 1993).

2.2.4. Vitamin and Minerals

Walnuts are high in various vitamins and minerals, including magnesium and phosphorus. Both minerals are essential to achieve optimal health. Walnuts contain more than 10% of the daily recommended intake for both magnesium and phosphorus. According to a study conducted on two walnut cultivars (Franquette and Hartley), potassium levels of the French walnuts were higher (Franquette: 487 and Hartley: 466 mg 100 g^{-1}), and high levels of magnesium were observed for the Franquette cultivars (French: 191 and Californian: 202 mg 100 g^{-1}). In addition, 28.35g of walnut has 450 mcg of copper, which is more than 20% of the recommended daily allowance for the mentioned mineral. Various other nutrients in walnuts include vitamins E, C, B6, and K, pantothenic acid, folate, calcium, choline, riboflavin, betaine, thiamin, niacin, iron, potassium, selenium, and zinc (Lavedrine et al., 2000).

Table 2.3. Detailed nutritional composition of walnut nutrition facts (weight:100g)

Nutrients	Amount	Nutrients	Amount
Water	3.14 g	**Vitamins**	
Carbohydrates	10.9 g	Thiamin	0.23 mg
Ash	1.64 g	Niacin	1.12 mg
Fat	69.7 g	Vitamin B-6	0.66 mg
Proteins	14.6g	Biotin	17.3 µg
Amino Acids		**Minerals**	
Tryptophan	0.127 g	Calcium	88 mg
Threonine	0.443 g	Iron	2.24 mg
Isoleucine	0.52 g	Magnesium	142 mg
Leucine	0.997 g	Phosphorus	365 mg
Lysine	0.38 g	Potassium	424 mg
Methionine	0.19 g	Sodium	<2.5 mg
Cysteine	0.377 g	Zinc	2.76 mg
Phenylalanine	0.607 g	Copper	1.21 mg
Tyrosine	0.43 g	Manganese	3 mg
Valine	0.597 g	Molybdenum	21 µg
Arginine	2.02 g		
Histidine	0.343 g		
Alanine	0.59 g		
Aspartic acid	1.29 g		
Glutamic acid	2.99 g		
Glycine	0.69 g		
Proline	0.78 g		
Serine	0.78 g		
Hydroxyproline	<0.01 g		

g: Gram, mg: Milligram, µg: Microgram
Source: U.S. food and drug administration's Food Data Central (USDA, 2022.)

2.3. Nutraceutical Potential of Walnut

2.3.1. Antioxidant Potential:

Numerous studies have demonstrated that walnut ingredients, mainly their fruits, leaves, and the alcoholic chemicals formed from green fruits, have powerful antioxidant properties, according to Delaviz et al. (2017). For example, walnuts have antioxidant characteristics that make them helpful in treating individuals with chronic diabetes. In addition, according to a few studies, the fruits of walnut trees have been demonstrated to contain polyphenols and Vitamin C, which have antioxidant benefits (Ahmad et al., 2012).

A study found that a handful of walnuts has a lot more phenols than a glass of red wine (372 mg), a bar of milk chocolate(205 mg), or a glass of apple juice(117 mg) (Anderson et al., 2001). Furthermore, compared to other commonly consumed plant foods, walnuts had the second highest antioxidant activity after rose hips (*Rosa canina*) (Halvorsen et al., 2002). Also, compared to other tree nuts, walnuts scored top in terms of the total radical-trapping antioxidant parameter, ferric-reducing antioxidant power, and Trolox equivalent antioxidant capacity (Pellegrini et al., 2006). The majority of the antioxidant property is attributed to the polyphenolic compounds, particularly the ellagitannins, which are primarily contained in the pellicles (Blomhoff et al., 2006).

Polyphenols extracted from walnuts, including polymeric tannins, ellagic acid monomers, and other phenolic substances, are potent inhibitors of plasma and LDL oxidation in vitro (Anderson et al., 2001). In addition, they decrease the biomarkers of oxidative stress in diabetic mice (Fukuda et al., 2004). Furthermore, melatonin, another antioxidant ingredient found in walnuts, has been linked to improved plasma antioxidant properties in rats (Reiter et al., 2005).

2.3.2. Antidiabetic Potential

Although the most common way to treat diabetes is insulin, a nutritional approach to the treatment of diabetes is very effective in developing countries. Regular consumption of walnuts aids in the treatment of type II diabetes by maintaining insulin metabolism and blood sugar. A study found that polyphenols and the polyphenolic constituents of walnut-like Tellimagradin I, Tellimagradin II, and Casuarictin have strong inhibition activity on several enzymes such as maltase, sucrose, amylase, and glycosidase. Researchers also discovered that a 200mg/kg/day dose of walnut polyphenol-rich fraction has a triglyceride-lowering and urine peroxide-lowering impact in genetically inherited Type II diabetic mellitus (db./db.) mice (Fukuda et al., 2004).

A study including 58 men and women between the ages of 35 and 75 revealed a favorable impact of a moderate-fat diet that included walnuts on the blood lipid profiles of type II diabetes patients. The researchers determined that 30 g of walnuts per day provides significant amounts of polyunsaturated fatty acid, improving the lipid profile of type 2 diabetic patients (Neale et al., 2017).

A prospective cohort study was done on 83,818 (34-59 yrs.) women, from 11 states, with no history of diabetes and discovered that women who ate one-ounce amounts of nuts, such as peanuts or walnuts, had a considerably lower risk of acquiring type II diabetes than women who ate nuts infrequently or never. The researchers also concluded that increased peanut butter and nut intake might help in lowering the chances of type II diabetes in these women. Further, nut consumption can be easily used as a snack in daily diet, which can remove refined carbohydrate products or processed or red meats and helps in reducing caloric intake, which can further help in solving many health problems (Jiang et al., 2002).

2.3.3. Heart Health Potential

Walnuts are gaining popularity as a healthy food since frequent intake has been shown to reduce the likelihood of developing heart disease (Lavedrine et al., 1999; J. Sabate and Fraser, 1993). Furthermore, walnuts are a good food/snack since they have a 4:1 ratio of n-3 and n-6 polyunsaturated fatty acids. The "Lyon Heart Study" has also been demonstrated to reduce the chance of sudden death. The n-3 fatty acids are heart-healthy because they reduce the bloodstream's low-density lipoprotein (LDL) cholesterol levels while maintaining or increasing high-density lipoprotein (HDL) cholesterol levels (Patel, 2005).

Although walnuts are high in fat, a walnut-enriched diet lowers blood cholesterol. In addition, it reduces the ratio of serum concentrations of low-density lipoprotein to high-density lipoprotein by 12% (Sabate and Fraser, 1993).

Researchers have shown a relationship between mineral intake and healthy blood pressure. The critical blood pressure-regulating minerals are potassium, with 375-500 milligrams per 100 grams; calcium, with 13-91 milligrams per 100 grams; and magnesium, with 189-278 milligrams per 100 grams, found in different varieties of walnut. These minerals aid in the maintenance of normal blood composition and blood vessel wall elasticity. Furthermore, walnuts are high in ALA, essential for enhancing cardiovascular functions such as blood pressure. It not only improves the good-to-bad cholesterol ratio but also protects cholesterol from forming plaque in the arteries and avoids irregular heartbeats. Furthermore, these acids successfully prevent heart attacks by decreasing the likelihood of blood clotting in arteries (Feldman, 2002).

2.3.4. Anticancerous Effect

Walnuts are a good source of flavonoids. Flavonoids have antioxidant properties, help improve the immune system and increase the anticancerous activity of the body (Shojaii et al., 2011). Many researchers have demonstrated that the consumption of walnut can help against cancer. Walnut consists of gamma-

tocopherol, which has been shown to help combat breast, prostate, and lung cancer. Walnut consumption has been related to a lower risk of breast cancer in mice, which has been connected to altered gene expression for cancer growth and survival. Hardman et al. (2019) conducted a study that activated pathways promoting cell adhesion and apoptosis and suppressing pathways promoting cell proliferation and migration.

Consuming nuts on a regular basis has been linked to improved weight management as well as lower levels of adiposity. In addition, a favorable effect was seen on cardiovascular disease risk factors; weight, and satiety in a clinical study performed on overweight men and women who were given a reduced-energy diet (15% of energy) rich with walnut constituents. The assessment was performed at clinical appointments every 3 to 6 months, with a feeling of emptiness, satisfaction, and eagerness (Rock et al., 2017). Furthermore, 30 grams of walnuts per day resulted in weight loss and positive changes in food choices (Neale et al., 2017).

2.4. Bioactive compounds in different parts of walnut

Studies have shown that the phytochemicals of edible walnuts, particularly phenolic compounds, can have several positive effects on health. The Walnut pellicle is the outer skin of walnut kernels. It primarily comprises phenolic acids, hydrolyzable tannins, polyphenolic compounds, many lipids, and a limited portion of the flavonoids family (Ni et al., 2022).

The primary constituent of the walnut pellicle is hydrolyzable tannin. The walnut pellicle contained 32 different phenolic compounds, with a high content of ellagic acid in the phenolic acid. The levels of hexahydroxydiphenoyl-glucose, bis-hexahydroxydiphenoyl-glucose in tannin, Galloyl-hexahydroxydiphenoyl-glucose, and other sugars were found to be high in the pellicle (Zhang et al., 2020).

Table 2.4. Phenolic acids in walnut peel and kernel (Trandafir and Cosmulescu, 2020; Zhang et al., 2020)

Compound	Walnut Pellicle (µg/g)	Walnut Kernel (µg/g)
Phenolic acid	Ellagic acid (1023.9)	Cinnamic acid (213.38)
	Azelaic acid (244.21)	Ellagic acid (65.07)
	Ferulic acid isomer (204.93)	Chlorogenic acid (29.68)
	Dihydrophaseic acid (100.02)	Neochlorogenic acid (1.89)
	Quinic acid (58.48)	Vanillic acid (7.2)
	p-hydroxybenzoic acid (19.48)	Syringic acid (7.26)
	Syringic acid (14.97)	Quinic acid (4.69)
	Coumaroylquinic acid (9.13)	Gallic acid (3.26)
	p-coumaric acid (4.3)	Caffeic acid (2.76)
	Caffeic acid (5.8)	*p*-hydroxybenzoic acid (1.38)
		Ferulic acid (0.98)
		p-coumaric acid (0.21)

2.5. Ultrasound

The food sector is constantly looking into emerging mild processing technologies such as high-pressure processing, pulsed electric, and magnetic fields, etc., not only to obtain high-quality food with "fresh-like" characteristics but also to obtain improved or even novel food functionalities (Ashokkumar et al., 2008). Among these emerging technologies, ultrasound (US) has received considerable attention in recent years, especially when paired with mild pressure and temperature (manothermosonication, MTS) (Demirdöven and Baysal, 2008).

Ultrasound technology is based on mechanical waves at a frequency above the threshold of human hearing (>16 kHz). These waves travel either through the bulk of a material or on its surface at a speed characteristic of the wave's nature and the material through which it propagates (Knorr et al., 2004; Povey and McClements, 1988; Malcolm Povey et al., 2010). Ultrasound, an example of sound waves, is depicted in Fig. 2.2

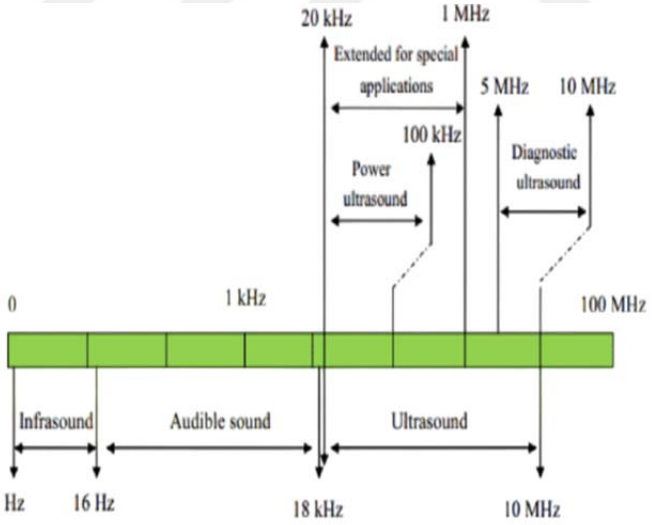

Figure 2.2. Classification of sound waves with their frequency (Cheng et al., 2015)

Ultrasound is classified into two categories: 1) low-intensity ultrasound (with power intensity of < 1 Wcm^{-2} and frequency of 5-10 MHz) and 2) high-intensity ultrasound (with power intensity of 10-100 Wcm^{-2} and frequency of 20-100 kHz), the former of which is employed in material science and the latter is utilized in food processing technologies. There are several applications for high-intensity ultrasound in the food and dairy industries, including homogenization, extraction, degassing, emulsification, frying, cutting, mixing, brining, pickling, degumming, and meat tenderization (Tiwari and Mason, 2012).

These days, drinkable extracts of plants, often known as plant-based milk, are becoming increasingly popular due to their nutritional benefits. These serve as an alternative to milk derived from dairy animals, particularly for individuals who cannot digest lactose and have an extreme sensitivity to cow's milk (Iorio et al., 2019). In addition, plant-based milk is highly recommended to reduce their chances of developing cardiovascular problems, which are relatively high (Aydar et al., 2020).

To restate, plant-based replacements, often known as plant milk, are aqueous extracts of legumes, grains, seeds, and nuts, among other plant-based foods, designed to mimic the animal-based texture and flavor of milk. According to a study, beverages made from plants are suspensions made up of plant materials that have been dissolved. The milling of raw material (wet or dry), which is followed by the preparation of slurry and the filtration of slurry until it reaches the consistency of cow's milk, followed by the addition of other ingredients such as sugar, flavor, etc., followed by homogenization and pasteurization to improve the microbial and storage stability of the beverage (Diarra et al., 2005).

Thus, this study was focused on processing and bringing out the value of walnut beverages which is not much explored. Hence this study broadly covered the effect of ultrasound on the various quality attributes of walnut beverages. Ultrasound, a relatively new method, is utilized mainly for the physical effects, referred to as acoustic cavitation (Shanmugam and Ashokkumar, 2017) (Fig.2.3).

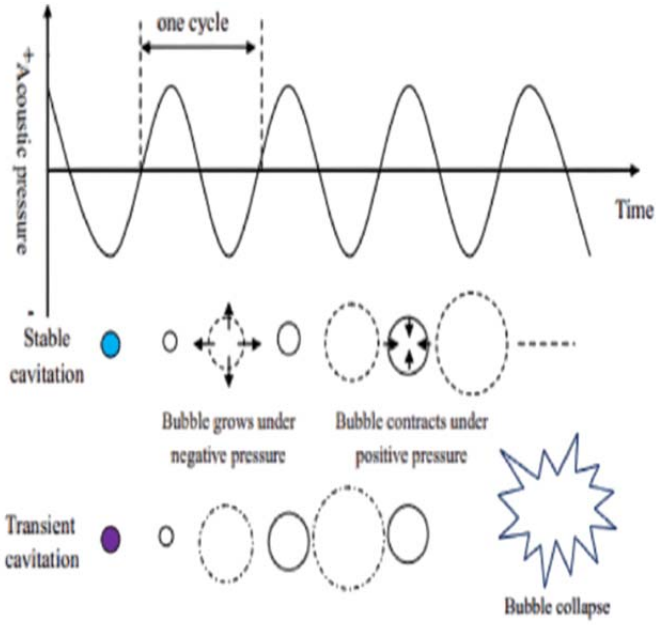

Figure 2.3. Cavitation induced by ultrasound (Cheng et al., 2015)

Acoustic cavitation produces changes in food matrices by the growth and collapse of microbubbles, causing localized incremental temperatures, turbulence, shockwaves, and pressures, as represented in Fig 2.3. The equipment primarily used for ultrasound applications includes sonotrode, as illustrated in Fig 2.4.

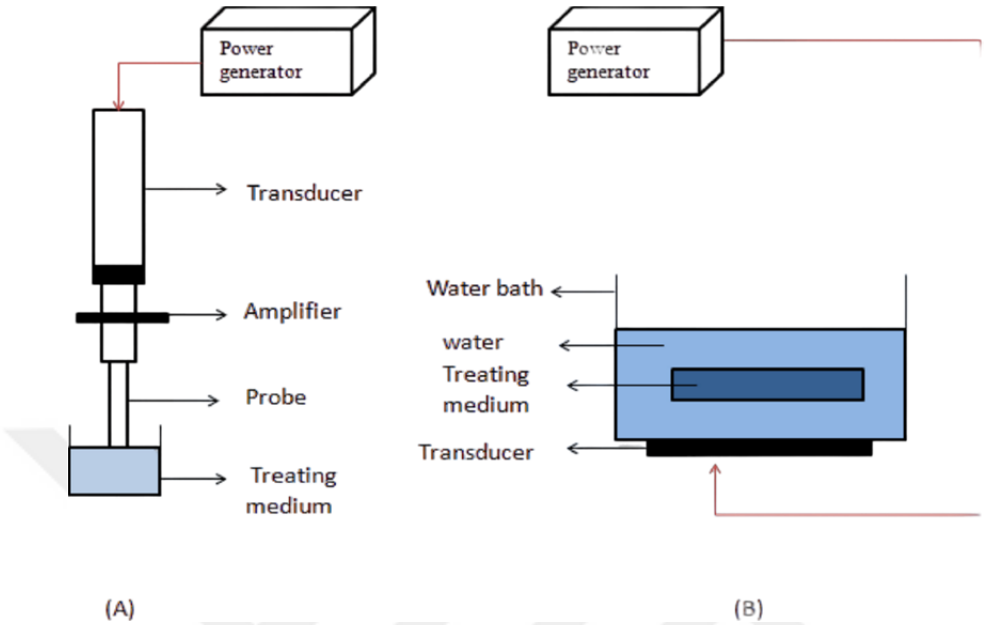

Figure 2.4. Different ultrasound systems (A) is a sonotrode, and (B) is an ultrasonic water bath (Sarangapany et al., 2022).

Thus, the main objective of this study was to determine the effect of ultrasound application on the physico-chemical properties, functional properties, and organoleptic properties of walnut beverages.

2.6. Previous studies

Popovici et al. (2017) conducted a study to design a walnut milk production technique. The basic quality properties, chemical, microstructure, and rheological behavior in storage were analyzed. In order to prepare walnut milk, the walnuts were broken, peeled, soaked, and ground into a powder. This powder was then extracted in water, decanted, and homogenized. This investigation showed that walnut milk had a higher fat concentration (18 g/100 mL) than its protein concentration (1.3 g/100 mL). On the first day, the normal distribution was seen for the dimensions of the oil drops in the walnut milk. The sizes of the particles ranged from 0.45 microns to 5.40 microns. Most of the oil's volume had an average diameter of approximately 2.70 microns. The sample of walnut milk showed a rise in viscosity after three days in storage.

Chen et al. (2022) undertook a study to assess the health benefits of eating raw, soaking, peeled, and roasted walnut kernels, as well as to better determine the advantages of walnut intake from the standpoint of bioactive proteins. Therefore, the walnuts were peeled by hand, and an extract was prepared using water. To further understand the solubility characteristics of bioactive proteins and their relationship to walnut kernels' primary components (storage proteins and oil bodies), the water extract was centrifuged to separate the floating, skim, and precipitate fractions. Bioactive proteins were detected using LC-MS/MS in the floating, skim, and precipitate fractions and the relative quantities of bioactive proteins such as exopeptidases, endopeptidases, SOD-related enzymes, SODs, protease inhibitors, and lipid-degrading enzymes were estimated. This study found that walnut kernels included a high concentration of proteases and antioxidant enzymes (such as SODs and catalases). Also, endopeptidases, aminopeptidases, carboxypeptidases, superoxide dismutase, catalases, and phospholipases with relative abundances of 2.73, 1.728, 0.477, 3.148, 0.743, and 0.173 %, respectively were identified.

Medic et al. (2021) did a study to investigate the phenolics content of walnuts, focusing on the content of various phenols and phenolic groups in the pellicle and peeled kernel to determine how much each segment of the kernel contributes to the total phenolic content. Kernels were gathered and immersed in water for 30 minutes to facilitate peeling in order to obtain peeled kernels and pellicles. The pellicles were ground into a fine powder using liquid nitrogen and a mortar. The remaining peeled kernels were mashed with a mechanical blender. 0.25 g of pellicle and 1 g of the peeled kernel were extracted separately with 100% methanol. The samples were vortexed, sonicated in cold water for 60 minutes, then centrifuged. The results revealed that walnut pellicles contained 12 ellagic acid derivatives, 19 ellagitannins, five phenols, and four anthocyanins. There was one phenol and 15 dicarboxylic acid derivatives in the walnut kernels. Peeled walnut kernels contained 13 compounds, while the pellicle had 14 compounds. The total phenolics in walnut kernels comprised 31% to 35% hydrolyzable tannins.

Peeled walnut kernels with deionized water (1:4.5) were blended, filtered, and mixed with Xanthan gum and nonionic emulsifiers to produce walnut beverage emulsions. This study aimed to investigate the impact of dietary antioxidants on the oxidation of lipids and the physical stability of walnut beverage emulsions. According to the findings, tea polyphenols may enhance the droplet size of emulsions and improve their physical stability during thermal storage at a temperature of 62 ± 1 °C. However, during the thermal storage process, the physical stability of the emulsion system was compromised due to the presence of water-dispersed oil-soluble vitamin E and enzymatically modified isoquercitrin. The physical stability of walnut beverage emulsions was not significantly altered by the BHT or natural antioxidant extracts (Liu et al., 2016).

In a study by Chen et al. (2014), a drink from walnut and raw soya milk was prepared by homogenization with subsequent heating. Original walnut milk was subjected to three different treatments. The first was heating the walnut milk at 120°C for 10 min, second to single-stage homogenization (40 MPa at each stage)

followed by heating at 120℃ for 10 min. The last was a two-stage homogenization (40 MPa at each stage) followed by heating at 120℃ for 10 min. This study uncovered that mixing walnut milk with raw soymilk in a ratio of 1/2 (w/w) resulted in approximately 90% of the walnut protein remaining in the supernatant after centrifugation (4,000 g, 30 minutes). The study indicates that this ratio should be recommended for improved walnut protein dispersibility.

A study conducted by Bolarinwa et al. (2018) prepared soy-walnut milk drinks by combining various quantities of freshly made soymilk (from soybeans or malted soybeans) and walnut milk (0:100, 10:90, 30:70, 50:50). In addition, the sensory qualities, proximate composition, mineral content, physicochemical characteristics of the soy-walnut drinks were also analyzed. The findings demonstrated that soy-walnut milk drinks made with 30% soymilk substitution significantly boosted protein and mineral content and were also consumer-acceptable.

A walnut kernel slurry was produced by combining walnut kernels that had been peeled with water that had been deionized at a weight ratio of 1:4.5. In order to remove the particles from the coarse slurry, a colloid mill was used for cleaning. Then the mixture was passed through three layers of gauze. This research aimed to determine how different pH levels, freeze-thaw cycles, and heat sterilization affect a walnut beverage emulsion's oxidative and physical stability while accounting for the inclusion of several emulsifiers and xanthan gum. When determining the emulsion's physical stability, the size of the droplets and the zeta potential were considered. After going through four cycles of freezing and thawing, the emulsion's stability was significantly improved using xanthan gum and a mixture of emulsifiers. In addition, blended emulsifiers with a pH range of 3-10 had improved stability by absorption at the oil-water interface (Liu et al., 2016).

Liu et al. (2022) investigated the effect of *Lactobacillus plantarum* on walnut milk after fermentation. Walnut milk was then put through a 10-minute sterilization process at 121 degrees Celsius and 35 and 45 MPa homogenizer

pressure. After that, strains of *lactobacillus plantarum* were introduced. Walnut milk that had been fermented contained a greater quantity of free amino acids than walnut milk that had not been fermented. Furthermore, fermented walnut milk had a more significant concentration of free fatty acids than non-fermented walnut milk. This finding lends credence to the theory that *Lactobacillus plantarum* LP56 boosts the synthesis of both amino acids and fatty acids. In addition, test samples that were fermented for 12 hours had the highest levels of sweet amino acids, umami amino acids, and polyunsaturated fatty acids, which contributed to an improvement in the quality and nutritional value of walnut milk.

Conjugated fatty acids were extracted from the walnut milk's abundant linoleic acid and linolenic acid-containing lipids through probiotics fermentation in a study conducted by Mao et al. (2022). Lactic acid bacteria were introduced to ferment walnut milk after lipase was added to liberate linoleic and linolenic acids. The study concluded that when 60 U/mL lipase was added, the total amount of conjugated fatty acids reached 1.33 0.01 mg/mL, with conversion rates of 11.3% and 5.0% for conjugated linolenic acid and conjugated linoleic acid, respectively. When 20 U/mL lipase was added to fermented walnut milk, the level of conjugated fatty acids increased to 1.58 mg/mL. Conversion rates for conjugated linolenic acid and conjugated linoleic acid increased to 21.6% and 7.2%, respectively. As a result, introducing lipase to walnut milk allows conjugated linolenic acid and conjugated linoleic acid to be converted into active forms.

Batun et al. (2017) determined the fatty acid and mineral composition of walnut genotypes growing in five locations around Van Lake. To measure the fatty acids in the walnuts, Fatty Acids Methyl Esters (FAMEs) were produced by dissolving 0.4 g of oil in 4 mL of isooctane and 0.2 mL of 2 M methanolic KOH. Gas chromatography was used to quantify the fatty acids (GC). Atomic absorption spectrometry directly detected minerals in the ash solution (dry ashing at 550°C). The oil content of walnuts increased dramatically throughout ripening, ranging from 65.7% to 70.7% in walnuts. The most abundant fatty acid in walnut was

linoleic acid, followed by oleic acid. During ripening, the levels of unsaturated fatty acids significantly rose in samples. In addition, during ripening, the tocopherol content of walnuts increased significantly. Initial and final tocopherol levels ranged from 8.3-18.5 to 121.9-135.4 mg/100 g oil. The most prevalent mineral in ripe walnuts was potassium, followed by magnesium and calcium.

In another study, the use of kefir grains as inoculum in the production of a walnut milk beverage was the subject of an investigation conducted by Cui et al. (2013). As a result, it was deduced that the ideal fermentation terms for the production of a walnut beverage inoculated with kefir grains were a fermentation temperature of 30 degrees Celsius, a fermentation time of 12 hours, an inoculum size of 3 gm of kefir grains, and a sucrose concentration of 8 gm/100 mL. Additionally, the inoculum size should consist of 3 gm of kefir grains.

Liquid chromatography combined with electrospray ionization hybrid linear trap quadrupole-Orbitrap mass spectrometry was used to identify the phenolic chemicals in walnuts (LC-LTQ-Orbitrap). Based on accurate mass measurements, retention periods, subsequent mass fragmentation data, or comparison with reference substances and literature, a total of 120 compounds, including hydrolyzable and condensed tannins, phenolic acids, and flavonoids, were identified or tentatively identified (Rgueiro et al., 2014).

A study isolated and characterized the primary phenolic compounds in walnut pellicles. Their interaction with walnut protein during extraction was investigated by Kong et al. (2023). In particular, the effect on the solubility of proteins in walnuts was studied in depth. Alterations in protein structure were characterized using SDS-PAGE with various staining techniques, particle size analysis, spectrum analysis, and zeta potential analysis. UPLC-MS/MS identified ellagitannins as the primary polyphenols in phenolic extracts of the walnut pellicle. Under pH-shifting conditions, phenolic extracts of walnut pellicle addition significantly enhanced the solubility of walnut protein (above 90% at pH 7.0) with 75 mg/g protein. This research confirms that the phenolic extracted portion of

walnut pellicles has a superior potential as a food additive to improve protein solubility. More research on the macromolecular hydrophilic protein aggregates produced by phenolic extracts of walnut pellicle and walnut protein is currently being conducted.

Wang et al. (2022). utilized tandem mass spectrometry and ultra-high-performance liquid chromatography (UHPLC-MS/MS) to determine the presence of free, esterified, and bound phenolic chemicals in walnuts with varying pellicle colors (2022). In the kernel (without pellicle), whole kernel (with pellicle), and pellicle, both free (62,6%) and bound (1.30%-12.2%) phenolics, were detected. Moreover, the flavonols, flavones, and flavonols contents of the phenolic compounds in free form in the whole kernel (with dark pellicle) were 2-153 times higher than other phenolic compounds in free form, specifically (+)-catechin (40.7 μg/g) and epicatechin (25.8g/g). In addition, the dark group of esterified phenolic acids, especially ellagic acid (428 μg/g) and gallic acid (130 μg/g) exhibited positive health effects.

Scopel et al. (2013) studied how ultra-high-pressure homogenization (UHPH) influences changes in the quality parameters of soymilk in order to generate a product with the same characteristics as pasteurized milk that has been kept in refrigeration settings. After 28 days of storage at 4 °C, soymilks treated with UHPH had a higher level of microbial inactivation than those pasteurized. The soymilk stored at 200 MPa and 75 °C was the most stable microbiologically.

Flores et al. (2013) examined the impact on the microbiological stability of almond milk caused by ultra-high-pressure homogenization (UHPH) in conjunction with thermal treatment. They discovered that the amount of protein in almond seeds was insufficient to produce physically stable almond beverages. As a result, adding an emulsifying agent (lecithin) was imperative, even to the conventionally homogenized almond beverages, which had the lowest volume fraction of oil droplets in the solution. This research concluded that ultrahigh-pressure homogenization (UHPH) is an effective alternative to conventional heat treatments

because it results in vegetable beverages that are microbiologically, chemically, and physically stable.

Kouchaksaraei et al. (2014) analyzed the effects of $NaHCO_3$ (used in order to reduce off-flavor/beany flavor) concentration in soaking water, roasting temperature, and blanching time on the chemical, physical, and sensory properties of sesame milk. According to the findings, the best preparation method involves soaking in 0.5 g/100 mL $NaHCO_3$ and blanching for 15 minutes. However, any roasting method cannot satisfy these three criteria to achieve the best physicochemical and sensory qualities in sesame milk.

Ouyang et al. (2022) formulated a walnut emulsion drink with the following ingredients: 85% deionized water, 8.5% walnut kernel, 0.1% monoglyceride, 8% sugar, 0.1% SP-60, 0.025% xanthan gum, 0.1% sucrose ester, 0.005% potassium sorbate, 0.083% konjac gum, and 0.067% guar gum. Ultrasonic emulsification was carried out using a dual-frequency ultrasonic combination of 28/68 kHz, an ultrasonic power density of 260 W/L, and an ultrasonic time of 50 minutes. The ultrasonic treatment created a walnut emulsion with a mean volume diameter of 2.05 μm, a Zeta potential absolute value of 40 mV, and an emulsion stability that lasted almost 14 days without undergoing phase separation. In addition, transmission electron microscopy and the laser scanning confocal microscope both confirmed the excellent stability of walnut emulsion produced using slit dual-frequency ultrasonic.

Mu et al. (2022) studied the effects of various ultrasound treatments (20 kHz at 400 W for 0 to 9 minutes) on the functional characteristics, flavor properties, and storage stability of soybean milk at 4 degrees Celsius. Soybeans (3:1 w/w) were soaked for 18 hours at 4 °C. The soaked soybeans were then ground with ultrapure water at a ratio of 1:10 w/w. The soybeans were then processed for 3 minutes in a high-speed blender to make raw soybean milk. Finally, the suspension was filtered through a two-layered 300-mesh screen to get the soybean milk. High-intensity ultrasound (HIU) at 20 kHz and 400 W output

power was used on the soybean milk samples for 0, 1, 3, 5, 7, and 9 minutes. Both control and sonicated samples were kept in the refrigerator (at 4 °C). The results showed that the largest particle size, $D_{4,3}$ in non-sonicated soymilk, was 2.47 ± 0.47 µm, whereas $D_{4,3}$ was reduced to 0.44 ± 0.01 µm after 9 minutes of high-intensity ultrasound (HIU). Using HIU for 9 minutes, the total number of microorganisms in soymilk dropped from 4.51 to 3.95 Log (CFU/mL). Hence, HIU is a potential technology for producing stable soybean milk with a mild bean flavor.

3. MATERIAL AND METHOD

3.1. Material

Shelled walnuts were humbly supplied by ZNT Gıda Tarım Adıyaman, Turkey.

Figure 3.1. Walnuts used for the production of walnut beverages

Chemicals used in the study; anhydrous sodium sulfate, chloroform, potassium hydroxide, sodium hydroxide, sodium carbonate, *p*-anisidine, 2,2-diphenyl-1-picrylhydrazil (DPPH), 2,4,6-Tripyridyl-S-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and Folin-Ciocalteau reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). Starch and gallic acid were purchased from Merck (Darmstadt, Germany). Potassium iodide and methanol (chromatographic purity) were purchased from Carlo Erba (Milan, Italy). Hexane (chromatographic grade), methanol (reactive grade), and acetic acid (analytical grade) were purchased from Isolab (Akron, OH, USA). Ethanol (analytical purity) and hexane (analytical purity) were purchased from Tekkim Kimya (Istanbul, Turkey).

3.2. Method

3.2.1. Production of walnut beverage

The walnut beverage was produced by following the steps presented in Fig 3.2. The dried walnuts were deshelled manually (Fig 3.3). Then, the kernels were divided into two parts; one was kept with the pellicles (unpeeled), and another was peeled.

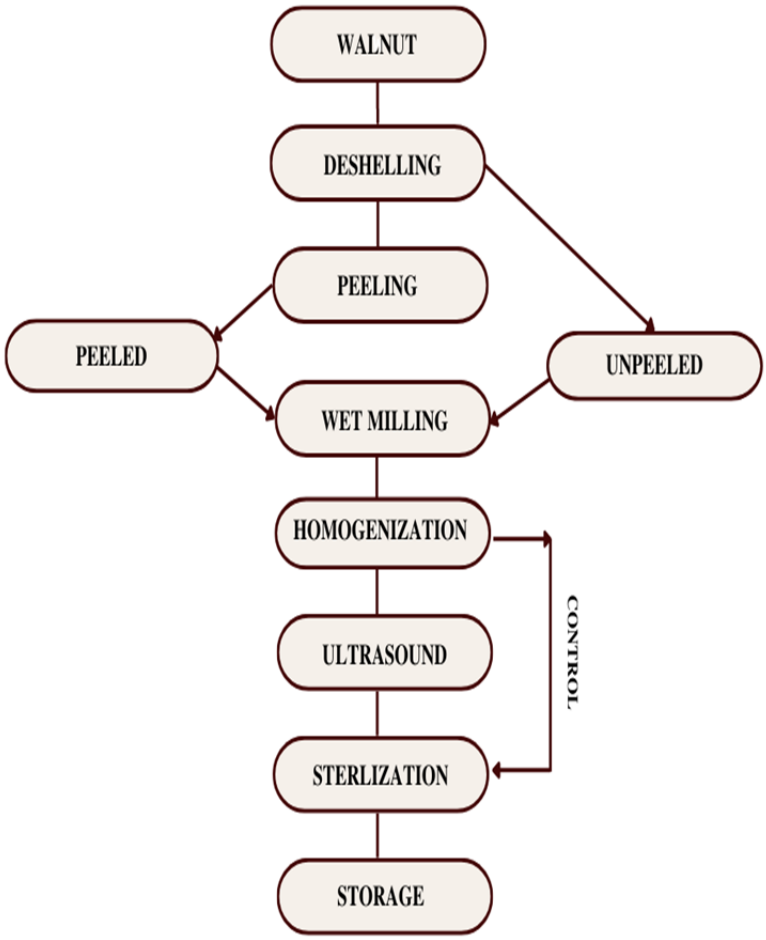

Figure 3.2. Walnut beverage production flow chart

Figure 3.3. Manual deshelling of walnuts

In preliminary trials, the optimization of the peeling process was done in which walnut kernels were dipped in a solution with various NaOH concentrations for different time duration, as mentioned below in Table 3.1

Table 3.1. Time and NaOH concentration for peeling

	30 sec	1 min	2 min	3 min
NaOH Concentration (%)	5	5	5	5
	7.5	7.5	7.5	7.5
	8	8	8	8
	10	10	10	10
	12	12	12	12
	15	15	15	15

According to preliminary studies and literature overview, 12 % NaOH and 3 minutes of application provided the best peeling results. Walnut kernels were weighed and dipped in the solution containing 12% NaOH on a heater, maintaining a temperature of 90°C for 3 minutes. Next, the kernels were removed from the solution and washed under a jet stream of tap water. An abrasive brush was used to clean the kernels from any remaining pellicles (Fig. 3.4).

Figure 3.4. Peeling of walnut pellicle

Further, the peeled kernels were soaked in distilled water in a jar overnight to neutralize the effect of NaOH (Fig 3.5).

Figure 3.5. Soaking peeled kernels in distilled water overnight.

After being soaked, the peeled walnut kernels used in this study were rehydrated due to the diffusion of water within the kernels. As a consequence of this process, the peeled samples have a lower ratio of total dry matter compared to unpeeled walnut kernels. The total dry matter of the kernels used in walnut beverage production is mentioned below in Table 3.2.

Table 3.2. Total solids of the kernels used in beverage production (%)

Samples	Total dry matter
Peeled 1	59.40
Peeled 2	58.79
Peeled 3	58.86
Average	59.02
Unpeeled 1	64.09
Unpeeled 2	64.65
Unpeeled 3	64.53
Average	64.42

For both walnut kernels (peeled and unpeeled), 4.5 times their weight of distilled water (50°C) was added. Then walnut kernels were ground in a blender (Waring, USA) for 2 min. The obtained slurry was homogenized using Ultra Turrax homogenizer for 2 minutes (IKA T 18 Digital Ultra-Turrax, Germany) (Fig.3.6).

Figure 3.6. Ultra-Turrax homogenizer (IKA T 18 digital Ultra-Turrax, Germany)

According to the preliminary trials, 10 minutes of ultrasonication was chosen the best, based on the effect on quality attributes of the walnut beverage, for example, smell, taste, and appearance. Around 450 mL of walnut beverage sample was poured into the double-jacketed 500 mL beaker. Ultrasound equipment with cool/hot water circulation was utilized throughout the process to maintain the desired temperature (standard deviation less than ± 2°C). The sonotrode was submerged 1 cm above the beaker's bottom. The samples were subjected to ultrasonic treatment for 10 minutes at 25 kHz. During the execution of the application, the total electrical power used was also calculated using ultrasound energy density instead of ultrasound time. The following equation describes the relationship between the ultrasonic energy density parameter (UED, J/g) and ultrasonic power (P, W), treatment time (t, s), and sample mass (m, g) (Eq. 3.1)

$$\mathbf{UED} = \frac{\mathbf{P.t}}{\mathbf{m}} \qquad \text{(Eq. 3.1)}$$

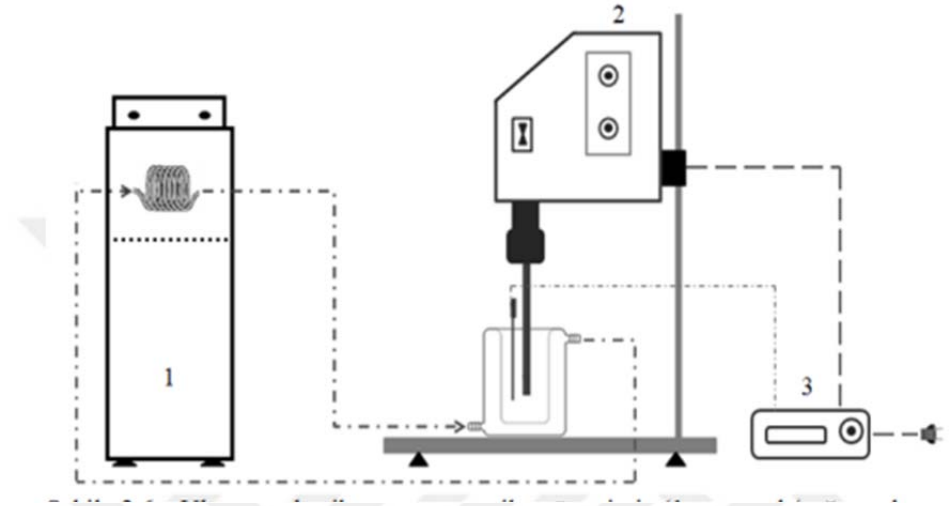

Figure 3.7. Systematic representation of ultrasound device: 1. Heated/Cooled water circulator, 2. Ultrasound generator, 3. Data logger for temperature and consumed energy (Ağçam, 2017).

After ultra-sonification, the walnut beverages were poured into bottles (Fig.3.8 and Fig 3.9) and autoclaved at 121°C for 15 minutes (Cui et al., 2013). Finally, the bottles were stored at 4°C for six months and analyzed at 45 days intervals.

Figure 3.8. Peeled walnut beverage samples

Figure 3.9. Unpeeled walnut beverage samples

3.2.2. Total solids

Each peeled and unpeeled walnut beverage sample's weight was recorded at the beginning and end of the drying process, and weight loss was calculated according to the AOAC method 934.06. The total solids of the samples were measured by weighing 2 g of each sample and heated in an oven (MMM, Venticell, Germany) at 105 °C for 4 hours and later in a vacuum oven (DZF-6030A, China) until a constant weight was obtained (AOAC, 1990). Moreover, the total solids were calculated using Eq. 3.2. The results are expressed as percent (%).

Calculations:

$$\text{Dry matter content} = \frac{(W2-W1)}{WS} * \mathbf{100} \quad \text{(Eq. 3.2)}$$

Where

W1 = Mass in gm of dish

WS = Mass in gm of dish + Sample

W2 = Mass in gm of the dish + sample after drying

3.2.3. Ash content

The AOAC Official Method 950.49 (AOAC 1997) was used to determine the ash level of peeled and unpeeled walnut beverage samples. Approximately 2 g of the sample was weighed in porcelain crucibles, which were brought to a constant weight beforehand, and burned at 550 °C in a muffle furnace (Protherm, PLF 110/15, Turkey) until white ash was obtained. The amount of ash remaining in the crucibles was calculated according to the initial sample amount using Eq. 3.3. The results are given as % ash content.

Calculations:

$$\textbf{Ash content} = \frac{(W2-W1)}{WS} * 100 \qquad \text{(Eq. 3.3)}$$

Where

W_1 = Mass in gm of the crucible

WS = Mass in gm of crucible+ Sample weight

W_2 = Mass in gm of the crucible + Sample weight after ashing

3.2.4. Protein

The protein content of the peeled and unpeeled walnut beverage samples was determined by the Kjeldahl method according to the AOAC Official Method 950.48 using the Kjeldahl distillation unit UDK 129 series (VELP Scientifica, Italy) (Fig.3.10).

The Kjeldhal method is divided into three main steps

1. Digestion
2. Distillation
3. Titration

Figure 3.10. Kjeldahl distillation unit UDK 129 series (VELP Scientifica)

3.2.4.1. Digestion

About 1g of the sample was weighed on an analytical balance, transferred to a Velp glass test tube, and homogenized properly. Approximately 12 mL of sulfuric acid and 1-2 capsules of K-tabs were added to these tubes. The tubes were lowered into the heater set at 480 °C. and closed with an exhaust manifold with suction cap glass bells attached to a continuous water supply to remove the fumes generated inside the glass test tubes. Next, digestion was carried out until a clear and colorless solution was obtained. During this phase, the sulfuric acid reacts with the sample, converting all nitrogen in organic form into an inorganic form that was stable and ready to be analyzed. After 1.5 hours, the velp glass tubes containing samples were raised from the heater and cooled. Finally, 50 mL of distilled water was added to the digested sample in each tube to dilute it.

3.2.4.2. Distillation

Using steam distillation, nitrogen from the digested mixture was separated in the Kjeldahl distillation unit. Next, the tube with digested samples was introduced and clamped into position in the distillation unit. On the receiver end, distilled vapors were collected in a 250 mL conical flask containing 30 mL of 4% boric acid and a few drops of Bromocresol Green-Methyl Red indicator. The color of the solution in the 250 mL conical flask was initially purple, which changed to crystal green in response to the distilled vapors. After completion of the distillation process, the tube with the digested sample was drained.

3.2.5. Titration

Titration was used to calculate the nitrogen or protein amount as a percentage by determining the amount of ammonia distilled from the digested solution. The distilled vapors were titrated against 0.1 N HCL in a conical flask until a purple/pink hue was achieved. The amount of acid-titrant solution required to attain the endpoint was measured. After determining the nitrogen content (N%) of the walnut samples, the results were calculated as % protein by multiplying the results with the recommended 6.25 factor for walnuts (Joshi et al., 2015). The results were calculated using Eq 3.4 and Eq 3.5. The protein content is expressed as %.

Calculation

$$\textbf{Nitrogen } \% = \frac{1.4 \times N \times TV}{S} \quad \text{(Eq. 3.4)}$$

$$\textbf{Protein } \% = \% \, N \times 6.25 \quad \text{(Eq. 3.5)}$$

Where

TV =Titration volume

N = Normality of HCl solution

S = Sample weight

3.2.6. Fat extraction

Fat from the walnut beverages was extracted using the procedure suggested by Gao et al. (2019) with some modifications. First, about 5 g of the sample was taken in a 50 mL centrifuge tube. Next, 20 mL ethyl acetate was added to the sample and mixed on a vortex followed by centrifugation at 6000 rpm at 4°C for 5 minutes in a centrifuge (Hettich Zentrifugen, 2205, Germany). Next, the mixture was filtered through a filter (Whatman No. 4) placed on top of a funnel containing sodium sulfate. The process was repeated three times, and the filtrates were mixed at the end in a preweighed round bottom flask. Finally, the solvent was removed from the extracts using a rotary evaporator (Heidolph, Hei-VAP, Germany) (Fig.3.11) under a vacuum with a vacuum control and thermostatic bath maintained at 30 °C until no solvent evaporated.

Figure 3.11. Vacuum rotary evaporator (Heidolph, Hei-VAP, Germany)

The round bottom flask used for evaporation was weighed at the end of the analysis. The amount of fat was calculated using the following Eq 3.6, and the results are expressed as %.

Calculation

$$\textbf{Fat content} = \frac{(W2-W1)}{WS} * \textbf{100} \quad \text{(Eq. 3.6)}$$

Where

W_1 = Initial mass in gm of the round bottom flask

WS =Mass in gm of sample

W_2 = Final assembly in gm of the round bottom flask

3.2.7. Dietary fiber

Samples of walnut beverages were analyzed for their dietary fiber content using the AOAC Method 985.29. About 10 mL of walnut beverage samples were dried in an oven (Memmert UM 400, Germany) at 70 °C overnight for dietary fiber measurement. The dried samples were blended into a powder to achieve a powder consistency (Waring, USA). About 1 gm from the dried sample was weighed into a 250 mL glass bottle with a lid. A magnetic and 40 mL MES-Tris buffer solution was added to each bottle. The bottles were placed on a magnetic stirrer with a hotplate. Two blanks were also run with each sample to determine the extent to which each reagent impacted the final residue concentration. After mixing the sample thoroughly into the solution, 50 µL α-amylase was added to each sample while stirring at low speed. Next, the bottles were capped and placed in a shaking water bath (Memmert WNB 22, Germany) at 98°C for 30 minutes. The bottle contents were swirled at 5 minutes intervals over the 30-minute incubation period. Next, the bottles were removed from the water bath and cooled to room temperature. About 50 µL protease was added to each sample and placed in a shaking bath at 60°C for 30 minutes. The bottles were taken out of the water bath

and cooled. Using a concentration of 4-5% HCl, the pH of the solution was adjusted to pH 4 - 4.7. The samples were re-stirred in a 60 °C, shaking water bath for 30 minutes after adding around 150 µL of amyloglucosidase.

By heating the fritted crucibles for an hour, they were brought to a consistent weight. The weight of the crucibles was recorded, and approximately 1g of celite was weighed in the preweighed crucible. The celite-filled crucible was put in the oven for 1 hour at 130 °C. Later, the celite-filled crucible was kept over a Buchner flask and connected to a vacuum pump. The contents of the sample bottles were vacuum-filtered after being decanted through the crucible with celite. The total fiber was recovered as crucible residue. The residues were washed three times with 15 mL 78% ethanol, twice with 10 mL 95% ethanol, and three times with 10 mL acetone. The crucibles containing the residue were put in a 105 °C oven overnight.

The weight of the crucibles was noted. From the residue, weighed amount was used for protein and ash determination. Ash was determined as mentioned in 3.3.3, and protein as in 3.3.4. The calculation of dietary fiber is mentioned below in Eq.3.7. However, the dietary fiber was calculated using Megazyme Mega-Calc^{TM}. The results are expressed as %.

$$Dietary\ Fiber = \frac{\frac{R_1 + R_2}{2} - p - A - B}{\frac{m_1 + m_2}{2}} \times 100 \qquad \text{(Eq. 3.7)}$$

Where

R_1 = Residue weight 1 from m_1

R_2 = Residue weight 2 from m_2

m_1 = Sample weight 1

m_2 = Sample weight 2

A = Ash weight from R_1

p = Protein weight from R_2 and

B = Blank

$$Blank = \frac{BR_1 + BR_2}{2} - \ BP - BA \quad \text{(Eq. 3.8)}$$

Where

BR = Blank residue; BP = Blank protein from BR_1

BA = Blank ash from BR_2

3.2.8. pH

The pH of peeled and unpeeled walnut beverage samples was determined using a pH meter (Mettler Toledo S220, China). Before the measurement, the pH meter was calibrated at room temperature to pH 4 and 7 using standard buffer solutions. Next, the sample was taken into a small beaker, and a magnetic bar was placed to stir the sample continuously on a magnetic stirrer. The standardized electrode tip was immersed into the sample and gently mixed using a "flea" to give a constant pH value. The pH values were recorded (Luana et al., 2020).

3.2.9. Acidity

The titratable acidity of peeled and unpeeled walnut beverage samples was carried out by the titration method suggested by Anonymous (2011) with some modifications. About 10 mL of the sample was mixed with 10 mL of distilled water in a 100 mL beaker with a flea for continuous stirring. The standardized pH (Mettler Toledo S220, China) electrode tip was immersed into the sample and titrated against 0.1 N NaOH solution using an automatic burette. The NaOH volumetric factor (F) to be used in acidity was determined by taking 0.1 gm of oxalic acid and titrating it against prepared NaOH, which was calculated using Eq.3.9. The acidity was calculated using Eq. 3.10 and is expressed as g/100 mL.

$$\text{Volumetric Factor Calculation (F)} = \frac{W*1000}{V*N*\ mEq} \quad \text{(Eq. 3.9)}$$

Where

N = Normality of NaOH

V = Titration volume (mL)

mEq = Equivalent weight of oxalic acid.

W = Sample weight of oxalic acid

$$\textbf{Acidity} = \frac{\text{N*V*F* mEq*}\mathbf{100}}{\text{S}} \qquad \text{(Eq. 3.10)}$$

Where

N = Normality of NaOH

V = Titration volume

F = NaOH Volumetric Factor

mEq = Equivalent weight of oxalic acid.

S = Sample weight

3.2.10. Viscosity

The viscosity of the peeled and unpeeled walnut beverage samples was determined using DV-II+Pro Viscometer in conjunction with Brookfield software (Fig. 3.12). The guard leg was first mounted on the DV-II+ProViscometer and inserted into the container. Probe no SC4-18 was selected on the software for peeled walnut beverage samples because of their thin consistency as compared to unpeeled for which the SC4-18 probe was used. Firstly 6.7 mL of peeled walnut beverage sample was taken into the sample holder. It was attached to the cylindrical sample adapter and locked by slightly twisting the sample holder. Next, the free-hanging spindle was attached to the coupling nut and inserted into the sample holder.

Figure 3.12. DV-II+Pro viscometer (Brookfield)

Finally, the temperature probe was attached to the cylindrical sample adapter. The viscosity was measured at different shear rates (13, 26, 53, 106, 158, 264)/Sec. For the unpeeled walnut beverage sample, probe no SC4-31 was used.

Furthermore, the probe selection on the software was also changed to SC4-31. For the viscosity measurement at different shear rates (13, 26, 53, 106, 158, 264)/Sec, 9.2 mL of unpeeled walnut beverage sample was used. The results are expressed as mPa·s or cP (Wang et al., 2018).

3.2.11. Color Measurement

Hunter Lab Spectro-colorimeter (Color Flex Hunter Lab, USA) was used to measure color. Standardization of a Color Flex hunter lab was done by first reading the black tile and then the white tile. After the standardization was complete, the sample was poured into the sample cup and covered with the opaque cover. The samples were read in daylight color setting (D65/10°C), and L*, a*, and b* color values were recorded for all peeled and unpeeled walnut beverage samples. The L* values correspond to lightness/ darkness and extend from 0 (black) to 100 (white), with higher values indicating lightness.

The a* and b* values correspond to an object's color with standards describing a sample's red (+a) to greenness (-a), while b* values represent a sample's yellow (+b) to blueness (-b). Larger values point out more redness, and larger b* values indicate yellowness. ΔE* value is a color parameter used to compare two colors and express the total color difference between them. The ΔE* value increases after the processing of foods and during storage.

The total color difference (ΔE*) between peeled and unpeeled walnut beverage samples was calculated using Eq. 3.13. In addition to these measurements, Hue*, Croma, and whiteness index were also determined in addition to these measurements. The chroma (C*) value defines the saturation of the color. While the L* value of the sample is fixed, the increase in C* value causes the color to become more vivid.

The C* value technically expresses the circle's radius created by the a* and b* values in the trigonometric color planes. In contrast, the Hue value mathematically expresses the angle in degrees of the slope line associated with

these values. Both of these values are related to the hue value. For example, the Hue * values a * and b * indicate base color (red, yellow, green, or blue). The point where the C * and Hue * values intersect gives the coordinates of the color of the analyzed sample in the plane. In addition to these measurements, Hue* was calculated using Eq.3.11, Croma (C*) was calculated using Eq.3.12, and the whiteness index of all the samples was calculated using Eq.3.14 (Rincon et al., 2020a).

$$\mathbf{Hue}^{*} = \mathbf{arctan}\left(\frac{\mathbf{b}^{*}}{\mathbf{a}^{*}}\right) \quad \text{(Eq. 3.11)}$$

$$\mathbf{C}^{*} = \sqrt{(\mathbf{a}^{*}) + (\mathbf{b}^{*})} \quad \text{(Eq. 3.12)}$$

$$\mathbf{\Delta E}^{*} = \sqrt{(\mathbf{\Delta L}^{*})^{2} + (\mathbf{\Delta a}^{*})^{2} + (\mathbf{\Delta b}^{*})^{2}} \quad \text{(Eq. 3.13)}$$

$$\mathbf{Whiteness\ Index(WI)} = \mathbf{100} - \sqrt{(\mathbf{100} - \mathbf{L}^{*})^{2} + \mathbf{a}^{2} + \mathbf{b}^{2}} \quad \text{(Eq. 3.14)}$$

3.2.12. Mineral composition

The mineralization of the samples was accomplished by carrying out wet acid digestion under intense pressure in a microwave digester (Berghof Speedwave MWS-2, Germany). The concentration of potassium (K), sodium (Na), calcium (Ca), Iron (Fe), copper (Cu), zinc (Zn), and magnesium (Mg) were determined in a Flame Atomic Absorption Spectrometer (FAAS).

3.2.12.1. Microwave Digestion

In Teflon tubes, a sample of approximately 2 mL of walnut beverage was placed, after which 2 mL of 65% nitric acid and 1 mL of 35% hydrogen peroxide acid were added. After ensuring that the tubes were sealed entirely, they were placed inside the microwave (1000W, Berghof MWS-2, Germany) (Fig. 3.13) and subjected to the microwave digester's programmed parameters which are mentioned below in Table 3.3.

Table 3.3. Microwave digestion procedure for walnut beverages.

Stage	Temperature (°C)	Power (%)	Time (min)
1	170	90	10
2	200	50	10
3	100	50	10

After digestion, 2 mL of the clear, colorless solution was poured into a 25 mL volumetric flask. The remaining space was filled with ultrapure water to the appropriate level. Certified standards were used in the calibration of the flame atomic absorption spectrometer (Perkin Elmer, Analyst 400, Massachusetts, USA) (Fig.3.14). Five different concentrations (0.25, 0.5, 1,2, and 4 ppm) of solutions were prepared for each standard, and calibration curves were created. The amount of the compounds was calculated as mg/L from the curves obtained (Uçan et al., 2014).

Figure 3.13. Microwave digestion system speed wave (Berghof MWS-2, Germany)

Figure 3.14. Flame atomic absorption spectrometer (FAAS)

3.2.13. Sugar Compounds

Using an HPLC-PDA/RID (Shimadzu, LC-20AT, Koyoto Japan,2006) system, the sugar components of peeled and unpeeled walnut beverage samples were evaluated following Sturm et al. (2003) with some modifications. First, sugar compound determination was performed by transferring 2 g of walnut beverage sample to a 15 mL centrifuge tube, adding 8 mL of ultrapure water, mixing on a vortex for 1 minute, then centrifuging the sample extracts at 6000 rpm, 4 °C for 10 minutes (Hettich 2205, Germany). Then, after passing through a 0.45 µm nylon membrane filter, the resultant clear fractions were finally injected into an HPLC-PDA/RID device (Shimadzu, Japan). Following are the chromatographic conditions applied during the analysis.

Chromatography conditions;

- Column: Core gel Ion 300 (Concise, USA)

- Column temperature: 30 ^{o} C
- Mobile phase: 5mM H2SO4, isocratic flow
- Mobile phase flow: 0.4 mL/min
- Injection volume: 20 µL
- Elution time: 55 min
- Detector: PDA/RID

The identification of sugar peaks was made with the use of approved standard materials. The retention time and spectra of the peaks were found by comparing the peaks to the standard. The system was injected with five different doses of reference substances, and the resultant calibration curves were utilized to calculate the peaks' concentrations. This was done so that the peaks' concentrations could be accurately determined. The results are expressed as mg/100mL (Sturm et al., 2003).

3.2.14. Fatty Acid Composition

The fatty acid composition of walnut beverages was determined using the oil extracted in step 3.3.4. The fatty acid methyl esters (FAMEs) were prepared using a procedure suggested by Geng et al. (2021) with slight modifications. First, in a 15 mL centrifuge tube, 0.1 g of previously extracted oil was weighed. Next, the samples were vortexed with 10 mL of n-hexane. The mixture was then treated with 0.5 mL 2 N potassium hydroxide diluted in methanol and left to stand for two hours. Later, the upper layer of the mixture was extracted and filtered in a glass vile using polytetrafluoroethylene (PTFE) syringe filters before GC-mass spectrometry analysis (GC-MS). The analysis was done using a Gas Chromatograph Agilent 7890A (Agilent Technologies, Palo Alto, California, USA) (Fig. 3.15).

Figure 3.15. Agilent 7890A Gas chromatograph (Agilent Technologies, Palo Alto, California, USA).

Fatty acid methyl esters (FAMEs) separation and quantification were accomplished using gas chromatography following Tapia et al. (2013) procedure with some modifications. A 60 m cis/trans-FAMEs column (Agilent Technologies, 60 m 0.320 mm ID 0.25 m film thickness) was used to separate fatty acids

The compounds were identified by correlating the retention times of the unknown compounds to those of the standards. The chromatographic separation was accomplished by heating the column to 165 °C for 35 minutes before slowly increasing the temperature to 220 °C at a rate of 5 °C per minute. All the examined FAMEs were adequately separated in 62 minutes under these conditions. The external standard calibration method was used to quantify fatty acids. The results are given as a percentage of each fatty acid.

3.2.15. Particle Size

Particle size distribution measurements were performed using a Malvern Panalytical instrument (Mastersizer 3000, UK) (Fig. 3.16) by following the method suggested by Vogelsang-O'Dwyer et al. (2022) with some modifications. Assuming spherical particles, the refractive index for walnut beverage fat was set to 1.46, and the absorption was calculated to be 0.1. The water-based dispersant had a refractive index of 1.33. Samples were introduced into the dispersing unit using ultrapure water as the dispersant until a laser obscuration of 19% was attained, and all particle size measurements were conducted in triplicate. The obtained results are expressed as the median diameter ($D_{0.5}$), surface weighed mean diameter ($D_{3,2}$), and volume-weighted mean diameter ($D_{4,3}$).

Mastersizer Conditions:

- Scattering Model: Mie
- Particle Refractive Index: 1.460
- Dispersant Refractive Index: 1.330
- Laser Obscurations: 19.33%
- Dispersant Name: Water
- Concentration: 0.0229%
- Span:20.294
- Uniformity: 5.872

Figure 3.16. Malvern Panalytical (Mastersizer 3000, UK)

3.2.16. Phenolic Compounds

About 2 mL of sample were mixed with 10 mL of 80% methanol and centrifuged at 4000 rpm and 4°C for 10 minutes in order to extract phenolic components from peeled and unpeeled walnut beverages (Hettich 2205, Germany). The supernatant was collected using 0.45 µm polytetrafluoroethylene (PTFE) syringe filters. The extract was transferred to vials and introduced to the HPLC machine (Shimadzu LC-20AT, Japan). The HPLC used for this analysis was fitted with a quaternary pump, column temperature control oven (CTO-10AS), an autosampler unit (SIL-20A), a degasser module (DGU-20A5), and a photodiode array detector (SPD-M20A). The C18 XTERRA (Waters, 4.6X250 µm) column was injected with 20 µL of supernatant. The flow rate was 1 mL/min, and the column temperature was 30°C. The wavelengths of the photodiode array detector were set to 280 and 320 nm. For mobile phases A and B, 5% formic acid (A) and 20:80 acetonitrile (ACN) solutions (B) were used. Phenolic compounds were

identified by comparing the UV–visible spectra and retention times of the approved standards. The phenolic components were measured using a conventional external method at 280 nm. Using commercially available standards, calibration curves were developed. The results are expressed as mg/L (Ağçam, 2022).

3.2.17. Total Phenolic Content (TPC)

About 2 mL of walnut beverage sample was mixed with 10 mL of 80% methanol and centrifuged at 4000 for 10 minutes at 4°C (Hettich 2205, Germany). The supernatant was collected and filtered through 0.45 µm polytetrafluoroethylene (PTFE) syringe filters. The TPC was determined using the Folin–Ciocalteau colorimetric method of Medic et al. (2021) with some modifications. First, 100 µL from the filtered extract, 100 µL of Folin-Ciocalteau, and 3000 µL of distilled water were taken in a test tube and left undisturbed for 10 minutes. Later 100 µL of 20% Na_2CO_3 was added to the solution and kept in the dark for 2 hours. The reading was taken at 765 nm in the spectrophotometer (Perkin Emler Lambda, 25 UVVE Massachusetts, USA).

The amount of TPC was calculated using standard gallic acid curve y = 0.0025x - 0.01933 with a correlation coefficient (R^2 = 0.99834) and expressed as mg gallic acid equivalents per L of the sample (mg GAE/L). Results are expressed as a mean value of three determinations per sample.

3.2.18. DPPH Scavenging Activity

The DPPH free radical-scavenging activity measurements were carried out according to the procedure of Fregapane et al. (2020) with some modifications. 2 mL of walnut beverage sample was mixed with 10 mL of 80% methanol and centrifuged at 4000 rpm for 10 minutes at 4°C. The supernatant was collected and filtered through 0.45 µm polytetrafluoroethylene (PTFE) syringe filters. Antioxidant activity was determined by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay. 0.1 mL of the extract was mixed with 2.9 mL of 0.05 gL^{-1} DPPH in 80%

methanol, incubated in the dark for 2 hours, and read at absorbance 515 nm against a blank of methanol. Antioxidant activity is expressed as TE mg/L.

3.2.19. Ferric-Reducing Antioxidant Power (FRAP)

In this study, total antioxidant activity was determined using a modified version of the ferric reducing antioxidant power (FRAP) assay developed by Benzie and Strain (1996). The FRAP assay is a redox-linked colorimetric technique that utilizes antioxidants as reductants in conjunction with a simple oxidant system. The assay is based on the principle that the reduction of ferric tripyridyl triazine (Fe^{3+} TPTZ) complex to ferrous form Fe^{2+} (which has a solid blue color) can be monitored at low pH by detecting the change in absorbance at 593nm.

The reaction is nonspecific because any half-reaction with a lower redox potential than the ferric half-reaction will promote the production of ferrous (Fe^{3+} to $Fe^{2+)}$ ions under reaction conditions. Given this, the change in absorbance is directly linked to the "total" reducing the power of the electron-donating antioxidants in the reaction mixture. The FRAP reagent comprised 10 mM/L of 2,4,6-tripyridyl-S-triazine (TPTZ) dissolved in 40 Mm/L of hydrochloric acid, 20 Mm/L of ferric chloride, and 300 mM/L of sodium acetate buffer pH 3.6 with hydrochloric acid. FRAP solution was made up of the ratio of 1:1:10 (v/v/v) of sodium acetate: $FeCl_3$: TPTZ, respectively. A 100 µL extract was added to 3 mL of FRAP reagent, mixed thoroughly, and kept in the dark for 2 hours. The absorbance at 593 nm was noted against the reagent blank. Results are defined as TE mg/L.

3.2.20. Sensory Analysis

The sensory evaluation was conducted in a dark, well-lit space free of odors. Panel cubicles were uniformly lighted with special daytime bulbs for color evaluation. The sensory evaluation was performed by a panel of eleven experienced judges from the Department of Food Engineering at Çukurova University in Adana. The samples were served in glasses bearing random three-digit numbers. On a scale from 1 to 9, 1 being "Extremely dislike" and 9 being " Extremely like," ranking was assigned to the attributes of color, taste, aroma, and overall acceptance. The average score was based on these individual sensory attributes and designated as "overall consumer acceptability." In addition, the organoleptic qualities of both peeled and unpeeled walnut drinks were evaluated on a 9-point Hedonic scale.

- Color
- Taste
- Aroma
- Overall acceptability

3.2.21. Statistical Analysis

Mean values, standard deviation, and analysis of variance (ANOVA) were computed using a commercial statistical package (IBM SPSS Statistics 23. Inc). These data were compared using critical difference tests at a 5% significance level ($p \leq 0.05$).

4. RESULTS AND DISCUSSION

4.1. Protein Content of Walnut Beverages

The results obtained for the protein content of walnut beverage samples are presented in Fig 4.1. The protein content was highest in unpeeled ultrasonicated (UPUS) samples (2.93%), followed by unpeeled control (UPC) samples (2.72%), peeled ultrasonicated (PUS)samples (2.29%), and peeled control (PC) samples (2.26%). The protein content in unpeeled walnut beverages was higher than the peeled walnut beverages. This difference in protein content can be attributed to the presence of pellicle-containing proteins in the unpeeled walnut beverages.

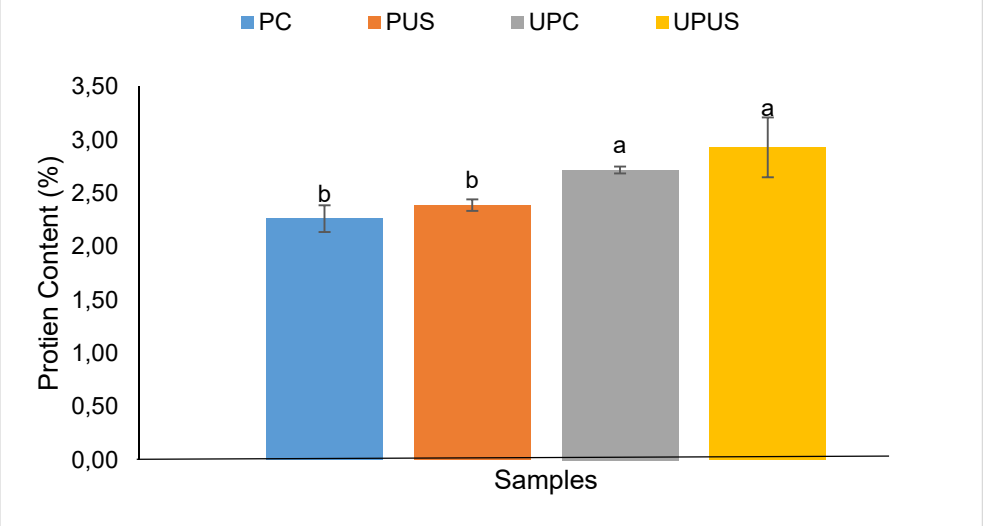

Figure 4.1. Protein content of walnut beverages
The lowercase letters on each bar indicate significant differences among the samples (p<0.05)

Furthermore, studies have demonstrated sonication to increase protein content (Ogunwolu et al., 2009). Therefore, the protein results determined in this study were similar to those obtained by Author et al. (2015), who reported 2.36 g/100 mL protein content and 1.70 g/100 mL in soymilk and hazelnut milk, respectively. Also, Tulashie et al. (2022) reported 2.22 g/100g protein content in coconut milk.

The protein content in ultrasonicated walnut beverages was more than the control beverages. The results showed that ultrasound treatment of walnut beverages increases the release of protein. The most probable governing mechanism for ultrasonic enhancement of extraction was cavitation. In the case of walnut beverages, shear forces generated by ultrasound treatment due to the collapse of cavitation bubbles, as well as shock waves, appear to physically disrupt the plant cell walls and increase the penetration of the solvent into the walnut cells, resulting in a more efficient mass transfer of intracellular proteins into the extractable walnut beverages (Fahmi et al., 2011).

4.2. Fat content of Walnut Beverages

The crude mixture of fat-soluble substances in the sample is referred to as "crude fat." It is also referred to as the free lipid content or the ether extract. It is of the utmost importance to determine the amount of fat that is contained in walnut beverages since fat plays an important nutritional role and is a key source of energy. (Erfanian and Rasti, 2019).

The results obtained for the fat content of walnut beverage samples are presented in Fig 4.2. The fat content was the highest in UPUS (12.68%), followed by UPC (12.22%), PUS (11.95%), and PC (11.83%). The fat content in unpeeled walnut beverages was slightly higher than the peeled walnut beverages. This difference in fat content can be attributed to pellicle-containing fat in unpeeled walnut beverages. However, these differences were statistically insignificant (p>0.05). Also, the difference between peeled and unpeeled walnut beverage

samples was negligible, indicating the uniform distribution of fat in kernel and pellicle; this was in agreement with Yan and Zhou (2021), who reported no significant difference in peeled and unpeeled defatted walnut flour.

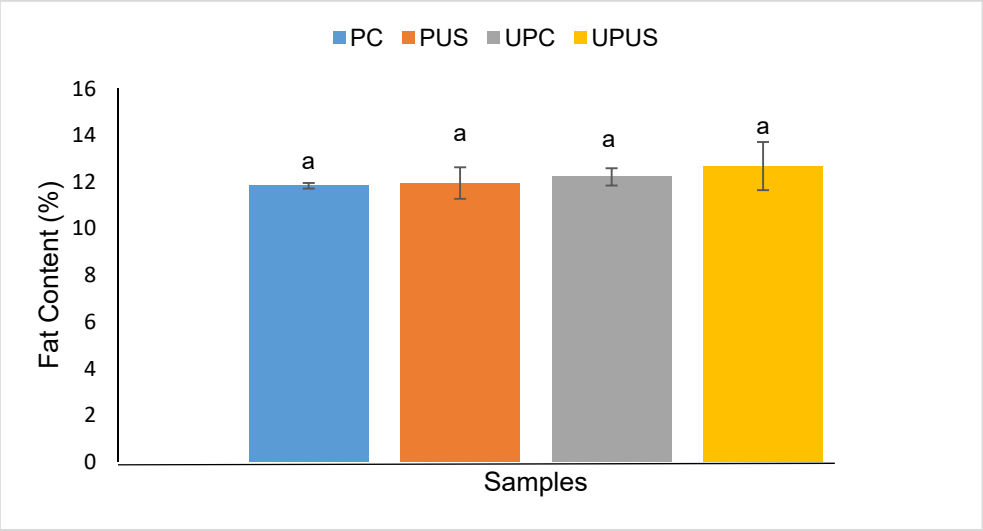

Figure 4.2. Fat content of walnut beverages
The lowercase letters on each bar indicate significant differences among the samples (p<0.05)

The average fat content in ultrasonicated walnut beverages was higher than the control beverages. However, the difference was negligible. This increase in the fat yield may be explained in terms of cavitational effects caused by the application of ultrasound (Li et al., 2004).

4.3. Dietary Fiber Content of Walnut Beverages

Plants are the primary source of dietary fiber. It is made up of complex, non-starch carbohydrates and lignin that cannot be broken down in the small intestine because mammals do not possess enzymes that can break them down into their basic monomers. Thus, these molecules are directed unchanged to the colon, where they can be fermented by the indigenous bacteria. Although dietary fiber is thought to be calorie-free, the metabolites produced by colonic bacteria are used as

a source of energy by humans and other mammals (Turner and Lupton, 2011). The dietary fiber of walnut beverages is presented in Fig. 4.3.

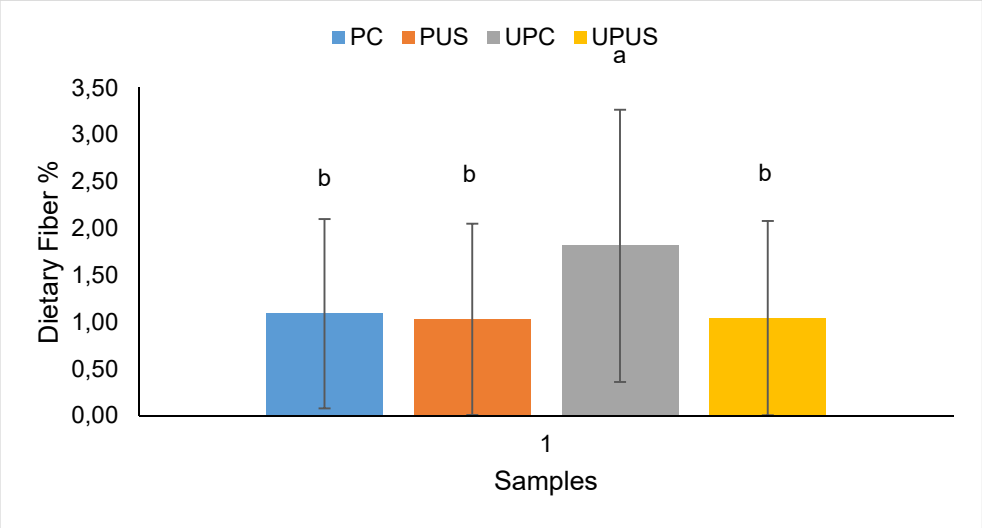

Figure 4.3. Dietary fiber of walnut beverages
The lowercase letters on each bar indicate significant differences among the samples (p<0.05)

The total dietary fiber content was highest in UPC (1.82%), followed by PC (1.09 %) and UPUS (1.04 %), while it was lowest in PUS (1.03%) followed. The increased dietary fiber is due to pellicles containing non-starch polysaccharides, lignin, and other substances like resistance starch, phenolic compounds, phytates, saponins, and waxes. Also, it may be due to residual lipids, such as phospholipids which are difficult to remove without saponification (Cardozo and Li, 1994).

On the other hand, the decrease in dietary fiber in ultrasonicated unpeeled and peeled walnut beverages is due to high ultrasound temperature (57°C), which is in agreement with Sun et al. (2018), who reported a significant decrease in the yield at the ultrasound temperature range from 30 °C to 80 °C since the temperature raised to 58°C during sonication. As a result, the dietary fiber values

obtained in this study were lower than almond milk (2.16 g/100mL) and oat milk (3.21g/100mL) but higher than soymilk (1.33g/100mL).

4.4. Mineral Content of Walnut Beverages

Plant foods can serve as dietary sources of all essential minerals required by humans (Grusak, 2013). Walnuts are considered a good source of dietary minerals because they are rich in potassium, which eliminates sodium and is highly implicated in the elevation of blood pressure. In addition, copper, chromium, iron, and zinc are essential micronutrients for human health and necessary for human metabolism (Juranović Cindrić et al., 2018). Mineral content values of walnut beverages are given in Table 4.1 in mg/L. The walnut beverages were analyzed for K, Na, Ca, Mg, Fe, Cu, and Zn minerals.

Table 4.1. Mineral content of walnut beverages (mg/L)

Mineral	PC	PUS	UPC	UPUS
Mg	74.38 ± 0.36^{c}	70.80 ± 0.84^{d}	95.92 ± 0.55^{a}	83.68 ± 0.24^{b}
Ca	71.90 ± 0.22^{d}	78.87 ± 0.38^{c}	81.75 ± 1.17^{b}	83.88 ± 0.48^{a}
K	40.42 ± 2.24^{d}	47.32 ± 1.38^{c}	52.23 ± 1.22^{b}	56.93 ± 1.47^{a}
Fe	1.58 ± 0.38^{bc}	1.18 ± 0.27^{c}	2.58 ± 0.44^{a}	2.07 ± 0.39^{ab}
Zn	1.41 ± 0.15^{b}	1.78 ± 0.06^{b}	1.74 ± 0.30^{b}	3.22 ± 1.00^{a}
Na	1.05 ± 0.13^{b}	1.41 ± 0.13^{a}	1.21 ± 0.12^{ab}	1.18 ± 0.14^{ab}
Cu	0.43 ± 0.08^{a}	0.35 ± 0.10^{ab}	0.20 ± 0.11^{b}	0.36 ± 0.06^{ab}

The lowercase letters in each row indicate significant differences among the samples ($p<0.05$).

The mineral content of the UPUS samples was found to be higher than that of the other samples in terms of potassium (K), calcium (Ca), sodium (Na), and copper (Cu). As seen in Table 4.1, when the results obtained were statistically evaluated, a significant difference was found in terms of K, Na, Mg, Fe, Zn, and Cu minerals in the samples. In terms of magnesium (Mg) content, the UPC sample (95.92 mg/L) had the highest value, followed by UPUS (83.68 mg/L), then PC

(74.38 mg/L) and the PUS sample (70.80 mg/L) had the lowest value. Calcium (Ca) content was found to be highest in UPUS samples (83.68 mg/L), followed by UPC samples (81.75 mg/L), then PUS samples (47.32 mg/L), and the lowest content was found in PC samples (71.90 mg/L).

The highest values of potassium (K) were found in UPUS samples (56.93 mg/L), while the lowest were found in PC samples (40.42 mg/L). UPC samples (2.58 mg/L) were found to be higher in iron (Fe), while PUS samples (1.18 mg/L) had the lowest value. Zinc (Zn), Sodium (Na), and Copper (Cu) were found in trace amounts in walnut beverages, and the highest values for all samples were detected in UPUS samples (3.22 mg/L), PC sample (1.41 mg/L) and PC sample (0.43 mg/L) respectively.

According to Gharibzahedi et al. (2014), walnuts have comparatively high levels of potassium (284 mg 100 g-1) and magnesium (79.6 mg 100 g-1), while they have relatively low levels of sodium and iron (0.34-2.94 mg 100 g-1). Amin et al. (2017) reported that post-mature walnut kernel had a relatively high concentration of potassium (325 mg 100 g-1) which was followed by calcium (160.15 mg 100 g-1) and magnesium (131 mg 100 g-1).

The mineral composition values in this study were lower than the mineral composition of coconut milk prepared by Tulashie et al. (2022), which contained magnesium (279.45 mg/L), potassium (805 mg/L), iron (149 mg/L), zinc (71 mg/L), calcium (92.5 mg/L), and copper (2.5 mg/L). However, the mineral content values of this study were higher for calcium (3.90 mg 100/mL) in soymilk (Author et al., 2015).

4.5. Sensory Evaluation of Walnut Beverages

Sensory Evaluation of the walnut beverages produced was sensorially analyzed by eleven panelists at the beginning of the storage (0 days). The mean scores for taste, aroma, color and overall acceptability are displayed in Table 4.2

for PC, PUS, UPC, and UPUS samples (Sensory evaluation form given in Appendix 1).

Table 4.2. Sensory analysis results of walnut beverages

Sample	Color	Taste	Aroma	Overall acceptability
PC	7.97±1.25[a]	5.00±2.53[a]	5.46±2.04[a]	7.77±1.79[a]
PUS	7.06 ±1.93[a]	3.86±1.80[a]	4.92±2.47[a]	4.43±2.00[a]
UPC	4.65±2.63[b]	3.12±3.15[a]	4.47±2.90[a]	3.60±3.34[a]
UPUS	4.57±2.43[b]	3.82±2.76[a]	4.67±2.04[a]	3.94±2.61[a]

Values are expressed as mean±SD. Means with different letters within the same column are significantly different ($p<0.05$).

As seen in Table 4.2, the statistical evaluation of the results obtained showed no significant difference in the sensory properties of the samples except color, which showed a significant difference between the values. Regarding color, the mean score was highest for PC samples and lowest for UPC lowest. Results indicated that ultrasound application did not significantly affect the color of peeled walnut beverages. The color of peeled samples was most liked, possibly due to the bright white color similar to cow's milk.

The most liked sample in terms of color in walnut beverages was the PC sample (7.97), while the least liked sample was the UPUS sample (4.57). When the results were evaluated for taste, PC was scored highest, and UPC was scored the least. Regarding the aroma, PC was liked by the panelist, while UPC was not liked.

The results indicate that the PC sample was the most preferred walnut beverage by the panelists in terms of aroma, taste, color, and overall acceptability, followed by PUS, UPUS, and UPC. Therefore, PC (peeled walnut beverage) could be a new type of beverage made from a mixture of walnut and water as it was well accepted by the panelists.

4.6. Total Solid Content of Walnut Beverage

Total solids refer to the amount of dry material that remains after removing moisture (Bradley, 2010). A product's total solid content is inversely proportional to the amount of moisture present; hence, total solid content is proportionally reduced when the moisture content is increased. Studying the total solid content of plant-based beverages is essential because it is related to the texture of the beverage. The total solids of the peeled and unpeeled walnut beverages obtained in this study are presented in Fig. 4.4.

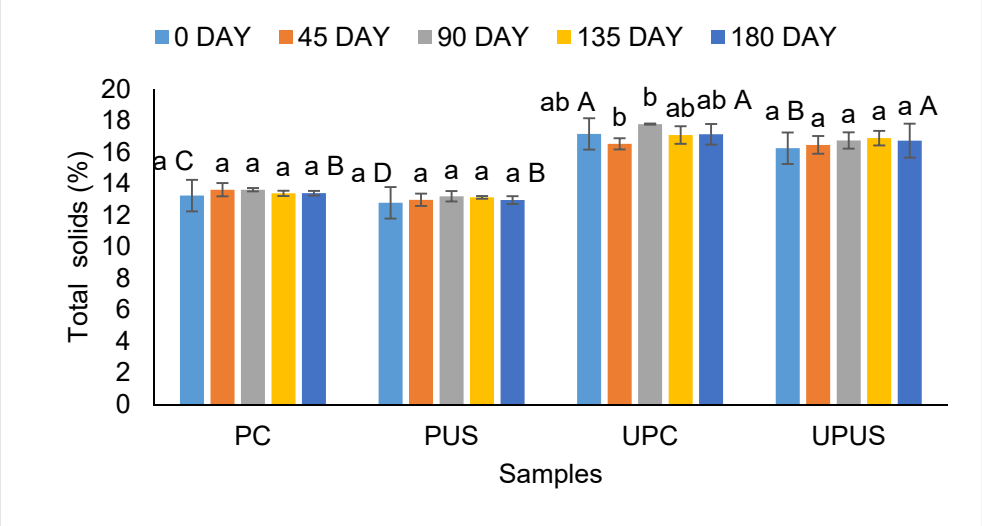

Figure 4.4. Total solid content of walnut beverages
The lowercase letters on each bar indicate significant differences among the samples during storage (p<0.05). The uppercase letters indicate the difference among the samples of 0 days and 180 days (p<0.05).

The mean total solids value obtained from PC samples for days 0, 45, 90, 135, and 180 were 13.26, 13.91, 13.44, 13.40, and 13.41 %, respectively. The mean total solids value obtained from PUS samples for days 0, 45, 90,135, and 180 were 12.80, 12.99, 13.21, 13.15, and 12.97 %, respectively. No statistical difference was detected in the samples during storage.

The mean total solids value obtained from UPC samples for days 0, 45, 90, 135, and 180 were 17.17, 16.11, 17.79, 17.05, and 17.15 %, respectively. No significant difference was observed in the samples during storage. However, the highest mean total solids were observed in the UPC sample at day 90.

The mean total solids value obtained from UPUS samples for days 0, 45, 90, 135, and 180 were 16.26, 16.47, 16.76, 16.90, and 16.75 %, respectively. Total solid differences among the samples of 0 days and 180 days were statistically significant (indicated by a capital letter at 0 and 180 days in Fig. 4.4).

The total solid value of unpeeled walnut beverage samples was higher than the peeled walnut beverage samples by 25.73% for control (PC-UPC) and 23.84% for ultrasonicated peeled and unpeeled walnut beverage samples (PUS-UPUS) at the start of the storage. This can be attributed to walnut pellicles in unpeeled walnut beverages. The total solid content of the walnut beverage (12.80 -17.79%) was higher than the range of total solid content (9.34 – 13.17%) obtained in a study on the development of soy-walnut milk drinks by Bolarinwa et al.(2018).

4.7. Ash Content of Walnut Beverages

The ash value is a measure of mineral content obtained from the ashing process. In other words, ash is the inorganic, white residue that forms when an organic food product has been completely oxidized or burned. Ash content varies among foods based on their natural characteristics (Erfanian and Rasti, 2019). There were no remarkable changes in the ash content of the walnut kernels during the whole storage period in all walnut beverage samples. The ash content of the walnut beverages is presented in Fig. 4.5.

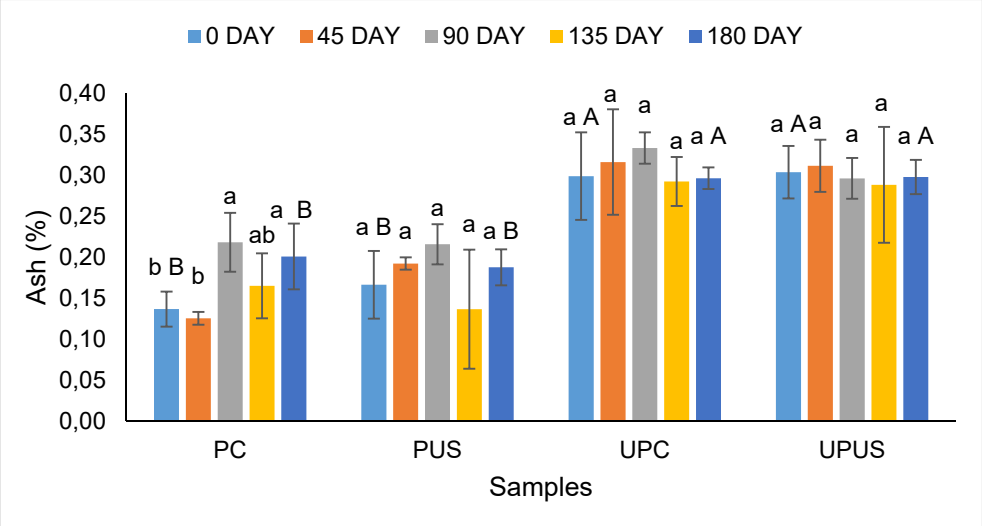

Figure 4.5. Ash content of walnut beverages
The lowercase letters on each bar indicate significant differences among the samples during storage (p<0.05). The uppercase letters indicate the difference among the samples of 0 days and 180 days (p<0.05).

The ash content was the highest for UPC samples (0.33%), followed by UPUS (0.31%), PC (0.22%), and PUS (0.22%). The final ash content of PC for days 0, 45, 90, 135, and 180 was 0.14%, 0.13%, 0.22%, 0.17%, and 0.20%, respectively. While the mean ash values for PUS samples for days 0, 45, 90, 135, and 180 were 0.17%, 0.19%, 0.22%, 0.14%, and 0.19%, respectively.

The ash content in UPC samples for days 0, 45, 90, 135, and 180 was 0.30%, 0.32%, 0.33%, 0.29%, and 0.30%, respectively. While the mean ash values for UPUS samples for days 0, 45, 90, 135, and 180 were 0.30%, 0.31%, 0.30%, 0.29%, and 0.30%, respectively. Total ash content differences among the samples of 0 days and 180 days were statistically significant (indicated by a capital letter at 0 and 180 days in Fig. 4.5).

Although significant differences (p<0.05) in ash content were noticed during storage among the walnut beverage samples, these differences were negligible. Furthermore, the ash content of unpeeled walnut beverage samples was

higher than the peeled walnut beverage samples, indicating a higher amount of minerals in the pellicles (Trandafir et al., 2016). The results for ash content (0.13 – 0.33%) in this study were lower than the ash content (0.37%) determined in chickpea milk and 0.4% ash content determined in coconut milk obtained by Rincon et al. (2020). However, the results in this study were similar to the results determined for hazelnut milk (0.32%) and almond milk (0.2%) obtained by Bernat et al.(2015).

4.8. pH of Walnut Beverages

The pH values obtained in this study for walnut beverages during storage are given in Fig. 4.6 The pH value of the walnut beverage ranged from 5.96 to 6.95. The PC samples' pH values were 6.59, 6.23, 6.85, 6.93, and 6.95 for days 0, 45, 90, 135, and 180, respectively. The PUS samples' pH values were 6.02, 6.70, 6.74, and 6.83 for days 0, 45, 90,135, and 180, respectively. The pH values differed significantly (p<0.05).

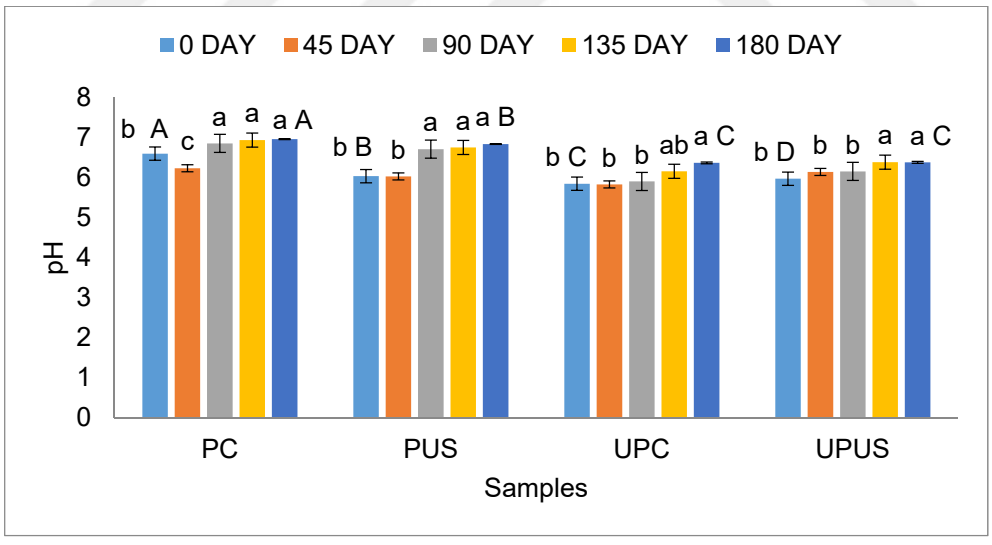

Figure 4.6. pH of walnut beverages
The lowercase letters on each bar indicate significant differences among the samples during storage (p<0.05). The uppercase letters indicate the difference among the samples of 0 days and 180 days (p<0.05).

The pH value obtained from UPC samples were 6.23, 5.82, 5.90, 6.15, and 6.36 for days 0, 45, 90,135 and 180. The pH value obtained from UPUS samples were 6.09, 6.13, 6.15, 6.38, and 6.37 for days 0, 45, 90,135, and 180, respectively. The results of pH were similar between the samples, and the pH values of all samples remained above 5.90. The pH differences among the samples of 0 days and 180 days were statistically significant (indicated by a capital letter at 0 and 180 days in Fig. 4.6).

There was a sudden decrease in the pH of peeled control samples after 0 days, which showed an increase in the pH after 180 days of storage. Likewise, in ultrasonicated walnut beverage samples, the pH was constant up to day 45 of storage which increased later during the storage, which can be attributed to the presence of pellicles containing phenolic acids and other compounds (Jahanban-Esfahlan et al., 2019b). The ultrasound did not affect the pH of the walnut beverage samples.

The pH values (5.96-6.95) determined in this study were similar to the pH value (6.68) obtained for walnut beverage inoculated with kefir by Cui et al. (2013) and higher than the pH values (6.23-6.5) obtained for chickpea and coconut milk by (Rincon et al., 2020).

4.9. Titratable Acidity of Walnut Beverages

The importance of determining factors like pH and titratable acidity is emphasized because these are crucial elements associated with the development of bacteria in food. For example, less acidic foods (pH > 4.5) are more prone to the formation of harmful microbes, in addition to molds and yeasts. pH, in particular, affects the presence and spread of germs (Jin and Kirk, 2018; Roberts, 2003).

The total titratable acidity values obtained in this study for walnut beverages during storage are given in Fig. 4.7. The total titratable acidity (TTA) of walnut beverages obtained in this study ranged from 0.05 to 0.15%.

The total titratable acidity (TTA) values obtained for PC and PUS samples for days 0, 45, 90, 135, and 180 were 0.05, 0.08, 0.09, 0.09, 0.06 % and 0.05, 0.09, 0.09, 0.10 and 0.05 % respectively. The TTA values of the walnut beverages were significantly different (p<0.05) during storage.

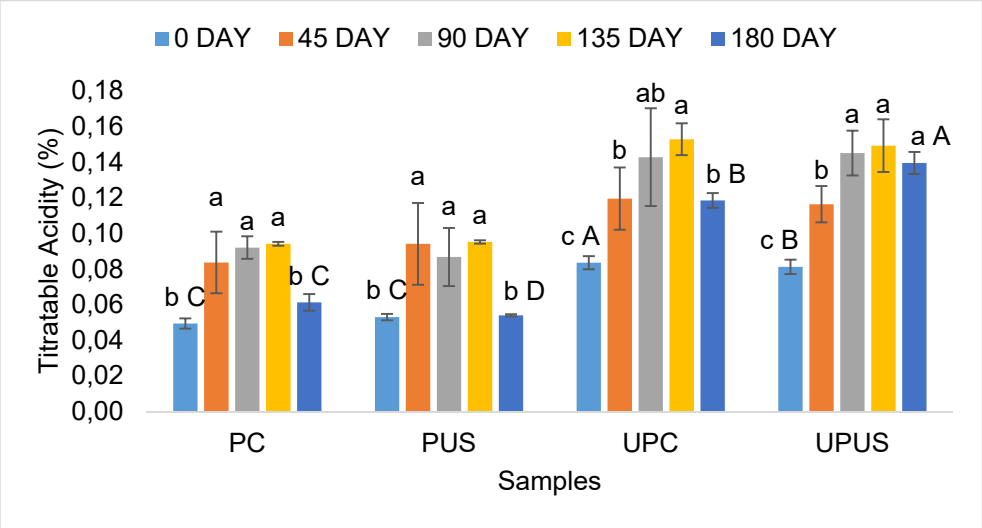

Figure 4.7. Titratable acidity of walnut beverages
The lowercase letters on each bar indicate significant differences among the samples during storage (p<0.05). The uppercase letters indicate the difference among the samples of 0 days and 180 days (p<0.05).

The total titratable acidity (TTA) values obtained from UPC samples for days 0, 45, 90, 135, and 180 were 0.08, 0.12, 0.14, 0.15, and 0.12 %, while the total titratable acidity (TTA) values obtained for UPUS samples for days 0, 45, 90, 135, and 180 were 0.08, 0.12, 0.15, 0.15, and 0.13 %. The TTA values of the walnut beverage were significantly different (p<0.05) during storage. The titratable acidity differences among the samples of 0 days and 180 days were statistically significant (indicated by a capital letter at 0 and 180 days in Fig. 4.7).

The acidity of peeled walnut beverage samples was lower than that of unpeeled walnut beverages, which indicates that the peel contains a high number of phenolic acids and other compounds (Jahanban-Esfahlan et al., 2019b). No effect of ultrasound was seen on walnut beverages.

The total titratable acidity content (0.02 – 0.15 %) of the walnut beverage reported in this study was lower than the TTA value (0.16 – 0.21%) of raw cow milk (Gemechu et al., 2015), also, was lower than the TTA (0.25 – 0.42%) of the soy-walnut milk drinks reported by Bolarinwa et al. (2018).

4.10. Viscosity of Walnut Beverages

Viscosity is an essential characteristic of foods that influences the mouthfeel and consistency of fluids such as beverages and other liquids (Yu et al., 2007). The apparent viscosity-shear rate correlation of walnut beverage samples revealed that viscosity decreased when the shear rate increased. As a result of this flow behavior, all of the walnut beverage samples displayed non-Newtonian shear-thinning flow behavior.

As seen in Fig 4.8, during the storage period, the viscosity values of the samples generally increased. At the same time, a continuous increase was noted in all samples during the first 45 days, which continued to day 180 of storage. For example, the viscosity of PC samples for day 0 was 6.5 mPa·s, which increased to 14.9 mPa·s at day 180 ($p<0.05$).

In comparison, the viscosity of PUS samples for day 0 was 6.1 mPa·s, which later increased to 10.1 mPa·s at day 180 of storage ($p<0.05$). For UPC samples, the viscosity increased from 43.0 mPa·s at day 0 to 139 mPa·s at day 180 ($p<0.05$), while the viscosity for UPUS samples 61.2 mPa·s at day 0 and increased to 105 mPa^{-1} at day 180 ($p<0.05$). According to the analysis, this viscosity increase indicates that the walnut beverages' thickness increased with storage.

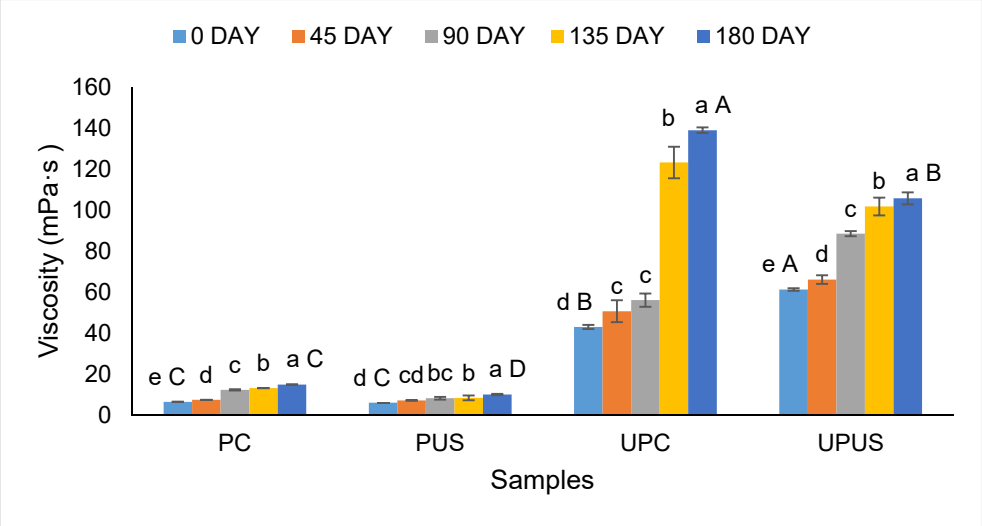

Figure 4.8. Viscosity of walnut beverages

The lowercase letters on each bar indicate significant differences among the samples during storage ($p<0.05$). The uppercase letters indicate the difference among the samples of 0 days and 180 days ($p<0.05$).

The viscosity differences among the samples of 0 days and 180 days were statistically significant (indicated by a capital letter at 0 and 180 days in Fig. 4.8). The viscosity values of PC samples varied between 6.5 to 14.9 mPa·s, which showed a gradual increase throughout the storage. In contrast, PUS samples ranged between 6.1 to 10.1 mPa·s. The viscosity of PC samples was higher as compared to control samples.

Unpeeled walnut beverage samples were denser than the peeled walnut beverage sample. The results indicated that ultrasound treatment caused a significant increase in the viscosity of walnut beverages during storage ($p<0.05$). The viscosity values of UPC samples varied between 43 to 139 mPa·s, which showed a gradual increase throughout the storage. In contrast, UPUS samples ranged between 61 to 105.7 mPa·s and showed increased viscosity during storage. The increase in viscosity during the storage of walnut beverages could be due to the protein rearrangement and protein–protein contact as reported in the literature

(Sahan et al., 2008). Additionally, when plant milk is heated, the non-polar amino acids come into contact with water, which increases their hydrophobicity. This leads to an increase in the interactions between proteins, which causes sedimentation or gelling, which leads to an increase in viscosity. (Mäkinen et al., 2016). Moreover, there was a positive correlation between sample viscosity and walnut beverage solids concentration (12.8 – 17.79%), meaning samples with a higher solids-to-water ratio had higher viscosity values (Bi et al., 2015).

The lower viscosity of PUS and UPUS samples compared to control samples can be attributed to the penetration of ultrasonic waves, which caused a decrease in the implosion force of the generated bubbles during cavitation and reduced the viscosity of the liquid (Villamiel and De Jong, 2000).

The shear viscosity of plant-based milk mainly depends on the particle concentration, such as oil bodies or fat droplets, which is why unpeeled walnut beverage samples had a higher viscosity than peeled walnut beverage samples due to the pellicles containing various oil bodies and fat content (McClements, 2020).

The viscosity results obtained in this study agree with Atalar et al. (2019), who observed the same increasing trend in the viscosity of almond and hazelnut milk applying various amplitude and times of sonication. Manzoor et al. (2021) found that increasing the treatment temperature and time for almond milk resulted in a considerable rise in viscosity, with the highest viscosity value (4.22 cP) obtained in the thermosonicated almond milk treated at 60 °C for 40 minutes.

4.11. Color Analysis of Walnut Beverages

Color is a significant parameter in assessing the visual quality of the walnut beverage. Another important quality criterion for walnuts is the surface color of the walnut kernels. Light yellow grains are preferred in walnut grains. Color, an essential quality parameter, can indicate structural defects, loss of nutritional value, and taste change. The pigmentation of the walnut shell, which is important for walnut color, can vary considerably depending on the variety and

maturation stages. It can also be affected by environmental stress factors (Donis-González et al., 2020). Table 4.3 shows the results of color parameters, L*, a*, b*, chroma, and hue of walnut beverage samples.

4.11.1. L* Values of Walnut Beverages

An object's ability to reflect or transmit light is referred to as its luminous intensity, which is related to the first color property known as the lightness value (L*). (Kwok et al., 1999).

During storage, the L* value for PC increased from 70.5 to 74.4 ($p>0.05$), while the value for PUS increased from 70.8 to 75.6 ($p<0.05$). For UPC samples, the L* value increased from 34.1 to 35.6 ($p>0.05$), while the L* value increased from 35.2 to 35.6 for UPUS samples. The highest L* value (75.6) was found in PUS samples indicating their light color. In contrast, the lowest L* value (34.1) was found in UPC samples indicating their dark color. As a result of the statistical analysis, the increase in L* value was found to be significant ($p<0.05$).

In the statistical comparisons, a significant difference ($p<0.05$) in terms of L* value was observed during storage among the samples of 0 days and 180 days. The L* values at the beginning of the storage ranged between 71.2 and 33.2, while the UPC sample had the lowest L* value. At the end of the storage, the L* values of the UPC were the lowest (indicated with a capital letter at 0 and 180 days in Table 4.3).

Table 4.3. Color values (L*, a*, b*), chroma, and hue of walnut beverage

Sample	Storage (Day)	L*	a*	b*	Chroma	Hue
PC	Day 0	$70.5\pm0.65^{a\ A}$	$0.3\pm0.30^{b\ C}$	$10.7\pm0.73^{a\ D}$	$10.7\pm0.73^{a\ D}$	$88.6\pm0.15^{a\ A}$
	Day 45	70.1 ± 6.13^{a}	0.3 ± 0.19^{b}	11.6 ± 1.11^{a}	11.6 ± 1.10^{a}	88.4 ± 1.15^{a}
	Day 90	72.2 ± 0.60^{a}	0.9 ± 0.42^{ab}	12.0 ± 0.82^{a}	12.0 ± 0.84^{a}	85.9 ± 1.76^{ab}
	Day 135	72.0 ± 0.44^{a}	1.0 ± 0.55^{a}	11.6 ± 0.59^{a}	11.7 ± 0.61^{a}	84.9 ± 2.58^{b}
	Day 180	$74.4\pm1.72^{a\ A}$	$0.9\pm0.20^{ab\ C}$	$8.6\pm0.87^{b\ B}$	$8.6\pm0.89^{b\ C}$	$84.0\pm0.73^{b\ A}$
PUS	Day 0	$70.8\pm0.50^{b\ A}$	$0.3\pm0.02^{b\ C}$	$12.8\pm2.45^{ab\ C}$	$12.8\pm2.45^{ab\ C}$	$88.5\pm0.56^{a\ A}$
	Day 45	72.1 ± 0.63^{b}	0.2 ± 0.05^{b}	11.1 ± 0.10^{ab}	11.1 ± 0.10^{b}	88.9 ± 0.27^{a}
	Day 90	74.2 ± 1.27^{a}	0.8 ± 0.33^{a}	12.8 ± 0.29^{b}	12.8 ± 0.31^{ab}	86.4 ± 1.41^{b}
	Day 135	74.6 ± 0.75^{a}	0.9 ± 0.08^{a}	13.1 ± 0.54^{a}	13.2 ± 0.54^{a}	86.0 ± 0.34^{b}
	Day 180	$75.6\pm0.17^{a\ A}$	$1.1\pm0.31^{a\ C}$	$11.7\pm2.18^{ab\ A}$	$11.7\pm2.20^{ab\ B}$	$84.5\pm0.53^{c\ A}$
UPC	Day 0	$34.1\pm1.53^{c\ B}$	$5.5\pm0.38^{c\ B}$	$15.4\pm0.11^{a\ A}$	$16.4\pm0.09^{a\ A}$	$70.2\pm1.35^{a\ B}$
	Day 45	38.2 ± 1.81^{ab}	6.9 ± 0.12^{ab}	14.1 ± 1.08^{a}	15.7 ± 1.02^{a}	63.8 ± 1.42^{b}
	Day 90	38.7 ± 1.68^{ab}	7.0 ± 0.23^{ab}	14.6 ± 0.78^{a}	16.2 ± 0.80^{a}	64.3 ± 0.63^{b}
	Day 135	40.4 ± 0.33^{a}	7.2 ± 0.2^{a}	14.8 ± 0.41^{a}	16.5 ± 0.46^{a}	64.1 ± 0.59^{b}
	Day 180	$35.6\pm2.46^{bc\ B}$	$6.7\pm0.09^{b\ A}$	$14.4\pm1.90^{a\ A}$	$15.9\pm1.75^{a\ A}$	$65.2\pm2.67^{b\ B}$
UPUS	Day 0	$35.2\pm1.01^{b\ B}$	$6.0\pm0.11^{b\ A}$	$14.4\pm0.07^{a\ B}$	$15.6\pm0.10^{a\ B}$	$67.4\pm0.31^{a\ C}$
	Day 45	39.8 ± 0.74^{a}	6.8 ± 0.18^{a}	12.9 ± 0.18^{b}	14.6 ± 0.08^{b}	62.1 ± 0.96^{c}
	Day 90	37.3 ± 1.68^{ab}	6.7 ± 0.17^{a}	12.7 ± 0.24^{b}	14.3 ± 0.14^{bc}	62.4 ± 1.01^{c}
	Day 135	37.1 ± 0.33^{ab}	6.7 ± 0.02^{a}	12.7 ± 0.10^{b}	14.3 ± 0.09^{bc}	62.0 ± 0.23^{c}
	Day 180	$35.6\pm2.46^{b\ B}$	$6.0\pm0.27^{b\ B}$	$12.8\pm0.12^{b\ A}$	$14.1\pm0.19^{c\ AB}$	$64.7\pm0.90^{b\ B}$

Means within the same column with different letters are significantly different ($p<0.05$). The uppercase letters indicate the difference among the samples of 0 days and 180 days ($p<0.05$).

Sterilization treatments caused a significant decrease in the L* values of both UPUS and UPC samples, indicating darkening compared to peeled (PC-PUS) walnut beverage samples, which showed the highest L* values as compared to unpeeled samples. Similar results were observed by sensory analysis, with unpeeled walnut beverage samples being darker than peeled walnut beverage samples in the panel opinion which can be possibly due to caramelization or Maillard reactions due to the presence of sugars, pigments in the pellicle and the conditions of elevated temperature during the process of autoclave-sterilization (Lepaus et al., 2022).

According to the analysis, this increase in L* value shows the lightning. In contrast, the decrease shows the darkening of the product with storage. This increase is possibly due to the homogenization process, as the number and size of particles contribute to the light reflection (Bernat et al., 2015a). However, the low L* value of unpeeled walnut beverages can be attributed to the Maillard reaction triggered by high temperatures above 50°C (Manzoor et al., 2021).

The L* value results obtained in this study were higher than the L* value (64.38) obtained by Manzoor et al. (2021) for almond milk and lower than the L* value (81.14) obtained by Lu et al. (2019).

4.11.2. a* Values of Walnut Beverages

The changes in the * values for walnut beverage samples during storage are given in Table 4.3. the a* value of PC samples for day 0 was 0.3, which increased to 0.9 at day 180, while the a* value of PUS samples for day 0 was 0.3, which later increased to 1.1 at day 180 of storage ($p<0.05$). For UPC samples, the * values increased from 5.5 on day 0 to 6.7 on day 180 ($p<0.05$), while the a* value for UPUS samples was 6.0 on day 0 and increased to 6.7 on day 135 ($p<0.05$). According to the analysis, this increase observed in a* value indicates that the redness in the color of the walnut beverages increases with storage.

Statistical comparisons between the samples were made at 0 and 180 days. The differences between the a* values of the samples at the beginning of storage (0 days) and end of storage (180 days) were found to be statistically significant (indicated with a capital letter at 0 and 180 days in Table 4.3). The a* values at day 0 of the storage ranged between 0.23 and 6.07, while the UPUS sample had the highest a* value. At the end of the storage, the a* values of the UPC were the highest. However, the a* values of the samples at the end of storage were higher.

The a* values of all UPC-UPUS samples were higher than the a* values of PC-PUS samples; this indicates that the sugars and phenolic compounds are more confined in the pellicles of walnut. In many studies, this increase in a* value during storage is associated with the Maillard reaction caused by sugars due to high temperature during sterilization (Manzoor et al., 2021).

The a* value results obtained in this study were higher than the a* value (-0.36 to -0.95) obtained by Bruno et al. (2020) cashew nut milk at a storage of 30 days and also higher than a* value (-1.16) of hazelnut milk treated at 40% amplitude for 10 minutes obtained by (Atalar et al., 2019).

4.11.3. b* Values of Walnut Beverages

The b*values of walnut beverages during storage are given in Table.4.3 A decrease in PC, UPC, and UPUS for b* values was observed with storage. While the b* value at 0 days was 10.7 for PC samples, this value decreased to 8.6 at day 180. The b* value at day 0 for UPC samples was 15.4, this value decreased to 14.4 while at day 180, while the b* value at day 0 for UPUS samples was 14.4, and at day 180 days, this value decreased to 12.8. In contrast, for PUS, the initial b* value was 9.8; eventually, this value increased to 11.7 during storage. The b* values, with all measurements above zero, confirmed that the yellow coloration dominates over the blue in all samples.

In the statistical comparison between samples at the beginning and end of the storage, the differences between the b* values of the samples at the beginning

of storage ranged between 10.17 and 15.54, while the UPC sample was with the highest b* value. At the end of storage, b* values of PC samples were found to be lower (p<0.05) (indicated with a capital letter at 0 and 180 days in Table 4.3).

According to the analysis, a slight decrease in the b* value was detected in all samples at the end of storage compared to the 0 days. It shows that the carotenoid compounds that give the product a yellow color decrease during storage. As a result of the statistical analysis, this decrease in the b* value was found to be significant for PC and UPUS samples (p<0.05) and insignificant for PUS and UPC samples (p>0.05). Therefore, this decrease in the b* value for walnut beverage samples may be associated with the reduction of carotenoids during storage (Contreras et al., 2008).

Results obtained in this study for the b* value are similar to the results obtained by Bruno et al. (2020) for the b* value (9.39) for cashew nut milk and were higher than the b* value (6.82) obtained by Dhakal et al. (2016) for almond milk.

4.11.4. Chroma of Walnut Beverages

The C* value (Chroma) indicates the saturation of the color. One further aspect of chromatic appearance is the chroma value (C*), which is the saturation, purity, or intensity of color and is calculated as $(a^{*2}+b^{*2})^{1/2}$. Changes in C* value in walnut beverages is shown in Table 4.3.

As seen in Table 4.3, C* values of walnut beverages decreased with storage. For example, while the C* value on day 0 was 10.7 for PC samples, this value increased to 11.7 on day 135 and dropped to 8.6 on day 180, while the C* value on day 0 for PUS samples was 12.8 on day 0, this value increased to 13.1 at day 135 and dropped to 11.7 at day 180. Also, the C* value at day 0 for UPC samples was 16.4, which first increased to 16.5 on day 135 and later slightly decreased to 15.9 on day 180. For UPUS samples, the initial C* value was 15.6, which decreased to 14.1 at the end of storage.

According to the analysis, C* values decreased in all samples during storage, and it was determined that the dull appearance of walnut beverages increased with storage. As a result of the statistical analysis, this decrease in C* value for the samples was significant (p<0.05) in PC and UPUS samples and insignificant for PUS and UPC samples (p>0.05). The lowest C* values were detected in the 180-day samples.

In the statistical comparisons between the samples (0 and 180 days), the C* values at the start of the storage ranged between 10.42 and 16.48. In contrast, the PC sample had the lowest C* values. When the C* values of the samples were compared at the end of storage, the lowest C* value was detected in the PC sample (p<0.05), and the highest value was detected in the UPC samples; this indicates that unpeeled walnut beverages had a denser color than peeled walnut beverages. (Indicated with a capital letter at 0 and 180 days in Table 4.3).

The C* values obtained in this study ranged between 8.6 and 16.2, which were lower than C* values (26.07 and 5.47) obtained by Rincon et al. (2020) for chickpea and coconut milk and were higher than C* values (5.92 - 4.62) obtained for blanched sesame milk at different temperatures (Ahmadian-Kouchaksaraei et al., 2014).

4.11.5. Hue* Value of Walnut Beverages

The angle created by the axis +a* and the line segment c is called the hue (i.e., color) and is denoted by the letter h*. The hue value, h*, ranges from 0°- 360° and corresponds to whether the object is red (0° and 360°), orange, yellow (90°), green (180°), blue (270°) Fig 4.9. The changes in hue* values of walnut beverages subjected to ultrasound treatment during storage are given in Table 4.3.

Hue* values of the samples obtained as a result of ultrasound application on the peeled walnut beverage for days 0, 45, 90, 135, and 180 were 88.0, 88.9, 86.4, 86.0, and 84.5, respectively. In addition, the statistical comparison detected a statistical difference in the PUS, UPC, and UPUS samples during storage (p<0.05).

While the decreases in PC hue* values for days 0, 45, 90, 135, and 180 were 88.6, 88.4, 85.9, 84.9, and 84, respectively (p>0.05).

While the hue* values obtained for UPC samples for days 0, 45, 90, 135, and 180 were 70.2, 63.8, 64.3, 64.1, and 65.2, respectively (p<0.05). A statistical difference was detected in the samples during storage (p<0.05). The lowest hue* value in UPC for day 45 (63.8) and the highest hue* value in the UPC sample (70.2) were determined.

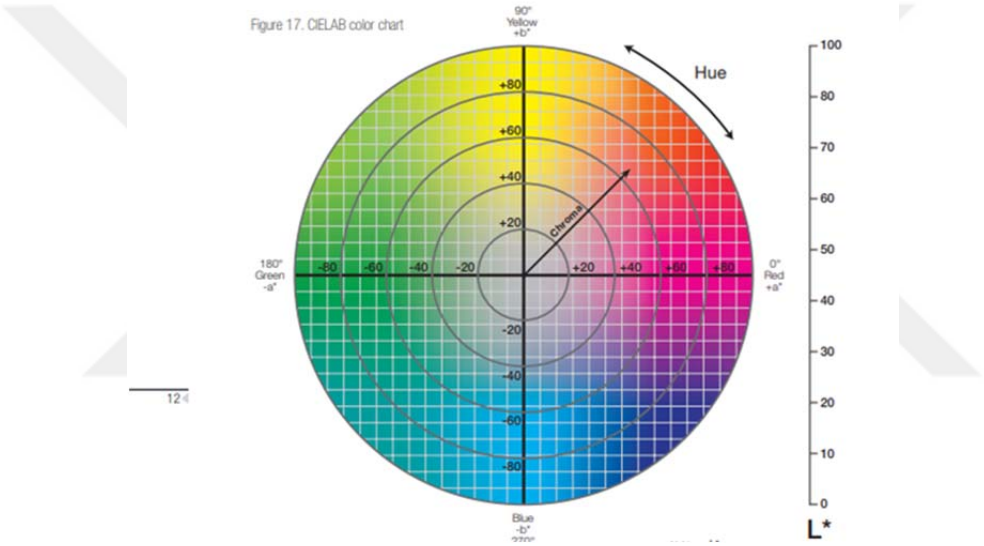

Figure 4.9. Hunter color system

Hue* values obtained for UPUS samples for days 0, 45, 90, 135, and 180 were 67.4, 62.1, 62.4, 62, and 64.7, respectively. The differences between the Hue* values were statistically significant (p <0.05). The lowest hue* value was found in the day 135-UPC sample (62), and the highest hue* value was found in the day 45-PUS sample (88.9). During storage, the decline started after day 45 of storage.

In the statistical comparisons between the samples (0 and 180 days), the hue* values on the 0-day of the storage ranged between 88.70 and 67.16. In contrast, the differences between the samples were not statistically significant.

However, the hue* values of the UPC samples at the end of the storage were lower, and the differences were statistically significant (indicated with a capital letter at 0 and 180 days in Table 4.3).

Although the hue* values of the UPUS samples were significantly lower, ranging from 62.1 – 70.2 during storage. This can be possibly attributed to the dark color of the walnut beverage samples, which was cream-colored but turned brown after sterilization, which activated the Maillard reaction. The hue* values (62.0 – 88.9) obtained in this study were found to be similar to hue* values (82.2 - 90.2) of hazelnut milk but lower than the hue* values (90.3 - 96.1) for almond milk obtained by Bernat et al. (2015). The results of the h* value obtained in this study were similar to the h* value (86.73) obtained by Rincon et al. (2020) for plant-based milk of chickpeas and coconut.

4.11.6. ΔE Values of Walnut Beverages

The L*, a*, and b* values were used to calculate the color difference (ΔE) value for comparing peeled and unpeeled walnut beverages. According to the L*a*b* system, the color difference between two colors is calculated according to the formula (Atalar et al., 2019).

$$\Delta E = [\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}]^{0.5}$$

The ΔE* values of walnut beverages during storage changes are given in Fig. 4.10. An increase was observed in the ΔE* values of all samples during storage. For example, while the ΔE* value was 2.24 on day 0 for the peeled samples (PC-PUS), this value increased to 3.55 on day 180, while the ΔE* value the 0 days was 1.68 for the unpeeled (UP-UPUS) samples on day 180, this value increased to 2.07 at day 180.

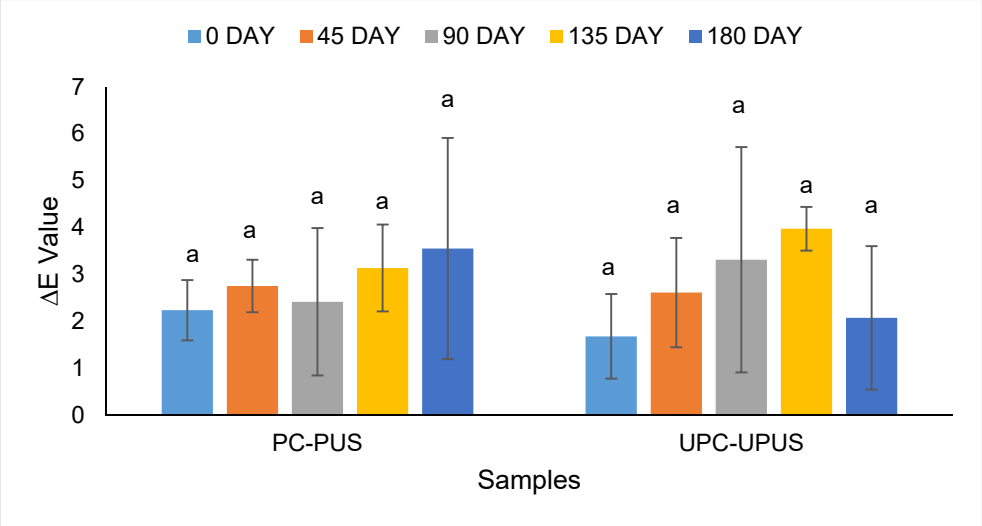

Figure 4.10. ΔE* of walnut beverages during storage.
The lowercase letters on each bar indicate significant differences among the samples during storage. (p<0.05). The uppercase letters indicate the difference among the samples of 0 days and 180 days (p<0.05).

According to the analysis, ΔE* values increased in all walnut beverage samples during storage. However, a slight decrease was seen in unpeeled samples on day 180. As a result of the statistical analysis, this increase in ΔE* value was found insignificant for the samples (p>0.05). Therefore, the lowest ΔE* value in the unpeeled sample (1.68) at day 0 and the highest ΔE* value in the unpeeled samples (3.98) were determined.

Atalar et al. (2019) found a minute color difference (0.12) in hazelnut milk treated with ultrasound; however, the color difference increased with an increase in process time due to the effect of temperature. The ΔE* values for thermosonicated hazelnut milk ranged from 0.12 to 1.26 with increased amplitude, time, and temperature. These values were less than the ΔE* values results (2.07- 3.98) obtained in this study. Cruz et al. (2007) determined ΔE* values in soymilk treated with ultra-high-pressure homogenization in the range of 3.35 and 5.83. Manzoor et al. (2021) reported that the ultrasound did not affect the ΔE* value (0.842) of the coconut milk system with maize additive.

4.11.7. Whiteness Index of Walnut Beverages

The whiteness of milk products is often the most critical color characteristic. The whiteness index (WI) indicates the degree of whiteness and mathematically combines lightness and yellow–blue into a single term. It is widely measured using the following equation (Milovanovic et al., 2020). The results for the whiteness index in walnut beverages are given in Fig. 4.11.

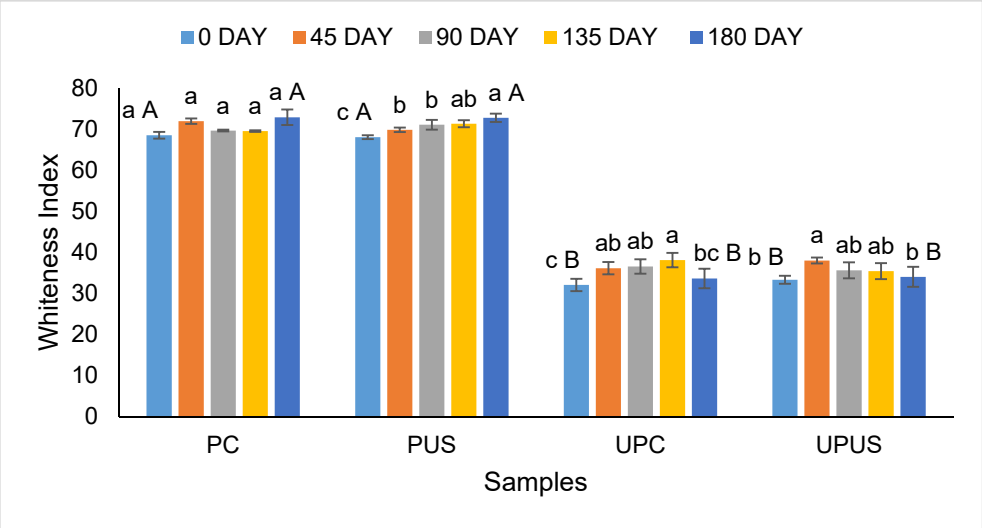

Figure 4.11. Whiteness index of walnut beverages
The lowercase letters on each bar indicate significant differences among the samples during storage (p<0.05). The uppercase letters indicate the difference among the samples of 0 days and 180 days (p<0.05).

The whiteness index (WI) of walnut beverages also followed a similar trend as that of the L* values. The whiteness index (WI) value of PC samples for day 0 was 68.60, which increased to 73.00 at day 180 (p>0.05), while the WI values of PUS samples for day 0 was 68.14 which later increased to 72.87 at day 180 of storage (p<0.05).

For UPC samples, the WI values increased from 5.5 on day 0 to 6.7 on day 180 (p>0.05), while the WI values for UPUS samples were 32.10 on day 0 and increased to 38.16 on day 135. According to the analysis, this increase observed in a* value indicates that the whiteness in the color of the walnut beverages increases with storage. However, a decrease was observed in the whiteness index (33.66) of UPUS samples on day 180.

Statistical comparisons between the samples were made at 0 and 180 days. The differences between the WI values of the samples at the beginning of storage (0 days) and end of storage (180 days) were found to be significant (indicated with a capital letter at 0 and 180 days in Fig. 4.11).

The highest WI value (73) was found in PC samples indicating their light color. In contrast, the lowest WI value (32.1) was found in UPC samples indicating their dark color. According to the analysis, this increase in WI value shows lightning. In contrast, the decrease shows the darkening of the product with storage. A high WI value was obtained in all peeled samples. The color remained the same after sterilization, indicating that the color change was due to the presence of sugars, phenolic compounds, and pigments in the pellicle. As a result of the statistical analysis, this increase in WI value was found to be significant (p<0.05).

Bernat et al. (2015) analyzed the effect of heat treatments and high homogenization pressures on almond and hazelnut beverages' physical properties and stability. The whiteness value obtained in this study for autoclaved almond milk at 121°C for 15 minutes was 77.5. The whiteness value obtained for autoclaved hazelnut milk was 74.3. These values are near the whiteness values (68.14 -73) obtained in this study. The whiteness values results in this study were similar to the whiteness value of almond milk (68.4), cashew milk (65.6), hemp milk (68.5), soy milk (69.3), and were lower than WI (81.9) of cow milk (McClements, 2020).

4.12. Total Phenolic Content of Walnut Beverages

The total phenolic content (TPC) was determined by the Folin-Ciocalteau method. The total phenolic content of the peeled and unpeeled walnut beverages obtained in this study is presented in Fig. 4.12 The mean total phenolic content value obtained for PC samples for days 0, 45, 90, 135, and 180 were 531.43, 516.88, 515.47, 475.76 and 433.96 GAE mg/L respectively. The mean total phenolic content value obtained for PUS samples for days 0, 45, 90, 135, and 180 were 564.51, 538.36, 514.27, 474.40, and 435.92 GAE mg/L, respectively. While the mean total phenolic content value obtained for UPC samples for days 0, 45, 90, 135, and 180 were 1556.26, 1725.00, 1438.70, 1442.71, and 1262.09 GAE mg/L, respectively.

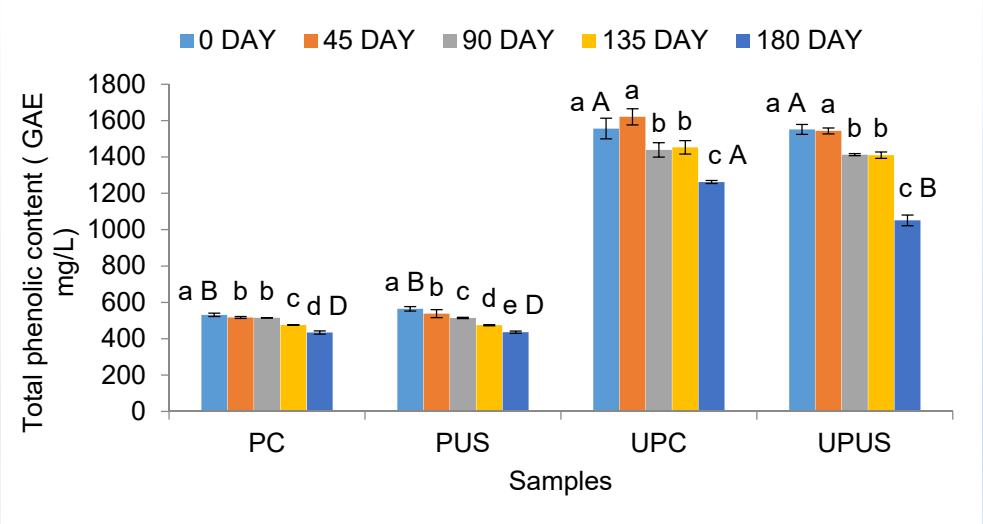

Figure 4.12. Total phenolic content of walnut beverage

The lowercase letters on each bar indicate significant differences among the samples during storage (p<0.05). The uppercase letters indicate the difference among the samples of 0 days and 180 days (p<0.05).

The mean total phenolic content value obtained for UPUS samples for days 0, 45, 90, 135, and 180 were 1317.11, 1777.47, 1256.48, 1409.64, and 1051.15 GAE mg/L, respectively.

In the statistical comparisons between the samples (those in 0 days and 180 days), the differences in total phenolic content at the beginning and end of storage (0 days) were found to be statistically significant (indicated by a capital letter at 0 and 180 days in Fig. 4.12). There was a decrease in total phenolic content at the end of storage.

While the total phenolic content (TPC) of the unpeeled walnut beverage samples showed a sudden increase in both ultrasonicated and control samples of 45-day storage, some compounds are likely generated during storage that reacts with the Folin-Ciocalteau reagent and dramatically increase the overall phenolic content of the unpeeled walnut beverage (Klimczak et al., 2007). After 45 days of storage decrease in TPC was seen in both ultrasonicated and control unpeeled samples. The decrease in total phenolic content is most likely attributed to the rise in Sulphur-containing compounds and terpenoid compounds in kernel essential oil.

Results showed a high value of total phenolic content of 1762.83 GAE mg/L in unpeeled walnut beverage samples than peeled samples, which can be attributed to the walnut pellicle present in unpeeled walnut beverage samples, walnut pellicle being the most abundant in total phenolic content as reported by Zhang et al. (2020).

The total phenolic content (TPC) of the control and ultrasonicated walnut beverage samples tended to decrease gradually with storage. Phenolic compounds are generally synthesized by the shikimate pathway in which phenylalanine ammonia-lyase (PAL) is the key enzyme (Shiri et al., 2011). During storage, the phenolic content decreases due to the decreased activity of enzymes phenylalanine ammonia lyase (Buranasompob et al., 2007). The degradation of phenolic compounds in the course of storage has been reported by Christopoulos and Tsantili (2011).

The total phenolic content (TPC) of all the walnut beverage samples varied between 433.96 (PC) to 1777.47 (UPUS) GAE mg/L. Therefore, the total phenolic content obtained in this study is close to the previously reported ranges of the total phenolic content of dry walnut (1020–2052 mg GAE/100 g) reported by (Kornsteiner et al., 2006) and is higher than the total phenolic content (80.97 - 142.91 mg/kg) reported by Ergun and Süslüoğlu (2021).

4.13. Antioxidant Activity of Walnut Beverages

Antioxidants may react differently to various sources of radicals or oxidants. Therefore, in order to measure the antioxidant activity of walnut beverages, two approaches based on distinct reaction processes were employed, and the relationship between them was studied (Fernández-Agulló et al., 2020).

4.13.1. DPPH

The degree to which the yellow test solution turns green and blue indicates the reducing power of the various components. The reduced DPPH could be quantified by measuring the decrease in absorbance at 517 nm (Pereira et al., 2008). The ability of extracts to scavenge radicals produced by DPPH can provide vital information regarding the antiradical activity of the extracts. The DPPH values of the peeled and unpeeled walnut beverages obtained in this study are presented in Fig. 4.13.

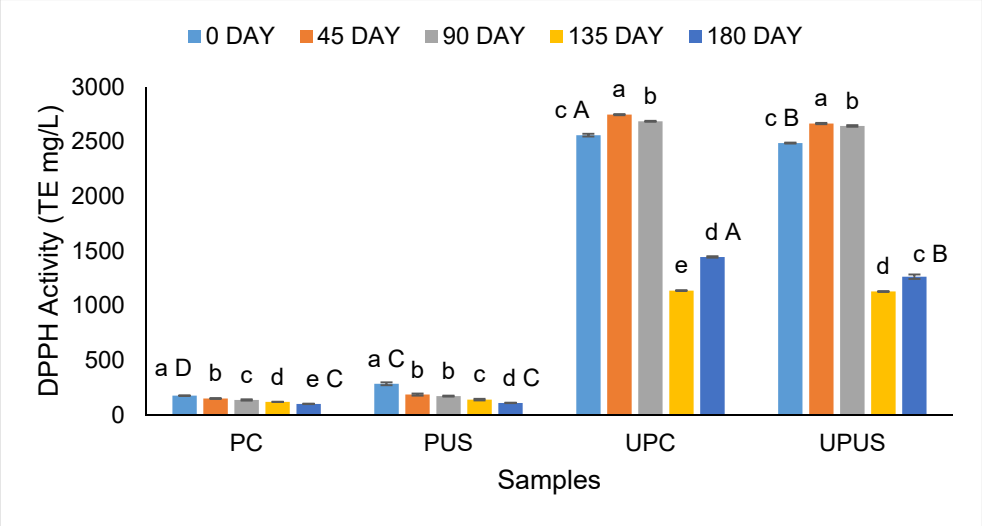

Figure 4.13. DPPH radical scavenging assay of walnut beverage
The lowercase letters on each bar indicate significant differences among the samples during storage (p<0.05). The uppercase letters indicate the difference among the samples of 0 days and 180 days (p<0.05).

The mean DPPH value obtained for PC and PUS samples for days 0, 45, 90, 135, and 180 ranged from 102.09 to 177.89 TE mg/L and 111.32 to 286.29 TE mg/L, respectively. While the mean DPPH activity value obtained for UPC and UPUS samples for days 0, 45, 90, 135, and 180 ranged from 1446.11 to 2748.24 TE mg/L and 1130.59 to 2666.80 TE mg/L, respectively. DPPH values showed a significant statistical difference between the samples during storage (p<0.05).

In the statistical comparisons between the samples (those in 0 days and 180 days), DPPH values at the beginning and end of storage (0 days) were found to be statistically significant (indicated by a capital letter at 0 and 180 days in Fig. 4.13). There was a decrease in DPPH values at the end of the storage.

As seen in Fig 4.13, the values of unpeeled walnut beverage samples are higher than peeled walnut beverage samples by 14.4-fold, and both ultrasonicated and control unpeeled walnut beverage extracts revealed a strong scavenging activity on DPPH radicals. The findings confirm that walnut pellicle, which is a

high source of phenolics, is what effectively scavenges free radicals (Samaranayaka et al., 2008). Furthermore, the values of DPPH radical scavenging activity for walnut beverages observed in this study were higher than the DPPH values of 64.14 to 70.52 % reported by Ergun (2021). These results support that walnut pellicles are a rich source of phenolics and could be responsible for effectively scavenging free radicals (Trandafir et al., 2016). However, no effect of ultrasound was seen on the DPPH activity of the walnut beverage samples. Also, the DPPH values (102.09 - 2748.24 TE mg/L) in this study are higher than 21.02 mM TE/g obtained in thermosonicated almond milk at 30°C for 10 minutes (Manzoor et al., 2021).

4.13.2. FRAP

In the presence of antioxidants, the FRAP assay quantifies the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron). Since the ferric-to-ferrous iron reduction occurs rapidly with all reductants with half-reaction reduction potentials above that of Fe^{3+} / Fe^{2+}, the values in the FRAP assay express the corresponding concentration of electron-donating antioxidants (Blomhoff et al., 2006).

FRAP technique showed a higher correlation with total phenolics. FRAP is a widely used method to determine the total antioxidant capacity of plant materials (Luximon-Ramma et al., 2002). The FRAP assay was originally designed to evaluate the antioxidant capacity of plasma. However, it has now gained widespread application in studying other biological samples, including food, plant extracts, juices, beverages, etc. (Pulido et al., 2000).

The lowest FRAP value was determined in PC samples ranging from 14.17 to 17.35 mgTE/L, while for PUS samples, FRAP values ranged from 14.23 to 18.43 mgTE/L. In addition, the FRAP values were significantly different ($p<0.05$) during storage.

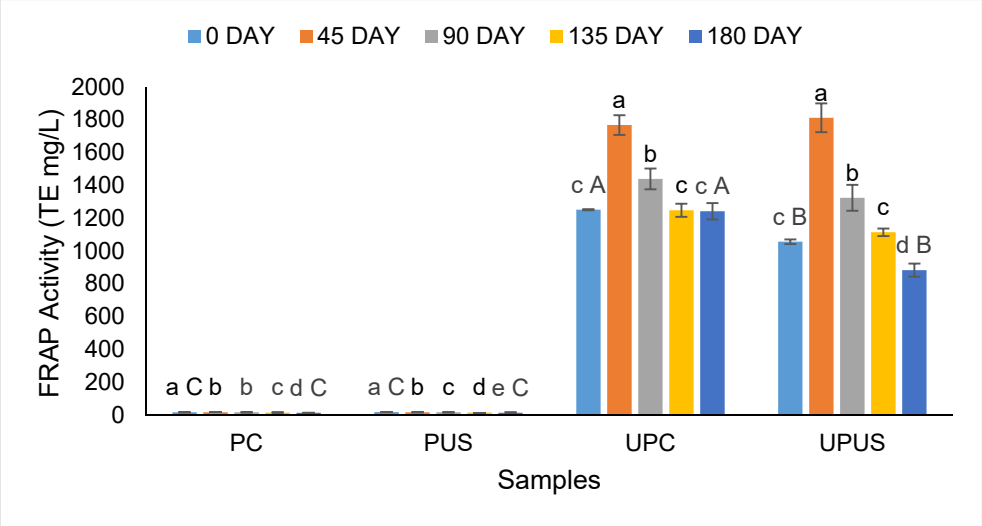

Figure 4.14. FRAP antioxidant activity of walnut beverages
The lowercase letters on each bar indicate significant differences among the samples during storage (p<0.05). The uppercase letters indicate the difference among the samples of 0 days and 180 days (p<0.05).

Correspondingly, the highest FRAP value was displayed in UPC samples, which also had the highest total phenolic content. The FRAP value of UPC samples ranged from 1243.11 to 1768.46 mgTE/L, and for UPUS samples, FRAP ranged from 883.86 to 1812.71 mgTE/L. The FRAP values were significantly different (p<0.05) during storage.

In the statistical comparisons between the samples (those in 0 days and 180 days), FRAP values at the beginning and end of storage (0 days) were found to be statistically significant (indicated by a capital letter at 0 and 180 days in Fig. 4.14). There was a decrease in FRAP values at the end of storage.

Furthermore, a 71.5% increase was observed in UPUS samples at day 45, possibly linked to the uprising unbound total phenolic content due to cavitation. Earlier similar trends were reported in almond milk treated with thermosonication at 40 kHz for various temperature and time combinations (Manzoor et al., 2021). FRAP value of the walnut beverage sample during storage decreased, indicating

that antioxidant activity became weaker as the storage period advanced with a trend almost similar to total phenolic content (Fig. 4.14), as was expected since phenol oxidation is responsible for the loss of antioxidant capacity (Manzocco et al., 2000).

4.14. Particle Size of Walnut Beverages

The particle size characteristics of walnut beverages are important parameters for evaluating their stability. The particle size distribution of walnut beverages processed at 25 kHz frequency and processing time 10 min and upon storage at 4 ± 2◦C, consisting of the values of cumulative percentile ($D_{0.5}$), volume-weighted mean diameter ($D_{4,3}$) and surface-weighted mean diameter ($D_{3,2}$) of the samples are given in Table 4.4.

Table 4.4. Particle size parameters ($D_{3,2}$, $D_{4,3}$, and $D_{0.5}$) of walnut beverages (µm)

Sample	Storage (Day)	D [3;2] µm	D [4;3] µm	Dv (50) µm
PC	0	3.80 ± 0.36$^{b\ C}$	140.67± 16.17$^{b\ A}$	112.00± 14.00$^{b\ A}$
	45	4.62± 0.77^{b}	150.67± 3.79^{b}	136.67± 10.50^{b}
	90	3.77± 3.15^{b}	150.00± 13.23^{b}	131.00± 19.47^{b}
	135	9.60± 0.56^{a}	195.67± 17.01^{a}	189.67± 15.18^{a}
	180	10.78± 3.30$^{a\ B}$	198.67± 11.14$^{a\ A}$	188.00± 9.17$^{a\ A}$
PUS	0	2.00± 0.26$^{a\ D}$	123.00± 11.14$^{a\ AB}$	86.10± 12.95$^{a\ B}$
	45	2.59± 2.38^{a}	144.67± 24.19^{a}	91.34± 10.77^{a}
	90	3.28± 2.60^{a}	122.33± 10.50^{a}	51.73± 30.75^{a}
	135	3.11± 5.13^{a}	110.80± 48.71^{a}	64.74± 96.45^{a}
	180	5.57± 0.39$^{a\ C}$	104.03± 18.22$^{a\ C}$	26.03± 20.16$^{a\ C}$
UPC	0	8.00± 1.44$^{a\ A}$	114.00± 5.29$^{a\ BC}$	85.00± 11.53$^{a\ B}$
	45	13.27± 2.14^{a}	141.67± 5.03^{a}	113.40± 36.14^{a}
	90	12.90± 1.45^{a}	126.33± 5.77^{a}	82.93± 11.45^{a}
	135	13.36± 5.13^{a}	150.67± 14.74^{a}	104.43± 16.84^{a}
	180	15.53± 2.87$^{a\ A}$	145.33± 25.72$^{a\ B}$	100.47± 24.35$^{a\ B}$
UPUS	0	5.80± 0.30$^{c\ B}$	96.00± 6.24$^{b\ C}$	65.67± 3.51$^{a\ B}$
	45	9.29± 5.28^{bc}	103.13± 81.19^{ab}	71.18± 54.27^{a}
	90	11.91± 2.24^{ab}	135.67± 11.59^{ab}	85.43± 10.36^{a}
	135	12.30± 0.95^{ab}	168.00± 18.25^{ab}	115.67± 13.65^{a}
	180	14.97± 2.06$^{a\ AB}$	178.33± 27.57$^{a\ AB}$	109.10± 23.37$^{a\ B}$

Means ± standard deviation with lowercase letters on the same line differed by Duncan test (p<0.05). The uppercase letters indicate the difference among the samples of 0 days and 180 days (p<0.05).

The $D_{3,2}$(μm), $D_{4,3}$ (μm), and $D_{0.5}$ (μm) values obtained for peeled control samples (PC) for 0, 45, 90, 135, and 180 days were 3.80, 140.67, and 112 μm; 4.62, 150.67 and 136.67 μm; 3.77, 150 and 131 μm; 9.60, 195.67 and 189.67 μm and 10.78, 198.67 and 188 μm respectively. The particle size values of the PC samples were significantly different (p<0.05) during storage.

While the $D_{3,2}$(μm), $D_{4,3}$ (μm), and $D_{0.5}$(μm) values obtained for peeled ultrasonicated samples (PUS) for 0, 45, 90, 135, and 180 days were 2, 123, and 86.10 μm; 2.59, 144.67 and 91.34 μm; 3.28, 122.33 and 51.73 μm; 3.11, 110.80 and 64.74 μm and 5.57, 104 and 26.03 μm respectively. The particle size value differences of the PUS samples were insignificant during storage.

The $D_{3,2}$(μm), $D_{4,3}$ (μm), and $D_{0.5}$(μm) values obtained for UPC for 0, 45, 90, 135, and 180 days were 8, 114, and 85 μm; 15.93, 141.67, and 113.40 μm; 12.90, 126.33 and 82.93; 13.36, 150.67 and 104.43 μm and 15.53, 145.33 and 100.47 μm respectively. While the $D_{3,2}$(μm), $D_{4,3}$ (μm), and $D_{0.5}$(μm) values obtained for unpeeled ultrasonicated samples (UPUS) for 0, 45, 90, 135, and 180 days were 5.80, 96, and 65.67 μm; 9.29, 103.13 and 71.18 μm; 11.91, 135.67 and 85.43 μm; 12.30, 168 and 115.67 μm and 14.97, 178.33 and 109.10 μm respectively. The differences in the particle size values of the UPC and UPUS samples were insignificant during storage.

The data in Table 4.4 shows a gradual increase in $D_{3,2}$ in control samples, whereas, in ultrasonicated samples, a non-uniform pattern was observed. The PC samples' $D_{4,3}$ value (198.67 μm) was the highest, while the PUS samples showed the lowest $D_{4,3}$ value (96 μm). Also, the highest $D_{3,2}$ value (15.53 μm) was determined in UPC samples, while the lowest value (2 μm) was determined in PUS samples. Volume-weighted mean diameter $D_{4,3}$ values showed similar results with average particle size values $D_{0.5}$.

In the statistical comparisons between the samples (0 and 180 days), the $D_{3,2}$ values at the start of the storage ranged between 1.7 and 9.6 (p<0.05)., while the PUS sample was with the lowest $D_{3,2}$ value. In contrast, UPUS samples had the

lowest $D_{4,3}$ and $D_{0.5}$ values. When the particle size values of the samples were compared at the end of storage, the lowest $D_{3,2}$, $D_{4,3}$, and $D_{0.5}$ values ($p<0.05$) were detected in the PUS sample, and the highest $D_{3,2}$ value was detected in UPC samples, The $D_{4,3}$ and $D_{0.5}$ value were found highest in PC samples (Indicated with a capital letter at 0 and 180 days in Table 4.4).

During storage, an increase in the $D_{4,3}$ and $D_{0.5}$ values was observed. These results indicated that aggregations were formed, leading to the increase in the particle sizes during the storage period, which is in agreement with Mu et al. (2022), who observed the same results for soymilk after the ultrasound treatment at 20 kHz at 400 W for 0 to 9 min.

As seen from Table 4.4, the values of $D_{3,2}$, $D_{4,3}$, and $D_{0.5}$ for ultrasonicated samples were lower than the control samples, indicating that ultrasound treatment reduces the $D_{3,2}$ and $D_{4,3}$ values of walnut beverages. The possible explanations include ultrasound's contribution to oil droplet disruption by acoustic cavitation, microstreaming, and turbulent forces. This study's results agree with Tiwari et al. (2009), who found that the sonication of orange juice caused a substantial shift in the particle size distribution due to the cavitation effect. In another study, a substantial reduction in particle size of coconut milk was noticed following the ultrasonic treatment at 20 kHz for 13 min (Lu et al., 2019).

4.15. Fatty Acid Composition of Walnut Beverages

Table 4.5 shows the fatty acid composition of walnut beverages as determined by capillary column GC. Over 70% of walnut kernels are lipids, recognized for their high polyunsaturated fatty acid content (PUFA). Linoleic acid (49.3–62.3g/100g), oleic acid (13.8–33.0g/100g), and -linolenic acid (8.0–15.4g/100g) are the predominant fatty acids in walnut (Savage, 2001). However, in the present study, the major fatty acids studied were palmitic acid (PA) and stearic acid (ST) in saturated fatty acids (SFAs), oleic acid (OL) in monounsaturated fatty

acids (MUFAs); linolenic acid (LL) and linoleic acid (LN) in polyunsaturated fatty acids (PUFAs).

Linoleic acid was the most abundant fatty acid in PC and UPC samples (64.65% and 63.12%, respectively). In contrast, palmitic acid was the major fatty acid in PUS samples (7.65%). The PC samples were also determined to be higher in oleic (14.58%). Linolenic acid and stearic acid were found abundant in PUS samples (15.81% and 3.02, respectively) (Appendix 2). The differences between the values of palmitic acid for PUS, UPC, and UPUS samples were significantly different ($p<0.05$) during storage.

In the Statistical comparisons between the 0 and 180-days samples, the differences between the fatty acid values of the samples at the beginning of storage (0 days) and the end of storage (180 days) were found to be insignificant. However, at the beginning of the storage, the oleic acid value was higher in the PUS sample. In contrast, at the end of the storage, it was higher in PC samples (indicated with a capital letter at 0 and 180 days in Table 4.5).

Table 4.5. Fatty acid composition of walnut beverages (%)

Sample	Storage (Day)	Palmitic acid C16:0	Stearic acid C18:0	Oleic acid C18:1	Linoleic acid C18:2	Linolenic acid C18:3
PC	Day 0	5.84±0.55$^{b\ C}$	1.83±0.49$^{a\ A}$	14.05±0.34$^{a\ A}$	62.07±0.44$^{b\ A}$	15.12±0.19$^{a\ A}$
	Day 45	7.15±0.34^{a}	2.51±0.63^{a}	13.58±0.55^{a}	62.34±0.98^{b}	14.67±0.38^{bc}
	Day 90	6.75±0.13^{a}	2.92±0.18^{a}	13.61±0.40^{a}	61.38±0.24^{b}	14.94±0.10^{ab}
	Day 135	6.60±0.05^{a}	2.82±0.07^{a}	13.22±0.02^{a}	61.59±0.17^{b}	14.83±0.09^{bc}
	Day 180	6.67±0.61$^{a\ A}$	2.56±0.37$^{a\ A}$	14.58±1.49$^{a\ A}$	64.65±1.50$^{a\ A}$	14.47±0.20$^{c\ A}$
PUS	Day 0	6.07±0.66$^{c\ A}$	2.87±0.04$^{a\ A}$	12.34±0.36$^{a\ B}$	63.10±0.60$^{a\ A}$	15.34±0.46$^{ab\ A}$
	Day 45	7.65±0.30^{a}	2.92±0.27^{a}	12.76±0.73^{a}	61.41±0.26^{a}	14.34±0.37^{b}
	Day 90	6.64±0.04^{bc}	2.98±0.15^{a}	13.21±0.19^{a}	61.76±0.38^{a}	14.40±0.44^{b}
	Day 135	6.60±0.07^{bc}	2.94±0.05^{a}	12.64±0.04^{a}	61.92±0.13^{a}	14.68±0.04^{ab}
	Day 180	6.75±0.62$^{b\ A}$	2.98±0.03$^{a\ A}$	13.36±1.89$^{a\ A}$	61.97±2.08$^{a\ A}$	15.81±1.46$^{a\ A}$
UPC	Day 0	6.00±0.56$^{c\ AB}$	2.46±0.26$^{b\ A}$	13.75±0.26$^{a\ A}$	62.21±0.56$^{ab\ A}$	15.20±0.33$^{a\ A}$
	Day 45	7.36±0.19^{a}	3.02±0.10^{a}	13.22±0.22^{a}	61.63±0.29^{b}	14.41±0.04^{c}
	Day 90	6.75±0.22^{b}	2.95±0.12^{a}	13.17±0.78^{a}	62.04±0.41^{ab}	14.81±0.08^{b}
	Day 135	6.63±0.08^{b}	2.96±0.05^{a}	13.36±0.14^{a}	60.96±0.42^{b}	14.66±0.25^{bc}
	Day 180	6.58±0.34$^{b\ A}$	2.93±0.12$^{a\ A}$	13.47±1.08$^{a\ A}$	63.12±1.02$^{a\ A}$	14.88±0.13$^{b\ A}$
UPUS	Day 0	5.96±0.47$^{c\ B}$	2.36±0.26$^{a\ A}$	14.11±0.40$^{a\ A}$	62.33±0.72$^{ab\ A}$	15.07±0.25$^{a\ A}$
	Day 45	7.10±0.38^{a}	2.93±0.03^{a}	13.41±0.07^{b}	60.71±0.37^{c}	14.52±0.44^{a}
	Day 90	6.64±0.06^{b}	2.86±0.08^{a}	13.96±0.17^{a}	61.02±0.23^{bc}	14.67±0.17^{a}
	Day 135	6.66±0.05^{b}	2.80±0.08^{a}	13.31±0.16^{b}	61.06±0.48^{bc}	14.58±0.15^{a}
	Day 180	6.88±0.08$^{ab\ A}$	2.33±0.94$^{a\ A}$	13.24±0.60$^{b\ A}$	63.00±1.61$^{a\ A}$	14.55±0.40$^{a\ A}$

Means ± standard deviation with lowercase letters on the same line differed by Duncan test (p<0.05). The uppercase letters indicate the difference among the samples of 0 days and 180 days (p<0.05).

The fatty acid profile of walnut beverages found in this study was evenly distributed in the kernel and pellicle since no difference was determined between them. Furthermore, the fatty acid profile of walnut beverages was nearly comparable in magnitude with the previously reported data (Eliseeva et al., 2016; Rabrenovic et al., 2011; Savage, 2001; Vidrih et al., 2012). However, the fatty acid profile in this study was found to be relatively (24.12%), higher in palmitic acid value (5.78%), stearic acid value (1.56%), linoleic acid value (24.12%) reported by Manzoor et al. (2020) for untreated almond milk. The difference in fatty acid profile may be attributed to the difference in cultivar, geographical location, cultural practices, and maturity stage (Akca et al., 2005).

4.16. Phenolic Compounds of Walnut Beverages

One of the most prevalent families of phytochemicals is phenolic compounds. They have been divided into the following subclasses: tannins, phenolic acids, flavonoids, stilbenes, and coumarins. Due to their antioxidant, anti-carcinogenic, antibacterial, anti-inflammatory, anti-aging, antifungal, and neuroprotective properties, phenolic compounds are significant for human health. (R et al., 2001; Voigt, 1993).

The chemical structure of phenolic chemicals isolated from walnut beverages was investigated using HPLC (Shimadzu LC-20AT, Japan). The phenolic compounds studied in this study include the most commonly reported phenolic compounds present in walnut, ellagic acid, and gallic acid (Kafkas et al., 2020; Liu et al., 2019) (Appendix 3).

Changes in the phenolic compound of walnut beverages are given in Table 4.6. Based on the average quantitative results from this study, ellagic acid ($C_{14}H_6O_8$, 38.74 - 274.23 mg/L) and gallic acid ($C_7H_6O_5$, 2.72 - 201.15 mg/L) were significantly ($p<0.05$) the higher phenolic compound values found in all the walnut beverage samples (chromatograms are given in Appendix 3). As seen in Table 4.6,

an increase was observed in these two phenolic compounds in all samples during the storage However, a decrease was seen in PUS samples at the end of storage.

Table 4.6. Phenolic compounds of walnut beverages (mg/L)

Sample	Storage (Day)	Gallic Acid	Ellagic Acid
PC	Day 0	$3.62 \pm 0.19^{a\ C}$	$38.74 \pm 2.30^{a\ C}$
	Day 45	4.37 ± 1.06^{a}	40.39 ± 0.40^{a}
	Day 90	4.58 ± 0.30^{a}	41.03 ± 0.33^{a}
	Day 135	3.49 ± 0.77^{a}	48.72 ± 10.91^{a}
	Day 180	$3.81 \pm 0.49^{a\ B}$	$45.31 \pm 2.46^{a\ B}$
PUS	Day 0	$5.34 \pm 1.57^{ab\ C}$	$44.35 \pm 1.46^{ab\ C}$
	Day 45	6.65 ± 0.53^{a}	52.73 ± 3.86^{ab}
	Day 90	6.34 ± 0.71^{a}	60.41 ± 2.63^{a}
	Day 135	2.72 ± 0.80^{c}	39.91 ± 10.37^{b}
	Day 180	$3.84 \pm 0.25^{bc\ B}$	$47.22 \pm 3.70^{ab\ B}$
UPC	Day 0	$145.87 \pm 10.72^{b\ A}$	$199.26 \pm 13.85^{b\ A}$
	Day 45	156.04 ± 34.14^{b}	172.45 ± 71.52^{c}
	Day 90	201.16 ± 13.96^{a}	274.23 ± 17.21^{a}
	Day 135	149.43 ± 20.56^{b}	236.50 ± 16.75^{ab}
	Day 180	$167.59 \pm 23.69^{ab\ A}$	$245.23 \pm 29.90^{a\ A}$
UPUS	Day 0	$108.67 \pm 19.27^{b\ B}$	$166.82 \pm 13.23^{b\ B}$
	Day 45	150.02 ± 21.42^{a}	142.28 ± 44.15^{b}
	Day 90	168.20 ± 2.48^{a}	262.60 ± 12.26^{a}
	Day 135	141.15 ± 8.15^{a}	231.38 ± 17.73^{a}
	Day 180	$167.84 \pm 18.33^{a\ A}$	$259.96 \pm 11.05^{a\ A}$

Means ± standard deviation with lowercase letters on the same line differed by Duncan test ($p<0.05$). The uppercase letters indicate the difference among the samples of 0 days and 180 days ($p<0.05$).

Gallic acid values of PC samples, as explained in Table 4.6, ranged from 3.62 to 4.58 mg/L, while the ellagic acid values ranged from 38.74 to 52.87 mg/L. For PUS samples, gallic acid values ranged from 2.72 to 6.65 mg/L ($p<0.05$). While the ellagic acid values ranged from 39.91 to 60.41 mg/L, the difference observed were insignificant.

Gallic acid values of UPC samples ranged from 145.87 to 201.16 mg/L. In comparison, the ellagic acid values ranged from 172.45 to 274.23 mg/L ($p<0.05$). For UPUS samples, gallic acid values ranged from 108.67 to 168.20 mg/L ($p<0.05$). At the same time, the ellagic acid values ranged from 142.28 to 265.31 mg/L ($p<0.05$).

Statistical comparisons between the samples were made at 0 and 180 days. The differences between the phenolic compounds of the samples at the beginning of storage (0 days) and end of storage (180 days) were found to be significant (indicated with a capital letter at 0 and 180 days in Table 4.6). The lowest value of gallic acid and ellagic acid at the beginning and end of the storage was observed in PC samples. Furthermore, an increasing trend was observed for ellagic acid and gallic acid in the walnut beverage samples. The ultrasound treatment enhanced the retention of phenolic compounds at the end of the storage.

Results showed a forty-fold and five-fold high value of gallic acid and ellagic acid in unpeeled walnut beverage samples than in peeled samples, respectively, which can be attributed to the walnut pellicle present in unpeeled walnut beverage samples. The pellicle is the main source of phenolic compounds in walnuts despite comprising just about 5-8% of the entire walnut kernel (Wang et al., 2022). The results agree with Zhang et al. (2020), who found the level of ellagic acid higher in the walnut pellicle.

As seen in Table 4.6, an increase in gallic acid and ellagic acid values was observed at 90 days of storage. According to Escarpa and González, (2001), phenolic chemicals can be detected either in a soluble state in the vacuole or bound to the traces of pectin, cellulose, hemicellulose, and lignin that are present in the

cell wall. The increase in gallic and ellagic acid can be explained by the release of these compounds from the cell wall due to the collapse caused by homogenization with Ultra Turrax. This process aims to extract compounds because of the high speed at which mass is transferred. It is convinced that Ultra-Turrax could pulverize plant materials by rapidly rotating blades (Xu et al., 2016). However, the increase was higher in the ultrasonicated sample. This is because cavitation causes the release of phenolic compounds from the vacuole and the cell wall. (de Souza Carvalho et al., 2020). Possible hydroxyl radical attachment to the aromatic ring of phenolic compounds during sonochemical events may also account for the observed enhancement. Moreover, it has been said that the phenolic compounds can be enhanced by adding a second hydroxyl group in the ortho, meta, or para positions. (Tomadoni et al., 2017). Later, the values declined, which can be explained by the storage effect.

Under ultrasound treatment, a similar rise in phenolic compounds was observed in carrot and orange juices (Jabbar et al., 2014; Khandpur and Gogate, 2015). The degradation of phenolic compounds in the course of storage has been reported by Christopoulos and Tsantili (2011).

4.17. Sugar Compounds of Walnut Beverages

The walnut beverage sugar composition is vital for good flavor and taste and consists of mainly sucrose, glucose, and fructose (Appendix 4). sugar composition consisted mainly of sucrose and raffinose and lower amounts of fructose, glucose, and galactose, and they are very valuable for good flavor and taste sugar composition consisted mainly of sucrose and raffinose and lower amounts of fructose, glucose, and galactose. They are very valuable for good flavor and taste.

The sugar contents of walnut beverages are given in Table 4.7. According to the analysis, the sucrose contents in the PC samples ranged between 3.53 and 42.10 mg/100mL, the glucose contents ranged between 0.88 and 3.45 mg/100mL,

and the fructose contents ranged between 1.98 and 4.95 mg/100mL. On the other hand, the sucrose contents in the PUS samples ranged between 9.84 and 110.15 mg/100mL, the glucose contents ranged between 0.42 and 6.23 mg/100mL, and the fructose contents ranged between 2.83 and 9.60 mg/100mL. The difference in sugar content values was statistically significant in all the samples ($p<0.05$).

Table 4.7. Sugar compounds of walnut beverages (mg/100mL)

Sample	Storage (Day)	Sucrose	Glucose	Fructose
PC	Day 0	$3.53 \pm 1.40^{d\ C}$	$0.88 \pm 0.42^{b\ B}$	$1.98 \pm 0.62^{c\ C}$
	Day 45	42.10 ± 6.28^{a}	2.68 ± 0.88^{a}	3.32 ± 0.58^{bc}
	Day 90	36.33 ± 2.06^{ab}	2.67 ± 0.34^{a}	2.77 ± 0.43^{c}
	Day 135	27.41 ± 6.13^{c}	3.45 ± 1.09^{a}	4.25 ± 1.34^{ab}
	Day 180	$31.00 \pm 0.74^{bc\ D}$	$2.53 \pm 0.20^{a\ B}$	$4.95 \pm 0.18^{a\ C}$
PUS	Day 0	$9.84 \pm 2.58^{c\ C}$	$0.42 \pm 0.21^{c\ B}$	$2.83 \pm 0.03^{d\ C}$
	Day 45	110.15 ± 1.63^{a}	6.03 ± 0.26^{a}	7.27 ± 0.40^{b}
	Day 90	90.05 ± 6.62^{b}	5.75 ± 0.09^{ab}	6.07 ± 0.57^{c}
	Day 135	88.71 ± 13.67^{b}	6.23 ± 0.94^{a}	7.36 ± 0.85^{b}
	Day 180	$86.83 \pm 4.76^{b\ C}$	$5.02 \pm 0.06^{b\ B}$	$9.60 \pm 0.48^{a\ C}$
UPC	Day 0	$61.23 \pm 0.25^{b\ B}$	$6.92 \pm 2.45^{c\ AB}$	$5.03 \pm 0.35^{d\ B}$
	Day 45	164.77 ± 23.50^{a}	32.47 ± 10.59^{b}	34.95 ± 11.48^{c}
	Day 90	168.28 ± 32.43^{a}	64.98 ± 9.44^{a}	78.78 ± 24.80^{b}
	Day 135	163.53 ± 16.09^{a}	81.14 ± 11.59^{a}	106.78 ± 15.10^{a}
	Day 180	$33.20 \pm 27.68^{a\ B}$	$70.78 \pm 17.78^{a\ A}$	$41.37 \pm 5.35^{c\ B}$
UPUS	Day 0	$246.00 \pm 34.66^{a\ A}$	$12.05 \pm 7.82^{c\ A}$	$7.80 \pm 1.87^{c\ A}$
	Day 45	295.22 ± 13.46^{a}	78.88 ± 10.68^{a}	74.30 ± 10.42^{ab}
	Day 90	298.38 ± 8.37^{a}	68.10 ± 9.70^{ab}	62.30 ± 8.24^{b}
	Day 135	245.18 ± 46.71^{a}	58.10 ± 17.35^{ab}	83.38 ± 11.36^{a}
	Day 180	$295.68 \pm 0.75^{a\ A}$	$54.20 \pm 11.73^{b\ A}$	$80.57 \pm 8.76^{a\ A}$

Means ± standard deviation with lowercase letters on the same line differed by Duncan test ($p<0.05$). The uppercase letters indicate the difference among the samples of 0 days and 180 days ($p<0.05$).

The sucrose contents in the UPC samples ranged between 61.23 and 168.28 mg/100mL, the glucose contents ranged between 6.92 and 81.14 mg/100mL, and the fructose contents ranged between 5.03 and 106.78 mg/100mL. On the other hand, the sucrose contents in the PUS samples ranged between 245.18 and 298.38 mg/100mL, the glucose contents ranged between 12.05 and 78.88 mg/100mL, and the fructose contents ranged between 7.80 and 83.38 mg/100mL. This difference was found to be significant ($p<0.05$).

As can be observed from Table 4.7, the sugar contents of unpeeled walnut beverage samples were higher as compared to peeled walnut beverage samples; this is due to the presence of pellicle, which contains abundant sugars. In addition, this study found that fructose, glucose, and sucrose levels were significantly ($p<0.05$) higher in almost all of the ultrasonicated samples than in the control samples. One possible explanation for the observed elevation in sugar concentration is acid hydrolysis.

On the other hand, the ultrasonication process may have contributed to the increase in sample volume by causing cell rupture and releasing carbohydrates from within the cell into the medium. Furthermore, ultrasound's mechanical effects, in the form of shear forces, increase the penetration power of the solvent into the sample matrix, facilitating a more rapid diffusion rate of the solute from the material to the solvent. (Rostagno et al., 2003). Therefore, the results of this study agree with those of Lieu and Le (2010), who reported that the ultrasonic treatment improved the extraction of sugars from grape juice. Also, the results obtained in this study for sucrose (3.53-298.38 mg/100mL), glucose (0.88 – 81.14 mg/100mL), and fructose (1.98 – 106.78 mg/100mL) were higher than the results obtained in oat milk with 0.1g/100g sucrose, 1.23g/100g glucose, and less than 0.1 g/100g fructose (Smith et al., 2022).

5. CONCLUSION

The present study was conducted by producing peeled and unpeeled walnut beverages to investigate the effects of ultrasound for 10 min at 25 kHz on various quality parameters of walnut beverages over 6 months. In addition, Walnut beverages that were sterilized and bottled were analyzed for physico-chemical properties (proximate composition, rheological behavior, color measurement, particle size, sensory evaluation), fatty acid profile characterization, total phenolic content, phenolic compounds, antioxidant activities (FRAP assay and DPPH radical scavenging activity). The conclusion of the findings of the present investigation is outlined as follows:

According to the findings of the changes during the storage period and the effect of ultrasound on the walnut beverages

- During storage, particle size, viscosity, ΔE* value, total solids, L* value, a* value, whiteness index, and titration acidity increased. On the contrary. b* value, C* value, antioxidant activity, total phenolic content, decreased; ash, total solids, pH, and fatty acids showed statistically minute significant changes.
- Changes in total solids values (12.80-17.17%) before storage were statistically significant, and an increase occurred with storage (12.97-17.18%). Ultrasound showed no effect on the total solids. At the beginning of storage, the ash values of the samples varied between 0.14% and 0.30%, and at the end of the storage varied between 0.19% and 0.30 %.
- During storage, the difference between the pH (6.09-6.99) and titration acidity (0.05-0.08 %) values at the 0 days of storage and the pH (6.36-6.95) and titration acidity (0.05- 0.16%) values measured at the end of the storage (180 days). Differences were found to be significant. Furthermore,

no effect of ultrasound was seen on the pH and acidity of the walnut beverages.

- At the end of storage, the viscosity of walnut beverage samples (PC, PUS, UPC, UPUS) compared to 0 days increased by 2.3, 1.7, 3.2, and 1.7-fold, respectively. The highest viscosity was observed in unpeeled walnut beverage samples. Ultrasound showed a positive effect on the viscosity of walnut beverages. Also, the viscosity of ultrasonicated samples was lower than the control samples
- When the color changes in the walnut beverage samples were examined during storage, L*, a* whiteness index, and ΔE* values increased. In contrast, hue, b*, and C* values decreased. Changes during storage were found to be statistically significant, with the lowest L* value on the 0 days in the UPC sample (34.1), the lowest a* value on day 45 in PUS (0.2) samples, the lowest b* value in the day 180 in PC sample (8.6), and the lowest C* value in the day 180 in PC sample (8.6). The increase observed with storage indicates that the redness in the color of the product increases with storage. When the color values of the ultrasonicated samples were examined at the end of storage, the L* and a* values in the samples were higher than in the control samples.
- High whiteness index and L* values in peeled walnut beverages are considered desirable from the consumer's point of view.
- L* value is a good indicator in determining the color of the walnut beverages, which increased remarkably with the course of storage. As the exposure time of storage duration increased, there was a significant increase in the L* value and whitening index of all the PC, PUS, and UPC. However, an insignificant decrease was observed in UPUS samples at day 180. No vivid effect of ultrasound was seen on L* value and whiteness index.

- During storage, a gradual decreasing tendency was determined in the total phenolic content of PC and PUS samples which decreased by 18.34% and 22.78%, respectively, at the end of the storage. However, the total phenolic content of UPC and UPUS samples showed the highest increase by 9.17% and 34.95%, respectively, on 45 days of the storage and later decreased at the end of the storage. The total phenolic content of unpeeled walnut beverages was notably higher (3 folds) than peeled walnut beverages. Also, phenolic content was higher in ultrasonicated samples at the beginning of the storage. The notable increase in TPC could be valuable as a potential antioxidant for consumers' health. However, the levels of total phenolic content decreased progressively with advanced storage time.
- DPPH values of the walnut beverages followed the same trend as total phenolic content. At the end of the storage, a gradual decrease was observed in PC and PUS samples by 42.61% and 61.12%. However, an increase was observed in the first 45 days of storage in UPC and UPUS samples by 7.35% and 7.20%, respectively, on 45 days of storage and decreased later. At the end of storage, the FRAP value for PC and PUS decreased gradually by 18.34% and 24.78%, respectively, compared to the beginning of the storage. However, an increase was observed in the first 45 days of storage in UPC and UPUS samples by 41.20% and 71.46%, respectively, on 45 days of storage and later decreased during storage.

  Antioxidant activity in terms of FRAP values was highest in UPUS samples, and as storage progressed, it decreased. The antioxidant activity of unpeeled walnut beverage samples was 72.2 folds higher than peeled walnut beverage samples. At the same time, DPPH showed the same trend as FRAP, with the highest value in UPUS samples.
- The value of $D_{3,2}$ for PC, PUS, UPC, and UPUS samples, compared to 0 days, decreased by 183.60 %, 178.80%, 94.17%, and 158.05 %, respectively, at the end of storage. On the other hand, the $D_{4,3\ and}$ $D_{0.5}$ value

showed an increase in PC, UPC, and UPUS samples by 41.23%, 27.49%, 85.76 %, and 67.86%, 18.20%, 66.14 %, respectively, with a decrease in PUS samples by 15.42% and 69.76% respectively.

- Particle size showed a gradual increase in $D_{3,2}$ in control samples, whereas, in ultrasonicated samples, a non-uniform pattern was observed. The PC samples' $D_{4,3}$ value (198.67 μm) was the largest, while the PUS samples showed the lowest $D_{4,3}$ value (96 μm). Also, the highest $D_{3,2}$ value (15.53 μm) was determined in UPC samples, while the lowest value (2 μm) was determined in PUS Samples. Volume-weighted mean diameter $D_{4,3}$ values showed similar results to average particle size values $D_{0.5}$.
- During storage, a moderate to medium surge in the contents of fatty acids was noticed. The highest increase was observed for stearic acid in PC and UPC samples by 40.17 % and 19.03 %, respectively, at the end of the storage. Furthermore, at the end of the storage, palmitic acid showed some increase in PC, PUS, UPC, and UPUS samples by 14.18%, 11.28%, 9.69%, and 13.39%, respectively. In addition, some saturated fatty acids showed a slight increase during storage. For example, linoleic acid increased from 62.07% before storage to 64.65% after six months in untreated peeled walnut beverage samples and from 62.21% to 63.12% in UPC samples.
- Similarly, palmitic acid increased from 5.96% before storage to 6.88% after six months in the UPUS samples. The major unsaturated fatty acid was linoleic acid (60.71 – 64.65%), being highest in PC samples and the minimum in UPUS samples. The PUS samples contained the highest (15.81%) linolenic acid. Oleic acid was found to be highest in PC samples.
- Ellagic acid ($C_{14}H_6O_8$) and gallic acid ($C_7H_6O_5$) were the most abundant phenolic compounds in all the walnut beverage samples. At the end of storage, compared to 0 days, an increase was observed in ellagic acid; in PC, PUS, UPC, and UPUS samples by 16.95%, 6.48%, 23.07%, and 55.83%, respectively. Similarly, an increase was observed in PC, UPC,

and UPUS samples in gallic acid by 5.40%, 14.89%, and 54.44%, respectively. A decrease in gallic acid in PUS samples was determined by 28.13%. While the total phenolic content of walnut beverages was between 3.62 to 199.26 mg/L at the beginning of storage, they showed significant increases. They decreased at the end of the storage. However, the differences were found to be insignificant. The most common phenolic components identified in walnut beverages are gallic acid and ellagic acid. There was an increase in the phenolic compounds at the beginning of storage. The contents of gallic and ellagic acid at the beginning and end of storage were the lowest in PC samples. The application of ultrasound increased the gallic and ellagic acid content of the walnut beverage, as shown in Table 4.6. As a result, from an industrial standpoint, ultrasound may be preferable to heat treatment for beverage processing since it enables more preservation of these compounds and higher antioxidant activity, which tends to rise with sonication time.

- The most abundant basic sugar component of walnut beverages was determined as sucrose, followed by fructose and glucose, which showed an increasing behavior at the end of storage compared to 0 days of storage. In PC samples, an increase was seen in sucrose, glucose, and fructose by 8.77, 2.87, and 2.50-fold, in PUS samples by 8.83, 12.04, 3.39-fold, in UPC samples by 2.18, 10.23,8.22-fold and in UPUS samples by 1.20, 4.50, 10.33-fold respectively. Also, the ultrasound showed a positive effect on the sugars.
- Nutritional analysis of walnut beverages revealed that UPUS contained 2.93 and 12.63 percent crude protein and fat, respectively, higher than the control samples. Also, dietary fiber was found to be the highest in UPC samples. In addition, total solids and ash content were highest in UPC samples. Thus, ultrasound showed a positive effect on some nutritional parameters.

- Among the determined minerals, walnut beverages were found to be richer in terms of Magnesium (Mg), Calcium (Ca), Potassium (K) contents, and Iron (Fe). In contrast, sodium (Na), copper (Cu), and zinc (Zn) were found in small traces. The highest magnesium (Mg) content was observed in the UPC sample at 95.92 mg/L, while the lowest was observed in the PUS sample at 70.80 mg/L. In walnut beverages, Na, Fe, Zn, and Cu were found in trace amounts. While the UPUS sample has the highest Ca content with 83.88 mg/L, the PC sample has the lowest Ca content with 71.90 mg/L.
- The sensory evaluation of the walnut beverages produced was carried out at the beginning of the storage by 11 panelists. The PC samples were more appreciated, and the most liked sample, with a score of 7.77.

In this study on the production of walnut beverages, a product with high nutritional value was obtained by processing walnut kernels into a product with added value. According to the findings of this research, sonication treatment significantly improved the phenolic compound, total phenolic content, DPPH free radical scavenging activity, FRAP, and particle size of walnut beverages without having any significant effect on the physicochemical parameters (pH, ash, titratable acidity). Furthermore, the total phenolic content and antioxidant activity of the unpeeled walnut beverage were found to be the highest, making it the ideal choice from a commercial perspective. In conclusion, the results indicate that sonication technology could be successfully applied to the production of walnut beverages with better health safety and quality for the consumer.

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APPENDIX

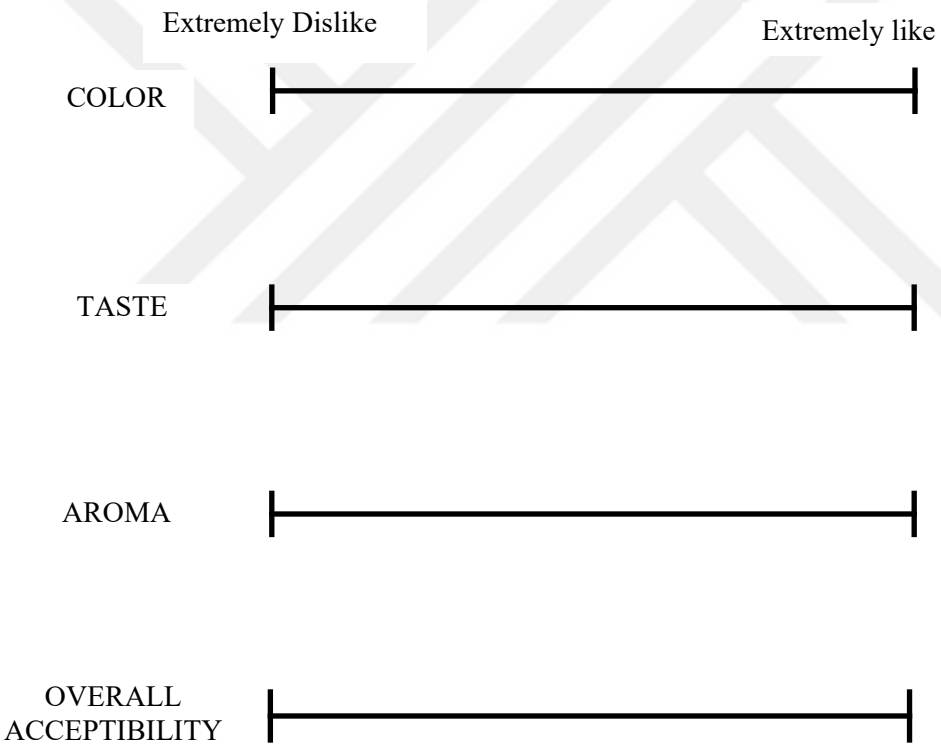
Appendix-1

SENSORY EVALUATION FORM

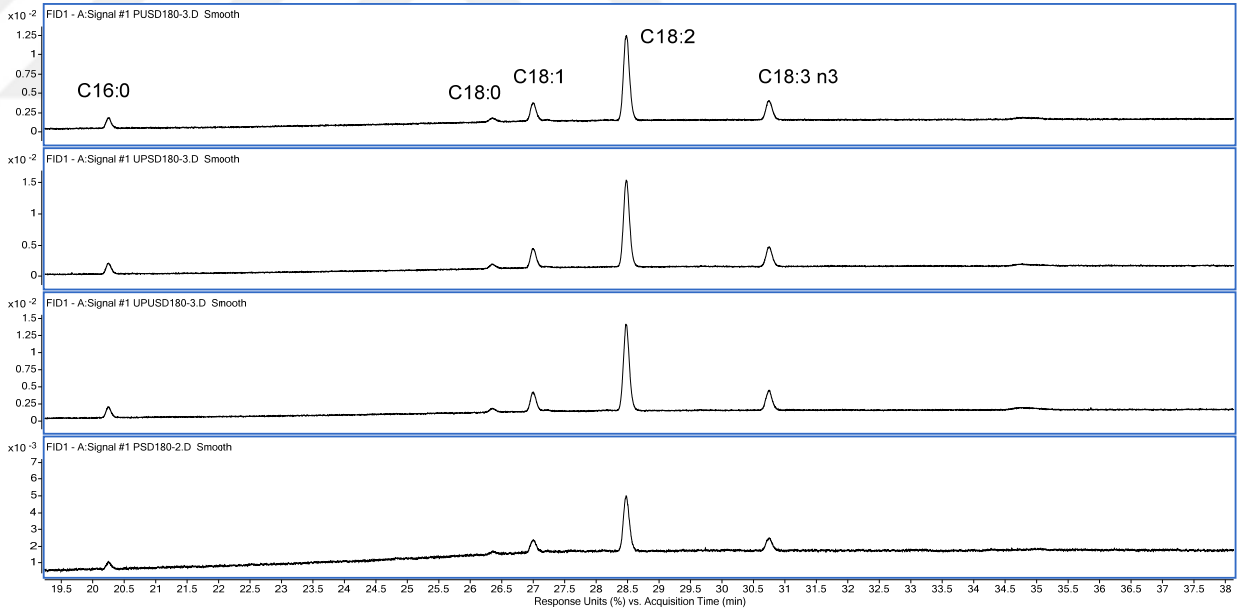
Name: **Date:**

In the scales given below, all characteristics increase from left to right. Evaluate the walnut beverage characteristics by placing an "X" in the appropriate place on the scale.

Sample Code:

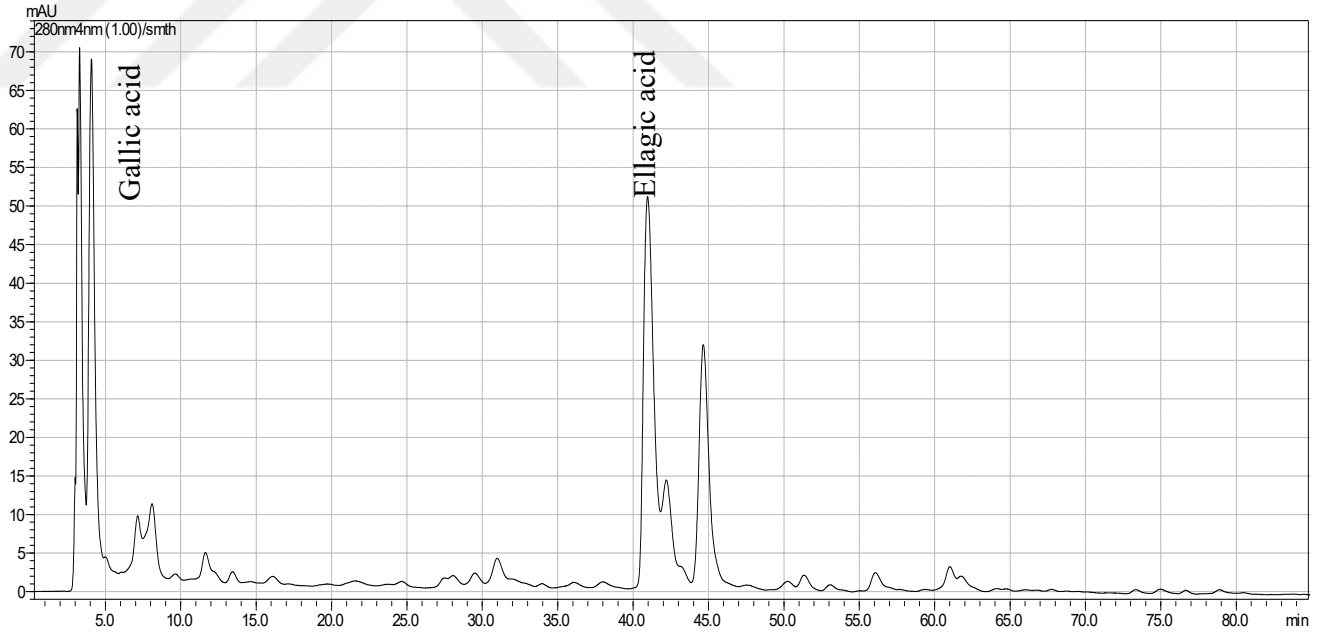


Appendix 2



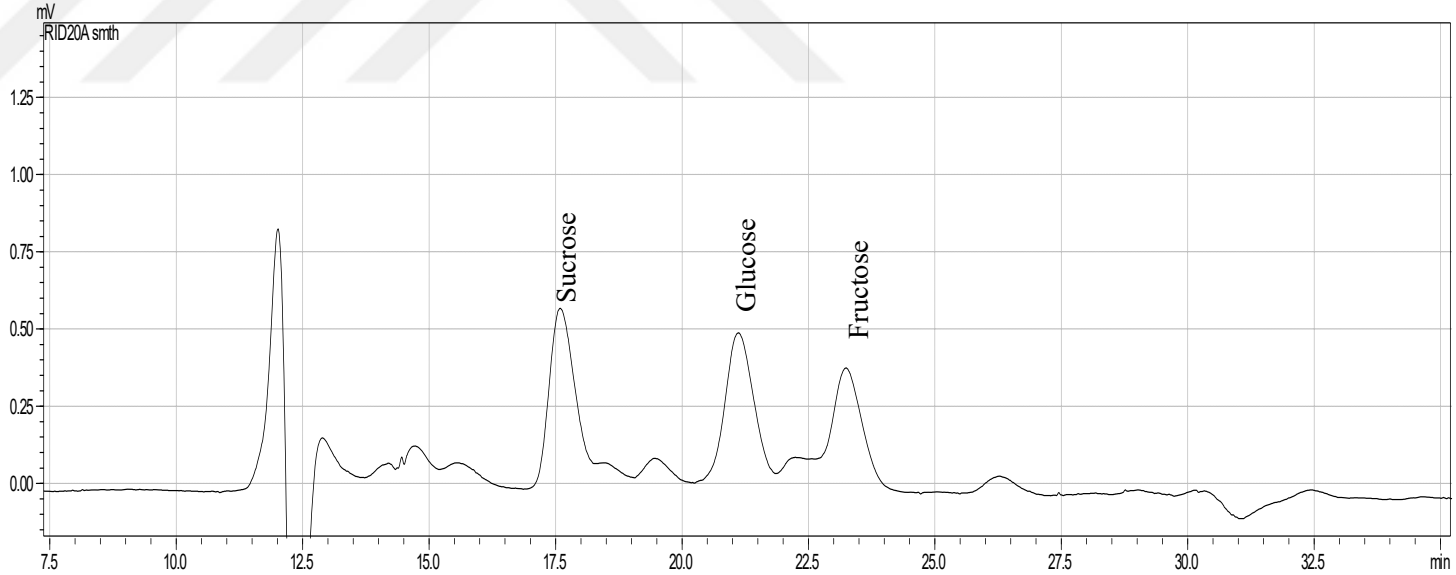
Appendix 2 GC- Chromatograms of Fatty Acids in Walnut Beverages (C18:0 Stearic acid, C18:1Oleic acid, C18:2 Linoleic acid, C18:3 Linolenic acid)

Appendix-3



Appendix 3. HPLC chromatograms of phenolic compounds in walnut beverages at 280 nm

Appendix-4



Appendix 4. HPLC chromatogram of sugar compounds