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

Research / Araştırma
GIDA (2024) 49 (3) 580-593
doi: 10.15237/gida.GD24039

GREEN EXTRACTION OF CAROTENOIDS FROM LEMON PEELS

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Received/Geliş: 31.03.2024; *Accepted*/Kabul: 20.05.2024; *Published online*/Online baskı: 05.06.2024

Meral, H., Demirdöven, A. (2024). Green extraction of carotenoids from lemon peels. GIDA (2024) 49 (3) 580-593 doi: 10.15237/ gida.GD24039

Meral, H., Demirdöven, A. (2024). Limon kabuklarından karotenoidlerin yeşil ekstraksiyonu. GIDA (2024) 49 (3) 580-593 doi: 10.15237/ gida.GD24039

ABSTRACT
Nowadays, there is a growing interest in fully utilizing agro-industrial wastes, with carotenoids gaining attention as valuable coloring agents. One of the potential sources for carotenoid extraction is lemon peel. The purpose of this study was to determine optimal extraction techniques for extracting as much carotenoids as possible from lemon peel. In this context, a comparison was conducted among extracts obtained via conventional, ultrasound-assisted (UAE), and ultrasound-assisted enzymatic (UAEE) extraction methods. The highest carotenoid content (0.792±0.01 mg/L) was achieved with UAEE, while the lowest (0.493±0.01 mg/L) was obtained conventionally. UAEE exhibited the highest antioxidant activity values among three methods: 753.80±5.79 mg TE/L (ABTS), 624.64±10.52 mg TE/L (DPPH), and 186.64±1.66 µmol TE/L (FRAP). In conclusion, UAEE showed promise in extracting carotenoids from lemon peel. Thus, by carotenoid extraction using green technology from waste lemon peels, with higher added value, richer in terms of phenolic composition and antioxidant properties, has been obtained.
Keywords: Ultrasound, enzyme, extraction, lemon peel

LİMON KABUKLARINDAN KAROTENOİDLERİN YEŞİL EKSTRAKSİYONU

ÖZ
Günümüzde, tarım endüstrisi atıklarının tam olarak kullanımına olan ilgi giderek artmaktadır ve karotenoidler, değerli bir renklendirici ajan olarak dikkat çekmektedir. Karotenoid ekstraksiyonu için potansiyel kaynaklardan biri limon kabuğudur. Bu çalışma, limon kabuğundan maksimum miktarda karotenoid elde etmek için optimal ekstraksiyon prosedürlerini belirlemeyi amaçlamıştır. Bu bağlamda geleneksel, ultrason destekli (UAE) ve ultrason destekli enzimatik ekstraksiyon (UAEE) yöntemleri ile elde edilen ekstraktlar arasında kıyaslama yapılmıştır. En yüksek karotenoid içeriği (0.792±0.01 mg/L) UAEE ile elde edilirken, en düşük içerik (0.493±0.01 mg/L) geleneksel yöntem ile elde edilmiştir. En yüksek toplam fenolik madde miktarı (TPC) UAEE ile elde edilmiştir. Benzer şekilde, UAEE, üç yöntem arasında en yüksek antioksidan aktivite değerlerini sergilemiştir: 753.80±5.79 mg TE/L (ABTS), 624.64±10.52 mg TE/L (DPPH) ve 186.64±1.66 µmol TE/L (FRAP). Sonuç olarak, UAEE, karotenoidlerin ekstraksiyonu için umut vaat etmektedir. Dolayısıyla, atık limon kabuklarından yeşil teknoloji kullanılarak karotenoid ekstraksiyonu ile daha yüksek katma değerli, fenolik bileşim ve antioksidan özellikler açısından daha zengin bir ürün elde edilmiştir.
Anahtar kelimeler: Ultrason, enzim, ekstraksiyon, limon kabuğu

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INTRODUCTION

Agriculture has been important to humanity in every age. The agricultural industry is responsible for generating a wide array of nutrients and enhancing their diversity through processing, fulfilling the nutritional requirements of individuals, and consequently playing a crucial role in the health and advancement of communities. Fresh fruits and vegetables constitute an integral part of agriculture. World fruit production recorded a growth of 63% from 2000 to 2022, with a total production volume in 2022 of 933 million tons. Turkey is the world's fourth-largest producer of fruits (FAO, 2022). In particular, increments became in the production of mandarin by 58.3%, 74.8% in oranges, 75.8% in lemons production in the citrus group of fruits (TUİK, 2023). Turkey is a major producer of citrus fruits: in the case of lemons (*Citrus limon* L.), more than 1.32 million tons were produced in 2023. Lemon (*Citrus limon* L.) is one of the important citrus plants among the fruit groups grown all over the world. Lemons include a variety of bioactive substances that have been linked to health benefits, including carotenoids, phenolic compounds, dietary fiber, essential oils, and vitamins (Benestante et al., 2023). Lemon fruit is used in many food and non-food products that are sold commercially because of its special qualities, which include increasing taste and flavor, supporting health, and having an appearance. Nonetheless, the leftover peel from these non-food and food industries is still a source of valuable bioactive components that can be used in related sectors such as the pharmaceutical, cosmetic, home care, and health care industries (Jagannath and Biradar, 2019). The peels from lemons make up between 50-70 % of the fresh fruit mass (John et al., 2017). It has been stated that the bioactive compounds in peels can help prevent diseases like diabetes, obesity, blood lipid reduction, cardiovascular disease, and some types of cancer (Li et al., 2023). These peels remaining bioactive components can be used as a source of vitamin C, carotenoids, and phenolic compounds, all of which have been shown to have a variety of health-promoting and antioxidant qualities (Magalhães et al., 2023). Lemon peels, which are waste from households, restaurants, and the processing industry, can be distributed free of charge, making them popular sources for extracting phenolic substances, essential oils, and producing natural colorants such as carotenoids (Güzel and Akpınar, 2017; Weldekidan et al., 2024).

Carotenoids are a group of pigments found in many plants, algae, and photosynthetic bacteria. They are responsible for the yellow, orange, and red colors in various fruits, vegetables, and other organisms. Carotenoids, which have an isoprene skeleton, are composed of 40 carbon atoms. In recent years, there has been a growing emphasis on research concerning plant pigments, particularly due to their provitamin A activity, and their recognition as natural antioxidants and bioactive compounds has elevated their significance (Ashokkumar et al., 2023). Fruits and vegetables high in carotenoid antioxidants are recognized to impact on human health. Citrus fruits are a staple in people's everyday diets and contain a considerable amount of carotenoids. This is why many of articles in recent years have focused on studying these fruits (González-Peña et al., 2023).

An important research area with potential implications for the chemical and pharmaceutical industries is the extraction of bioactive compounds from lemon peels. At this point, the conventional approach utilized in the pharmaceutical industry involves extracting bioactive constituents from peels through a solvent-based method, typically using Soxhlet extraction or maceration processes. Conventional extraction methods, which utilize diverse solvents to extract a range of bioactive compounds from natural sources, encounter several drawbacks including excessive solvent usage, extended extraction durations, and suboptimal extraction efficacy (Karne et al., 2023). Researchers have recently proposed several new extraction methods, like supercritical fluid extraction, microwave, enzymatic, and ultrasonic to assess the comparatively large amount of peels produced. One of these new techniques, ultrasound assisted extraction (UAE) is an inexpensive and simple in comparison with

traditional extraction methods. Ultrasound is identified as sound waves with frequencies above the threshold for human hearing (>16 kHz). It means to pressure waves with a frequency of 20 kHz and/or more and in food industry, ultrasound equipment is used in frequencies from 20 kHz to 10 MHz (Demirdoven et al., 2021). The primary mechanism of ultrasound extraction is associated with the cavitation phenomenon, where small bubbles form within a liquid solvent, rapidly expand to a critical size, and subsequently implode (Wang et al., 2015). The utilization of UAE has the potential to increase the value of these bioactive compounds, as it represents an efficient and environmentally friendly process. The method is characterized a more successful extraction and the use of moderate extraction temperatures, which are particularly advantageous for heat-labile chemicals (Junaid et al., 2023). Numerous variables are considered critical during UAE, such as applied ultrasonic power, frequency, extraction temperature, reactor properties, and solvent-sample interaction. It is believed that the majority of bioactive compounds are extracted during the first few minutes of the process (Siddiqui et al., 2023).

Enzymes are regarded as green chemicals and are a flexible type of biocatalyst because of their environmental beneficial. Enzymatic extraction of bioactive substances presents another viable alternative to conventional extraction techniques. Given that pectin constitutes a significant portion of the cell wall in many fruits, including lemon peel, pectinase is employed for this purpose. Pectinase is an enzyme that can be utilized to extract bioactive compounds from a variety of sources and to maximize fruit juice clarity (Chen et al., 2023; Radziejewska-Kubzdela, 2023). The hydrolysis of α-1,4-glycosidic linkages in polygalacturonic acid substance (Tapre and Jain, 2014) or pectic acid is catalyzed by the enzyme pectinase. Pectinase facilitates the breakdown of pectin in cell walls, leading to a more efficient release of β-carotene from chloroplasts. Recent studies have shown that, under specific ultrasonic intensity conditions, low-frequency ultrasonication can enhance the activity of enzyme preparations (Le and Nguyen, 2013; Wu et al., 2014; Wang et al., 2017; Osete-Alcaraz et al., 2019; Shahram et al., 2019; Larsen et al., 2021; Gamage and Choo, 2023). Therefore, ultrasound-assisted enzymatic extraction (UAEE), which employs the synergistic interaction of enzymatic hydrolysis and ultrasound, could fulfil the requirement for extracting carotenoids from lemon peels.

In the existing literature, numerous studies have focused on extracting essential oils and bioactive compounds, such as carotenoids and phenolics, from lemon peel, employing various methodologies. However, to date, no research has been conducted on UAE combined with enzymatic treatment for carotenoid extraction from lemon peel, nor on the synergistic extraction models between ultrasound and enzymatic treatment. Therefore, this study investigates the extraction of carotenoids as color pigments from lemon peel, a by-product of the food industry, utilizing three environmentally friendly methods: ethanol maceration (conventional), ethanol + UAE (combined), and UAEE. The aim is to determine the most effective method for carotenoid extraction, yielding higher quantities and antioxidant activity. As far as we know, no prior research has explored this specific domain, and the findings from this study regarding β-carotene extraction from lemon processing waste could potentially be applicable at an industrial level.

MATERIALS AND METHODS

Reagents and Chemicals

Pectinex Ultra Color (Novozymes-Denmark) enzyme preparate was used for enzymatic extraction. The enzyme preparate having pectin lyase and polygalacturanase activity (7700 PECTU/ml) was stored at +4 °C until the extraction. Folin–Ciocalteau reagent (PubChem CID 516996); sodium carbonate (Na_2CO_3) (PubChem CID 10340); potassium persulfate ($K_2S_2O_8$) (PubChem CID 24412); hexane (C_6H_{14}) (PubChem CID 8058); acetone (C_3H_6O) (PubChem CID 11434908), ethanol (CH_3CH_2OH) (PubChem CID 702) was provided from Merck Chemicals (Darmstadt, Germany). Gallic acid (PubChem CID 370); Iron (III)

chloride (PubChem CID 24380); 2,2- Diphenyl-1-picrylhydrazyl (DPPH) (PubChem CID 2735032); 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (PubChem CID 9570474); 2,4,6-Tripyridyl-*s*-triazine (TPTZ) (PubChem CID 77258); Trolox (PubChem CID 40634) was purchased from Sigma-Aldrich (Steinheim, Germany). All chemicals used in this study were of analytical grade.

Plant Materials

The lemon fruits were provided by a local supermarket (Tokat, Turkey). The lemons were washed with plenty of water to remove possible impurities on the surface. The skins were then peeled by hand, cut into smaller pieces, and then dried in a drying oven (Memmert 100-800, Germany) at 70°C for 8 hours. After being dried and ground into a powder using a laboratory grinder (Sinbo, SCM 2934, Turkey), the samples were stored at room temperature (20±5 °C) until the extraction step.

Extraction Processes

Conventional Extraction

In the extraction process, 1 g of lemon peels was mixed with 20 mL of the extraction solvent (ethanol 75% v/v) in a glass beaker and homogenized with ultra-turrax (IKA T18, Staufen, Germany) at third level speed (approximate 21.000 rpm/min) for three minutes. Then, extraction was carried out using a magnetic stirrer at 300 rpm and at a temperature of 40°C for 60 minutes. Following the extraction, the mixture was centrifuged at 6000 rpm for 10 minutes, and the clear liquid remaining on the sediment was kept at -18°C until it was further examined (Chatzimitakos et al., 2023).

Ultrasound-Assisted Extraction Processes (UAE)

The conventional extraction was combined with ultrasonication to demonstrate the effectiveness of ultrasound treatment. The ultrasound-assisted extraction (UAE) was performed with a laboratory scale ultrasonic bath (365x278x264 mm, WxDxH, Elmasonic S100H, 37 kHz, Singen, Germany). The tank of device (281x222x149 mm, WxDxH) is made of cavitation-resistant stainless steel the volume of 9.50 liters. Total power consumption of the device is 550W and maximum ultrasonic peak performance is 600 W. The temperature of bath can adjust by rotary switch from 30 to 80°C and the change of temperature was controlled continuously with water-resistant digital thermometer with a cable probe during analysis. The lemon peel was mixed with extraction solvent (ethanol solution, 75% v/v) at a solid-to-solvent ratio of 1:20 and homogenized using ultra-turrax (IKA T18, Staufen, Germany) at third level speed (approximate 21.000 rpm/min) for 3 minutes. It was subjected to UAE in the ultrasonic bath and sonicated with frequency of 37 kHz for 60 minutes at 40 °C. After UAE process, the mixture was centrifuged at 6000 rpm for 10 minutes, and the resulting supernatant was stored at −18°C until analysis. The extraction process was carried out by considering similar working conditions in the literature and supported by preliminary experiments (Boukroufa et al., 2017; Chatzimitakos et al., 2023).

Ultrasound-Assisted Enzymatic Extraction Processes (UAEE)

The interaction between enzyme macromolecules and ultrasonic has a major impact on the bioprocess's efficiency (Rokhina et al., 2009; Yun et al., 2023). Therefore, enzymolysis and ultrasonication synergistic models were investigated in this study. UAEE was carried out with a laboratory scale ultrasonic equipment (365x278x264 mm, WxDxH, Elmasonic S100H, 37 kHz, Singen, Germany). The sample (1 gram) was mixed with 7 mL of enzyme (pectinase solution, 10% v/v) and stirred with ultra-turrax (IKA T18, Staufen, Germany) at third level speed for 3 minutes. It was immersed in the ultrasonic bath and sonicated at a constant temperature of 40°C with frequency of 37 kHz for 60 min at constant power (550 W). At the end of extraction, all samples were kept in a water bath at 90°C for three minutes for enzyme inactivation, and then cooled approximately at 25°C. Finally, the samples were centrifuged (Universal 320 R, Tuttlingen, Germany) at 6000 rpm for 10 minutes. The carotenoid extract was obtained as

explained for UAE following the centrifugation and stored at −18°C until further analysis.

Analytical determinations

Total Carotenoid Content

The carotenoid content of the lemon peel extracts was determined by spectrophotometric method developed by Lee and Castle (2001). Firstly, 15 mL extract was mixed with 30 mL extraction solvent (hexane/acetone/ethanol; 50/25/25 v/v) and homogenized with ultra-turrax for 30 seconds. The mixture was centrifuged at 1968×*g* (4000 rpm having 12 × 15 mL tube vessels, EBA 21, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) for 10 min at 5°C. And then samples were measured at 450 nm wavelength. The carotenoid content was calculated in ppm β-carotene according to the formula Eq. (1), considering the molar absorption coefficient ($E^{1\%}$; E_{1cm}=2505).

$$c=\left({}^{a}/_{E}\times b\right)\times 1000 \quad (1)$$

c: unit concentration (w/v)
a: absorbance value
E: molar absorption coefficient, 2505
b: unit optical path length, 1 cm

Antioxidant Properties

Total Phenolic Compounds

The total phenolic compounds (TPC) of the samples were measured by the Folin–Ciocalteau method (Singleton and Rossi, 1965). Approximately 500 µL of sample was mixed with 2 mL of Folin–Ciocalteau reagent (10% v/v). The mixture was stirred with 1 mL of Na_2CO_3 solution (7% v/v) and stored in a light-free environment at 25°C for 30 minutes. Following this incubation period, the mixture was analyzed at a wavelength of 760 nm using a T80+ spectrophotometer (PG Instruments, Leicestershire, United Kingdom). Standard curves were generated based on the concentrations of gallic acid by correlating absorbance values measured at 760 nm. The concentration of samples was determined based on the absorbance values obtained from the standard curve created with different dilutions of gallic acid solutions, and expressed as milligrams of gallic acid equivalent (GAE) per liter of sample.

Antioxidant Activity

The antioxidant activity of the lemon peel extracts was conducted in accordance with 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-Diphenyi-l-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods. For ABTS method, ABTS radical stock solution was first prepared. Equal volumes of 2.45 mM $K_2S_2O_8$ solution and 7 mM ABTS stock solution were combined, and the mixture was then incubated for 16 hours at 25°C in darkness to prepare radical stock solution. Subsequently, the solution was adjusted to an absorbance of 0.700 at a wavelength of 734 nm by mixing 1 mL of this solution with 50 mL of sodium acetate buffer (20 mM sodium acetate, pH 4.5). Then, 100 µL of the extract was added to 2900 µL of the adjusted solution and allowed to stand at room temperature in darkness for 30 minutes. Following incubation, the absorbance of the extracts was measured at 734 nm. The obtained data were then calculated as milligrams of Trolox equivalents (TE) per liter using a standard curve (Pająk et al., 2019).

The DPPH radical is among the commonly used stable radical sources, widely employed for assessing the ability of antioxidants to donate electrons and scavenge free radicals. DPPH is commonly recognized as a method for assessing the free radical scavenging activities of natural compounds. The DPPH radical scavenging assay was evaluated using a previously employed methodology (Brand-Williams et al., 1995). In summary, 100 µL of carotenoid extract was mixed with 3900 µL DPPH solution (0.1 mM) and vortexed. The absorbance of the mixture was measured using a spectrophotometer at 517 nm after it was left at room temperature and in the dark for 30 minutes. The samples were analyzed for free radical scavenging activity (mg TE/L) using the calibration curves created for various concentrations of standard Trolox solutions.

The analysis of the FRAP was conducted according to Benzie and Strain (1996). In brief, TPTZ (10 mM), Iron (III) chloride (20 mM), and buffer solution (0.3 M sodium acetate; pH 3.6) were mixed in a 10:1:1 ratio to prepare the FRAP

reagent. Afterward, 2900 µL of FRAP reagent was blended with 100 µL of the extract and incubated at room temperature for 30 minutes in a dark environment. The absorbance was measured at 593 nm, and the results were calculated based on a standard curve as µmol TE/L.

Physicochemical Analyses

The pH, titratable acidity (TA) and total soluble solids (TSS) of carotenoid extracts were determined according to the AOAC methods (AOAC, 1995). The carotenoid extracts' pH values were measured with a pH meter (WTW Inolab, Germany). To calculate the TA, the titrimetric method was employed, and the obtained TA values were expressed as a percentage of citric acid (%). The TSS of extracts were recorded with a digital refractometer (RFM 330; Bellingham Stanley Limited, Atago-Palette, PR-101, Tokyo, Japan) at 20 °C, and the results were expressed in ºBrix.

Color

CIELAB color co-ordinates were used to determine the color parameters L* (darkness, brightness), a* (redness, greenness), and b* (blueness, yellowness) in the study. The color measurements were conducted using a Minolta colorimeter (CR-300, Osaka, Japan). The chroma (ΔC) values were determined using lemon peel as reference material for comparison. The ΔC and hue angle values of extracts were calculated according to Eq. (2) and Eq. (3).

$$\Delta C=[(a-a_{ref})^2+(b-b_{ref})^2]^{1/2} \quad (2)$$

$$\text{Hue angle}=\tan^{-1}\frac{b^*}{a^*} \quad (3)$$

Statistical Analysis

Statistical analysis was performed to assess the significance of differences among the obtained analysis results using ANOVA variance analysis followed by Duncan Tests. The mean ± standard deviation of three separate experiments was used to express all results. The statistical software package SPSS 17.0 for Windows (SPSS Inc., Chicago, USA) was employed for result evaluation. The coefficient of determination (R^2) indicated the fit of the polynomial model equation, and an F-test was used to determine its statistical significance.

RESULTS AND DISCUSSIONS

Extraction of Carotenoid from Lemon Peel

After extraction by conventional, ultrasound-assisted extraction (UAE) and ultrasound-assisted enzymatic extraction (UAEE) methods, 16.1 mL, 5.8 mL, and 17.1 mL of extract were obtained, respectively. The total carotenoid contents of these lemon peel extracts are presented in Table 1. The highest total carotenoid content was found to be 0.792±0.01 mg/L ß-carotene following ultrasound-assisted enzymatic extraction (UAEE). The lowest value of total carotenoids was determined as 0.493±0.01 ppm ß-carotene with conventional extraction. The results demonstrated statistically significant differences between the carotenoid contents of lemon peel extracts subjected to different extraction methods ($P < 0.05$). In our study, the total carotenoid content of the lemon peel extracts tended to increase as the ultrasonication process. This could be elucidated by the cavitation phenomenon, where microbubbles develop within a liquid solvent and rapidly enlarge until they reach a critical size, causing internal rupture. Under these conditions, the mixture environment experiences significant shear stress, physically decompose the cell walls (Lin et al., 2023). Consequently, they enhance the interface between the solvent and the targeted chemicals, facilitating the permeation of the lemon peels by the solvent. Throughout the extraction process, this occurrence leads to a more substantial transfer of pigment mass from within the cell to the solvent, resulting in β - carotene being more easily released from the matrix into the extraction medium (Xu et al., 2023). Sun et al. (2011) reported that extending the ultrasonication time from 20 to 120 minutes resulted in a significant ($P < 0.05$) enhancement in the efficiency of trans β-carotene extraction from the peel of Bendizao mandarin fruit. Similarly, Shahram and Dinani (2009) investigated the extraction of β-carotene pigment from orange processing waste by combining ultrasonic and enzymatic processes using ethanol solvent. They found that the increase in β-carotene content

extraction with the increase of ultrasonication can be attributed to the cavitation phenomenon induced by ultrasound. On the other hand, the use of enzymes in UAE has become widespread to perform the extraction process more effectively and quickly in recent studies (Ricarte et al., 2020; Umair et al., 2021; Gao et al., 2022; Suo et al., 2023). In this sense, the synergistic effect resulting from the using enzymes led to the highest amount of carotenoids being attained in the extracts obtained through the UAEE method. Studies on the increase of extraction efficiency with the use of enzymes also support the results obtained (Kumar et al., 2023; Singla et al., 2023). Pectin lyase and polygalacturonase, which are used as enzyme in this study, due to their pectin-degrading activity, increase the breakdown of the pectic substance. This study hypothesized that the degradation of pectic substances increases with the utilization of pectin lyase and polygalacturonase, enzymes known for their pectin-degrading activities. Consequently, the flow of extracts and the carotenoid content increased with the ultrasound process.

Table 1. Antioxidant Analysis Results of Extracts from Lemon Peel

	Carotenoid Extract		
	Conventional Extraction	UAE	UAEE
Total Carotenoid Content (TCC)	0.493±0.01^{c}	0.589±0.01^{b}	0.792±0.01^{a}
Total Phenolic Content (TPC)	504.01±5.11^{c}	559.10±3.53^{b}	1118.31±2.10^{a}
Antioxidant Capacity (ABTS)	418.41±9.40^{c}	473.28±6.41^{b}	753.80±5.79^{a}
Antioxidant Capacity (DPPH)	412.46±10.45^{c}	507.25±5.79^{b}	624.64±10.52^{a}
Antioxidant Capacity (FRAP)	138.01±1.36^{b}	130.31±0.96^{c}	186.64±1.66^{a}

UAE, ultrasound-assisted extraction; UAEE, ultrasound-assisted enzymatic extraction; TCC, mg/L ß-carotene; TPC, mg gallic acid/L; ABTS, mg Trolox/g; DPPH, mg Trolox/L; FRAP, μmol Trolox/L.
Results are given as mean ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$).

Antioxidant Properties

The total phenolic content (TPC) in the carotenoid extracts from lemon peels was statistically significantly influenced by the applied extraction techniques and parameters used, as shown in Table 1. The TPC of the extracts were ranged from 504.01±5.11 to 1118.31±2.10 mg GAE/L. UAEE-extracted carotenoids showed the highest TPC, followed by UAE and conventional-extracted carotenoids respectively. These findings are consistent with other studies utilizing UAEE, where authors generally concur that the enhanced polyphenol yields result from the complementary effects of cavitation and enzymatic hydrolysis (Athanasiadis et al., 2023; Lin et al., 2023; Mapholi and Goosen 2023). Three different methods were employed to evaluate the antioxidant capacities of the extracts obtained in this study, namely ABTS, DPPH, and FRAP assays. The antioxidant capacity (ABTS) content of lemon peel extracts using conventional, UAE, and UAEE methods were found to be 418.41±9.40, 473.28±6.41, and 753.80±5.79 mg TE/L, respectively. In the study, the carotenoid extracts obtained by UAEE extraction demonstrated the highest antioxidant capacity value. Similarly, the extract obtained with the UAEE also showed excellent DPPH radical scavenging ability (624.64±10.52 mg TE/L) compared with other extraction methods. The FRAP assay relies on antioxidants' capability to convert Fe^{3+} to Fe^{2+} in the presence of TPTZ, indicating of the potential antioxidant activity of natural products. As shown in Table 1, the FRAP values of the extracts obtained by conventional, UAE, and UAEE methods are 138.01±1.36, 130.31±0.96, and 186.64±1.66 μmol TE/L, respectively. All results in this section demonstrated statistically significant differences in the antioxidant properties of the carotenoid extracts subjected to different extraction procedures ($P < 0.05$). These results agree with those previously reported studies for carotenoid extraction with different extraction procedures

(Tchabo et al., 2015; Jagannath and Biradar, 2019; Karne et al., 2023; Suri et al., 2023). Dong et al (2019) similarly investigated phenolic compounds and antioxidant capacity of Eureka lemon fruits harvested at different months of the year. The TFC value in lemon peel was determined to range from 6.35 ± 0.24 to 7.96 ± 0.17 mg GAE/g, the ABTS value ranged from 25.21 ± 0.27 to 40.55 ± 0.32 µmol TE/g, and the DPPH value ranged from 8.28 ± 0.19 to 16.49 ± 0.56 µmol TE/g. In a study conducted by Chatzimitakos et al. (2023) aimed at defining optimal extraction procedures and parameters to obtain bioactive components from lemon peel by-products, antioxidant activity values were determined as 128.9 µmol TE/g (FRAP) and 30.3 µmol TE/g (DPPH). The results of these related studies and ours indicate that ultrasound plays a critical role in extracting the maximum amount of antioxidants, and enzyme utilization has a positive effect. In addition, ultrasonication enhances enzymatic hydrolysis by physically breaking down particles, increasing substrate-enzyme interaction, boosting the frequency of collisions between them, and overcoming the mass transfer limitations (Mercado-Mercado et al., 2018). In present study, this ensured better release and more efficient removal of bioactive compounds, resulting in an increase in TPC and antioxidant capacity values. Furthermore, our study revealed that carotenoid extracts obtained from fresh lemon fruit peels exhibited a higher concentration of antioxidants compared to fresh lemon fruit peels.

Physicochemical Analyses

The analysis results of physicochemical properties (pH, titratable acidity, total soluble solids) are shown in Table 2. The pH values were determined to be 4.45±0.02, 4.63±0.04, and 3.16±0.01 for the conventional, UAE, and UAEE extraction methods, respectively. As expected, the titratable acidity value (reported as % citric acid) of UAEE extracts with a low pH value was determined to be higher compared to the extracts obtained by other extraction methods, and the difference between them is statistically significant ($P < 0.05$). It is believed that the reason for this phenomenon is attributed to a synergistic effect resulting from the application of ultrasound and enzymes. This effect facilitates extraction through cell wall disruption and enhances mass transfer of organic acids by increasing cell membrane permeability (Dalagnol et al., 2017; Ladole et al., 2018; Ma et al., 2022). In this study, a statistically significant difference was observed in all physicochemical analysis results among the three extraction methods ($P < 0.05$). The pH, TA, and TSS values obtained in this study were consistent with the expected ranges based on similar investigations conducted on this subject (Bagde et al., 2017; Chatzimitakos et al., 2023).

Table 2. Physicochemical Analysis Results of Extracts from Lemon Peel

	Carotenoid Extract		
Analyses	Conventional Extraction	UAE	UAEE
pH	4.45±0.02[b]	4.63±0.04[a]	3.16±0.01[c]
Titratable Acidity (TA)	0.46±0.04[b]	0.28±0.03[c]	2.80±0.01[a]
Total Soluble Solids (TSS)	21.25±0.06[a]	19.96±0.00[b]	15.85±0.06[c]

TA, citric acid%; TSS, Brix; UAE, ultrasound-assisted extraction; UAEE, ultrasound-assisted enzymatic extraction. Results are given as mean ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$).

Color

The CIELAB color coordinates were used to define the color parameters L* (darkness, brightness), a* (redness, greenness), and b* (yellowness, blueness) (Meral et al., 2024). When examining the color values of the carotenoid extracts (Table 3), it was determined that the difference between the extraction methods was statistically significant for all color parameters ($P < 0.05$). The lightness factor, L*, generally decreased from the carotenoid extracts obtained via the UAE method to those obtained via the

UAEE method in the analysis of the color values. The decrease in the L* value reflects darkening caused by carotenoid accumulation in extracts obtained through various extraction methods (Ruiz et al., 2005; Sebdani and Abbasi, 2023). The a* value is associated with colors ranging from green to red, with negative values representing green tones and positive values representing red tones (Aghajanzadeh et al., 2023). In the present study, the positive a* values of the carotenoid extracts resulted in an increased red color of the extract. The b* color value took a positive value in all carotenoid extracts, indicating an increase in yellow color, with the highest b* value (32.83±0.09) being reached by the UAEE method, where the combination of ultrasound and enzyme was applied. Research on fruit color and quality has shown that the use of the color space, which includes hue and chroma values, is effective in characterizing fruit color (Garcia et al., 2016). For example, lemon peel gradually changes color from green to yellow. In this process, where the lemon peel color changes from green to yellow, the chroma value increases and the hue angle decreases with the accumulation of carotenoids (Baruah and Kotoky, 2018). In the extracts obtained from lemon peels there was an increase in chroma value due to the higher carotenoid content. While the Hue angle was lower in the extract obtained by UAE compared to that of the conventional method, the lowest value was determined in the UAEE method. In summary, the color value results determined within the scope of the study were found to be consistent with the total carotenoid composition of the extracts. While the L* and hue angle values of the extract obtained by UAEE, which had the highest carotenoid content, were found to be lower, the a*, b* and chroma values were found to be higher. Similarly, in a study on the effect of carotenoid extracts obtained from the flesh and peel of different apricot varieties on color components, it was found that there was a good correlation between carotenoid composition and L*, a*, b*, chroma value, and hue angle values (Ruiz et al., 2005).

Table 3. Color Analysis Results of Extracts from Lemon Peel

	Carotenoid Extract		
	Conventional Extraction	UAE	UAEE
*L**	45.06±0.04^{b}	52.65±0.10^{a}	33.02±0.10^{c}
*a**	7.89±0.13^{b}	0.72±0.10^{c}	13.87±0.07^{a}
*b**	14.02±0.12^{c}	29.75±0.17^{b}	32.83±0.07^{a}
Croma Value	16.09±0.14^{b}	29.76±0.12^{a}	35.64±0.14^{c}
Hue°	75.14±0.13^{b}	88.75±0.17^{a}	45.32±0.19^{c}

UAE, ultrasound-assisted extraction; UAEE, ultrasound-assisted enzymatic extraction.
Results are given as mean ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$).

CONCLUSIONS

This study investigated, for the first time, the extraction of β-carotene from lemon processing waste powder using conventional extraction, ultrasound-assisted extraction, and a combination of ultrasound and enzymatic processes. To achieve this goal, the β-carotene content, antioxidant properties, physicochemical analysis and color parameters of L*, a*, and b*, chroma value, hue angle of the extracts were evaluated. The findings indicate that both UAE and UAEE are effective methods for extracting carotenoids from lemon peel. Comparative analysis of the extraction methods revealed notable differences in the obtained results. In UAEE, the β-carotene content was found to be approximately 61% higher than that of the conventional method. Furthermore, UAEE exhibited a remarkable 121% increase in TPC compared to the conventional method and a 100% increase compared to UAE. Regarding antioxidant capacity, UAEE outperformed conventional extraction across all assays: 80% higher in ABTS, 51% higher in DPPH and 35% higher in FRAP.

The increase in ABTS, DPPH and FRAP content observed with UAEE is consistent with the higher total phenol concentration estimated by UAEE, which confirms the typical correlation between antioxidant activity and TPC. The UAE, compared to conventional extraction, offers the advantage of promoting the rupture of the material's cells and the expansion of the cell walls' pores, thereby contributing to higher mass transfer and increased antioxidant capacity of the extract. These results demonstrate that the bioactive chemicals' extraction efficiency and yield can be significantly enhanced by carefully selecting the extraction methods and solvent composition, thus offering a more effective and energy-efficient approach. In conclusion, these findings underscore the superior efficiency of UAEE in extracting bioactive compounds from lemon peel, presenting UAEE as a promising approach for enhancing the value and quality of extracted compounds for various applications in the food and pharmaceutical industries. In particular, carotenoid extracts from lemon peel within the scope of study have a potential application as natural antioxidant and coloring agent for food formulations compatible with their acidic structure and it will contribute to the product protection and minimization of color losses. In the future, exploring the valorization of lemon by-products and devising an integrated and sustainable process for recovering lemon fractions would be of great interest. This research will serve as a reference study in the literature, contributing to the advancement of the 'zero waste' concept. It aims to reduce the environmental footprint and promote a circular economy, offering sustainable solutions applicable in the food industry.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

AUTHOR CONTRIBUTIONS

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

ETHICAL APPROVAL

Ethical approval is not required for this research.

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