

Article

Effect of Sonication Time on Bioactive Compounds, Fatty Acid Profile, and Lipid Quality of Tea Seed Oil

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Abstract

In this study, the effect of sonication on the bioactive components, antioxidant capacity, phenolic components, and fatty acid composition of tea seeds sonicated for different periods of time was investigated. The total phenol content (TPC) and total flavonoid content (TFC) of non-sonicated (raw) and sonicated tea seeds were established to be between 58.31 (15 min) and 75.55 mg GAE/100 g (control) to 85.00 (15 min) and 127.95 mg/100 g (5 min), respectively. With the increase in sonication time, TPC and TFC of the tea seeds decreased, while the antioxidant capacities of the seeds increased partially. In general, an increase in the antioxidant value of tea seeds was observed with increasing sonication time. Catechin and *p*-coumaric acid contents of the tea seeds were assessed to be between 123.69 (25 min) and 360.87 (5 min) to 7.29 (15 min) and 13.26 mg/100 g (5 min), respectively. The amount of palmitic acid, the highest saturated fatty acid in tea seed oils, was identified to be between 13.51 (15 min) and 13.77% (5 min). While the amount of oleic acid of the tea seed oils was assessed to be between 61.72 (15 min) and 62.98% (25 min), linoleic acid results of the tea seed oils were characterized to be between 19.32 (5 min) and 19.69% (control). Nutritive Value Index (NVI) and Atherogenic Index (AI) results of sonicated tea seed oils were calculated to be between 4.74 (5 min) and 4.81 (25 min) to 0.161 (15 min) and 0.178 (control), respectively. Considering its overall fatty acid profile and other chemical properties, tea seed oil appears to be a promising alternative edible oil with characteristics similar to commonly consumed cooking oils such as olive and sunflower oil.

Keywords: tea seed; ultrasound treatment; phytochemicals; antioxidant activity; oxidative stability; fatty acid composition; edible oil quality



Academic Editor: Carlos Vilchez

Received: 2 June 2026

Revised: 3 July 2026

Accepted: 8 July 2026

Published: 16 July 2026

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1. Introduction

The tea plant belongs to the genus *Camellia* within the family *Theaceae* and the order *Parietales* [1]. Among the species of *Camellia*, *Camellia japonica* is widely recognized as an important ornamental plant, whereas *Camellia sinensis* is the most economically significant species due to its use in tea production [2]. Seed production in tea plants increases as they age, and without rejuvenation pruning or leaf harvesting, excessive flowering and fertilization significantly boost seed yield [3]. Tea seed oil is considered a high-quality edible oil and has a fatty acid composition comparable to that of olive oil [4,5]. Tea seed oil is widely used not only as a cooking oil but also as a raw material in the cosmetic industry for products such as creams, lipsticks, hair conditioners, sunscreens, and makeup removers, as well as in industrial applications including the production of soap, margarine, and

paint [3,4]. Numerous seeds employed in the chemical industry are potential sources of edible vegetable oils [6]. Although tea seeds are often regarded as agricultural waste in many tea-producing regions worldwide, they have been identified as a source of exceptionally high-quality oil with potential applications as an oleochemical feedstock or chemical intermediate [7]. Tea seeds, which are produced in large quantities as waste each year, contain about 30–32% high-quality edible oil and are rich in oleic and linoleic acids [5]. In addition, the antioxidant activity of phenolic compounds in tea seed oil has been evaluated using chemical antioxidant methods [8]. Fattahi et al. [9] conducted a study on the production of margarine from tea seed oil, in which tea seed oils obtained from tea plants grown in Iran were converted into margarine via interesterification using a NaOH catalyst under varying temperature and time conditions, and the resulting product was reported to be comparable to commercially produced margarines. Tea seed oil is regarded as an alternative to canola and olive oil due to its fatty acid composition, and is also noted for its high antioxidant content, with reported benefits including reductions in blood pressure and low-density lipoprotein (LDL) levels [10,11]. Haiyan et al. [12] compared the fatty acid profile, total phenolic content, phenolic compounds, and volatile components of avocado, pumpkin, soybean, sesame, and tea seed oils. Tea oil has a more favorable fatty acid profile than other oils, with a high proportion of monounsaturated fatty acids (especially oleic acid) and low levels of polyunsaturated fatty acids, and it is also rich in natural antioxidants and phenolic compounds, which give it good oxidative stability [13,14]. Tea seed oil, which has characteristics similar to high-quality edible oils such as olive oil, contains large amounts of polyphenols. In studies comparing ultrasound power, duration, and temperature parameters in ultrasound-assisted extraction, as well as different extraction methods for obtaining oil from various plant materials such as watermelon seeds [15] and pomegranate seeds [16], it was found that ultrasonic extraction is more time-efficient and provides higher extraction efficiency in terms of the resulting oils. Ultrasound-assisted extraction is an alternative method that works by mechanically disrupting cell walls and enhancing mass transfer, making it faster than other extraction techniques and adaptable to both small and large scales [17]. The main mechanism of this method is the behavior of cavitation bubbles during the propagation of acoustic waves [18]. Previous studies have applied conventional solvent extraction and mechanical pressing to recover oil and bioactive compounds from tea seeds; however, these methods often suffer from low extraction efficiency, prolonged processing time, and limited recovery of valuable phytochemicals. Ultrasound-assisted extraction enhances mass transfer through acoustic cavitation, improving extraction yield and bioactive compound recovery. Nevertheless, the specific influence of sonication time on tea seed bioactive compounds and oil quality remains insufficiently investigated, representing a significant research gap [19,20]. Although previous studies have investigated the physicochemical properties and bioactive characteristics of tea seed oil, the available literature provides limited and fragmented evidence regarding the influence of sonication processing, particularly the effect of different sonication durations on its overall quality attributes. In particular, a comprehensive evaluation of the simultaneous changes in bioactive compounds, antioxidant capacity, phenolic profile, and fatty acid composition remains lacking. Addressing this knowledge gap is important for optimizing ultrasound-assisted processing and improving the functional quality of tea seeds. Therefore, the present study aimed to investigate the effect of different sonication times on the bioactive components, antioxidant capacity, phenolic composition, and fatty acid profile of tea seeds.

2. Material and Methods

2.1. Material

In 2023, tea seeds were supplied from Rize (Çayeli–Büyüktaşhane) in Türkiye (Figure 1). Following their collection, the outer shells were carefully removed, and the seeds were subsequently left to dry under natural open-air conditions for 30 days.



Figure 1. Tea seeds.

2.2. Methods

2.2.1. Moisture Content

The moisture amounts of tea seeds were assigned at 105 °C using an oven (Nüve FN055 Ankara, Türkiye) until a constant weight was observed, utilizing the modification method found in [13].

2.2.2. Oil Yield

The tea seeds were ground and passed through a 0.5-mesh sieve before being used for oil extraction. A 10 g portion of powdered tea seed sample was placed into an extraction cartridge and mixed with 25 mL of petroleum ether. The mixture was then subjected to ultrasonic treatment in an ultrasonic bath (35 kHz; Bandelin Sonorex, Berlin, Germany) for different extraction periods of 5, 15, and 25 min in order to evaluate the effect of sonication time on oil recovery. For comparison, the total oil content of non-sonicated tea seed samples (control group) was extracted using a conventional rinsing bath with 25 mL of petroleum ether for 25 min under the same extraction conditions. Following the extraction process, the solvent was removed by evaporation using a rotary evaporator maintained at 50 °C, leaving the extracted oil for subsequent analysis and quantification [10].

2.2.3. Extraction Procedure

A 5 g portion of ground tea sample was extracted with 15 mL of methanol and subjected to ultrasonic treatment for varying durations (0 min as control, 5 min, 15 min, and 25 min). Following sonication, the mixture was centrifuged (Hermle Z-200A, Wehingen, Germany) at 6000 rpm for 10 min and subsequently filtered (Cytiva, Marlborough, MA, USA 0.45 µm). The filtrate was then combined with 15 mL of n-hexane and thoroughly mixed using a vortex mixer (DZG SCIENCE MIX 2000 (Türkiye)). Methanol and hexane phases were separated using a separatory funnel, and the extraction with n-hexane was repeated twice more using 10 mL portions of n-hexane each time. The methanolic fractions collected from all separation steps were combined and evaporated to dryness at 40 °C. Finally, the dry residue was reconstituted with 10 mL of methanol and

prepared for subsequent analysis [21]. The extracts were stored at -18°C until analysis. Extracts taken from the deep freezer were analyzed immediately.

2.2.4. Total Phenolic Content (TPC)

The total phenolic amount of tea seeds was established using the modified Folin–Ciocalteu (FC) assay according to the study reported by Yoo et al. [22]. FC (1 mL) and Na_2CO_3 (10 mL) were added to the extract (0.1 mL) and mixed via vortex. Deionised water was added until the final volume was 25 mL. The samples were kept in the dark at room temperature for 2 h. Then, 5 mL of distilled water was introduced into each sample, and the absorbance was recorded at 725 nm. A calibration curve was prepared with gallic acid (0–200 mg/mL) as the standard. The results are shown as mg gallic acid equivalent (GAE)/100 g (dw).

2.2.5. Total Flavonoid Content (TFC)

The total flavonoid quantities of tea seed extracts were determined using a modified version of the method described by Hogan et al. [23]. The extract (1 mL) was mixed with 0.3 mL of NaNO_2 , 0.3 mL of AlCl_3 , and 2 mL of NaOH, respectively, and kept in the dark for 15 min. Afterward, the absorbance of the prepared solution was recorded at 510 nm using a spectrophotometer (Shimadzu UV mini 1240, Kyoto, Japan). The total flavonoid content in the tea seed samples are given as mg quercetin (QE)/100 g.

2.2.6. Antioxidant Activity

DPPH Free Radical Scavenging Activity

The antioxidant capacity of the tea seed extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method according to the procedure reported by Lee et al. [24]. In brief, 0.1 mL of the extract was mixed with 0.9 mL of buffer solution and 2 mL of DPPH solution in test tubes. The mixtures were then vortexed thoroughly to achieve homogeneity. The samples were subsequently kept in the dark for 30 min to enable the reaction to occur. Following incubation, absorbance values were measured at 517 nm using a spectrophotometer (Shimadzu UV mini 1240, Japan), and the results were assigned in mmol Trolox equivalent (TE)/kg.

ABTS Free Radical Scavenging Activity

Antioxidant activities of the extracts were determined using the 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method used in the report conducted by Köseoğlu et al. [25]. After pretreatments, the absorbance was defined at 734 nm using a spectrophotometer (Shimadzu UV mini 1240, Japan), and the findings were assigned in mmol Trolox equivalent (TE)/kg.

2.2.7. Determination of Phenolic Compounds

A Shimadzu HPLC (SCL-10A VP-Shimadzu, Kyoto, Japan) system fitted with a photo-diode array (PDA) detector was employed to detect and measure phenolic compounds in tea seed samples. Chromatographic separation of phenolic compounds was determined by a HPLC equipped with a PDA detector and an Inertsil ODS-3 (5 μm ; 4.6×250 mm) column. Separation of the compounds was performed using a binary mobile phase made up of 0.05% acetic acid in water (solvent A) and acetonitrile (solvent B). An aliquot of 20 μL of the sample extract was injected and analyzed at a steady flow rate of 1 mL/min, while the column temperature was kept at 30°C . Detection of compounds was performed at a wavelength of 280 nm using the PDA detector. The elution was programmed according to a gradient system as follows: from 0 to 0.10 min, 8% B; from 0.10 to 2 min, 10% B; from 2 to 27 min, 30% B; from 27 to 37 min, 56% B; from 37 to 37.10 min, 8% B; and finally

from 37.10 to 45 min, the system was maintained at 8% B to re-equilibrate the column. Each sample required a total runtime of 60 min for complete analysis. The obtained results are expressed as mg/100 g.

2.2.8. Fatty Acid Composition

Tea seed oils were converted into their corresponding fatty acid methyl esters through an esterification process using potassium hydroxide (KOH) in a mixture of n-hexane and methanol according to the study described by Multari et al. [26], with modifications. The resulting fatty acid methyl esters were subsequently analyzed by gas chromatography using a Shimadzu GC-2010 system. Detection was carried out using a flame ionization detector (FID), together with a capillary column (Teknokroma TR CN100, P/N TR 882162 fused silica column, 60 m × 0.25 mm × 0.20 µm). The oven temperature was initially held at 90 °C for 7 min, then ramped at 5 °C/minute to 240 °C, and maintained at that temperature for 15 min.

2.2.9. Calculation of Lipid Indices of Sonicated Tea Seed Oils

Oleic Desaturation Ratio (ODR) and Linoleic Desaturation Ratio (LDR) values were defined according to the study recommended by Pleines and Friedt [27].

The fatty acid composition was used to establish several nutritional values of lipids in tea seed oils. They were calculated via the following lipid indices:

- **NVI** (Nutritive Value Index) = (C18:0 + C18:1)/C16:0 [28].
- **AI** (Atherogenic Index) = (C12:0 + 4 × C 14:0 + C16:0)/Σ UFA [29].
- **TI** (Thrombogenic Index) = (C14:0 + C16:0 + C18:0)/[(0.5 × Σ MUFA) + (0.5 × Σ PUFA n−6) + (3 × Σ PUFA n − 3) + (Σ PUFA n − 3/Σ n − 6)] [29].
- **OFA**: (C14:0 + C16:0) dietary fatty acids which have an undesirable hypercholesterolemic effect in humans.

2.3. Statistical Analyses

Analysis of variance (ANOVA) was performed using JMP statistical software (SAS Institute, Cary, NC, USA). Differences among group means were evaluated using Duncan's Multiple Range Test (DMRT). Results are presented as mean ± standard deviation. Statistical significance was accepted at $p < 0.05$.

3. Results and Discussion

3.1. The Chemical Properties of Tea Seeds

The chemical composition and bioactive characteristics of tea seeds are presented in Table 1. The findings indicated noticeable variations depending on the duration of sonication. The initial moisture content of tea seeds was $6.10 \pm 0.40\%$. Oil yield from both untreated (raw) and sonicated tea seeds ranged from 4.00% (at 5 min of sonication) to 10.93% in the control group. Similarly, TPC and TFC were found to vary between 58.31 mg GAE/100 g (15 min) and 75.55 mg GAE/100 g (control) for TPC, and between 85.00 (15 min) and 127.95 mg/100 g (5 min) for TFC, respectively. In addition, antioxidant activity measured by DPPH and ABTS assays showed values ranging from 1.59 (control) to 1.61 (25 min) for one parameter, and from 0.55 (15 min) to 0.63 mmol/kg (25 min) for the other. The results show that ultrasonication time has a clear, non-linear effect on both total phenolic content (TPC) and total flavonoid content (TFC). In the control sample, TPC and TFC were 75.55 mg/100 g and 121.10 mg/100 g, respectively. Phenolic acids were the most abundant class [30]. Ultrasonic treatment caused notable time-dependent changes in these values. A short treatment (5 min) increased TFC to its maximum value of 127.95 mg/100 g, likely due to cavitation-induced cell wall disruption enhancing the release

of bound flavonoids into the solvent. However, at the same time, TPC slightly decreased compared to the control (73.31 mg/100 g), suggesting that some phenolic compounds may be sensitive to localized heat and pressure generated during cavitation, leading to partial degradation. With longer exposure (15 min), both TPC and TFC dropped significantly (58.31 and 85.00 mg/100 g), indicating that excessive ultrasonication may promote oxidation and degradation of bioactive compounds through increased radical formation and structural damage. At 25 min, partial recovery was observed (TPC: 71.88 mg/100 g; TFC: 120.43 mg/100 g), likely due to further matrix breakdown enabling additional extraction, although values did not fully return to control levels. Overall, the findings indicate that ultrasonication requires an optimal balance between enhanced extraction and compound stability. Short processing times, particularly around 5 min, maximize flavonoid extraction, whereas prolonged treatment can reduce yields due to degradation processes, consistent with previous reports on ultrasound-assisted extraction optimization. Overall, oil yield significantly decreased at shorter sonication durations, and none of the applied sonication treatments resulted in a higher oil yield compared to the untreated control sample [19].

Table 1. Oil content and bioactive properties of the tea seeds sonicated at different times.

Sonication Time	Oil Yield (%)	TPC (mgGAE/100 g)	TFC (mgQE/100 g)	Antioxidant Activity (mmolTE/kg, DPPH)	Antioxidant Activity (mmolTE/kg, ABTS)
Control	10.93 ± 0.03 * a	75.55 ± 3.92 a	121.10 ± 1.57 b	1.55 ± 0.00 c	0.60 ± 0.01 b
5 min	4.00 ± 0.01 d **	73.31 ± 3.26 b	127.95 ± 1.08 a	1.60 ± 0.00 a	0.59 ± 0.01 bc
15 min	8.00 ± 0.02 c	58.31 ± 1.50 d	85.00 ± 1.71 d	1.59 ± 0.00 ab	0.55 ± 0.00 d
25 min	10.40 ± 0.04 ab	71.88 ± 0.68 c	120.43 ± 2.29 c	1.61 ± 0.00 a	0.63 ± 0.00 a

TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TE: Trolox equivalent. * Standard deviation; ** values within each column followed by different letters are significantly different at $p < 0.05$.

The lowest levels of bioactive compounds were generally observed at 15 min of sonication. As sonication time increased, a reduction in TPC and TFC was observed, while antioxidant capacity showed a partial improvement. When compared with previous studies investigating ultrasound-assisted extraction parameters such as power, time, and temperature for oil recovery from plant materials like watermelon seeds [15] and pomegranate seeds [16], it has been reported that ultrasound-assisted extraction can improve extraction efficiency while reducing processing time. However, the results obtained in the present study for tea seeds differed from those findings. These discrepancies may be attributed to differences in ultrasound power, sonication duration, and the degree of particle size reduction (grinding) applied to the samples. Variations in ultrasound-assisted extraction outcomes are likely related to the incomplete disruption of cell walls, which limits mass transfer. As a result, intracellular oil cannot easily diffuse out, particle size remains relatively large, the contact surface area between solid and liquid phases is insufficient, and solvent penetration into plant tissues occurs slowly [15,16]. Furthermore, it was reported that 25.3% oil was obtained from tea seeds using the Soxhlet extraction method, while a higher yield of 29.2% was achieved under optimized supercritical CO₂ (SC-CO₂) extraction conditions [31]. In addition, Kenyan tea seeds (*Camellia sinensis*), widely cultivated for black tea production, have been documented to contain approximately 16–25% oil [6]. Zhenggang et al. [32] reported that the oil content of *C. hainanica* and *C. sinensis* ranged from 37.60% to 41.60% and from 31.04% to 33.20%, respectively. In a more recent study, Meng et al. [33] identified tea seed (*C. oleifera*) oil as a traditional edible oil. Zhenggang et al. [32] measured the TPC of *C. hainanica* seeds and found it to be 345.73 µg/g. For tea seed oil, which is recognized as a natural source of antioxidants, the TPC and antioxidant activity (DPPH) were reported to range between 0.036 and 0.043 mg GAE/L and between 14 and 21%, respectively [6].

Liu et al. [14] further determined the antioxidant capacity of tea seed oil to be 101.05 μmol QE per 100 g of oil. In the present study, however, the obtained results regarding the oil content and bioactive properties of tea seeds differed from those reported in the literature. These discrepancies are most likely due to variations in ultrasound power, sonication duration, sample grinding, and the degree of cell wall disruption, all of which affect extraction efficiency. Incomplete mechanical disruption of cell walls limits mass transfer, resulting in restricted release of intracellular compounds, larger particle sizes, reduced solid–liquid interfacial contact, and slower solvent diffusion into plant tissues, thereby decreasing extraction efficiency.

3.2. The Phenolic Profiles of Tea Seeds

The phenolic profiles of the tea seeds are displayed in Table 2. Significant changes were monitored in the phenolic component results of the tea seeds depending on sonication time.

Table 2. Phenolic compounds of tea seeds sonicated at different times (mg/100 g).

Phenolic Compounds	Control	5 min	15 min	25 min
Gallic acid	2.40 $\pm$ 0.22 * c	4.72 $\pm$ 7.28 b	2.39 $\pm$ 0.46 c	12.09 $\pm$ 1.21 a
3,4-Dihydroxybenzoic acid	3.81 $\pm$ 0.88 c **	4.49 $\pm$ 0.58 b	3.20 $\pm$ 0.43 cd	5.24 $\pm$ 1.01 a
Catechin	270.34 $\pm$ 27.64 b	360.87 $\pm$ 32.41 a	189.08 $\pm$ 12.94 c	123.69 $\pm$ 8.37 d
Caffeic acid	0.83 $\pm$ 0.99 b	1.72 $\pm$ 1.81 a	0.79 $\pm$ 0.13 d	0.81 $\pm$ 0.51 bc
Syringic acid	0.64 $\pm$ 0.99 c	1.65 $\pm$ 0.73 a	0.94 $\pm$ 0.04 b	0.61 $\pm$ 0.04 cd
Rutin	11.10 $\pm$ 1.34 b	6.51 $\pm$ 2.81 d	15.06 $\pm$ 1.79 a	10.86 $\pm$ 2.03 c
<i>p</i> -Coumaric acid	13.07 $\pm$ 0.32 a	13.26 $\pm$ 1.35 a	7.29 $\pm$ 03.89 c	11.91 $\pm$ 0.61 b
Ferulic acid	1.22 $\pm$ 0.60 a	0.96 $\pm$ 0.93 c	1.01 $\pm$ 01.18 b	0.75 $\pm$ 0.06 d
Resveratrol	0.17 $\pm$ 0.12 ab	0.10 $\pm$ 0.07	0.10 $\pm$ 0.16 b	0.06 $\pm$ 0.09 c
Quercetin	0.14 $\pm$ 0.04 b	0.15 $\pm$ 0.09 b	0.19 $\pm$ 0.19 a	0.12 $\pm$ 0.13 bc
Cinnamic acid	0.01 $\pm$ 0.00 b	0.04 $\pm$ 0.09 a	0.01 $\pm$ 0.02 b	0.01 $\pm$ 0.01 b
Kaempferol	0.05 $\pm$ 0.05	0.06 $\pm$ 0.09	0.12 $\pm$ 0.16	0.06 $\pm$ 0.07

* Standard deviation; ** values within each row followed by different letters are significantly different at $p < 0.05$.

Catechin, *p*-coumaric acid, and rutin were identified as the predominant phenolic compounds present in both raw and sonicated tea seed samples. The concentrations of catechin in the tea seeds ranged from 123.69 mg/100 g (after 25 min of sonication) to 360.87 mg/100 g (after 5 min of sonication), whereas *p*-coumaric acid levels varied between 7.29 mg/100 g (15 min) and 13.26 mg/100 g (5 min). Gallic acid content was observed to fluctuate between 2.39 mg/100 g (15 min) and 12.09 mg/100 g (25 min). Similarly, 3,4-dihydroxybenzoic acid levels were identified to be between 3.20 mg/100 g (15 min) and 5.24 mg/100 g (25 min). Rutin concentrations varied from 6.51 mg/100 g (5 min) to 15.06 mg/100 g (15 min), while ferulic acid levels ranged between 0.75 mg/100 g (25 min) and 1.22 mg/100 g (control sample). No statistically significant differences were found in the amounts of gallic acid and cinnamic acid between the control group and the samples sonicated for 15 min. The variation in phenolic compounds with sonication time is mainly driven by the balance between ultrasound-assisted extraction (UAE) enhancement and compound degradation. With a short treatment time (5 min), cavitation improves mass transfer by disrupting cell walls and increasing solvent penetration, leading to higher extraction of several phenolics such as catechin and caffeic acid, as commonly reported for UAE systems [17,19]. However, with prolonged sonication (15–25 min), the amount of some compounds decrease due to degradation caused by ultrasound-induced radicals, as well as oxidation and structural breakdown, particularly for more labile flavonoids like catechin and rutin. The strong decline in catechin supports its sensitivity to extended acoustic exposure, while the increase in gallic acid at 25 min may reflect continued release from bound forms and possible transformation from larger phenolics. Rutin shows a peak at 15 min, indicating an optimal extraction time before degradation becomes dominant. Overall, the results follow a typical biphasic UAE pattern,

where initial enhancement due to cell disruption is followed by degradation effects under excessive sonication, consistent with previous reports on ultrasound processing of plant phenolics [18]. Compared with the control, gallic acid and 3,4-dihydroxybenzoic acid levels generally increased with sonication duration, except with the 15 min treatment. Catechin, caffeic acid, and *p*-coumaric acid concentrations increased after 5 min of sonication compared to the control; however, these compounds decreased with longer sonication times. In contrast, ferulic acid and resveratrol contents decreased in all sonicated samples when compared with the control group. Other identified phenolic compounds were present only in trace amounts. Wang et al. [30] reported the identification of 24 compounds, including flavanols, cinnamic acids, and dihydroflavonoids, using HPLC-Q-TOF-MS analysis of tea seed oils derived from three species collected across 15 regions in China. Their tea seed oil extract contained compounds such as hydroxytyrosol (4.59), gentisic acid (4.95), 3,4-dihydroxyphenyl glycol (11.23), pyrocatechol (2.37), *p*-hydroxyphenylacetic acid (1.17), *p*-coumaraldehyde (1.37), homovanillic acid (2.53), ferulic acid (0.61), quercetin (0.01), isorhamnetin (0.01), *trans*-cinnamic acid (1.13), naringenin (15.47), apigenin (0.31), hesperetin (0.03), and 7-hydroxycoumarin (2.65 mg/kg). These variations are likely attributed to differences in tea species and environmental and climatic conditions, as well as the type of solvent used and extraction methods and durations applied.

3.3. Fatty Acid Profiles of the Tea Seed Oils

The fatty acid profiles of the tea seed oils obtained from the tea seeds sonicated at different times (control, 5, 15, and 25 min) are illustrated in Table 3 and Figure 2. At the 15th minute of sonication, lauric, myristic, and behenic acids could not be determined in the tea seed oil. In addition, behenic and erucic acid could not be determined at the 25th min of sonication. Looking at the results of the fatty acid profile of the tea seed oil, similar results were obtained with regard to the fatty acid profile of olive oil. Palmitic and stearic acid amounts in the tea seed oils were defined to be between 13.51 (15 min) and 13.77% (5 min) and between 2.48 (5 min) to 2.71% (15 min), respectively. Also, oleic acid amounts in the tea seed oils were assessed to be between 61.72 (15 min) and 62.98% (25 min), and linoleic acid results of the tea seed oils were stated to be between 19.32 (5 min) and 19.69% (control). The amount of fatty acids in the tea seeds depended on sonication time, showing changes when compared to the control. These changes were at minor levels, but statistically significant changes were observed in the quantity of fatty acids compared to the control, depending on sonication time ($p < 0.05$). However, there were no statistically significant changes in the quantities of linolenic acids in the control and in tea seeds sonicated for 5 min. The very low amount of erucic acid in tea seed oil is positive for consumer health. Table 3 demonstrates that ultrasonic treatment had only a limited effect on the overall fatty acid profile of tea seed oil, although several statistically significant changes were observed depending on the sonication duration. The predominant fatty acids, namely oleic, palmitic, and linoleic acids, remained relatively stable throughout the treatments, indicating that the ultrasonic process did not substantially alter the major lipid composition. In contrast, minor fatty acids exhibited greater sensitivity to sonication. Lauric and myristic acids increased after 5 min of treatment, while a pronounced increase in linolenic acid was observed after 15 min of sonication. However, these changes were not maintained at 25 min, suggesting that prolonged sonication did not produce a cumulative effect on fatty acid composition. Overall, despite statistically significant variations in some minor fatty acids, the preservation of the dominant fatty acids confirms that the ultrasonic conditions applied in this study maintained the fundamental fatty acid composition of tea seed oil. These findings indicate that the selected sonication parameters can enhance processing without compromising the nutritional quality associated with its characteristic fatty acid

profile. The amounts of other fatty acids in the tea seeds were detected at minor levels: SC-CO₂ and solvent contained 15.3 and 17.7% palmitic acids, 3.3 and 3.8% stearic acids, 57.5 and 52.9% oleic acids, 22.3 and 24.2% linoleic acids, and 0.9 and 0.7 (C:1) % [31].

Table 3. Fatty acid composition of the oils extracted from the tea seeds sonicated at different times (%).

Fatty Acids	Control	5 min	15 min	25 min
Lauric	0.09 ± 0.04 * c	0.32 ± 0.07 a	- ***	0.21 ± 0.01 b
Myristic	0.08 ± 0.02 c **	0.17 ± 0.02 b	-	0.13 ± 0.00 a
Palmitic	13.66 ± 0.42 ab	13.77 ± 0.82 a	13.51 ± 0.57 cd	13.60 ± 0.02 c
Stearic	2.54 ± 0.04 b	2.48 ± 0.06 cd	2.71 ± 0.05 a	2.50 ± 0.02 bc
Oleic	62.90 ± 0.34 ab	62.89 ± 0.70 ab	61.72 ± 0.40 c	62.98 ± 0.04 a
Linoleic	19.69 ± 0.08 a	19.32 ± 0.13 c	19.58 ± 0.05 ab	19.59 ± 0.09 ab
Arachidic	0.11 ± 0.01 a	0.09 ± 0.00 bc	0.10 ± 0.00 b	0.10 ± 0.00 b
Linolenic	0.87 ± 0.09 c	0.87 ± 0.07 c	2.35 ± 0.02 a	0.89 ± 0.01 b
Behenic	0.05 ± 0.00 b	0.09 ± 0.00 a	-	-
Erucic	0.07 ± 0.00 a	-	0.07 ± 0.00 a	-

* Standard deviation; ** values within each row followed by different letters are significantly different at $p < 0.05$; - ***: not detected.

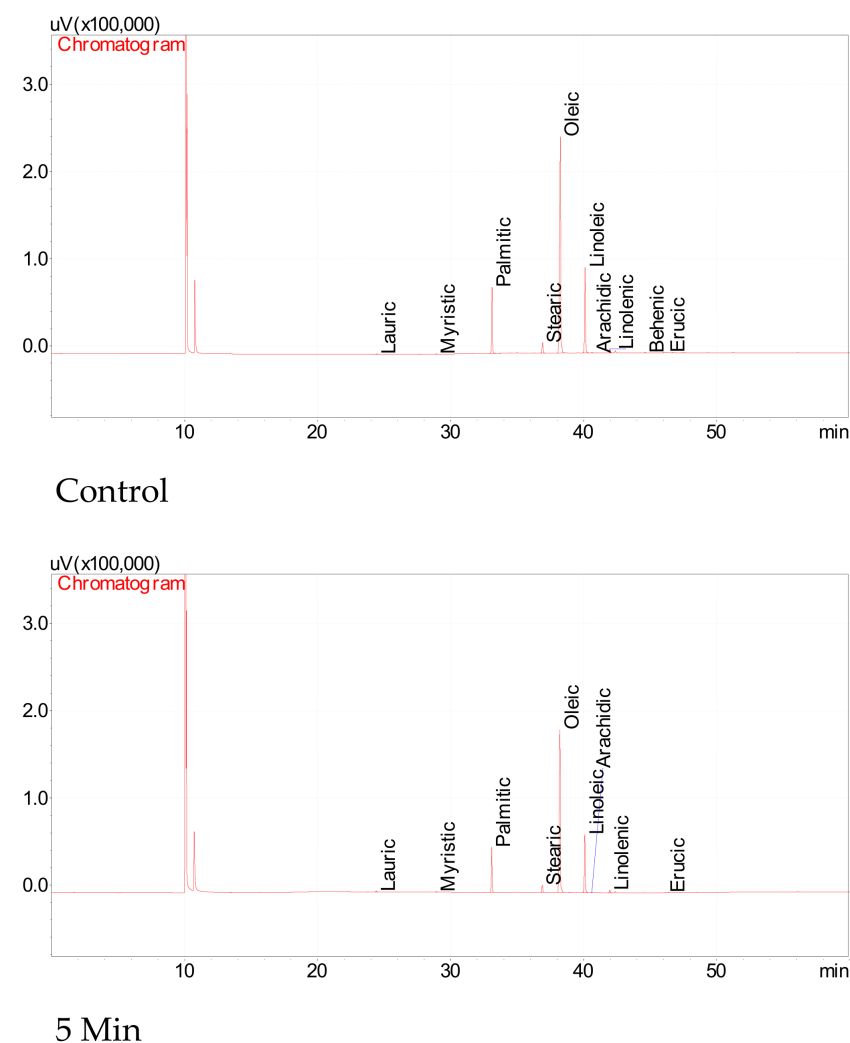


Figure 2. Cont.

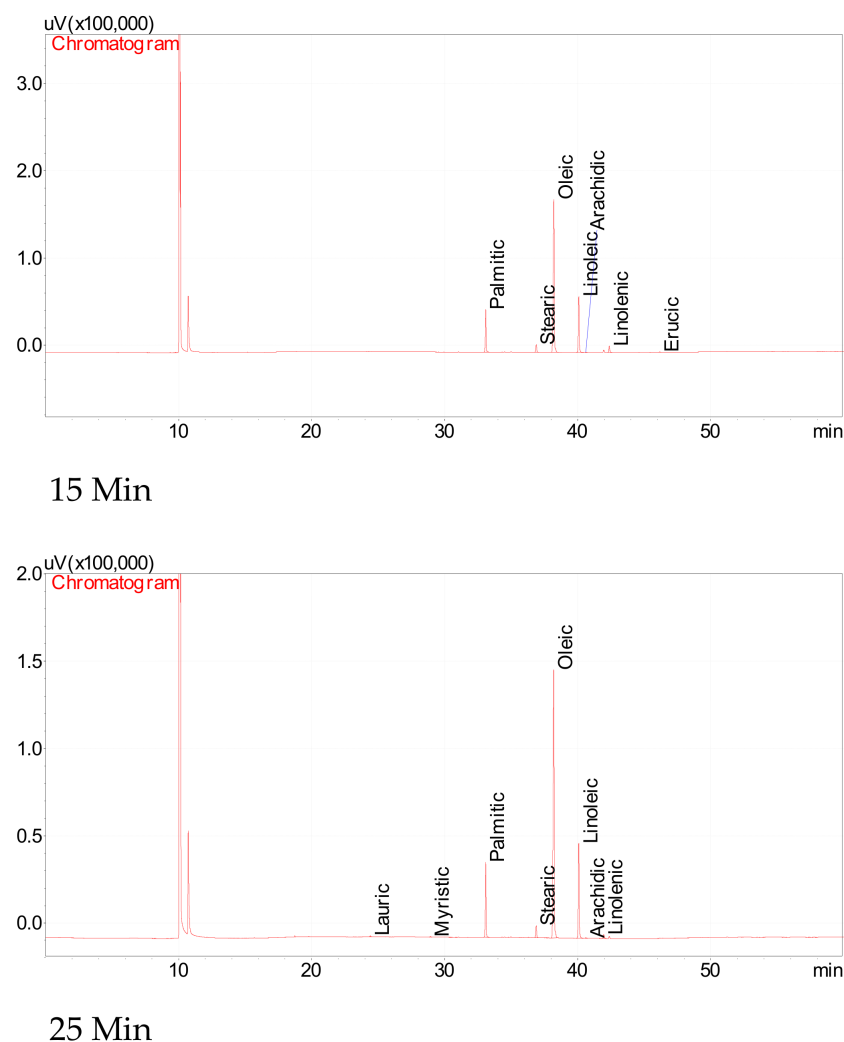


Figure 2. Fatty acid chromatograms of the oils extracted from tea seeds.

Zarringhalami et al. [32] reported that the oleic acid content of tea seeds increased from 46.64% to 59.01% during the plant's development stage between August and October. Among the fatty acids present in different tea seed oils, oleic acid is the most abundant, with reported contents of 79.62% in *C. hainanica* and 59.21% in *C. sinensis* tea seed oils [32]. Hu and Yang [34] identified the following fatty acid composition in tea seed oils: 7.07–9.52% palmitic acid, 0.09–0.22% palmitoleic acid, 1.45–2.94% stearic acid, 77.02–84.33% oleic acid, 5.19–11.23% linoleic acid, 0.53–0.70% linolenic acid, and 0.22–0.70% arachidic acid. Previous studies have also indicated that cultivar type, harvest time, and climatic conditions significantly influence both the oil content and fatty acid composition of tea seeds [35]. Overall, the predominant fatty acids observed in our study were consistent with those reported in the literature, although some variations in their proportions were noted.

3.4. Calculation of Lipid Indices, Oxidizability, and Oleic and Linoleic Desaturation Ratios of Sonicated Tea Seed Oils

In this study, the sonication times affected the lipid index values of the tea seed oils (Table 4). Nutritive Value Index (NVI) and Atherogenic Index (AI) results of sonicated tea seed oils were characterized to be between 4.74 (5 min) and 4.81 (25 min) to 0.161 (15 min) and 0.178 (control), respectively.

Table 4. Calculation of lipid indices, oxidizability, and oleic and linoleic desaturation ratios of sonicated tea seed oils.

Lipid Indices	Control	5 min	15 min	25 min
Nutritive Value Index (NVI)	4.79	4.74	4.77	4.81
Atherogenic Index (AI)	0.168	0.178	0.161	0.172
Thrombogenic Index (TI)	0.216	0.375	0.339	0.342
OFA	13.74	13.94	13.51	13.73
Oxidizability value (Cox)	2.84	2.81	2.84	2.67
Oleic Desaturation Rate value (ODR)	0.246	0.243	0.262	0.245
Linoleic Desaturation Rate value (LDR)	0.02	0.043	0.107	0.043

Additionally, the Thrombogenic Index (TI) and OFA values of sonicated tea seed oils ranged between 0.216 (control) and 0.375 (5 min), and between 13.51 (15 min) and 13.94 (5 min), respectively. The NVI, AI, TI, and OFA index values of the sonicated tea seed oils showed slight variations depending on the duration of sonication. The Atherogenic and Thrombogenic Index values of the sonicated tea seeds were found to be marginally higher than those of the control group. Interactions between raw and roasted samples were observed in terms of PUFA n-3/n-6 ratios. The type of heat treatment influenced the proportion of unsaturated fatty acids (UFAs) in the seed samples depending on the raw material. Overall, heat treatment affected NVI, AI, and TI values across all samples [28]. The considerable variation in index values according to the fatty acid composition of the material may be attributed to genetic factors, growing conditions, and the application of different roasting methods and parameters.

The differences in oleic and linoleic desaturation ratios are presented in Table 3. Across all samples, LDR values were very low due to the low level of linolenic acid in tea seed oil. The mean ODR value was 0.249, while the mean LDR value was 0.058. The minimum ODR value observed in tea seed germplasm was 0.243 (5 min), whereas the maximum was 0.262 (15 min). In this context, the 5 min treatment showed the highest oxidizability value (13.94), while the 15 min treatment showed the lowest value (13.51) (Table 3). Mondal et al. [36] reported average ODR and LDR values of 0.5 and 0.01, respectively. El-Beltagi et al. [37] found that oxidizability, ODR, and LDR values for sesame seed oil roasted using different methods ranged between 4.97 and 5.06, 0.5143–0.5172, and 0.0115–0.0136, respectively. Peanut oil exhibits a high degree of oleic unsaturation (ODR), as determined by evaluating unsaturation pathways using the oleic desaturation ratio (ODR) and linoleic ratio (RL), indicating a high level of oleic acid in the germplasm. This study examined the effects of different sonication durations on oxidizability (Cox value), ODR, and LDR indices in tea seed oil. Sonication was found to increase the oxidizability of the oil. However, the Cox values showed only minor differences which may be useful for evaluating the oxidative stability of vegetable oils [38]. The average ODR value was considerably higher than the LDR value, indicating a strong conversion of C18:2 and limited synthesis of C18:3. Similar ratios were reported by Meriem et al. [39] and Kurt [40], although the mean ODR values (0.372–0.87) in this study were somewhat lower than those reported by Buhunia et al. [41] (0.43). A high ODR indicates a greater conversion of oleic acid to linoleic acid, suggesting higher efficiency [36,42]. The estimation of oxidizability (COX) is important for assessing an oil's resistance to oxidation and its overall stability [43]. The fatty acid ratios determined in this study may help estimate the efficiency of desaturation pathways and support the development of future food strategies.

3.5. Principal Component Analysis (PCA)

Figure 3 shows the principal component analysis (PCA) of tea seeds sonicated at different times in relation to phenolic constituents, total phenolic content, total flavonoid quantities, and antioxidant capacity.

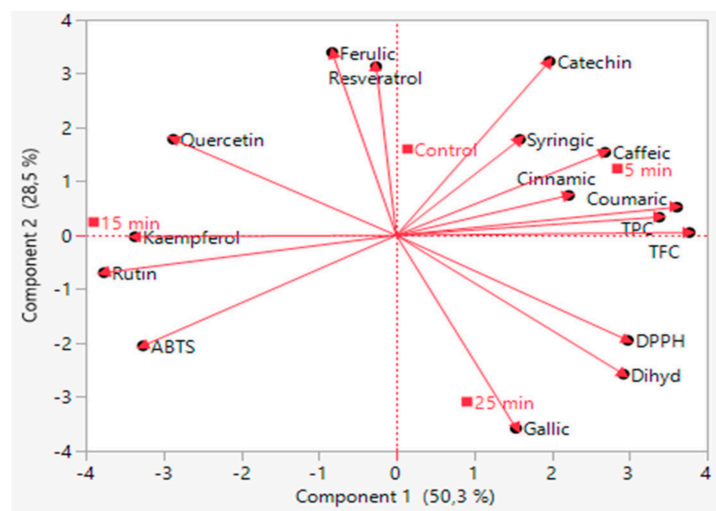


Figure 3. Biplot graph drawn with results of PCA (TPC: Total phenolic content, TFC: Total flavonoid content, Dihyd: 3,4-Dihydroxybenzoic acid).

The first two PCs explain 50.276% and 28.540% of the total variance, respectively (Table 5).

Table 5. PCA results in relation to the bioactive properties of tea seeds.

	PC1	PC2
Eigenvalue	8.044	4.566
Variability (%)	50.276	28.540
Cumulative %	50.276	78.816
Correlation		
Gallic	0.395	−0.919
3,4-Dihydroxybenzoic acid	0.750	−0.661
Catechin	0.505	0.829
Caffeic	0.689	0.396
Syringic	0.408	0.457
Rutin	−0.966	−0.178
Coumaric	0.926	0.134
Ferulic	−0.212	0.871
Resveratrol	−0.067	0.804
Quercetin	−0.737	0.458
Cinnamic	0.569	0.190
Kaempferol	−0.864	−0.006
TPC	0.868	0.087
TFC	0.968	0.014
DPPH	0.764	−0.499
ABTS	−0.837	−0.525

PCA results in relation to bioactive properties of olives.

A strong positive correlation with PC1 was found for coumaric acid (0.926), total phenolic content (0.868), and total flavonoid content (0.968). In contrast, rutin (−0.966) and kaempferol (−0.864) showed strong negative correlations with PC1. PC2, on the other hand, was mainly associated with catechin (0.829), ferulic acid (0.871), and resveratrol

(0.804), indicating that these compounds contributed more strongly to the second principal component. Regarding sample distribution, the control sample and the seeds sonicated for 5 min were positioned in the positive quadrant of both PC1 and PC2. These samples also exhibited higher levels of total phenolic content (TPC), total flavonoid content (TFC), catechin, and coumaric acid, which were clustered in the same region of the analysis. In contrast, the sample treated with 15 min of sonication was located in the negative region of PC1 but remained in the positive region of PC2. This sample was characterized by the lowest levels of most phenolic compounds, except for kaempferol, rutin, and quercetin, and it also showed reduced antioxidant activity as measured by the ABTS assay.

4. Conclusions

This study demonstrates that sonication is an effective processing strategy for modifying the chemical characteristics of tea seeds, with treatment duration playing a decisive role in determining extraction performance and oil quality. Although prolonged sonication did not improve oil yield, the results indicate that ultrasonic treatment can selectively influence the retention and transformation of bioactive compounds and fatty acids, highlighting the importance of optimizing processing conditions to achieve specific quality objectives. These findings suggest that ultrasound should be considered not only as a tool for extraction enhancement but also as a means of regulating the nutritional and functional properties of tea seed oil. Despite the compositional changes induced by sonication, tea seed oil maintained a favorable fatty acid profile comparable to that of widely consumed edible oils such as olive and sunflower oils, while its very low erucic acid content further supports its potential suitability for food applications. Overall, this work provides valuable insights into the application of ultrasonic-assisted processing as a green and sustainable technology for producing value-added edible oils. The approach may also be extended to other oilseed materials, offering opportunities to improve extraction efficiency and tailor oil composition for applications in the food, nutraceutical, and functional ingredient industries.

Author Contributions: M.E.E.-D.I.: Investigation, methodology, validation, editing; M.M.Ö.: Formal analysis, writing, editing; N.U.: Methodology, formal analysis; M.N.A.: Data curation, editing. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [GRANT: KFU263551].

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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