

## The Effect of Sonication Times on the Bioactive Compounds, Antioxidant Activity, Fatty Acid Profiles and Health Indices of the Oat Seeds

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### ABSTRACT

Oil yields of raw and sonicated oat seeds varied between 6.20 (raw, control) and 12.55% (30 min). Total phenol and flavonoid contents of raw (control) and sonicated oat seeds were determined between 42.01 (control) and 50.11 mgGAE/100g (30 min) to 12.83 (control) and 16.83 mg/100 g (30 min), respectively. The antioxidant activities of oat seeds depending on the sonication times varied between 4.88 (control) and 4.96 mmol/kg (30 min). Fluctuations in the phenolic compounds of oat seed extracts were observed depending on the sonication duration. The dominant phenolic acids and flavonoids in raw (control) and sonicated oat seeds were gallic acid, protocatechuic acid, caffeic acid, quercetin, and kaempferol. The main fatty acid in oat seed oils was linoleic acid, followed by linolenic, oleic, and behenic acids. Linoleic and linolenic acid contents in oils obtained via ultrasound-assisted extraction ranged from 53.28% (30 min) to 72.42% (control) and 21.46% (control) to 42.71% (30 min), respectively.

**Keywords:** oat, sonication, phytoconstituents, antioxidant activity, phenolics, fatty acids.

### INTRODUCTION

Oat (*Avena sativa* L.), which grown in cool and humid weather conditions, is a biennial herbaceous plant belonging to the Gramineae (Poaceae) family (Kim et al., 2021). Oat, which ranks 7<sup>th</sup> in production after corn, wheat, rice, barley, sorghum and millet among oat plant cereal crops, is grown as an annual crop in various regions of the world and grain products are widely grown around the world to meet the needs of people (Özcan et al., 2006; Mao et al., 2022). The most widely cultivated the *Avena* genus is the type of oat with peeled grains, common oat (*Avena sativa* L.) (Kourimska et al., 2018). Food oat, which has excellent moisture retention properties that keep baked goods fresh, is used primarily in hot and instant cereals, baked goods, snack foods, granola, cookies, bread, and other grain products (Özcan et al., 2006). As interest in the possible use of oats in human nutrition

increases, its use as animal feed is gradually decreasing (Ahmad et al., 2010). The oil amounts of oat seeds change depending on the variety, oil contents of oat grain can vary from 3 to 11% (Ciolek et al., 2012). The main elements that make oat oil a healthy food are that it is rich in fat-soluble vitamin E and polyunsaturated fatty acids (Zhou et al., 1999). Since oat grains have high nutritional and energy values, oat-based foods have a high nutritional value because they are rich in slowly digestible starch and protein and contain high amounts of exogenous amino acids, unsaturated fatty acids, fiber and minerals (Rasane et al., 2015; Allès et al., 2017). While oleic, linoleic and linolenic acid in oat seeds constitute the main unsaturated fatty acids of the oil, myristic, palmitic and stearic acids are the main saturated fatty acids and constitute approximately 90-95% of all fatty acids (Bagcı et al., 2019; Culetu et al., 2020; Nogala-Kalucka et al., 2020; Martin-Diana et al., 2021). Recently, oats have

received more attention as a functional food, considering that unsaturated fatty acids lower blood cholesterol (Varma et al., 2016; Diaz et al., 2020; Shvachko et al., 2021). Oats are a cereal rich in unsaturated fatty acids and polyphenols (Paudel et al., 2021). Phenolic acid and its derivatives show antimicrobial, anticancer and antimutagenic effects because they inhibit superoxide formation (Kumar and Goel, 2019; Zhang et al., 2021).

While traditional techniques are used for the extraction of various bioactive compounds from plant products, many of these techniques require high temperatures, require long extraction times, and use of high solvents, which can destroy some bioactive compounds (Wen et al., 2019; Aryanti et al., 2021). Ultrasound-assisted extraction significantly reduces extraction time, solvent usage, and energy while preserving the natural properties of biomolecules (Carreira-Casais et al., 2021; Zhao et al., 2024). Ultrasound-assisted extraction is known for its effectiveness in disrupting cell walls and improving mass transfer, leading to higher extractable yields (Staicu et al., 2023; Khalid et al., 2024; Siol et al., 2025). Oats and oat-based products are gaining increasing popularity in today's healthy food market

because they contain high levels of functional food components that reduce the risks of disease and metabolic disorders when consumed regularly (Shvachko et al., 2021). This study aimed to evaluate the bioactive properties, phenolic compounds, and fatty acid profiles of raw and sonicated oat seeds. The effects of different sonication durations on bioactive compounds, antioxidant capacity, and fatty acid composition were investigated, with the goal of establishing usage standards based on the compositional characteristics of the seeds.

## MATERIAL AND METHODS

### Material

Oat (*Avena sativa* L.) seeds (Diriliş cv) were provided from a farmer in Konya district in Turkey in 2025. The oat seeds were cleaned of foreign substances such as stem, leave and dust.

### Methods

#### Moisture content

The KERN & SOHN GmbH infrared moisture analyser was used for the moisture amounts of oat seed samples.

	Moisture content (%)
Oat sample	7.16 ± 0.25

### Extraction procedure

The method recommended by Vaher et al. (2010) with some modifications was used for the extraction of bioactive and phenolic compounds from oat seed samples. After the ground samples (2 g) were mixed with 20 ml of methanol:water (80:20 v/v), the mixture was treated separately in an ultrasonic water bath for 15 and 30 min and then centrifuged at 6000 rpm for 10 min. The control sample was kept in a shaking water bath for 30 min. After the pretreatments were completed, the supernatants were filtered through a filter paper before analysis.

### Total phenolic content

Total phenolic amount of oat seed extracts was determined using Folin-Ciocalteu (FC)

reagent according to the method suggested by Yoo et al. (2004).

### Total flavonoid content

Total flavonoid amount of oat seed extracts was determined using 0.3 ml of NaNO<sub>2</sub>, 0.3 ml of AlCl<sub>3</sub> and 2 ml of NaOH reagents for 1 ml extract according to the study recommended by Hogan et al. (2009). The findings are described as mg quercetin (QE)/100 g.

### DPPH free radical scavenging activity

The free radical scavenging activities of extracts were determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) according to Lee et al. (1998). The findings were stated as mmol trolox (TE)/kg.

### Determination of phenolic compounds

HPLC (Shimadzu) equipped with a PDA detector and an Inertsil ODS-3 (5  $\mu$ m; 4.6 x 250 mm) column was applied for analysis and chromatographic separation of phenolic compounds of oat seeds. The peaks were taken at 280 using a PDA detector. The total running time per sample was 60 min.

### Oil extraction

The ground oat seeds (10 g) in filter paper were added into 180 mL of petroleum benzine in a flask, and they were kept separately in ultrasonic bath for 15 min and 30 min. Control sample was kept in shaking water bath for 30 min. The solvent was removed with a rotary vacuum evaporator at 50°C.

### Fatty acid composition

Fatty acid methyl esters of oat oils esterificated according to method of Multari

et al. (2019) were analysed by gas chromatography (Shimadzu GC-2010) equipped with flame-ionization detector (FID) and capillary column.

### Statistical analysis

JMP version 9.0 was used for analysis of variance (ANOVA). The results are mean $\pm$ standard deviation (MSTAT C) of independent sonication times, and the divergences in means were calculated utilizing Duncan's multiple rate tests with  $p < 0.05$ .

## RESULTS AND DISCUSSION

### *Phytoconstituent contents and antioxidant activities of oat seeds sonicated for different times*

Oil yields and phytochemicals and antioxidant activities of oat seeds sonicated for different times are given in Table 1.

Table 1. Oil content and phytochemical properties of oat seeds sonicated at different times

Sonication time	Oil content (%)	Total phenolic content (mgGAE/100 g)	Total flavonoid content (mg/100 g)	Antioxidant activity (mmol/kg)
Control	6.20 $\pm$ 0.00c*	42.01 $\pm$ 0.72c	12.83 $\pm$ 0.30c	4.88 $\pm$ 0.04c
15 min	10.05 $\pm$ 0.07b	45.62 $\pm$ 0.51b	16.17 $\pm$ 3.41b	4.91 $\pm$ 0.04b
30 min	12.55 $\pm$ 0.35a	50.11 $\pm$ 0.72a	16.83 $\pm$ 0.70a	4.96 $\pm$ 0.06a

\*standard deviation and values within each column followed by different letters are significantly different at  $P < 0.05$

The applied sonication durations influenced both the oil yield and the bioactive properties of oat seeds. Oil amounts of raw and sonicated oat seeds varied between 6.20 (raw, control) and 12.55% (30 min). Total phenol and flavonoid contents in raw (control) and sonicated oat seeds ranged from 42.01 mgGAE/100 g (control) to 50.11 mgGAE/100 g (30 min) for total phenols, and from 12.83 mg/100 g (control) to 16.83 mg/100 g (30 min) for flavonoids.

Antioxidant activity varied slightly with sonication time, ranging from 4.88 mmol/kg (control) to 4.96 mmol/kg (30 min).

### *The phenolic compounds and their amounts of oat seeds extracted at different sonication times*

The phenolic compounds in oat seeds sonicated for different durations are shown in Table 2.

Table 2. Phenolic compounds of oat seeds sonicated at different times

Phenolic acids (mg/100 g)	Control		15 min		30 min	
Gallic acid	3.60	± 0.32a*	2.68	± 0.29c	2.97	± 0.45b
Protocatechuic acid	0.49	± 0.13c	2.13	± 0.27a	1.91	± 0.25b
Chlorogenic acid	1.19	± 0.42a	0.26	± 0.08c	0.90	± 0.28b
Caffeic acid	2.22	± 0.02a	2.09	± 0.31b	1.73	± 0.53c
Coumaric acid	0.28	± 0.03b	0.26	± 0.03c	0.31	± 0.07a
Ferulic acid	0.05	± 0.01b	0.21	± 0.03a	0.05	± 0.01b
Cinnamic acid	3.69	± 0.27a	0.21	± 0.07b	0.16	± 0.01c
Flavonoids (mg/100 g)	Control		15 min		30 min	
Catechin	0.47	± 0.04a	0.31	± 0.10b	0.19	± 0.04c
Rutin	0.96	± 0.43a	0.17	± 0.02c	0.27	± 0.01b
Hesperidin	0.45	± 0.12b	0.26	± 0.09c	4.58	± 0.19a
Quercetin	8.06	± 1.11a	3.50	± 0.11b	2.30	± 0.24c
Kaempferol	0.77	± 0.14c	1.66	± 0.29b	1.97	± 0.62a

\*standard deviation and values within each row followed by different letters are significantly different at  $P < 0.05$

Fluctuations in the phenolic constituents of oat seed extracts were observed depending on the sonication duration. The dominant phenolic acids and flavonoids in raw (control) and sonicated oat seeds were gallic acid, protocatechuic acid, caffeic acid, quercetin, and kaempferol. Gallic and protocatechuic acid amounts of the raw (control) and sonicated oat seeds were described to be between 2.68 (15 min) and 3.60 (control) to 0.49 (control) and 2.13 mg/100 g (15 min), respectively. Caffeic acid contents of the oat seeds were identified to

between 1.73 (30 min) and 2.22 mg/100 g (control). Quercetin and kaempferol amounts of the raw (control) and sonicated oat seeds were assessed to be between 2.30 (30 min) and 8.06 (control) to 0.77 (control) and 1.97 mg/100 g (30 min), respectively.

#### *Fatty acid profiles of the oils extracted from oat seed at different sonication times*

The fatty acid compositions and amounts of oils obtained from oat seeds after sonication for various durations are assigned in Table 3.

Table 3. Fatty acid composition of the oils extracted from oat seed sonicated at different times

Fatty acids (%)	Control		15 min		30 min	
Stearic	0.26	± 0.01a*	0.23	± 0.01b	0.19	± 0.00c
Oleic	4.07	± 0.03a	2.82	± 0.03b	2.46	± 0.00c
Linoleic	72.42	± 0.09a	53.30	± 0.03b	53.28	± 0.01bc
Arachidic	0.36	± 0.03	-**		-	
Linolenic	21.46	± 0.03c	42.38	± 0.03b	42.71	± 0.01a
Behenic	1.42	± 0.05a	1.26	± 0.02c	1.35	± 0.02b

\*standard deviation and values within each row followed by different letters are significantly different at  $p < 0.05$

\*\* -: Not detected

The fatty acid found most abundantly in oils extracted from oat seeds was linoleic, followed in decreasing order by linolenic, oleic, and behenic acid. Linoleic and linolenic acid amounts of the oils obtained from oat seeds by ultrasound-assisted extraction system at different times were characterized to be between 53.28 (30 min) and 72.42% (control) to 21.46 (control) and 42.71% (30 min), respectively. While oleic acid amounts of the oils vary between 2.46

(30 min) and 4.07% (control), behenic acid amounts of the oat oils were detected to be between 1.26 (15 min) and 1.42% (control). Arachidic acid was detected only in the oil of the raw (control) oat seed sample.

#### *Oxidizability, oleic and linoleic desaturation ratios of oat seed oils*

The differences in oleic and linoleic desaturation values are shown in Table 4.

Table 4. Oxidizability, oleic and linoleic desaturation ratios of the oils extracted from oat seed sonicated at different times

Indices	Control	15 min	30 min
Oxidizability (Cox)	12.14	14.67	14.71
Linoleic desaturation ratios (LDR)	0.958	0.971	0.975
Oleic desaturation ratios (ODR)	0.229	0.443	0.445

The average ODR value was 0.372, while the average LDR value was 0.968. The minimum ODR value determined for oat germ plasm was 0.229 (Control), while the maximum ODR value was 0.445 (30 min). In this context, 30 min sonication process has a maximum oxidizability value of 14.71, while the control sample has a minimum value of 12.14 (Table 4).

Considering the control group, a gradual increase was monitored in the oil, total phenol, total flavonoid amounts and antioxidant activities of oat seeds with increasing sonication times, and unroasted linseed seeds showed a linear increase with time in both extraction systems. This increase may be due to the deformation of the cell wall structure, which contains phenolic compounds, and the increased release of phenolic compounds with the extraction time in both extraction systems. In addition, this increase may be due to the reaction of Maillard reaction products formed during roasting with Folin-Ciocalteu reagent and the increase in color pigments resulting from caramelization. Crude lipid content of oat seeds is 6.9% on average and varies from 5.91 to 7.87% (Krasilnikov et al., 2018). The oil contents of oat seeds changed between 2.06 to 12% (Özcan et al., 2006; Kim et al., 2021). In addition, the oil contents of some naked oat varieties were established to be between 4.14 g/100 g (dw) (Santini) to 6.68 g/100 g (dw) (Kamil) (Pokhrel et al., 2025). Since the optimum extraction time depends on the stability of molecules during sonication, longer extraction time (60 min) was needed to obtain the optimum total phenol content of *Polygonum aviculare* leaves (Wu et al., 2021). Total phenolic amounts and antioxidant activity values of rapeseed extracts extracted from ultrasound-assisted and conventional extraction were

determined to be between 79.25 and 86.08 mgGAE/g and 99.28 and 110.81 mgTE/g, respectively (Cisneros-Yupanqui et al., 2023). In another study, total phenol and flavonoid amounts of oat varieties were assessed to range from 36.07 to 101.56 mgGAE/g and 754.16 to 1147.08 mgQE/g, respectively (Ibrahim et al., 2020). Total phenolic contents of all oat varieties ranged 50.88-101.56 mg/100 g (Jiang et al., 2021). The total phenol amount of oat seeds ranged from 36.07 to 101.56 mgGAE/100 g DW (Jiang et al., 2021). Smuda et al. (2018) determined the radical scavenging activity values of different grain milling products as  $39.3 \pm 0.1\%$  to  $70.6 \pm 2.00\%$ . Furthermore, the radical scavenging activity values of all oat varieties varied between 24.33 and 55.88% (Jiang et al., 2021). Varietal differences, such as analytical protocols and temperature, may account for slight differences in radical scavenging activity among oat cultivars (Jiang et al., 2021). Total flavonoid amount of oat seeds has been pointed out to be affected by seed color and temperature (Jiang et al., 2021). It increases the extraction efficiency by combining the acoustic cavitation created by ultrasonic sounds with the extraction solvent (Rodsamran and Sothornvit, 2019; Rao et al., 2021).

In general, decreases in the phenolic acid and flavonoid amounts of oat seed extracts were observed compared to the control, parallel to increases in sonication time. However, gallic acid, coumaric acid, hesperidin, and kaempferol contents of the extracts increased compared to the control. Furthermore, gallic acid, chlorogenic acid, coumaric acid, rutin, hesperidin, and kaempferol contents of oat seeds sonicated for 30 min were higher than those of oat samples sonicated for 15 min. The primary

reason for the increase in phenolic constituents in oils with ultrasound-assisted extraction systems is that these technologies disrupt cell structure or improve the extraction environment, increasing the contact of phenolic compounds with the solvent, thus allowing for greater phenolic release. Furthermore, ultrasound waves create more microscopic bubbles in the liquid medium, which rapidly grow and burst, leading to high temperature and pressure differences that disrupt plant cell walls, leading to an increase in phenolic compounds. These fluctuations in phenolic compound amounts may be due to factors such as seed maturity, structural composition of phenolic-containing cell tissues, and temperature and pressure differences during sonication. Oat cultivars contained 2.99-5.19 vanillic acid, 15.03-16.33 gallic acid, 13.18-16.68 caffeic acid, 0.36-0.60 4-Hydroxyphenylacetic acid, 42.26-45.18 protocatechuic acid, 143.95-147.13 ferulic acid, 2.24-4.56 *p*-coumaric acid and 1.35-2.40 mg/100 g cinnamic acid (Ibrahim et al., (2024). Jiang et al. (2021) reported that seven oat cultivar seeds contained between 2.99-5.19 vanillic acid, 29.94-103.00 gallic acid, 13.18-16.68 caffeic acid, 42.26-45.18 syringic acid, 143.95-147.13 ferulic acid, 2.24-4.56 *p*-coumaric acid and 0.43-3.64 mg/100 g chlorogenic acid. The key polyphenolic compounds of oat seeds were protocatechuic, syringic, vanillic, *p*-hydroxybenzoic, gallic, *p*-coumaric, *o*-coumaric, and caffeic acids (Kumar and Goel, 2019; Soycan et al., 2019). Various studies have shown that the phenolic compounds detected in oats are caffeic acids, coumaric acids, gallic acids, hydroxybenzoic acids, protocatechuic acids, syringic acids and vanillic acids (Wilson et al., 2017; Martin-Diana et al., 2021). Manzoor et al. (2023) reported that varieties were highly effective on the phenolic contents of plant materials.

With increasing extraction time, only the linolenic fatty acid content of the oils increased significantly, while the amounts of other identified fatty acids decreased. Therefore, the highest amounts of fatty acids

(except linolenic) were detected in the raw (control) oil. Changes in the fatty acid composition of the extracted oat oils were observed depending on the sonication duration. These changes are likely due to the maturity of the seed and the oxidation of unsaturated structures during extraction. Capouchova et al. (2021) reported that the oil extracted from oat cultivars contained 0.26-0.37 myristic, 17.10-19.80 palmitic, 0.22-0.34 palmitoleic, 1.42-1.62 stearic, 34.76-38.47 oleic, 37.80-40.00 linoleic, 0.15-0.16 arachidic, 2.02-2.28 linolenic, 0.11-0.13% behenic acids. The predominant fatty acids of oat seed oils were palmitic (18.67-21.14%), stearic (1.21-2.46%), linoleic (36.03-41.16%) and oleic (27.8-37.1%) (Kourimska et al., 2018). The predominant fatty acids in cereal oils were palmitic (15.3-17.8%), stearic (1.05-1.87%), oleic (33.5-36.7%) and linoleic (36.2-38.7%) acids (Krasilnikov et al., 2018). The oil extracted from oat seed contained palmitic (15.72%), oleic (33.97-51.26%) and linoleic (22.80-35.90%) acids (Özcan et al., 2006). The naked oat oils contained 36.2-38.7% linoleic, 33.5-36.7% oleic and 15.3-17.8% palmitic (Batalova et al., 2019). Pokhrel et al. (2025) determined 0.46 and 0.57% myristic, 21.58 and 24.81 palmitic, 2.46 and 2.68 stearic, 35.99 and 34.85 oleic, 33.91 and 35.43 linoleic, 2.22 and 1.75% linolenic acids in Atego and Korok oat cultivar seed oils. Our findings regarding the amounts of fatty acids showed partial differences with the findings of Sterna et al. (2016), who found that the oleic acid content in oat varieties varied between 36.2% and 40.0% and the linoleic acid content varied between 38.4% and 41.6%, or with Saastamoinen et al. (1989), who found that the oleic acid content varied between 37.2% and 42.1%, the linoleic acid content varied between 38.6% and 42.5%, and the palmitic acid content varied between 15.5% and 17.4%. Martinez et al. (2010) reported that the composition of fatty acids is more influenced by the environment than by the genotype.

The present study examined the effects of different sonication times on oxidizability (Cox value) and ODR and LDR indices on

oat oil. Across all samples, ODR values were established to be very low due to the low oleic acid level in oat oil. Mondal et al. (2010) reported an average ODR value of 0.5 and an average LDR value of 0.01. El-Beltagi et al. (2022) reported that Oxidizability, ODR, and LDR indices for oil recovered from sesame seeds roasted by different methods were determined to be between 4.97-5.06, 0.5143-0.5172 and 0.0115-0.0136, respectively. Oat oil has a low degree of oleic unsaturation (ODR) as revealed by estimating the activity of unsaturation pathways using oleic unsaturation ratio (ODR) and linoleic ratio (RL). This indicates that the amount of oleic acid in the germplasm is low. Sonication times were found to increase the oxidizability values of oat oil. The Cox score for the oil showed only minor differences and should be used to protect vegetable oils from oxidative degradation (Biglar et al., 2012). The average value of ODR is considerably higher than that of RDL, explaining the strong increase in C18:2 and the weak synthesis of C18:3. Similar ratios were reported by (Meriem et al., 2015; Kurt, 2018). The oleic unsaturation ratio (ODR) and linoleic unsaturation ratio (LDR) were developed to overcome this problem, as the fatty acid biosynthetic pathway is highly interconnected, making it difficult to assess suitable varieties with beneficial health traits based solely on phenotypic characteristics (Pleines and Friedt, 1988; Mondal et al., 2010; Bhunia et al., 2015). Estimating oxidizability (COX) helps determine an oil's ability to resist oxidation and, therefore, its stability (Coni et al., 2004). Oils with higher oxidizability values have a higher tendency to self-oxidize (Dar et al., 2019). The fatty acid ratios established in this study will help in estimating the relative efficiency of desaturation pathways and in designing strategies for future foods.

## CONCLUSIONS

This study provides insight into the effects of sonication duration on the oil content, phytochemical composition, and fatty acid

profile of oat grains. Given their high phytochemical content, oat seeds are recommended for use in health-promoting food products. The results indicate that oat lipids are rich in biologically important fatty acids, particularly linoleic acid. Oats represent an affordable source of high-quality phenolic and other bioactive compounds, and their extraction through sonication or ultrasound-assisted techniques can produce high-value-added compounds with both technological and biological potential.

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