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Quality attributes of oil extracted from hazelnuts treated with gaseous ozone

A.S. Demirci[a,✉], G. Tirpanci Sivri[a] and M. Tunc[a]

[a]Department of Food Engineering, Tekirdag Namik Kemal University, 59030 Tekirdag, Turkey
[✉]Corresponding author: ademirci@nku.edu.tr

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SUMMARY: In this study, the impact of ozonation on hazelnut oil quality was investigated. Hazelnuts were exposed to gaseous ozone at different concentrations (3.3 and 10 mg·L^{-1}) and exposure times (30, 60, and 120 min). The fatty acid value and composition remained unchanged. β–sitosterol, campesterol, and Δ5–avenasterol contents were unaffected. With increasing ozone levels and exposure times, there was a slight rise in peroxide value and γ–tocopherol, and a decrease in α–tocopherol. The total phenolic content and antioxidant activity were lower in oil extracted from hazelnuts which had been ozonated for more than 60 min at both doses, compared to the control. Overall, the quality and composition of hazelnut oil remained stable with ozone treatments, depending on the treatment conditions

KEYWORDS: *Hazelnut oil; Lipid profile; Oil quality; Ozonation.*

RESUMEN: ***Atributos de calidad del aceite extraído de avellanas tratadas con ozono gaseoso***. En este estudio, se investigó el impacto de la ozonización en la calidad del aceite de avellana. Las avellanas fueron expuestas a ozono gaseoso en diferentes concentraciones (3,3 y 10 mg·L^{-1}) y tiempos de exposición (30, 60 y 120 min). El valor y la composición de los ácidos grasos se mantuvieron sin cambios. Los contenidos de β-sitosterol, campesterol y Δ5-avenasterol no se vieron afectados. Con el aumento de los niveles de ozono y los tiempos de exposición, hubo un ligero aumento en el valor de peróxidos y γ-tocoferol, y una disminución en el α-tocoferol. El contenido fenólico total y la actividad antioxidante fueron menores en el aceite extraído de avellanas ozonizadas durante más de 60 minutos en ambas dosis, en comparación con el control. En general, la calidad y composición del aceite de avellana se mantuvieron estables con los tratamientos con ozono, dependiendo de las condiciones del tratamiento.

PALABRAS CLAVE: *Aceite de avellana; Calidad del aceite; Ozonización; Perfil lipídico.*

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1. INTRODUCTION

Hazelnuts are usually used in chocolate, confectionary and bakery products (Alasalvar *et al*., 2008). It is also used in the production of hazelnut oil because of its high oil content. Hazelnut oil is generally used for various purposes such as cooking, salad dressings and flavorings (Król *et al*., 2021). The total unsaturated fatty acid rate found in hazelnut oil is over 90%, particularly oleic (77.8%) and linoleic (10.1%) acids. Tocopherols, squalene, phytosterols, carotenoids and other bioactive substances are also available in hazelnut oil (Alasalvar *et al*., 2006). There is a growing interest on the part of consumers in hazelnut oil because of its beneficial effects on health. The primary concern of hazelnut oil is its oxidative stability because of its high unsaturated fatty acid level.

Previous researches have established that the application of gaseous ozon is a very effective method to provide food safety to nuts (Atakan *et al*., 2021, Davies *et al*., 2021). Since ozone can inactivate microorganisms, it prevents the growth of molds which cause aflatoxin problems (Babaee *et al*., 2022). In a previous study, Demirci *et al.* (2023) evaluated the potential of gaseous ozone to degrade aflatoxin in hazelnuts and stated that it accomplishes a decline in aflatoxins below the legal limits. However, one of the greatest challenges faced by gaseous ozone application to hazelnut is the oxidation of unsaturated fatty acids since ozone has high oxidation capacity (Pandiselvam *et al*., 2018). Uzun *et al.* (2018) revealed that longer ozone treatment time applied directly to the hazelnut oil resulted in the production of oxidation products and the disappearance of double bonds of unsaturated fatty acids. Moreover, the bioactive compounds in hazelnut may be deteriorated by ozone reactions (Taş and Gökmen, 2015). It is important to determine the proper processing conditions to have oil extracted from ozone-applied hazelnuts without the deteriotion of quality attributes.

On the other hand, there are many studies which affirmed that ozon can help to maintain the quality of the hazelnut oil by inactivating enzymes that can cause rancidity, off-flavors, and off-odors, and thus extend the shelf life of the oil (de Oliveira *et al*., 2020). In addition, ozonation is an environmentally-friendly process because ozone is a natural gas which decomposes into oxygen after use and does not leave any harmful residues (Pandiselvam *et al*., 2018).

The objective of this research is to investigate the effect of ozone treatment which is applied to hazelnuts with the aim of providing food safety, on the quality of hazelnut oil. For the purpose of research, hazelnut samples were treated with 6 different gaseous ozone applications (2 concentrations and 3 time periods). Following the ozone treatment of the hazelnuts, the samples were used to obtain hazelnut oil. The hazelnut oil samples were then carefully analyzed to determine quality parameters, including fatty acid profile and bioactive compounds.

2. MATERIALS AND METHODS

2.1. Materials

The hazelnut samples (Tombul cultivar) were supplied from a local producer in Giresun in 2019. The hazelnut kernels with testas (outer skin) were unroasted and stored in vacuum packs under refrigeration (4 °C) until use. All chemicals were purchased from commercial sources (Sigma Aldrich and Merck Chemical Co.). The gaseous ozone was generated on site using a corona discharge ozone device (PSC Ozon., Istanbul, Turkey). Ozone concentration was monitored throughout the experiment with the use of an ozone detector (PSC Ozon., Istanbul, Turkey).

2.2. Ozone treatment

The unshelled hazelnut samples were packed in 200-g portions for each ozone treatment. A total of 6 ozone treatments in different conditions were applied. Gaseous ozone treatment was conducted in an airtight chamber attached with an ozone detector. In order to treat the samples with ozone gas, the hazelnut samples were placed inside the chamber with gas inlet and outlet parts. The internal relative humidity of the chamber was kept at 70±5%. The hazelnut samples were kept in the chamber for 30, 60 and 120 minutes with the flow of gaseous ozone at concentrations of 200 and 600 $mg \cdot h^{-1}$ which resulted in 3.33 and 10 $mg \cdot L^{-1}$ ozone concentration inside the chamber. The residual ozone was destructed in a thermal unit. One portion of hazelnut samples was not treated with ozone and kept as the control.

2.3. Hazelnut oil extraction

Hazelnut samples exposed to ozone and control samples were grounded by a Waring grinder (WSG60, Conair Corporation, USA) separately, for 2 minutes. The ground samples were dried in the oven at 105 °C for 4 hours prior to lipid extraction. The dried samples were subjected to Soxhlet extraction with hexane (AOAC, 2000a). The solvent was removed from the extracts by evaporation under reduced pressure using a rotary evaporator (Heidolp, Hei-Vap Precision ML/G3B, Germany).

2.4. Peroxide value (PV) and free fatty acids (FFA) analysis

The iodometric titration method was used to determine the peroxide value in compliance with the International Union of Pure and Applied Chemistry (IUPAC) standard method 2. 501 (Dieffenbacher and Pocklington, 1992). The oil sample (1 g) was dissolved in a 10-mL of acetic acid:chloroform mixture (3:2). After the addition of 1 g of potassium iodide, the tube was stirred steadily for 1 min and then kept in the dark for 5 min. 50 mL of distilled water and 1 mL of 1% freshly prepared starch solution were added to the flask. The mixture was then gradually titrated against a 0.002-N sodium thiosulfate ($Na_2S_2O_3$) solution until the color changed to white. The peroxide values were expressed as milliequivalents of $O_2 \cdot kg^{-1}$ ($meq \cdot kg^{-1}$) oil.

The FFA was determined using the titration method stated in AOAC (940.28) with some modifications (AOAC 2000b). The hazelnut oil samples were mixed with a 1% phenolphthalein indicator (prepared in 70% ethanol) and then titrated with 0.05 mol/L sodium hydroxide (NaOH; prepared in 70% ethanol) until the mixture turned pink. The concentration of free fatty acids was expressed as oleic acid molar mass in g/mol.

2.5. Fatty acid methyl ester (FAME) preparation

Methyl esters of fatty acids were prepared primarily by the procedure described by Dieffenbacher and Pocklington (1992) with some modification for the determination of fatty acid composition. Methyl esters are required to make derivatives volatile for a GC analysis of fatty acids. To perform the derivatization, 100 mg of oil extract and 10 mL of hexane were mixed at room temperature until completely dissolved. Then, 0.5 mL KOH 2 N (in MeOH) were added to the solution and mixed by vortex for 30 s. The solution was kept in the dark for 1-2 h until the supernatant was transparent. The supernatant was collected in a GC vial.

2.6. Fatty acid composition analysis with gas-chromatography (GC-FID)

The methyl ester forms of fatty acids were injected into a TR-CN100 column (100 m × 0.25 mm I.D., 0.20 μm film thickness; Teknokroma, Spain) for seperation. The column was connected to a Shimadzu 2010 Series (Shimadzu, Tokyo, Japan) GC with a flame ionization detector (FID). The carrier gas used was helium at a flow rate of 0.5 mL min^{-1}. The temperature of the oven was set at 120 °C and kept for 10 min, then raised to 240 °C at a rate of 5 °C·min^{-1}. The flame ionization detector temperature was 280 °C. The identification of the fatty acid methyl esters in hazelnut oils was carried out by the comparison of the retention time of 37 FAME standard solution (Nu-Check-Prep, Inc., Elysian, MN, USA; Supelco, Inc., Bellefonte, PA, USA). The percent content of dominant fatty acids including palmitic, stearic, oleic and linoleic acids were calculated (Koç *et al*. 2019).

2.7. Extraction of hazelnut oil for bioactive compounds

The oil samples were extracted in three steps with methanol (1:1 v/v). Each 2 mL of oil extract were mixed with 20 mL of methanol and then homogenised at 10,000 rpm for 3 minutes. Subsequently, the solution was placed in a shaker water bath (Büchen, Germany) at ambient temperature for one hour to extract the phenolic compounds. Following the phenolic extraction process, the extracts were centrifuged (Hettich, Universal Germany) at 2,500 g for 10 min and filtered through Whatman No. 1 and 0.45 μm filters (Whatman Ltd., Maidstone, UK).

2.8. Total phenolic content

Total phenolic compounds in the hazelnut oil extracts were quantified by the Folin-Ciocalteu method. Their absorbence was measured at 760 nm in a spectrophotometer (Shimadzu, Japan). The results are expressed as mg gallic acid equivalents (GAE)·g^{-1} (Koç *et al*. 2019).

2.9. Antioxidant activity

2.9.1. DPPH free radical scavenging activity

Free radical scavenging activity was performed according to the procedure stated in previous research (Prior *et al*., 2005). A mixture of 0.1 mL extract and 2 mL methanolic DPPH solution was vortexed and incubated at room temperature for 30 min. The absorption was recorded at 517 nm (Schimadzu, Japan). The EC_{50} value, which indicates 50% reduction of the free radicals, was reported as g oil·g^{-1} DPPH.

2.9.2. ABTS radical decolorization assay

The blue-green radical cation ABTS+ can convert colorles ABTS molecules because of the presence of antioxidants. The stock solution was prepared by dissolving the ABTS in distilled water at a concentration of 7 mmol·L^{-1}. The ABTS+ working solution was made by diluting the stock solution with ethanol until an absorbance of 0.700 ± 0.020 at 734 nm was observed. The oil sample was mixed with equilibrated ABTS and after 6 min the absorbance at 734 nm was determined with a spectrophotometer. Trolox was used as standard. The experiment was performed in three replicates and the results were presented as µmol Trolox·g^{-1} oil (Prior *et al*., 2005).

2.10. Total tocopherol content

The total tocopherol content in the hazelnut oil samples was determined according to AOAC (AOAC, 2000a). The oil samples were dissolved in hexane and injected into aa 5-µm silica-filled column (250 × 4.6 mm) using the mobile phase of ethyl acetate/acetic acid/hexane (1:1:98 (v:v:v)) at a flow of 1.5 mL·min^{-1}. The samples were analyzed using a Schimadzu HPLC system (Schimadzu, Japon). The fluorescence detector at 290 nm (excitation) and 330 nm (emission) wavelengths were used for detection.

2.11. Sterol composition

The sterol composition was analyzed by using gas chromatography according to ISO (2014). The oil samples were saponified with 1 mol·L^{-1} methanolic potassium hydroxide solution. The isolation and quantitation of the sterol fractions were carried out by gas chromatography (Agilent USA) equipped with a capillary column (25 m, 0.20 mm i.d. and 0.33 µm film thickness) and a flame ionization detector (FID). The detector and injector temperatures were set at 300 °C, while hydrogen was used as the carrier gas at a flow rate of 1.5 mL·min^{-1}. The individual assessment of sterols was calculated in %.

2.12. Statistical analysis

The research findings were analyzed with the statistical computer program JMP (5.0.1). Duncan's multiple comparison test was applied to important factors, and significant differences were determined at the $p < 0.05$ level.

3. RESULTS AND DISCUSSION

The quality properties of oils extracted from non-ozonated and ozonated hazelnuts at different doses and treatment times are shown in Table 1.

3.1. Effect of ozone treatment of hazelnuts on FFA value of extracted oil

The FFA value is a sign of an oil's acceptability for industrial use and its suitability for human consumption. In addition, FFA is an important quality index which is consistantly used as a shelf-life monitoring parameter for oil. The FFA of the hazelnut oil samples was determined in terms of both mg KOH·g^{-1} and % oleic acid and is shown in Table 1. The free fatty acids remained below the 4.0 mg KOH·g^{-1} limits established by the *Codex Alimentarius* for edible oils, and ranged from 0.99 to 1.37 mg KOH·g^{-1} (FAO, 1999). These values were in the range recently reported by Krol *et al*. (2021) for oils extracted from six hazelnut cultivars. The results obtained from this work indicated that hazelnut oils present low FFA values (both in terms of mg KOH·g^{-1} and oleic acid %), which suggested low levels of hydrolytic and lipolytic activities in the oil. It is possible to conclude that the obtained oils can be stored for a long time without deterioration.

As seen in Table 1, the FFA value was not significantly ($p > 0.05$) influenced by the gaseous ozone treatment, although a slight decrease was observed which is in line with the findings of de Alencar *et al*. (2011) for oil extracted from ozonated peanuts. Our results also support those reported by de Oliveira *et al*., (2020), who showed no alteration in the level of free fatty acids in the raw oil extracted from

Table 1. Effect of exposure time and ozone concentration on some quality properties of hazelnut oil

Parameter	Ozone treatment						
		3.33 mg·L^{-1}			10 mg·L^{-1}		
	Control	30 min	60 min	120 min	30 min	60 min	120 min
FFA							
mg KOH·g^{-1}	1.36±0.21^{a}	0.99±0.18^{a}	1.17±0.12^{a}	1.20±0.09^{a}	1.37±0.22^{a}	1.01±0.14^{a}	1.29±0.11^{a}
Oleic acid (%)	0.68^{a}	0.50^{a}	0.59^{a}	0.61^{a}	0.69^{a}	0.51^{a}	0.65^{a}
PV (meq·kg^{-1})	3.60±0.44^{d}	4.21±0.21^{d}	5.03±0.51^{c}	8.73±0.80^{b}	5.33±0.42^{c}	5.59±0.35^{c}	12.18±0.92^{a}
TPC (mg GAE·g^{-1})	1858.3±105^{a}	1881.1±92^{a}	1929.7±85^{a}	1269.7±79^{b}	1818.3±114^{a}	1949.7±138^{a}	449.71±35^{c}
Antioxidant activity							
DPPH (EC_{50})	10.38±0.75^{d}	9.81±0.89^{d}	10.37±0.48^{d}	30.80±2.42^{b}	10.07±0.94^{d}	15.48±1.02^{c}	79.48±3.12^{a}
ABTS (µmol troloks·g^{-1})	16.42±1.86^{a}	17.91±3.15^{a}	14.27±2.66^{a}	5.84±0.40^{c}	8.81±1.55^{b}	10.11±1.04^{b}	1.92±0.09^{d}

Each value in the table represents the mean ± standard deviation of triple analyses. Values with the same superscript letter within each row are not significantly different at $p < 0.05$ according to Duncan's comparison test.

Brazil nuts that had been ozonized at concentrations of 2.42, 4.38, 8.88 and 13.24 mg·L^{-1} for exposure times of up to 240 min. These results may be caused by inactivation of lipase or lipoxygenase during the ozone treatment. It is known that prolonged ozone exposure may lead to changes in the active sides of enzymes (Khanashyam *et. al*., 2023).

3.2. Effect of ozone treatment of hazelnuts on peroxide value (PV) of extracted oil

The PV indicates the degree to which primary oxidation products of oil are formed. PV is also related to organoleptic characteristics and reflects the oil's freshness (Uquiche *et al*., 2008). As shown in Table 1, the PV of the non ozonated oil (control) was measured as 3.60 meq·kg^{-1} and ranged from 4.21 to 12.18 meq·kg^{-1} for ozonated hazelnut oils, indicating that ozone concentration and treatment time showed a pronounced effect on the PV of oil from hazelnuts ($p < 0.05$) (except at 3.33 mg·L^{-1}, 30 min). In addition, when hazelnuts were exposed to gaseous ozone at 3.33 and 10 mg·L^{-1}, the PV of the oils reached its peak at 120 min with 8.73 meq·kg^{-1} oil and 12.18 meq·kg^{-1} oil, respectively, which are still lower than the limit established by the *Codex Alimentarius* of 15 meq·kg^{-1} (FAO, 1999).

The increase in PV may be caused by the release of free radicals, which significantly accelerated oil oxidation in various foods containing fat. These ozonation-generated free radicals impacted the oil's unsaturated fatty acids and caused the formation of peroxides and hydroperoxides. Oxidative rancidity occurs as a result of the reaction of oxygen with unsaturated fatty acids in the ester structure and free fatty acids. More oil molecules can come into interaction with ozone molecules as application dose and time are increased. The results with respect to PV are in accordance with the observations of other authors for ozonated pistachios and whole wheat flour (Babaee *et al*., 2022). Ozone treatment was observed to increase the PV of hazelnut oil samples exponentially, according to Uzun and Ibanoglu (2017). Additionally, Chen *et al*. (2014) and de Oliveria *et al*. (2020) observed an increase in the peroxide index of oils extracted from ozonized peanuts and Brazil nuts, respectively.

3.3. Effect of ozone treatment of hazelnuts on phenolic content and antioxidant activitiy of extracted oil

Phenolic compounds are significant in plants for their scavenging capacity because of their hydroxyl groups. The TPC of the oils extracted from hazelnuts exposed to different doses and times of ozone was determined using the Folin Ciocalteu's phenol reagent. The TPC is shown in Table 1 and is given as mg GAE·g^{-1} oil. The phenol content of the oil obtained from untreated hazelnuts was 1858.3 mg GAE·g^{-1} and that of the oils from treated hazelnuts were between 449.71 and 1949.7 mg GAE·g^{-1}.

As can be observed in Table 1, lower application times of ozonation (30 and 60 min) at both doses did not lead to a significant difference ($p > 0.05$) in the content of phenolics, which indicated that phenols were not affected by ozonation under these condi-

tions. However, the application of both 3.33 and 10 $mg \cdot L^{-1}$ ($p < 0.05$) gaseous ozone doses per 120-min period resulted in a significant reduction in the TPC of the oils by 31.70 and 75.83%, respectively.

In this present study, to corroborate the measured antioxidant activity, radical scavenging was evaluated by the ABTS method in addition to the DPPH method as shown in Table 1. The ability of antioxidants to transfer hydrogen or electrons, resulting in a more stable species, is shown by their ability to scavenge DPPH radicals (Prior *et al*., 2005). There was no significant change ($p > 0.05$) in the DPPH radical scavenging actvity of hazelnut oils exposed to low-dose (3.33 $mg \cdot L^{-1}$) ozone for up to 60 min in parallel with ABTS. However, increasing the time to 120 min at this dose resulted in a significant decrease ($p < 0.05$) in antioxidant capacity.

The rate of antioxidant activity loss due to the increase in time was faster in 10 $mg \cdot L^{-1}$ ozonated samples than 3.3 $mg \cdot L^{-1}$ ozonated according to the both methods evaluated. The sample treated with 10 $mg \cdot L^{-1}$ ozone for 120 min exhibited the lowest potency in scavenging free DPPH radicals, as indicated by the highest EC_{50} value of 79.48 $mg \cdot mL^{-1}$. A high EC_{50} value indicates low antiradical activity. The maximum ABTS radical scavenging activity was observed in control sample (16.42 µmol troloks$\cdot g^{-1}$). The ABTS radical scavenging activity of the samples treated with gaseous ozone at 3.3 and 10 $mg \cdot L^{-1}$ reached the minimum at 120 min in parallel with DPPH, which were 64.41% and 88.33% respectively, and lower than the control group's ABTS radical scavenging activity.

A relationship between total phenol contents and the antioxidant activity of the hazelnut oil was observed, while total phenol and antioxidant activities of oils ozonized at 3.33 and 10 $mg \cdot L^{-1}$ decreased with the prolongation of the time applied. The significant decrease in antioxidant activity with long-term ozone gas application could be due to the decrease in the amount of phenolic substances in hazelnuts. The vulnerability of phenolics and other antioxidant compounds to oxidative reactions is most likely influenced by the nature of these compounds and their location in the cell.

The fact that the phenolic compounds in hazelnut are predominantly found in the outer membrane (Alasalvar *et al*., 2009) and that ozone gas does not interact with the phenols in the hazelnut due to the weak penetration into the hazelnut matrix in low time applications may explain the results of our study. However, the removal of ozone decomposition products by phenolic compounds in hazelnuts may also explain the reason for phenolic reduction, especially in long-term application. The phenol content in strawberries (Allende *et al*., 2007) was reported to decline after gaseous ozone applications. By contrast, the phenolic content and antioxidant activity of grain flour oil increased with ozone treatment (Obadi *et al*., 2018). Also, according to Chen *et al*. (2014), ozonation had no effect on the polyphenol or resveratrol contents in peanuts ($p > 0.05$).

3.4. Effect of ozone treatment of hazelnuts on fatty acid composition of extracted oil

The oxidative activity of edible oil is linked to the fatty acid composition. Table 2 reports the fatty acid profiles of lipid extracts from non-ozonized and ozonized hazelnut samples. The major fatty acids identified in non-ozonized (control) hazelnut oil were 5.17% palmitic acid (C16:0), 2.45% stearic acid (18:0), 85.57% oleic acid (C18:1) and 6.82% linoleic acid (C18:2) (less than 0.001% of other fatty

Table 2. Effect of exposure time and ozone concentration on fatty acid composition of hazelnut oil

Fattty acids (%)	Ozone treatment						
		3.33 $mg \cdot L^{-1}$			10 $mg \cdot L^{-1}$		
	Control	30 min	60 min	120 min	30 min	60 min	120 min
Palmitic acid C16:0	5.17±0.21[a]	4.92±0.18[a]	4.98±0.26[a]	5.19±0.16[a]	5.27±0.41[a]	5.02±0.38[a]	4.92±0.20[a]
Stearic acid C18:0	2.45±0.05[a]	2.37±0.09[a]	2.46±0.1[a]	2.44±0.05[a]	2.32±0.18[a]	2.35±0.2[a]	2.28±0.12[a]
Oleic acid C18:1	85.57±0.5[a]	85.71±0.6[a]	85.04±0.9[a]	85.17±0.5[a]	85.49±0.6[a]	85.61±0.5[a]	85.19±0.8[a]
Linoleic acid C18:2	6.82±0.4[a]	7.01±0.56[a]	7.52±0.7[a]	7.20±0.55[a]	6.92±0.45[a]	7.01±0.6[a]	7.61±0.28[a]

Each value in the table represents the mean ± standard deviation of triple analyses. Values with the same superscript letter within each row are not significantly different at $p < 0.05$ according to Duncan's comparison test.

acids were not detectable). When the ozone concentration of 10 mg·L^{-1} was used for 120 min, mean values of 4.92, 2.28, 85.19 and 7.61% were obtained for the C16:0, C18:0, C18:1 and C18:2 fatty acids, respectively. The high levels of unsaturated fatty acids particularly oleic and linoleic acids found in hazelnuts reported in this study are generally consistent with the literature. The fatty acid profile of hazelnut oil displays significant amounts of oleic acid, which is beneficial for human health.

Any treatment that changed the ratio of its fatty acids would be undesirable because it would decrease the food's nutritional value. Regarding the effect of ozonation on the fatty acid composition of the hazelnut oil samples, it can be observed in Table 2 that ozone treatment did not significantly alter ($p > 0.05$) the fatty acid composition of the oils. This may be due to the poor penetration of ozone gas into hazelnuts at the applied doses and times. Furthermore, it was found that hazelnut oil contains a large amount of antioxidants, such as tocopherols (Table 4), which might scavenges the radicals generated by ozonation. De Oliveira *et al*. (2020) also found no significant alteration in the lipid fraction of the oil obtained from the ozonized Brazil nuts and pistachios, respectively. On the other hand, Uzun *et al*. (2018) stated that ozone treatment caused a reduction in the percentage of unsaturated fatty acids in both hazelnut oil and soy bean oil. After longer ozone treatment periods, oleic acid was destroyed completely in both hazelnut oil and soy bean oil since the presence of double bonds increased the likelihood of oxidation reactions occurring.

As a result, despite the fact that ozone had a significant potential for oxidation, ozonation was unable to oxidize the oil in a way that would change its lipid composition. The unsaturated fatty acids contained in the lipids of many foods are vulnerable to chemical degradation when exposed to oxygen. As a consequence, a more stable form of unsaturated fat had the capability to substantially enhance the shelf life and added value of a large number of foods. Since hazelnut oil is primarily composed of unsaturated fatty acids, this finding is critical for maintaining the quality of hazelnuts.

3.5. Effect of ozone treatment of hazelnuts on sterol composition of extracted oil

Phytosterols are natural endogenous antioxidants which have high stability against oxidation and heat and can be very effective in the inhibition of oil rancidity. As shown in Table 3, among the phytosterols and phytostanol found in hazelnut oils and quantified, (campesterol, stigmasterol, β−sitosterol, sitostanol, Δ5−avenasterol, Δ7−stigmastenol, and Δ7−avenasterol), β−sitosterol contributed the most, accounting for 84.69–86.44% to the total, followed by campesterol (4.27–5.07%) and Δ5−avenasterol (2.32–3.65%), respectively (Table 3). Amaral *et al*. (2006) and Alasalvar *et al*. (2009) found the same numbers of phytosterols and phytostanol in hazelnut oils. The total amount of phytosterols and phytostanol detected in the current study varied from 1407 to 1590 mg kg^{-1} oil, which is in the range previously reported for hazelnut oils (Amaral *et al*., 2006)

Table 3. Effect of exposure time and ozone concentration on sterol composition of hazelnut oil

Sterol content (%)		Ozone treatment					
		3.3 mg·L^{-1}			10 mg·L^{-1}		
	Control	30 min	60 min	120 min	30 min	60 min	120 min
Campesterol	4.27±0.3^{a}	4.86±0.25^{a}	4.69±0.4^{a}	4.67±0.36^{a}	4.85±0.28^{a}	5.07±0.5^{a}	4.67±0.22^{a}
Stigmasterol	0.72±0.09^{a}	0.46±0.08^{b}	0.42±0.06^{b}	0.61±0.08^{ab}	0.50±0.05^{b}	0.59±0.03^{b}	0.37±0.01^{c}
β−Sitosterol	85.52±0.6^{a}	86.44±0.52^{a}	85.48±1.2^{a}	85.13±0.8^{a}	84.85±1.5^{a}	86.33±0.9^{a}	84.69±1.1^{a}
Sitostanol	2.12±0.32^{a}	1.72±0.05^{b}	2.79±0.35^{a}	2.24±0.18^{a}	2.64±0.22^{a}	1.61±0.06^{b}	2.61±0.19^{a}
Δ5−Avenasterol	3.65±0.36^{a}	2.97±0.3^{a}	3.42±0.15^{a}	3.03±0.28^{a}	2.99±0.15^{a}	2.32±0.17^{b}	3.23±0.17^{a}
Δ7−Stigmastenol	2.63±0.21^{b}	0.76±0.05^{c}	0.69±0.06^{c}	3.50±0.34^{a}	2.88±0.29^{a}	3.43±0.22^{a}	3.19±0.18^{a}
Δ7−Avenasterol	1.06±0.11^{b}	2.77±0.2^{a}	2.49±0.2^{a}	0.81±0.09^{c}	1.27±0.09^{b}	0.62±0.09^{c}	1.23±0.1^{b}
Total Sterol (mg·kg^{-1})	1483.48±86^{b}	1409.06±56^{b}	1412.74±95^{b}	1475.21±35^{b}	1482.35±64^{b}	1407.76±98^{b}	1590.61±12^{a}

Each value in the table represents the mean ± standard deviation of triple analyses. Values with the same superscript letter within each row are not significantly different at $p < 0.05$ according to Duncan's comparison test.

The total sterol content reached its maximum value at 10 mg·L^{-1} for 120 min, which was 107.13 mg kg^{-1} higher than that of the control group. However, other ozone gas applications did not cause any significant changes in the total sterol contents of hazelnut oil ($p > 0.05$). The campesterol, β-sitosterol, and Δ5−avenasterol contents of the oils extracted from hazelnuts exposed to ozone were not significantly different from the control group ($p > 0.05$), however, the sitostanol, Δ7−stigmastenol, and Δ7−avenasterol showed a fluctuating tendency. To our knowledge, this is the first study to evaluate how ozone treatment affects the sterol content in hazelnut oils.

3.6. Effect of ozone treatment of hazelnuts on tocopherol composition of extracted oil

Vegetable oils are a common source of tocopherols, which are lipid-soluble substances. Tocopherols are known to be strong lipophilic antioxidants and to provide significant health benefits, among other biological functions in the human body (Amaral *et al*., 2006). They serve as chain-breaking antioxidants during autoxidation by scavenging free radicals, which prevents the start or slows the development of oxidation. Hazelnut oils were determined to be a superior source of tocols (Alasalvar *et al*., 2009). Therefore, all processes used in the production and/or storage of hazelnuts should attempt to preserve these antioxidants.

The tocopherols content of the oil samples extracted from ozonized and non-ozonized hazelnuts is presented in Table 4. In this study, α- and γ-tocopherol were found in hazelnut oil, with α-tocopherol having the highest content and consitiutingbetween 88 and 96% of the total tocopherol. The levels of α-tocopherol ranged from 456.68 to 525.45 mg·kg^{-1} whereas the concentration of γ-tocopherol varied between 20.97 and 58.95 mg·kg^{-1}. These values are in accordance with those reported in the literature (Alasalvar *et al*., 2008). However, minor values of α-, β-, and γ-tocotrienols, together with two tocopherols in hazelnut oils, have been reported (Alasalvar *et al*., 2006).

The level of α-tocopherol and γ-tocopherol in non-ozonized hazelnut oils were 525.45 and 20.97 mg·kg^{-1}, respectively. The results in Table 4 also indicate that ozone application slightly reduces the tocopherol content in the oil extracted from hazelnuts but increases the γ-tocopherol content in oil, extracted from hazelnuts. The rise in γ-tocopherol content with ozonation may be due to the release of oil and fat-soluble compounds such as γ-tocopherols owing to cell membrane destruction (Lee *et al*., 2004)

There was general agreement that α-tocopherol has more potent antioxidant activity *in vivo* than other tocopherols (Kamal-Eldin and Appelqvist, 1996). However, it is important to note that the other forms of tocopherols, such as γ-tocopherol, also have important biological functions and may have unique benefits.

In the process of ozonation at 10 mg·L^{-1}, the *α*-tocopherol content decreased to the minimum value (456.68 mg·kg^{-1}) after 60 min of ozone application; while γ-tocopherol content increased to the maximum value (58.95 mg·kg^{-1}) after 30 min of ozonation. Vettraino *et al*. (2020) reported a decrease of the concentration of *α*- and γ-tocopherol in chestnuts when the nuts were treated with 300 ppb of gaseous ozone. However, as can be seen in Table 4, ozonation at low doses (3.3 mg·L^{-1}) had no significant effect on the concentration of total tocopherols in the hazelnut oil samples, while treatment at higher doses (10 mg·L^{-1}) caused a considerable reduction after 30 minutes. The oxidation processes and cell damage that occur during ozonation could be responsible for the decline in α- and total tocopherols in oils.

Table 4. Effect of exposure time and ozone concentration on tocopherol composition of hazelnut oil

W		Ozone treatment					
		3.3 mg·L^{-1}			10 mg·L^{-1}		
	Control	30 min	60 min	120 min	30 min	60 min	120 min
α-Tocopherol	525.45±12.1^{a}	494.86±10.9^{b}	483.00±18.6^{b}	512.84±28^{ab}	474.38±26.2^{b}	456.68±35.2^{c}	472.57±19.1^{b}
γ-Tocopherol	20.97±1.2^{c}	28.61±2.16^{b}	32.43±3.85^{b}	25.15±4.01^{b}	58.95±3^{a}	34.79±3.2^{b}	31.73±2.95^{b}
Total Tocopherol	546.42±28.2^{a}	523.47±12.5^{a}	515.43±5.4^{a}	537.99±22.8^{a}	533.33±17.5^{a}	491.46±18.1^{b}	504.30±5.9^{b}

Each value in the table represents the mean ± standard deviation of triple analyses. Values with the same superscript letter within each row are not significantly different at $p < 0.05$ according to Duncan's comparison test.

4. CONCLUSIONS

The effect of ozone treatment on the quality attributes of hazelnut oil was investigated in this research. It is important to note that the ozone treatments had no effect on the FFA value and fatty acid composition of oils, and the high concentrations of oleic and linoleic acid maintained even after exposure to ozone doses as high as 10 $mg \cdot L^{-1}$ for 120 min. Furthermore, ozone exposure for up to 60 minutes at either of the treatment doses had no impact on the TPC and antioxidant activity. Phenolic content and antioxidant capacity of hazelnut oils were substantially lost in the last phase at higher ozonation times. Although the peroxide values of hazelnut oil increased with exposure to ozone, these values were below the limit of 15 $meq \cdot kg^{-1}$ determined by Codex Alimentarius. The predominant sterol compound in hazelnut oils was β-sitosterol and the total sterol content of oils did not change with ozonation except 10 $mg \cdot L^{-1}$, 120 min application. The high α-tocopherol level in hazelnut oil may have assisted in preserving the oxidative stability of the oils, even though a minor reduction in α-tocopherol concentration was noted as a response to the ozone treatments. An unexpected significant increase in γ-tocopherol was observed in ozone-treated hazelnuts

Consequently, the results of this study showed that despite the fact that ozone had a significant potential for oxidation, ozonation was unable to oxidize the oil in a way that would change its lipid profile and quality.

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DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

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