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Chemical and structural variations in hazelnut and soybean oils after ozone treatments

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SUMMARY: In the present work, the effect of ozone treatments on the structural properties of soybean oil (SBO) and hazelnut oil (HO) were investigated. The study presents the findings and results about the oxidation of HO and SBO with ozone, which has not been fully studied previously. The HO and SBO were treated with ozone gas for 1, 5, 15, 30, 60, 180 and 360 min. The ozone reactivity with the SBO and HO during the ozone treatment was analyzed by ^{1}H, ^{13}C NMR, FTIR and GC. The iodine value, viscosity and color variables (L*, a* and b*) of untreated and ozone treated oils were determined. Reaction products were identified according to the Criegee mechanism. New signals at 5.15 and 104.35 ppm were assigned to the ring protons of 1,2,4- trioxolane (secondary ozonide) in the ozonated oils in ^{1}H and ^{13}C NMR, respectively. Ozonated oils exhibited peaks at 9.75 and 2.43 ppm in ^{1}H and NMR, which corresponded to the aldehydic proton and α-methylene group and to the carbonyl carbon, respectively. The peak at 43.9 ppm in ^{13}C NMR was related to the α-methylene group and to the carbonyl carbon. The new signals formed in the ozonation process gradually increased with respect to ozone treatment time. After 360 min of ozone treatment, the carbon-carbon double bond signal, which belongs to the unsaturated fatty acids, disappeared completely in the spectrum. An increase in viscosity, a decrease in iodine value and a dramatic reduction in b* of the oil samples on (+) axis were observed with increased ozone treatment time.

KEYWORDS: *FTIR; GC; Hazelnut oil; NMR; Ozone; Soybean oil; Viscosity*

RESUMEN: ***Variaciones químicas y estructurales de aceites de avellana y soja después de tratamientos con ozono***. En el presente trabajo se investigó el efecto de tratamientos con ozono sobre las propiedades estructurales de aceites de soja (SBO) y avellana (HO). El estudio muestra hallazgos y resultados sobre la oxidación de HO y SBO con ozono que no se habían presentado previamente. Los aceites HO y SBO son tratados con ozono gaseoso durante 1, 5, 15, 30, 60, 180 y 360 min. La reactividad del ozono con SBO y HO durante el tratamiento se analizó mediante ^{1}H, ^{13}C NMR, FTIR y GC. Se midieron las variables: índice de yodo, viscosidad y color (L *, a * y b *) de los aceites no tratados y tratados con ozono. Los productos de reacción se identificaron de acuerdo con el mecanismo de Criegee. Se asignaron nuevas señales a 5,15 y 104,35 ppm a los anillos protónicos de 1,2,4-trioxolano (ozónido secundario) en aceites ozonizados en ^{1}H y ^{13}C RMN, respectivamente. Los aceites ozonizados también mostraron picos a 9,75 y 2,43 ppm en ^{1}H NMR correspondientes al protón aldehídico y al grupo α-metileno, respectivamente. El pico a 43,9 ppm en ^{13}C RMN se relacionó con el C carbonilo del grupo α-metileno. Las nuevas señales formadas en el proceso de ozonización aumentaron gradualmente con respecto al tiempo de tratamiento con ozono. Después de un tratamiento de 360 minutos con ozono, la señal del doble enlace C=C que pertenece a los ácidos grasos insaturados desapareció por completo en el espectro. Se observó un aumento en la viscosidad, una disminución en el índice de yodo, una reducción drástica en b* de muestras de aceite en el eje (+) con el aumento del tiempo de tratamiento con ozono.

PALABRAS CLAVE: *Aceite de avellana; Aceite de soja; FTIR; GC; NMR; Ozono; Viscosidad*

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1. INTRODUCTION

While thermal methods can effectively eliminate pathogens, non-thermal technologies provide the potential advantages of maintaining physical, chemical, and sensory attributes and ensuring the raw characteristics of food products preferred by some consumers (Prakash, 2013). Ozone application is a relatively new method in food processing. It is a viable disinfectant for maintaining the microbiological safety of food products because of its substantial reactivity, penetrating ability and ability to decompose spontaneously to a nontoxic product (i.e., O_2) (Atungulu and Pan, 2012). It received the GRAS (generally recognized as safe) status for use as disinfectant and sanitizer in 1997 (USDA, 1997). Suggested applications of ozone in the food industry include food surface hygiene, sanitation of food plant equipment (Greene *et al.*, 1999), reuse of waste water (Rice *et al.*, 1981), cleaning of shellfish and disinfection of poultry carcasses and chill water (Yang and Chen, 1979), increasing shelf-life of fruit and vegetables (Beuchat, 1992), and providing hygienic conditions in storage atmosphere of nuts and cereal (Chen *et al.*, 2014). In addition, the ozone treatment of unsaturated triglycerides present in vegetable and nut oils has been suggested recently due to the use of ozonated oils in several applications (Soriano *et al.*, 2003). Ozone treated oils have been used in the food, cosmetic and pharmaceutical industries as antibacterial and fungicidal agents (Zanardi *et al.*, 2008). The chemical reactions of ozone with unsaturated fatty acids present in oils are very complex. The analyses related to these reactions provide useful information on the functional group changes during ozonation as well as the identification of the products, according to the Criegee mechanism regarding the formation of ozonides from alkenes and ozone. The reaction of ozone with the unsaturated bonds in the lipid fraction generates the formation of a mixture of oxygenated compounds such as ozonides, peroxides and aldehydes according to the mechanism described by Criegee (1975). The initial pathway of formation of the primary ozonide is a 1,2,3 trioxolane. Pryor, (1994) suggests that a diradical intermediate is produced from the primary trioxolane by a O-O bond scission. The diradical can decompose into an aldehyde and a carbonyl oxide. In an aqueous environment, the carbonyl oxide can form a hydroxyhydroperoxide, which breaks down to an aldehyde and hydrogen peroxide. In the relatively anhydrous environment, the primary ozonide rearranges to form the secondary ozonide (1,2,4 trioxolane), which can decompose to a hydroperoxide and an aldehyde. The hydroperoxide can initiate lipid peroxidation (Figure 1). Several oxygenated compounds such as hydroperoxides, ozonides, aldehydes, peroxides, and polyperoxides are produced by the Criegee mechanism. Various methods are used for studying the characterisation of ozonated vegetable oils e.g. ^{1}H and ^{13}C NMR, FT-IR, GC, determination of peroxide and acidity values and viscosity measurements (Soriano *et al.*, 2003; Sega *et al.*, 2010; Diaz *et al.*, 2005, Rodrigues de Almeida Kogawa *et al.*, 2015).

Physicochemical changes in the oxidation reaction of ozone with hazelnut oil and soybean oil have not been studied extensively. Hazelnut oil and soybean oil have been selected due to their high amounts of unsaturated fatty acids. In addition, there is a growing interest in evaluating the role of hazelnuts in human nutrition and health (Alasalvar *et al.*, 2006). This is related to their special fatty acid composition which consists of more than 90% oleic and linoleic acids and small amounts of palmitic and stearic acids, along with health-promoting components including tocopherols, phytosterols, polynenols and squalene (Alasalvar *et al.*, 2006; Xu *et al.*, 2007). Hazelnut oil is becoming increasingly popular in Turkey and elsewhere and is widely used for cooking, deep frying, salad dressing, and flavoring ingredients (Alasalvar *et al.*, 2006). Soybean oil is used to produce margarine, shortening, and cooking oil in the food industry (Wang, 2002). They both are rich in monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (approximately, 83% MUFA and 9% PUFA in hazelnut oil and 20.32% MUFA and 61.99% PUFA in soybean oil) (Alasalvar *et al.*, 2006; Gunstone, 2002).

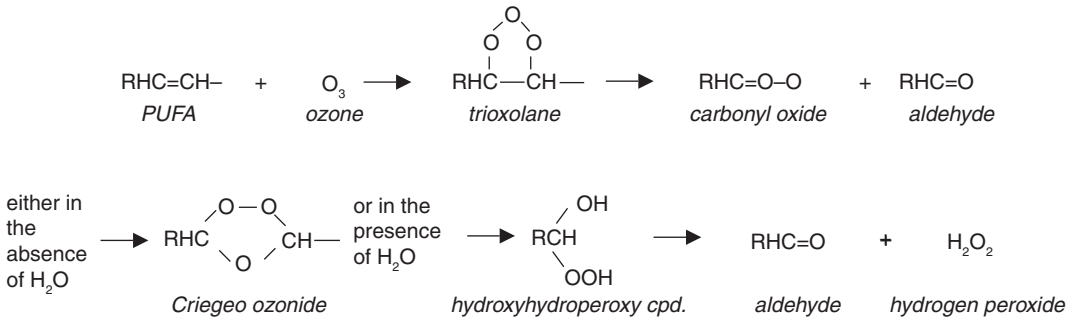

FIGURE 1. Classic ozone additions to carbon-carbon double bond of polyunsaturated fatty acids.

In the present study, the effect of ozone gas on the oxidation of hazelnut oil and soybean oil were investigated for different ozonation periods. ^{1}H and ^{13}C NMR (Nuclear Magnetic Resonance) and FTIR (Fourier Transform Infrared Radiation) were used to detect the chemical changes in ozone treated oil samples. The fatty acid compositions of the oil samples were analyzed using GC (Gas Chromatography) to determine the variation in the fatty acid compositions of untreated and ozone treated oil samples. The changes in viscosity and color of ozone treated oil samples were determined.

2. MATERIALS AND METHODS

2.1. Materials

Hazelnut oil and soybean oil were purchased from a local manufacturer (FISKOBIRLIK Company, Giresun, Turkey; Nadir Yağ Company, Gaziantep, Turkey). Acetic acid (100%) and chloroform (99.5%) were purchased from Merck Company. Potassium iodide, sodium thiosulfate, starch, wijs solution, and cyclohexane were supplied from Riedel de Haen. All chemicals used were of analytical grade.

2.2. Ozonation of hazelnut and soybean oils

Ozone gas was generated by an ozone generator (Ozone Marine, OMS Model, Izmir, Turkey) operating according to the corona-discharge method at a constant flow rate of 1L/min with an ozone concentration of 40 mg/L. The oxygen required for ozone generation was provided from the air. A 100 mL oil sample was placed in a glass bottle. Ozone gas was directed into the bottle and bubbled through the oil sample for different periods of time (1, 5, 15, 30, 60, 180 and 360 min). The ozonated samples and a control sample were stored in a hermetically sealed glass bottle kept in the dark at room temperature up to 120 days and analyzed at specific time intervals (5, 10, 15, 20, 30, 40, 50, 60, 75, 90 and 120 days).

2.3. Iodine value (IV)

The iodine value (IV) represents the quantity of iodine (in grams) that will react with the double bonds in 100 g of oil sample. The IV was determined according to the European Pharmacopoeia, 2004. The IV was calculated by means of the following equation:

$$IV = \frac{1.269 \times (A - B)}{m}$$

where A is the volume in mL of thiosulphate solution used for carrying out a blank test, B is the volume in mL of thiosulphate solution used for the titration, and m the quantity, in grams, of substance.

2.4. Hunter colorimeter

The color of untreated and ozone treated oil samples was measured using a Hunter Lab colorimeter (Color Flex, Hunter Associates Laboratory Inc., Reston, Virginia, USA). The instrument (65°/0° geometry, D25 optical sensor, 10° observer) was calibrated using white and black reference tiles. The color values were expressed as L* (whiteness or brightness/darkness), a* (redness/greenness) and b* (yellowness/blueness). The Color measurements were reported as the average of triplicate measurements for each sample.

2.5. Gas chromatography analysis

The fatty acid compositions of hazelnut and soybean oil were determined using an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) with a split/splitless injector, flame ionization detector and HP-88 capillary column (88% cianopropylaryl; 100 m × 0.250 mm, 0.20 μm i.d.). The injector and detector temperatures were 250 and 260 °C, respectively. The oven temperature was set as follows: 1 min at 120 °C, from 120 to 175 °C at 10 °C/min, 10 min at 175 °C, from 175 to 210 °C at 5 °C/min, 5 min at 210 °C, from 210 to 230 °C at 5 °C/min and 5 min at 230 °C. Helium was the carrier gas with a flow rate of 1.5 mL/min.

For fatty acid methyl esters (FAME) were prepared as follows: 2 mL n-hexane and 2 mL KOH (in methanol) were added to 1 drop of oil and the mixture was shaken vigorously. The supernatant (FAME) was taken and a 1μL of FAME mixture was injected into the GC system.

2.6. Viscosity measurements

The viscosity of the untreated and ozone treated oil samples was measured using a controlled stress rheometer (Bohlin CVO-R, Malvern Instruments Limited, UK) with cone and plates (2°/ 20 mm). Shear rates in the range of 0–300 s^{-1} under steady shear conditions were applied to the oil samples and the resulting shear stress was measured at 25 °C.

2.7. FT-IR analysis

FT-IR analyses were applied to investigate the alterations in the functional groups found in hazelnut and soybean oil after ozone treatment. About 2 μL of sample were deposited between two disks of KBr, avoiding air bubble formation, and then IR spectra were recorded using a FT-IR spectrometer (FT-IR 100, Perkin Elmer Incorporation, USA). The FTIR spectra of the samples were measured in the 4000–650 cm^{-1} region at room temperature.

2.8. NMR analysis

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Minispec spectrometer 400 MHz at 25 °C in $CDCl_3$. All the experiments were performed under the same experimental conditions and at the same sample concentration (about 100 µL of oil sample in 750 µL $CDCl_3$). The reaction of ozone with HO and SBO was studied by ^{1}H and ^{13}C NMR at certain time intervals (1, 60, 180 and 360 min).

2.9. Statistical analysis

The data from the chemical analyses were analyzed using ANOVA. Statistics analyses were applied to the data using the SPSS statistical package (2013), (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.)

3. RESULTS AND DISCUSSION

3.1. Iodine value (IV)

IV is often used to determine the amount of unsaturated bonds in the oils which react with iodine compounds. A high iodine number indicates the presence of a large number of C=C bonds (Thomas, 2000). The IV of HO before ozone treatment was measured as 95.4 ± 0.1 (Figure 2A). This value correlates well with previously reported values ranging from 90.6 to 97.4 (Xu *et al*., 2007). The IV of HO reduced dramatically as the samples were treated with ozone (Figure 2A) and the IV of the untreated and ozone treated HO samples were significantly different ($p < 0.05$). As the treatment time is extended, the number of ozone molecules contacting a constant volume of the oil sample increases, causing an extensive reduction in the amount of unsaturated bonds. Although a sharp reduction in the IV of samples was observed with respect to the ozone treatment period, the variation in IV with respect to storage (Figure 2B) was not statistically significantly ($p > 0.05$). Since ozone reacted with carbon-carbon double bonds in the unsaturated fatty acid, this aspect may represent a measurement of residual double bonds. The IV of the ozone treated oil samples correlated well with data from previous studies on different vegetable fats and oils in relation to ozone treatment time (Skalska *et al*., 2009; Zanardi *et al*., 2008). Zanardi *et al*., (2008) reported that the IV of sesame oil samples decreased as ozone treatment time was extended. Skalska *et al*., (2009) stated that almost all unsaturated bonds in the oils were saturated depending on ozonation time.

3.2. Color measurement

Table 1 represents the variation in L*, a*, and b* of untreated and ozone treated HO and SBO. The L*, a*, b*, or CIE Lab is an international standard, indicating lightness (L=0-100), chromaticity on a blue to yellow (−a to +a) and on a green to red (−b to +b) axes (Rocha and Morais, 2003). While the b* value of ozone treated oil samples was reduced significantly on the (+) axis with increased ozone treatment period ($p < 0.05$), the a* and L values did not exhibit significant changes ($p > 0.05$). Changes may arise from the pronounced effect of ozone treatment on carotenoid pigments which give their yellow color to the oils. The color of hazelnut oil and soybean oil turned pale as ozone treatment time increased. A loss in the color of the oils was more evident after 360 min ozone treatment. This was correlated well with previously reported studies.

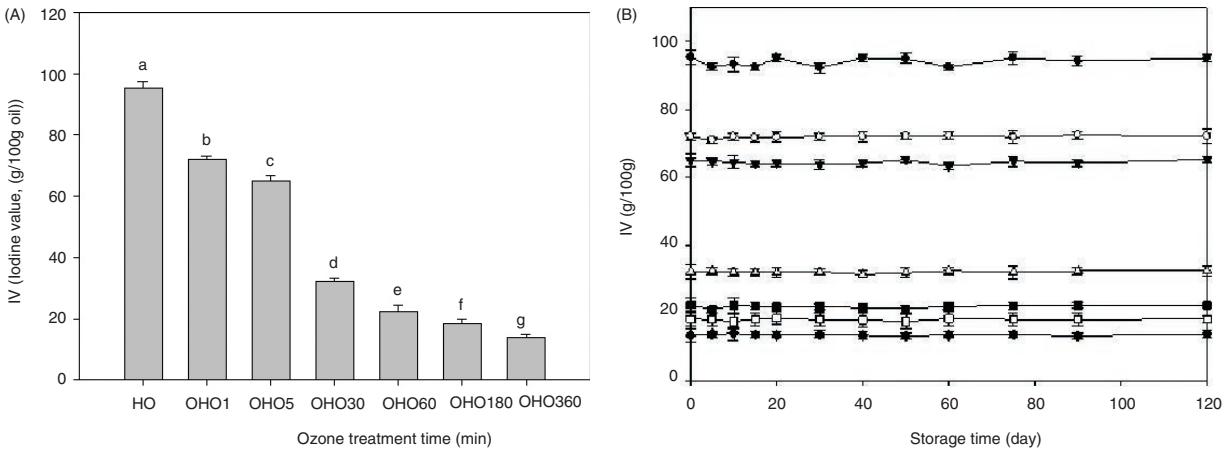

FIGURE 2. Changes in iodine value (IV) (g/100g oil) of untreated and ozone treated hazelnut oil with respect to ozone treatment time (A) and storage time (120 days) (B) (●; HO, ○; OHO1, ▼; OHO5, Δ; OHO30, ■; OHO60, □; OHO180, ♦; OHO360. Abbreviations: HO; hazelnut oil, OHO1, OHO5, OHO30, OHO60, OHO180 and OHO360 ozone treated hazelnut oil for 1, 5, 30, 60, 180 and 360 min, respectively. Each value in figure 2, A represents the mean ± standard deviation of triple analyses. Means with different letters in each column are significantly different ($p < 0.05$).

TABLE 1. L*, a*, and b* of untreated and ozone treated SBO and HO

Ozone treatment time (min)	Soybean oil (SBO)			Hazelnut oil (HO)		
	L*	a*	b*	L*	a*	b*
0	59.06±3.3a	−3.70±1.5a	80.10±2.5a	63.37±2.2b	−3.18±0.2b	57.16±1.5a
1	61.54±2.2a	−4.92±1.0a	48.48±0.7b	61.54±2.5b	−3.25±0.5b	28.52±1.8b
5	59.46±3.3a	−4.08±0.7a	45.07±3.7c	58.75±3.7b	−3.71±0.5b	15.22±0.8c
30	62.83±3.4a	−3.72±0.8a	15.64±2.4d	57.56±2.3b	−3.28±0.8b	13.13±0.7d
60	63.12±2.7a	−4.20±0.5a	12.26±0.8e	58.34±3.2b	−2.61±0.5b	9.06±1.3e
180	61.72±1.5a	−3.89±0.8a	3.63±0.8f	57.99±2.7b	−2.33±0.8b	7.37±0.9f
360	62.94±1.3a	−4.38±0.5a	3.94±0.9g	61.44±3.2b	−2.61±0.7b	2.63±1.3g

[a]Each value in the table represents the mean ± standard deviation of triple analyses. Abbreviations: HO; hazelnut oil, OHO1, OHO5, OHO30, OHO60, OHO180, and OHO360 ozone treated hazelnut oil for 1, 5, 30, 60, 180, and 360 min, respectively. SBO; soybean oil, SBO1, SBO5, SBO30, SBO60, SBO180, and SBO360 ozone treated soybean oil for 1, 5, 30, 60, 180, and 360 min, respectively. Means with different letters in each column are significantly ($p < 0.05$) different.

Carotenoids in nut oils are β-carotene, lutein, and zeaxanthin (King *et al*., 2008). Soybean oil also contains lutein, β-carotene, and chlorophyll pigments (Gunstone, 2002).

The basic chemical structure of carotenoids consists of a polyenic chain ranging from 3 to 15 conjugated double bonds (Britton, 1995). The carotenoids can be influenced by reactions of oxidation and hydrolysis, which modify their biological actions due to the highly unsaturated polyenic chain (Rodriguez and Rodriguez-Amaya, 2007). The oxidation of carotenoids leads to the formation of trace quantities of several compounds with a low molecular weight. Depending on the concentration of ozone, the percentage decay of β-carotene increases, possibly due to the Criegee mechanism (Benevides *et al*., 2011).

3.3. Gas chromatography analysis

The fatty acid compositions in HO and SBO were determined by gas chromatogram. The main unsaturated fatty acid present in HO was oleic acid (C18:1); whereas SBO contained linoleic acid (C18:2) as well as oleic acid (C18:1) as unsaturated fatty acids. The unsaturated fatty acid composition of crude HO was calculated as about 90.76%. A high proportion of unsaturated fatty acids (79.15% oleic acid, 11.43% linoleic acid and 0.18% gondoic acid) and a small proportion of saturated ones (5.31% palmitic acid and 2.75% stearic acid) were contained in HO. SBO contained a high amount of linoleic acid (56.25%) followed by oleic acid (21.93%), and linolenic acid (7.40%). The total unsaturated fatty acid content of SBO was about 85.58%. The polyunsaturated fatty acid (PUFA) contents of HO and SBO were about 11.43% and 63.65%, respectively; while HO and SBO contained about 79.33% and 21.93% monounsaturated fatty acids (MUFA), respectively.

Table 2 illustrates the changes in fatty acid composition of HO and SBO after being treated with ozone. The percentage of oleic acid and linoleic acid was observed to decrease after ozone treatment. The reductions in percentages of linoleic acid an oleic acid in OHO60 were about 48.9% and 7.0%, respectively; while the decrease in percentages of linoleic acid and linolenic acid in SBO60 were about 20.7% and 45.5%, respectively. The reduction in the percentage of linolenic acid was higher than linoleic acid and oleic acid. The percentage of oleic acid in the SBO60 was altered slightly with 60 min ozone treatment since linolenic acid is more prone to oxidation than linoleic and oleic acids, due to its number of double bond. It was determined that the relative percentages of unsaturated fatty acids in the oils was reduced after 60 min ozone treatment while the relative percentages of saturated fatty acids increased, which is consistent with a previous study (Liu and White, 1992).

As seen in Table 2, the percentages of unsaturated fatty acids in the oil samples decreased rapidly after 180 and 360 min ozone treatment. The percentages of oleic acid in the HO and SBO decreased by 86.34% and 95.44%, respectively, and linolenic and linoleic acids were destroyed completely after 360 min ozone treatment. The increase in the relative percentages of saturated fatty acids reached its highest value with 360 min ozonation. Thereafter, new peaks with different numbers of carbon atoms (4-24) were observed in the chromatograms of the ozonated samples. Considering the predominant unsaturated fatty acids in HO and SBO as oleic and linoleic acids, respectively, it could be presumed that the new peaks formed mainly from the reaction of ozone with those fatty acids. In the oleic acid, the double bond was present in the C_9 position; while the linoleic acid contained two double bonds in the C_6 and C_9 positions. Therefore, the highest probability of the ozone reaction was with the double bond in the C_9 position. The oxidation of oleic acids with ozone involved the cleavage of the carbon-carbon

Table 2. Reduction in fatty acid composition of HO and SBO

	Reduction in fatty acid composition (%)					
	$C_{18:1}$ (Oleic acid)	$C_{18:2}$ (Linoleic acid)	$C_{18:3}$ (Linolenic acid)	$C_{20:1}$ (Gondoic acid)	Saturated/ unsaturated	$C_{18:2}/C_{16:0}$
OHO60	7.0±0.08	48.90±0.22	-	100±0.99	0.10±0.25	1.09±0.05
OHO180	42±0.30	84.77±0.87	-	100±0.85	0.16±0.41	0.32±0.08
OHO360	86.34±0.55	100.00±1.12	-	100±1.42	0.74±0.65	-
SBO60	0.13±0.11	20.72±0.77	45.54±0.57	-	0.20±0.72	4.21±0.74
SBO180	66.48±0.32	90.63±0.81	100±1.35	-	1.13±0.23	0.49±0.68
SBO360	95.44±0.43	100±0.91	100±1.42	-	14.39±0.88	-

[a]Each value in the table represents the mean ± standard deviation of triple analyses. Abbreviations: OHO60, OHO180, and OHO360 ozone treated hazelnut oil for 60, 180, and 360 min, respectively. SBO60, SBO180, and SBO360 ozone treated soybean oil for 60, 180, and 360 min, respectively.

double bond at position nine of the carbon backbone (Zahardis *et al*., 2006). The oxidation products, which are so-called Criegee intermediates, are highly reactive and can undergo ester and acid formation and hydroperoxide formation. In the literature, the products formed from the cycloaddition of a Criegee intermediate with a double bond of fatty acids has been detected from the reaction of ozone with oleic acid in an aqueous medium (Zahardis *et al*., 2006). The reaction mechanism of ozone with unsaturated fatty acids and formed oxidative products changes in relation to the nature of the solvent (Nishikawa *et al*., 1995). In an aprotic media secondary ozonide (1,2,4-trioxolane) and peroxide oligomers are formed by the reaction of zwitterions and aldehydes; whereas in a protic medium, the zwitterions react with water to produce different peroxidic species (hydro-peroxides, oligomeric peroxides) and carboxylic acids (Murray, 1968).

The results of the gas chromatography analysis were consistent with the IV analysis in that the decrease in IV showed the reduction in double bonds. As a result of 360 min ozone treatment, the percentage of oleic acid in the HO decreased by 86.34%, while a dramatic reduction in IV was seen at 85.5%.

The ratio of $C_{18:2}/C_{16:0}$ for HO and SBO was 2.15 and 5.31, respectively. Table 2, also shows that the ratio of $C_{18:2}/C_{16:0}$ decreased from 2.15 to 0.32 for HO and from 5.34 to 0.49 for SBO as ozone treatment time increased. After 360 min ozone treatment, the ratio could not be calculated due to the complete disappearance of linoleic acid. The ratio of linoleic acid ($C_{18:2}$) to palmitic acid ($C_{16:0}$) can be used to determine the extent of oil deterioration because linoleic acid is more sensitive to oxidation while palmitic acid is more stable to oxidation (Tan and Che Man, 1999). The ratio of saturated to unsaturated fatty acids is used to indicate changes in the nutritional value of oils (Tsanev *et al*., 1998) in such a way that a small ratio is considered to show a high nutritional content of oils. The ratio of saturated to unsaturated fatty acids for HO and SBO was 0.08 and 0.16, respectively. It can be seen in Table 2 that the nutritional values of the oil samples decreased as ozone treatment progressed. This may result from the increase in the relative percentages of saturated fatty acid and the decrease in that of unsaturated fatty acids after ozone treatment. The untreated HO and SBO had the highest nutritional value while the nutritional values of OHO360 and SBO360 were minimal.

3.4. Viscosity measurement

Figure 3 represents the flow behaviors of ozone treated and untreated HO and SBO. The flow behaviors of the oil samples were determined by steady shear viscosity measurements at 25 °C. Figure 3 shows a linear increase in shear stress with increased shear rate, characterizing Newtonian flow for all the samples. The viscosity of the untreated and ozone treated oils was calculated from the slope of the curve. It was revealed that fresh vegetable oils showed Newtonian flow behavior (Santos *et al*., 2004). However, the viscosity of the oil samples varied distinctly depending on the ozone treatment time. The highest viscosity was observed for 360 min ozone treated oil samples followed by 180, 60 and 30 min. However, the viscosities of 1 and 5 min ozone treated samples were observed as close to the viscosity of crude oil. The increase in viscosity of HO and SBO with respect to ozone treatment time could be explained by the decrease in unsaturation (Sega *et al*., 2010). Adhvaryu *et al*., (2000) reported that as the oxidation reactions proceed, the products formed lead to oil thickening. This was explained by increased van der Waals interactions due to the disappearance of the double bonds (Zanardi *et al*., 2008). The modification of the unsaturated acyl chains affects the mobility and the reactivity of the molecules involved in the reaction. The reaction of ozone with unsaturated fatty acids produces different types of peroxidic compounds as described by the Criegee mechanism. Van der Waals

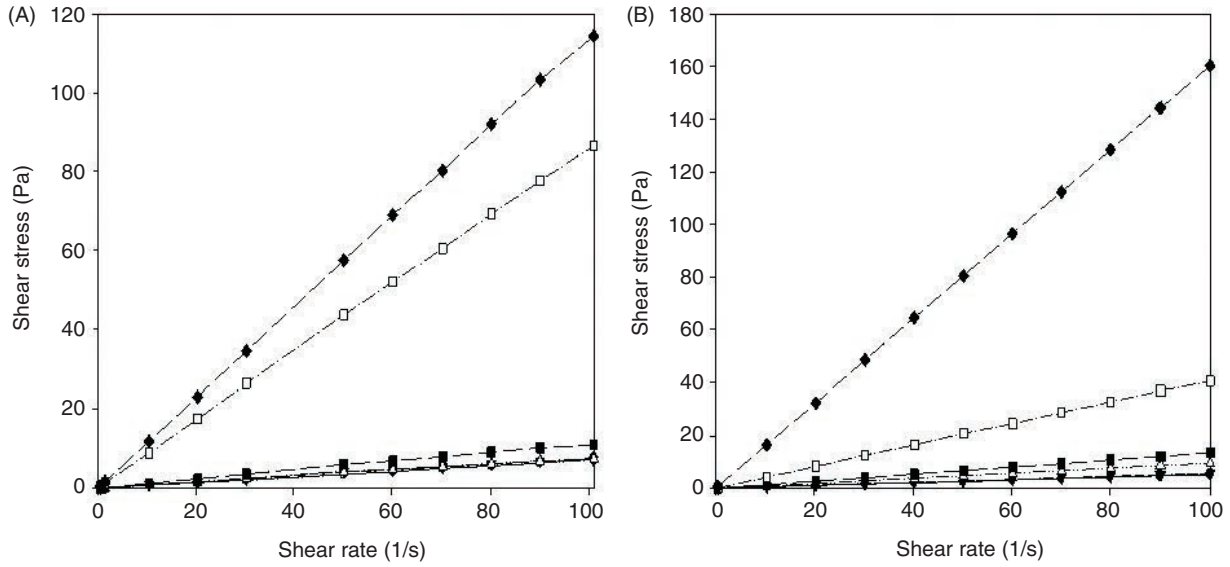

FIGURE 3. Curves of shear stress vs. shear rate of untreated and ozone treated HO (A) and SBO (B). (●; HO, SBO ○; OHO1, SBO1 ▼; OHO5, SBO5, Δ; OHO30, SBO30 Δ; OHO60, SBO60, ■; OHO180, SBO180, ♦; OHO360, SBO360. Abbreviations: HO; hazelnut oil, SBO; soybean oil. OHO1, OHO5, OHO30, OHO60, OHO180, and OHO360, SBO1, SBO5, SBO30, SBO60, SBO180, and SBO360 ozone treated HO and SBO for 1, 5, 30, 60, 180, and 360 min, respectively.

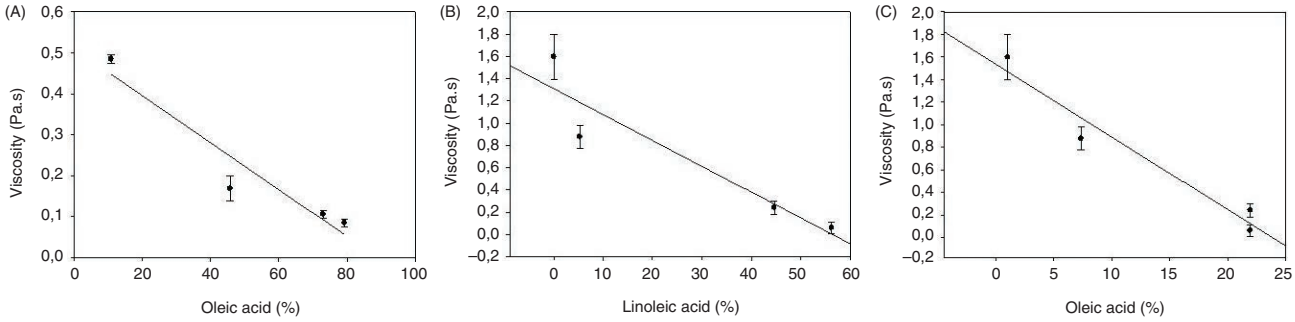

FIGURE 4. Correlations of oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) compositions of untreated and ozone treated oils with their viscosity. A: 18:1 fatty acid in HO; B: 18:2 fatty acid in SBO; C: 18:1 fatty acid in SBO. For A, B, and C, $R^2 = 0.951$, $R^2 = 0.87$, $R^2 = 0.953$, respectively.

interactions of these peroxidic compounds increase to form species having high molar masses. Therefore, the ozonation of double bonds in oils increases their viscosity due to those high molar mass species. However, the viscosities of the untreated and ozone treated HO and SBO were not observed to change with storage time.

Figures 4A, 4B and 4C show the relationship between the viscosity and oleic acid, and linoleic acid contents in HO and SBO. The viscosity of untreated and ozone treated oils were plotted against the percentages of oleic acid and linoleic acid found that in the oils. There was a high correlation between them (Figures 4A, 4B, and 4C, $R^2 = 0.951$, $R^2 = 0.87$, $R^2 = 0.953$, respectively). The oleic acid content had more effect on the flow behavior of HO. An increase in viscosity was observed when a decrease in the oleic acid composition of HO occurred with respect to ozone treatment time. It was seen that the viscosity of HO increased by 5.7 times after 360 min ozone treatment. The rise in viscosity of SBO was by about 20 times at the end of 360 min ozone treatment. Kim *et al*., (2010) revealed that double bonds which form a twist in the fatty acid chain do not allow fatty acids to come together. The dimensions and orientations of oil molecules influence the viscosity of oils. The unsaturated oils converted to saturated forms after ozonation lose their fluidity and their viscosity increases. Fatty acids with more double bonds do not have a rigid and fixed structure, and are loosely packed and more fluid-like (Kim *et al*., 2010).

3.5. FTIR (Fourier transform infrared radiation) analysis

The variations in frequency and absorbance of the FTIR spectra bands especially belonging to unsaturated bonds were observed after ozone treatment (Guillen and Cabo, 2000). The intensity and frequency of the bands related to the carbon-carbon double bonds in unsaturated oil decreased or these bands became overlapping and broadened as ozone treatment time was extended. The double bonds finally disappeared completely with increasing ozone treatment time. The broad and relatively strong bands corresponding to secondary ozonide were observed as ozone treatment proceeded. Figures 5A and 5B show the FTIR spectra of untreated and ozone treated HO and SBO. As seen in these figures, the weak bands that corresponded to the C=C-H stretch at 3006 reduced when ozone treatment time increased. In an earlier study, a higher band frequency was observed (=CH stretching vibration assigned to around 3006 cm^{-1}) for a large proportion of polyunsaturated fatty acid content in the oil than for monounsaturated fatty acid content in the oil (Guillén and Cabo, 2000). We observed that the frequency of the band of HO and SBO was sharply reduced as ozone treatment proceeded. The reduction in frequency of around 3006 cm^{-1} in the FTIR spectrum belonging to SBO was more pronounced for 180 and 360 min ozone treatment due to its higher polyunsaturated composition than HO. A dramatic reduction in band intensity at around 722 cm^{-1} corresponding to overlapping methylene (-CH_2) and rocking vibration of olefins was observed as ozone treatment continued (Silverstein *et al*., 1974).

The bands at 2922 and 2853 cm^{-1} corresponded to asymmetrical and symmetrical stretching of the methylene (-CH_2) group of mainly unsaturated bonds in the lipids (Dogan *et al*., 2007). It was observed that intensities of the bands decreased slightly during ozone treatment. However, the reduction in frequencies of the bands related to SBO was more pronounced than HO.

The untreated HO and SBO showed a relatively sharp band due to the carbonyl stretch of the triglyceride ester group at 1743 cm^{-1} (Figure 5A and 5B). It could be observed that the broadening of the band and decrease in frequency occurred due to the formation of new carbonyl compounds as ozone treatment was extended (Soriano *et al*., 2003).

A small band assigned at 1463 cm^{-1} corresponds to the bending vibrations of CH_2 and CH_3 groups (Che Man and Setiowaty, 1999). It was detected that the absorbance value of the band decreased as ozone treatment time increased (Figure 5, A and B). The bands at 1237 and 1160 cm^{-1} related to (=CH stretching vibration) was assigned at around 3006 cm^{-1} with stretching vibrations of the C-O group in esters were observed in the spectrum of HO and SBO (Figures 5A and 5B) (Guillen and Cabo, 1997).

The band at 1105 cm^{-1}, which was broader and stronger in the FTIR spectrum of HO and SBO as the ozonation period increased, was assigned in accordance with the C-O stretch of the secondary ozonide, as is consistent with previous research (Wu *et al*., 1992).

3.6. NMR (Nuclear magnetic resonance) analysis

Nuclear Magnetic Resonance (NMR) spectroscopy analysis for untreated and ozone treated HO and SBO were performed in ^1H spectra to identify variations in the structure of HO and SBO with respect to ozone treatment time.

Table 3 shows the assignments of the main groups of untreated and ozone treated HO and SBO and variations in the intensities of those main groups with ozonation period in the ^1H NMR spectra. The signals related with olefinic protons from fatty acids were determined at 5.30 ppm. The signals at 2.00 and 2.75 ppm belong to the methylenic group in both sides of olefinic protons and the methylenic group between olefinic protons, respectively. The methylenic group in the α-position with respect to the carbonylic group was assigned at 2.30 ppm. The double doublet belonging to glycerol protons in the sn 1,3 position of the glycerol moiety was assigned at 4.30 ppm. The intensities of olefinic proton signals at 5.30 ppm and the methylenic group between olefinic protons at 2.75 ppm gradually decreased with the increase in ozone treatment. The reduction in the intensity in the signal at 5.30 ppm for HO was determined as 70.29% after 360 min ozone treatment; while a 79.40% reduction was observed for SBO. The signal at 2.75 ppm for HO disappeared completely; while the intensity of the signal for SBO was reduced by 94.50% after 360 min ozone treatment. It was concluded that the methylenic group corresponding to linolenyl chains in HO was reacted with ozone completely due to a lower concentration of linoleic acid in HO than SBO. The intensity of the signal at 2.00 ppm for HO and SBO diminished when ozone treatment progressed. The reductions were observed to be 92.35 and 63.58% for HO and SBO, respectively. The intensity of the methylenic group in the α-position with respect to the carbonylic group which is assigned at 2.30 ppm remained almost the same as the ozone treatment time changed. A similar trend was observed for the signal at 4.30 ppm, belonging to glycerol protons in the sn 1,3 position of the glycerol moiety.

Similar assignments to ^1H NMR spectra were also determined in the 13C NMR spectrums of HO and SBO. Two signals at 127 and 130 ppm which corresponded to the unsaturation of fatty acids disappeared completely after 360 min ozonation. The signal at 174.3 ppm was related to carbonyl

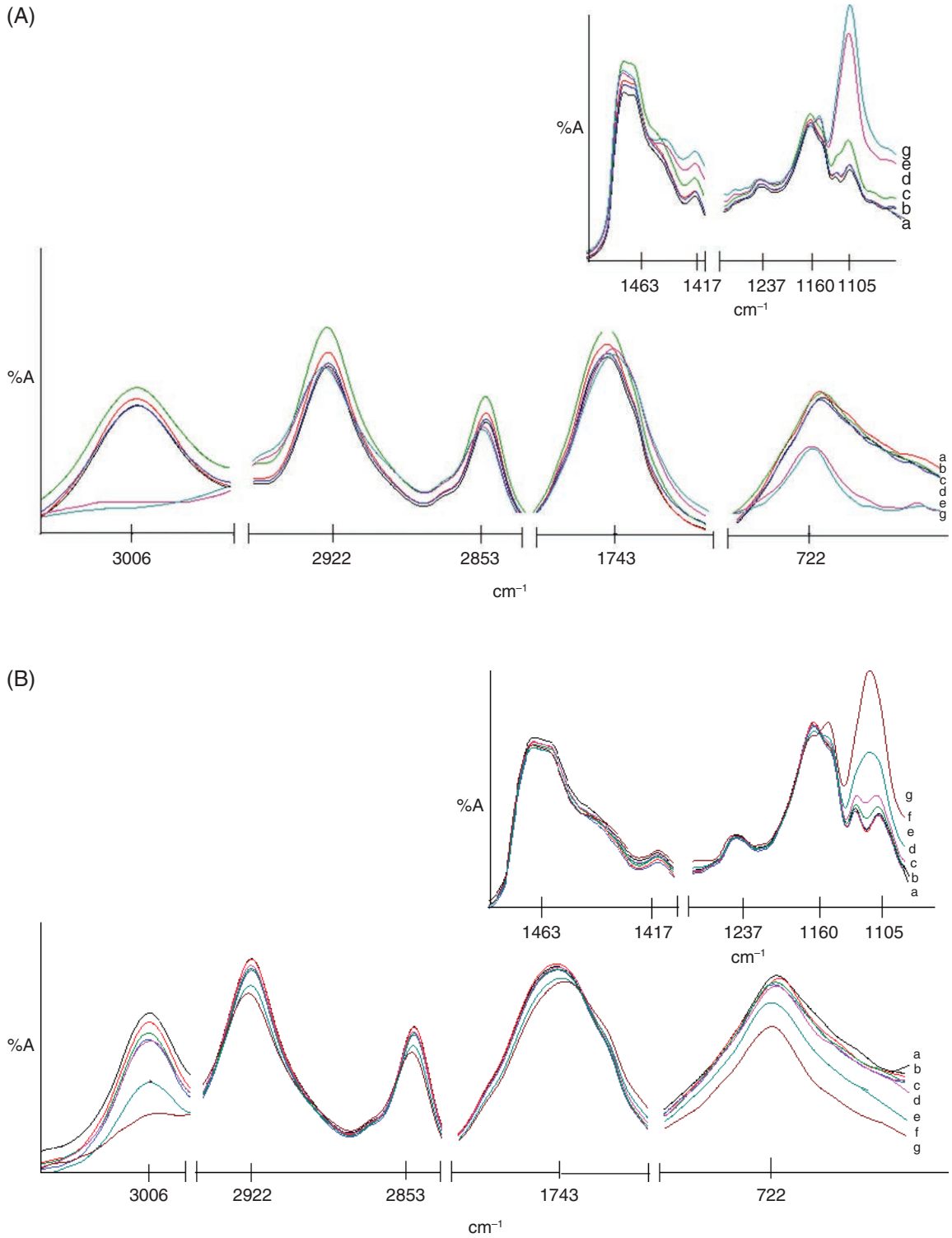

FIGURE 5. Major bands of FTIR spectra for HO (A) and SBO (B). Abbreviations: HO; hazelnut oil, SBO; soybean oil. OHO1, OHO5, OHO30, OHO60, OHO180 and OHO360, SBO1, SBO5, SBO30, SBO60, SBO180 and SBO360 ozone treated HO and SBO for 1, 5, 30, 60, 180 and 360 min, respectively. a: HO, b: OHO1, c: OHO5, d: OHO30, e: OHO60, f: OHO180, g: OHO360 and a: SBO, b: SBO1, c: SBO5, d: SBO30, e: SBO60, f: SBO360, g: SBO360.

TABLE 3. ^{1}H NMR intensities for the main groups of ozone reaction with HO and SBO

Ozone treatment time		HO	OHO60	OHO180	OHO360	SBO	SBO60	SBO180	SBO360
Groups	**δ^{1}H**	**^{1}H NMR intensities**							
α-metyhlene group to the carbonyl carbon	1.39	---	2.35	4.77	5.04	---	1.33	3.57	3.78
CH_2-CH_2COOH (All acyl chains)	1.65	---	3.19	3.49	8.12	---	1.06	3.33	3.71
CH_2CH=CH (All unsaturated acyl chains)	2.00	5.37	3.47	0.74	0.41	4.97	3.64	2.07	1.81
CH_2-COOH(All acyl chains)	2.30	2.93	3.06	3.04	3.05	2.96	3.00	3.13	3.02
α-metyhlene group to the carbonyl carbon	2.43	---	0.34	0.28	0.21	---	0.78	1.23	0.71
CH=CHCH_2CH=CH (Linolenyl and linolenyl chains)	2.75	0.36	0.15	---	---	2.04	0.85	0.13	0.11
CH_2-OCOR (Triglycerides)	4.30	0.97	1.00	0.97	0.91	1.00	0.98	0.99	1.00
1,2,4-trioxolane	5.15	---	0.93	1.09	1.07	---	0.84	1.90	1.91
CH=CH (All unsaturated fatty acids)	5.30	3.03	1.87	1.09	0.90	4.96	2.97	1.37	1.02
Aldehyde	9.75	---	0.04	0.07	0.07	---	---	0.05	0.07

δ ^{1}H (ppm): Chemical shifts
[a]Abbreviations: HO; hazelnut oil, OHO60, OHO180, and OHO360 ozone treated hazelnut oil for 60, 180, and 360 min, respectively. SBO; soybean oil, SBO60, SBO180, and SBO360 ozone treated soybean oil for 60, 180, and 360 min, respectively.

carbons of esters. The signals assigned at 61.8 and 68.7 corresponded to the carbons of glycerol, CH_2 and CH, respectively. The other carbons in the structure were assigned between 24.8 and 33.8 ppm (Diaz et al., 2005).

The new signals found in the ^{1}H NMR spectra of ozone treated HO and SBO are summarized in Table 3. A new signal at 5.15 ppm was assigned to the ring proton of 1,2,4-trioxolane (Sadowska *et al*., 2008). The result was confirmed by the appearance of the signal at 104.35 ppm in ^{13}C NMR spectra (Wu *et al*., 1992). Ozone treated HO and SBO also exhibited new peaks belonging to the aldehydic proton and α-methylene group to the carbonyl carbon at 9.75 and 2.43 ppm in the ^{1}H NMR spectra. In the ^{13}C NMR spectrum, α-methylene group related to the carbonyl carbon was determined at 43.9 ppm. In the ^{1}H NMR spectrum of HO and SBO, a weak signal which corresponded to aldehydes was identified at 9.75 ppm. Furthermore, the protons of the α-methylene group which were assigned at 2.00 and 2.75 ppm in the ^{1}H NMR spectrum of the untreated oils shifted to 1.39 and 1.65 ppm in the ^{1}H NMR spectrum of the ozone treated oil samples due to the formation of 1,2,4-trioxolane (Anachkov *et al*., 2000).

CONCLUSIONS

The oxidation of HO and SBO with ozone resulted in some oxidation products which have been detected by FTIR and NMR spectroscopic analyses. These spectroscopic analyses involve useful and convenient techniques to explain structural variations in the oil samples during ozone treatment. The NMR and FTIR analyses exhibited evidently, that new signals corresponded to ozonation products which gradually increased with respect to ozone treatment time. After 360 min ozone treatment, the carbon-carbon double bond signal which belongs to unsaturated fatty acids disappeared completely in the spectrum. The results of gas chromatography showed that all of the carbon-carbon double bonds had been consumed completely as ozone treatment progressed. Also, the results of the gas chromatography analysis were consistent with the IV analysis in that a decrease in IV resulted in a reduction in double bonds.

The relationship between ozone treatment time and structural and viscosity changes in HO and SBO were evaluated. We observed an increase in the viscosity of HO and SBO with respect to ozone treatment time due to the decrease in unsaturation. Moreover, ozone treatment may result in a significant variation in color parameters due to the degradation of carotenoid pigments.

The results of this work are consisted with the previously proposed mechanism related to the reaction of ozone with vegetable oils (Soriano *et al*., 2003; Sega *et al*., 2010; Diaz *et al*., 2005). Also, it was demonstrated that the applied ozone dose is an important parameter to determine the effects of ozone on the structural properties of HO and SBO.

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