SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 22(2) (2024) 104-114, DOI: 10.24323/akademik-gida.1542583

Research Paper / Araştırma Makalesi

Emulsion Stability and Rheological Properties of Emulsions Prepared with Ozonized Hazelnut Oil

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Received (Geliş Tarihi): 27.02.2024, Accepted (Kabul Tarihi): 03.08.2024
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ABSTRACT

In this study, physical and rheological characteristics of the emulsion stabilized with whey protein isolate (WPI) at a concentration of 0.25% (w/w) and containing ozone applicated hazelnut oil (OHO) were studied. The hazelnut oil was subjected to ozone gas exposure for durations of 1, 5, 30, 60, 180, and 360 min. The emulsions made with both control (HO) and OHO were analysed to assess emulsion properties. It was observed that as the duration of ozone application increased, the emulsion activity index (EAI) experienced a decrease. Specifically, ozone applications of 30, 60, and 180 min significantly reduced emulsion stability index (ESI). The creaming ratio of emulsions formulated with OHO for durations of 1, 5, 30, 60, and 180 min were found to be 23%, 20%, 18%, 16%, and 44%, respectively. Optical microscopy revealed that significant flocculated droplet formed in emulsions including OHO. The longer time the ozone application resulted that the emulsions exhibited more solid-like behaviour. Furthermore, differential scanning calorimeter (DSC) was utilized to assess the changes in the melting and cooling profiles of hazelnut oil after being applicated with ozone. An increase in ozone application duration led to the melting peaks broadening and shifting towards lower temperatures. Also, ozone application caused that the sharp exothermic crystallization shifted towards lower temperatures and become broader and less pronounced as the duration of the application increased.

Keywords: Ozone, hazelnut oil, and emulsion stability, rheology, differential scanning calorimeter (DSC)

Ozonlanmış Fındık Yağı ile Hazırlanan Emülsiyonların Emülsiyon Kararlılığı ve Reolojik Özellikleri

ÖZ

Bu çalışmada, %0,25 (w/w) konsantrasyonda peynir altı suyu protein izolatı (WPI) ile stabilize edilen ve ozon uygulanmış fındık yağı (OHO) içeren emülsiyonun fiziksel ve reolojik özellikleri incelenmiştir. Fındık yağı, 1, 5, 30, 60, 180 ve 360 dakikalık sürelerle ozon gazına maruz bırakılmıştır. Hem kontrol (HO) hem de OHO ile yapılan emülsiyonlar, emülsiyon özelliklerini değerlendirmek için analiz edilmiştir. Ozon uygulama süresi arttıkça emülsiyon aktivite indeksinin (EAI) azaldığı görülmüştür. Özellikle 30, 60 ve 180 dakikalık ozon uygulamaları emülsiyon stabilite indeksini (ESI) önemli ölçüde azaltmıştır. 1, 5, 30, 60 ve 180 dakika süreyle OHO ile formüle edilen emülsiyonların kremalaşma oranları sırasıyla %23, %20, %18, %16 ve %44 olarak bulunmuştur. Optik mikroskopi, OHO içeren emülsiyonlarda önemli miktarda topaklaşmış damlacık oluştuğunu ortaya çıkarmıştır. Ozon uygulamasının süresi uzadıkça emülsiyonlar daha katı benzeri davranış sergilemiştir. Ayrıca, ozon uygulandıktan sonra fındık yağının erime ve soğuma profillerindeki değişiklikleri değerlendirmek için diferansiyel taramalı kalorimetre (DTK) kullanılmıştır. Ozon uygulama süresindeki artış, erime tepe noktalarının genişlemesine ve daha düşük sıcaklıklara doğru kaymasına yol açmıştır. Ayrıca ozon uygulaması, uygulama süresi arttıkça keskin ekzotermik kristalleşmenin daha düşük sıcaklıklara doğru kaymasına ve daha geniş ve daha az belirgin olmasına neden olmuştur.

Anahtar Kelimeler: Ozon, fındık yağı, emülsiyon stabilitesi, reoloji, diferansiyel taramalı kalorimetre (DTK)

INTRODUCTION

The utilization of ozone as a novel, non-thermal technique in the food industry has been preferred over thermal methods. In thermal methods, heat procedures have damage or diminish the physical, chemical, and sensory qualities of food products despite their efficacy in disinfection. Ozone has been recognized as a reliable chemical disinfectant in food processing in several developed countries, including the United States, Japan, Australia, France, and Canada, since receiving an approvement as the GRAS (Generally Recognized as Safe) from the US FDA in 1997 [1-3]. It's use have cover various sectors of the food industry, including the sanitation of food surfaces and plant equipment [4], the reuse of wastewater [5], the disinfection of poultry carcasses and chill water [6], the inactivation of microbial load in nuts and cereal storage atmospheres [7], and the disinfection of fruits and vegetables [7]. Additionally, ozone application has gained attention in the pharmaceutical and cosmetic industries [8]. Application of ozone to vegetable oils, which are a key component in numerous products in the food and pharmaceutical industries, has become popular due to its antibacterial and fungicidal properties. Recent research indicates that the structural, chemical, and physical characteristics of vegetable oils undergo changes following ozone application. This study focuses on the effects of ozone on vegetable oils, specifically hazelnut oil. Hazelnut oil has been used as a food ingredient in food formulations at the food industry due to its beneficial properties. It has been obtained through the cold pressing of hazelnuts and utilized in personal care and cosmetic products, food preparations, and the pharmaceutical industry, finding application in industries such as chocolate, confectionery, and pastry.

Ozone, as an oxidizing substance, affects food constituents, especially proteins, lipids, vitamins, and other substances which are containing double bonds in their structure. The reaction of ozone with unsaturated lipid bonds generates a mixture of oxygenated molecules like ozonides, peroxides, and aldehydes [9]. Limited research exists on the rheological and functional behaviour of emulsions made with ozone-exposed hazelnut oil. Therefore, the work presents fundamental findings on how ozone applicated hazelnut oil affects the rheological and emulsifying characteristics of emulsions stabilized with WPI. Furthermore, effects of ozone application on thermal stability of hazelnut oil were investigated. Hazelnut oil was exposed to ozone for various durations (1, 5, 30, 60, 70, 180, and 360 min), and subsequently, both applicated and control samples were stored at room temperature in hermetically sealed glass bottles in a dark place. Emulsions were formed by mixing OHO with WPI solutions and then homogenizing. The EAI, ESI, and creaming properties of emulsions containing control and OHO were measured. Optical microscopy was used to observe the changes in droplet size, structure, and flocculation in emulsions after ozone application. The rheological behaviour of emulsions also examined, and differential scanning calorimetry (DSC) was used to determine the variations in melting and cooling profiles of hazelnut oil due to ozone application.

MATERIALS and METHODS

Materials

Hazelnut oil was acquired from a local producer (FISKOBIRLIK Company, Giresun, Turkey). Buffer salts, potassium dihydrogen orthophosphate, disodium hydrogen orthophosphate and sodium dodecyl sulphate (SDS) were purchased from Analar analytical reagent. WPI (98%) (WPI, BIOPRO, Lot 86 No. JE 030-3-420) were sourced from Davisco Foods International (Le Sueur, MN, USA). Used chemicals utilized were of analytical grade.

Ozone Application of HO

Ozone generator (Ozone Marine, OMS Model, Izmir, Turkey) was used to produce ozone gas. The generator operates on the corona-discharge method (flow rate: 1L/min; concentration of ozone: 40 mg/L). The oxygen necessary for generating ozone was supplied from the air. Firstly, 100 mL of hazelnut oil was poured into a gas washing bottle. The ozone gas generated by ozone generator was passed through the bottle including hazelnut oil for different durations (1, 5, 15, 30, 60, 180, and 360 min). The ozone dosages were calculated as 0.0004, 0.002, 0.006, 0.012, 0.024, 0.072, 0.144 g ozone/mL oil for the ozone application times of 1, 5, 15, 30, 60, 180, and 360 min, respectively [10].

Emulsion Properties

Emulsion Preparation

Phosphate buffer was prepared by dissolving potassium dihydrogen orthophosphate (3.76 g) and disodium hydrogen orthophosphate (3.44 g) in 2 liters distilled water and pH was adjusted to 7.0 by using 0.1M NaOH and 0.1M HCl. 0.01 % (w/w) 28 sodium azide was added to the buffer as an anti-microbial agent for emulsion experiments. WPI was dissolved in phosphate buffer (ionic strength 0.05M and pH 7.0) or double distilled water at room temperature while stirring with magnetic stirrer for 1 hour.

The modified method [11] was employed to evaluate the emulsifying properties of WPI. Emulsions were created by homogenizing hazelnut oil (50.0% (v/v)) and protein solution (50.0% (v/v)) for 30 seconds using an Ultrasonic Disintegrator (Soniprep 150) at 72 kHz. The last protein content in the emulsions was 0.25% (w/w). To examine the impact of ozone gas on activity and stability of emulsion, ozone applicated oils were used to prepare emulsions. The emulsions were produced in triplicate.

Emulsion Stability and Activity

The modified method [11] was employed to evaluate the emulsifying stability and activity of WPI stabilised emulsions. At different intervals, a 0.05 ml of the emulsion which taken from the bottom part of the beaker was diluted with 50 ml of a 0.1% (w/v) SDS solution. After emulsion formation, absorbance of the sample was measured immediately for every minutes for 2 hours. Later, absorbance of the emulsions were measured daily for 10 days. The absorbance of this diluted emulsion was then measured at 500 nm using a spectrophotometer (Pharmacia Biotech, Novaspec II, UK). Emulsion Stability Index (ESI) was calculated by monitoring the variation in absorbance over time. Emulsifying Activity Index (EAI) was measured instantly after the formation of the emulsion based on the absorbance. The calculation provided the surface area (m^2) / amount of protein (mg) used in the emulsions. These emulsion tests were conducted three times.

$$EAI = 2T/\emptyset C \qquad (1)$$

where C is the amount of protein per unit volume of aqueous phase before forming the emulsion. T represents turbidity, with T = 2.303 A/l, where A is the absorbance and l is the path length of the cuvette. Ø is the volumetric fraction of the dispersed phase.

$$ESI = \Delta t/\Delta T \qquad (2)$$

where ΔT is the variation in turbidity during the time interval Δt.

Creaming Analysis

Emulsions prepared from OHO were used to investigate the influences of ozone on creaming. A freshly made 10 mL emulsion was placed in a test tube and kept at room temperature (22-24°C). The length of the serum layer was measured every 10 hours to observe its change over time. The volume of the creamed emulsion at the top and the initial volume of the emulsion were calculated, and the results were expressed as a percentage of creaming.

$$\%creaming = 100 - \left[\left(\frac{Vc}{Vt}\right)\right] \qquad (3)$$

where Vc is the volume of the creamed phase and Vt is the total volume [12].

Optical Microscopy

At room temperature, images of the emulsions belonging to droplets were assessed using polarized light microscopy (PLM). 1 mL of 0.1% (w/v) SDS solution was used to dilute emulsions, utilizing a volume of 100 μL. Subsequently, a little part of the emulsion was applied to a microscope slide, and a cover slip was carefully positioned over it. After a brief period of acclimatization, photomicrographs were captured at a 40x magnification using an Olympus BX51 microscope (Tokyo, Japan) and a camera (Pixera PVC, 100C, USA).

Rheological measurement

Bohlin CVO-R controlled stress rheometer (Bohlin Instruments, Gloucestershire, UK) was used for dynamic rheological measurements at 25±0.01°C (plate-to-plate geometry: 40 mm diameter; 0.105 mm gap). 2.0 ml of emulsion was attentively placed on the rheometer plate. A thin layer of silicone oil was applied to the exposed surface of the sample to avoid evaporation during the measurement, once the plate made contact with the sample. Elastic (G′) and viscous (G″) behaviour were determined against frequency. Frequency sweep tests (1Pa stress value at 25°C) was conducted within a linear viscoelastic region from 0.01 to 10 Hz frequency range. The experimental data was collected using Reowin Pro Data Manager Version 2.64, a data analysis program.

DSC Analysis of Heating and Cooling Thermogram

Differential scanning calorimetry (DSC) was utilized to investigate the melting and cooling profiles of both control and OHO using a Perkin-Elmer DSC 6 equipped with Pyris software (Perkin-Elmer Inc., Wellesley, USA). Pure indium was used to calibrate DSC, and a baseline was established using an empty open aluminium pan. Nitrogen was utilized as the purge gas, flowing at a rate of 40 ml/min. Oil samples, weighing between 5-10 mg, were put in aluminium pans and sealed. The aluminium pan which was an empty, hermetically sealed utilized as the reference. To eliminate any prior thermal history, the sample was heated to 80°C in the DSC instrument and maintained at this temperature for 10 minutes. It was then cooled to -60°C at a rate of 5°C/min and held at this temperature for 10 minutes. Following this, the sample was heated up to 80°C (heating rate: 5°C/min). The onset (T_{on}) and offset (T_{off}) temperatures, as well as the enthalpy (ΔH) related to melting were detected from the DSC thermogram.

Statistical Analysis

Statistical analysis was conducted using SPSS Statistics 16.0, version 2.0 (2006) (SPSS Inc., Chicago). Analysis of variance (ANOVA) was used to evaluate the experimental results. In order to determine statistically significant differences between the data sets, The Duncan multiple range test was applied. Differences were evaluated that there were a significant level at the $p < 0.05$.

RESULTS and DISSCUSSION

Emulsifying Properties

The study investigated the emulsifying properties of emulsions containing control and OHO and stabilized with WPI. An ultrasound disintegrator (15 amplitudes for 30 seconds) was employed for emulsion preparation. As suggested by prior research [13], severe intensity ultrasonic waves were utilized to eliminate any droplets which is flocculated in the emulsions.

EAI and ESI were measured over an extended period (approximately 10 days) to observe droplet behaviour in the emulsion. For control emulsion, EAI was 917 m^2/mg, while for OHO containing emulsions for 1, 5, 30, 60, 180, and 360 min, EAI values were 893, 880, 812, 791, 616, and 177 m^2/mg, respectively. Figure 1 indicates the EAI of the control (HO) emulsion was similar with the emulsions containing OHO1and OHO5. However, EAI values for the other emulsions containing OHO30, OHO60, OHO180, and OHO360 were reduced with ozone application time. The result was revealed that a negative impact of ozone application on EAI ($p<0.05$). The adverse effect of ozone on EAI was more apparent emulsions including OHO360. As the ozone application time increased, it was noticed that the pH of the emulsions changed. The emulsions were prepared with buffer solution (pH: 7.0). pH value of the emulsion containing HO was measured as 6.80. The pH values of emulsions containing OHO were 6.75, 6.00, 5.22, 4.86, 3.00, and 2.25 for 1, 5, 30, 60, 180, and 360 min ozone application. This might be due to pH variation influenced the solubility of WPI in the emulsions. WPI is a protein mixture and contains a high amount of β-lactoglobulin. Previous research [14] proposed that the reduction in β-lactoglobulin adsorption at low pH was due to structural changes in the molecule, reducing its flexibility and, consequently, it's emulsifying characteristics. The pH of the solution also impacts the net charge distribution of proteins, affecting their solubility. Generally, a decrease in protein solubility has a negative impact on functionality. Some researchers stated that the protein showed the lowest solubility near its isoelectric point (pI) [15, 16].

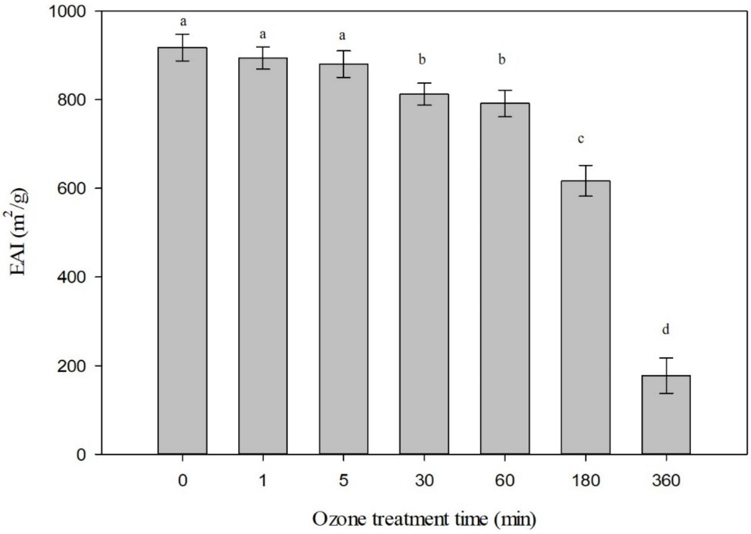


Figure 1. EAI of emulsions prepared with HO and OHO. (Abbreviations: HO; control hazelnut oil, OHO; ozone applicated hazelnut oil at different times (1, 5, 30, 60, 180 and 360 min).

The oxidative interaction of ozone on proteins affects their structural characteristics. Especially, this interaction negatively impacting on their nutritional value (loss of key amino acids), charge, enzyme activity, and structure (exposure of lipophilic groups, alterations in secondary structure, and disulfide groups). Additionally, the functional properties of proteins had been affected by ozone application. It was also observed that the lowest solubility did not occur at the isoelectric point of β-lactoglobulin. This could be due to whey proteins contains a mixture of some proteins such as β-lactoglobulin, α-lactalbumin serum albumin [17].

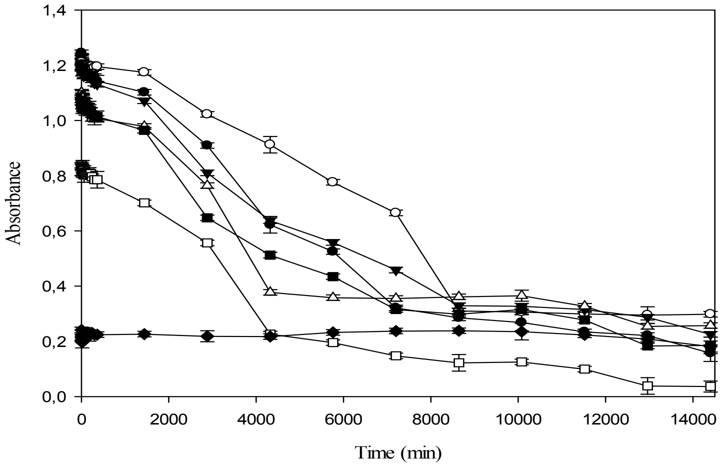


Figure 2. ESI of emulsions prepared with HO and OHO. (●; HO; ○; OHO1, ▼; OHO5, Δ; OHO30, ■; OHO60, □; OHO180, ♦; OHO360. Abbreviations: HO; control hazelnut oil, OHO; ozone applicated hazelnut oil at different times (1, 5, 30, 60, 180 and 360 min).

The changes in absorbance of emulsions prepared with control and OHO were utilized to calculate the ESI. As depicted in Figure 2, the ESI of ozone applicated samples rapidly decreased within the first 4000-6000 minutes, followed by a gradual slowdown approaching a plateau value. Figure 2 indicates a significant impact of ozone application on ESI ($p<0.05$). Notably, as the ozone application duration increased, ESI experienced a more pronounced decline. Significant drops in ESI were determined after ozone applications for 30, 60, and 180 min. As reported earlier [18], the pH values of emulsions made with ozone applicated oils decreased with increasing ozonation duration. The emulsions showed the lowest stability near the isoelectric point of WPI, which has an isoelectric value around pH 5 [18]. This phenomenon is associated with a minimal net charge, leading to reduced electrostatic and steric repulsions, causing flocculation and creaming extensively, accelerating emulsion destabilization [18]. The stability of the emulsion including OHO360 exhibited slow alterations over time. This could be attributed to the increase in oil sample viscosity with the duration of ozone application. The rise in viscosity over ozone application time is explained by ozone oxidizing unsaturated fatty acids in the oil, leading to a reduction in the proportion of unsaturated fatty acids and an increment in saturated fatty acids. The viscosity of ozonated oils increases with the ratio of saturated fatty acid to unsaturated ones, and the most substantial increase in viscosity was observed after 360 min of ozone application. Consequently, it can be argued that as oil viscosity increased, the movement of oil droplets in the emulsion decelerated. Previous researchers [19] have mentioned that an increment in the viscosity of the continuous phase can delay the gravitational separation of emulsions.

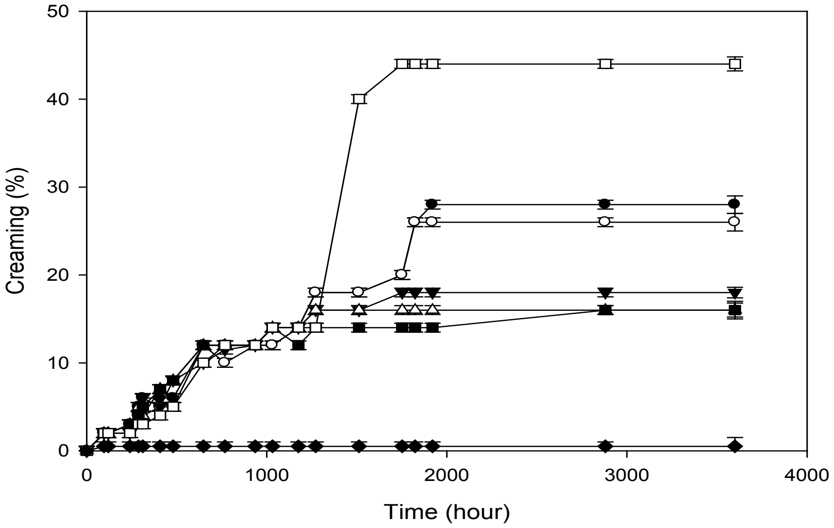


Figure 3. Creaming behaviour of emulsions prepared with HO and OHO. (●; HO; ○; OHO1, ▼; OHO5, Δ; OHO30, ■; OHO60, □; OHO180, ♦; OHO360. Abbreviations: HO; control hazelnut oil, OHO; ozone applicated hazelnut oil at different times (1, 5, 30, 60, 180 and 360 min).

Figure 3 illustrates the creaming behaviour of control emulsion and made with OHO. The duration of ozone application had a significant impact on the creaming behaviour of the samples ($p<0.05$). Creaming, the migration of droplets to upward due to their lower density than the surrounding liquid, progressed prominently for approximately 75 days, after which it decelerated and reached a plateau value except the including OHO360. Emulsions including OHO 5, OHO30, and OHO60 exhibited comparable creaming behaviours. While control emulsion showed 24% creaming after 75 days of storage, emulsions including OHO1, OHO5, OHO30, OHO60 and OHO180 exhibited 23%, 20%, 18%, 16%, and 44% creaming, respectively. Consequently, the creaming ratio of the emulsion including short-term ozone applicated oils was found to be slower than the creaming behaviour of the control, whereas, the creaming ratio of the emulsion made with OHO180 increased dramatically. This may be due to a variation in the pH of emulsions including ozone applicated oils. The acid value of the hazelnut oil increased after ozone application, leading to a decrease in the pH of the emulsions depending on the ozone application period. pH of the emulsion containing OHO180 was 3.35. Previous studies have shown that pH affects the physical stability of emulsions, with emulsion stability studies indicating fast creaming and droplet aggregations at low pH values (pH: 3) [20]. The creaming in emulsion containing OHO360 was not observed. Due to the increment in viscosity and acid value of the oil after 360 min of ozone application, there might be insufficient interaction between the oil droplets and continuous solution of the emulsion system. It can be inferred that the lack of noticeable creaming after 360 min of ozone application was due to a significant increase in oil viscosity.

Polarised Light Microscope (PLM)

Polarized light microscopy at 40x magnification was utilized to capture images of control and emulsions including OHO to discern any microstructural disparities between the emulsions. In figure 4A, a well-formed structure of generally small and well-isolated spherical oil droplets was observed in the emulsion made with control. In figure 4 B, C and D, the similar results were obtained with control emulsion. As seen in these figures (Figures 4 B, C and D), spherical, small, well-formed droplets were observed in the emulsions including OHO1, OHO5, OHO30. The limited packing volume of emulsion droplets, coupled with electrostatic repulsion between protein molecules at the interface, prevented droplet flocculation, resulting in a well-stabilized emulsion in liquid form. However, optical microscope observations revealed extensive droplet flocculation in emulsions containing oil exposed to ozone for prolonged periods. The size of emulsion oil droplets increased as the duration of ozone application lengthened. Specifically, in figures 4E, F and G, the microscopic measurements of emulsion microstructure verified extensive flocculation of droplet in emulsions including OHO 60, OHO180 and OHO360. The results showed that protein-coated droplets at the isoelectric point is more susceptible to formation of flocculated droplets. Consequently, aggregated droplets have been formed from insufficient electrostatic repulsion between droplets. Furthermore, as the ozone application period increased, the pH of the emulsions reduced. The distribution of size of the emulsion droplets moved to larger sizes with increasing ozone application time, as depicted in Figure 4E, F and G, due to a decrease in emulsion pH. Near the isoelectric point, electrostatic repulsion between oil droplets has reduced result in droplet aggregation was further facilitated. Ultimately, the microstructure of the emulsion including OHO360 appeared as coarse droplets (Figure 4 G). Despite the presence of coarse droplets, the creaming ratio was observed at a low level due to an increment in the viscosity of OHO360. As the viscosity of the emulsion increases, the motion of the oil droplets slows down, contributing to increase in the emulsion stability [21].

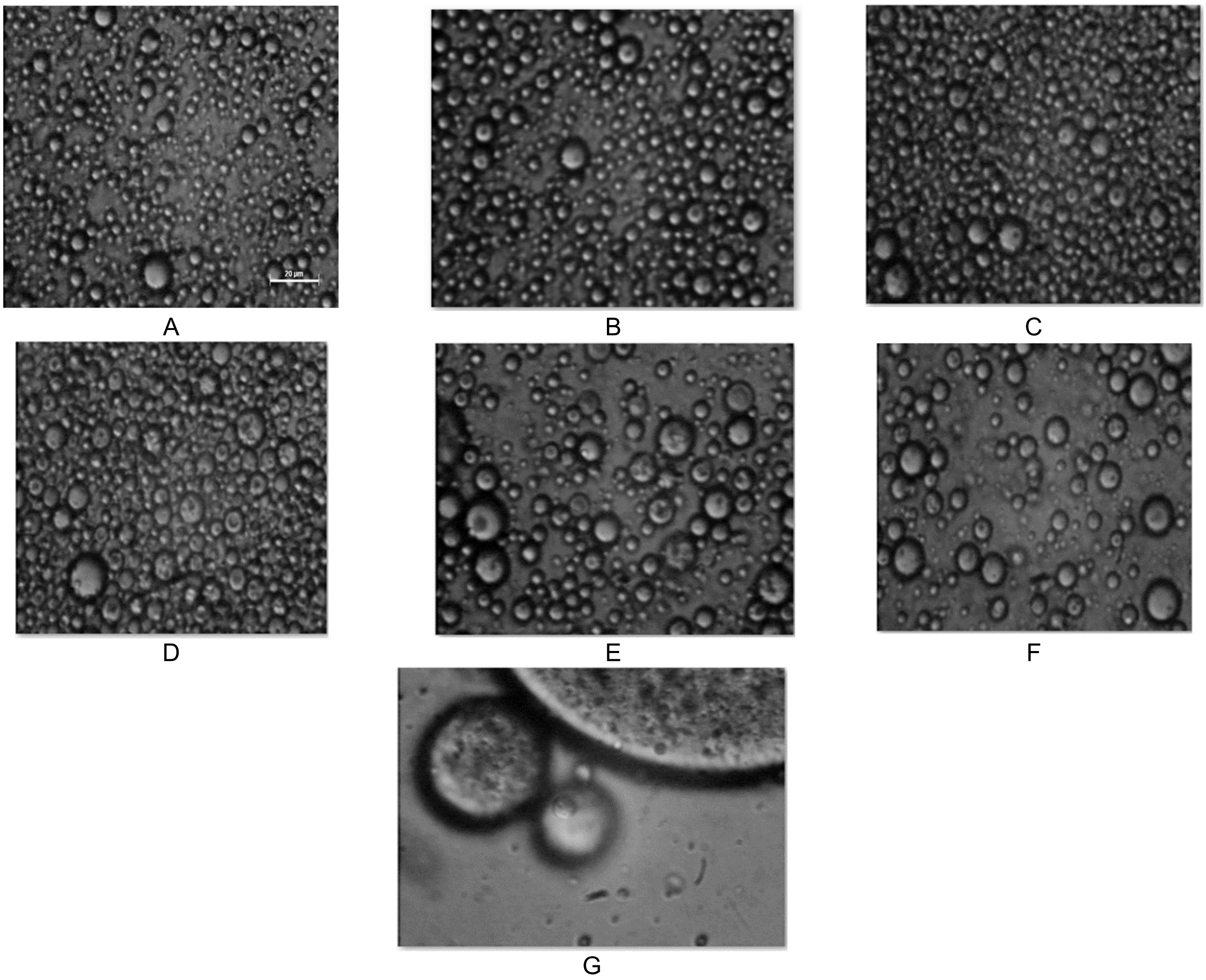


Figure 4. Polarized light micrographs of emulsions prepared with HO and OHO. (HO: control hazelnut oil; OHO: ozone applicated hazelnut oil for 1, 5, 30, 60, 180, and 360 min.) A: HO; B: OHO1; C: OHO5; D: OHO30; E: OHO60; F: OHO180; and G: OHO360. Scale bar = 20μm.

Rheological Properties of Emulsions

The mechanical spectra of emulsions made with control and OHO are illustrated in Figure 5. In this context, *G′* represents the energy stored in an oscillation cycle, while *G″* reflects energy scattered as viscous flow in an oscillation cycle [22]. At low frequencies, it is observed that the loss modulus (*G″*) exceeded the storage modulus (*G′*). The meaning of this the sample showed a more fluid similar behaviour (Figure 5). Depending on the ozone application time, the crossing between *G″* and *G′* curves occurred at distinct frequency ranges. For instance, the crossing between the *G″* and *G′* curves of the control emulsion was observed at 7.88 Hz, while the crossing for an emulsion including OHO360 was seen at 2.39 Hz. For emulsions including OHO1, OHO5, OHO30, OHO60, 0H0180 and OHO360, the crossing frequencies were altered to 3.85, 4.89, 3.85, 3.03, and 2.39 Hz, respectively. The shift of the crossover towards lower frequencies could be associated with an increase in the ozone application time, and emulsions including OHO showed a more similar solid behaviour with a rise in the ozone application period. The similar solid behaviour was observed at upward frequencies than the crossing frequency, where *G′* exceeded *G″*. This representation of the mechanical spectrum, [23] is characteristic of viscoelastic fluids. Another crucial parameter to consider is the loss tangent, expressed as $\tan \delta = G''/G'$ [18]. The behaviour of phase angle (tan *δ*) with time is also characteristic of a viscoelastic liquid at short times (around 90^{0}) and reaches a value characteristic of an elastic solid (near 0^{0}) at larger times [24]. Our findings indicated that, at shorter durations, the phase angle of all emulsions was around 90°. Accordingly, it was determined that all emulsions made with both control and OHO exhibited viscoelastic liquid behaviour.

DSC Heating and Cooling Thermogram

Heating thermograms of vegetable oils are often more intricate during the heating process than during cooling due to the overlapping of numerous polymorphic crystal melting events [25]. The thermograms of control heating at -5.6°C exhibited a distinct endothermic peak, as depicted in Figure 6. The melting point of hazelnut oil was reported to be -6.1°C in previous DSC reports [26]. According to earlier studies [25], the heating thermogram of hazelnut oil shows either a single melting peak or an extensive endothermic incident with four overlapped peaks, corresponding to triacylglycerol (TAG) melting [27]. The hazelnut oil heating thermograms exhibited noticeable changes, particularly after 30 min of ozone application. Endothermic peaks shifted slightly towards lower temperatures. After 30 min of ozone application, the melting curve of hazelnut oil widened and became less distinct. Following exposure to ozone for 180 and 360 min, the melting curves either disappeared or became less pronounced. Previous studies suggested that the significant increase in free fatty acids and lipid oxidative products may have hindered TAG crystallization, resulting to the formation of noticeable and less stable crystal polymorphic forms compared to those of control [28]. These mixed crystals, composed of TAG and molecules resulting from oxidative changes (monoacylglycerols, diacylglycerols, free fatty acids, primary and secondary oxidation products), likely melted at a lower temperature due to their decreased stability.

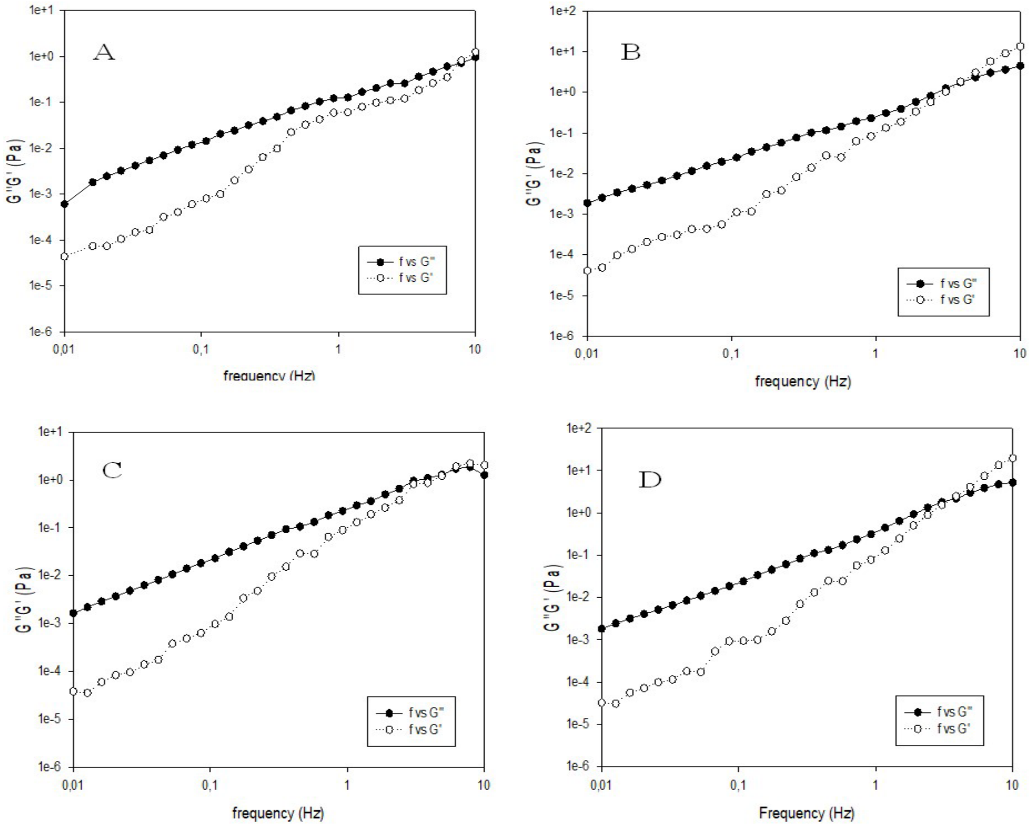
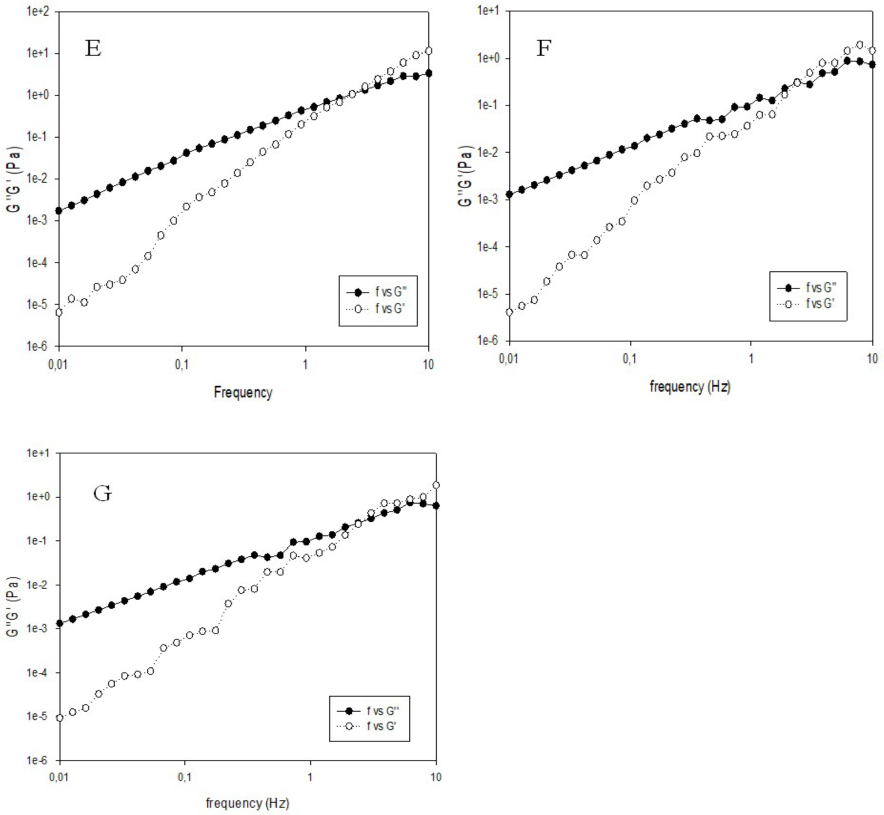


Figure 5. Viscoelastic moduli for emulsions prepared with HO and OHO. (HO: control hazelnut oil; OHO: ozone applicated HO for 1, 5, 30, 60, 180, and 360 min) A: HO; B: OHO1; C: OHO5; D: OHO30; E: OHO60; F: OHO180; and G: OHO360.)



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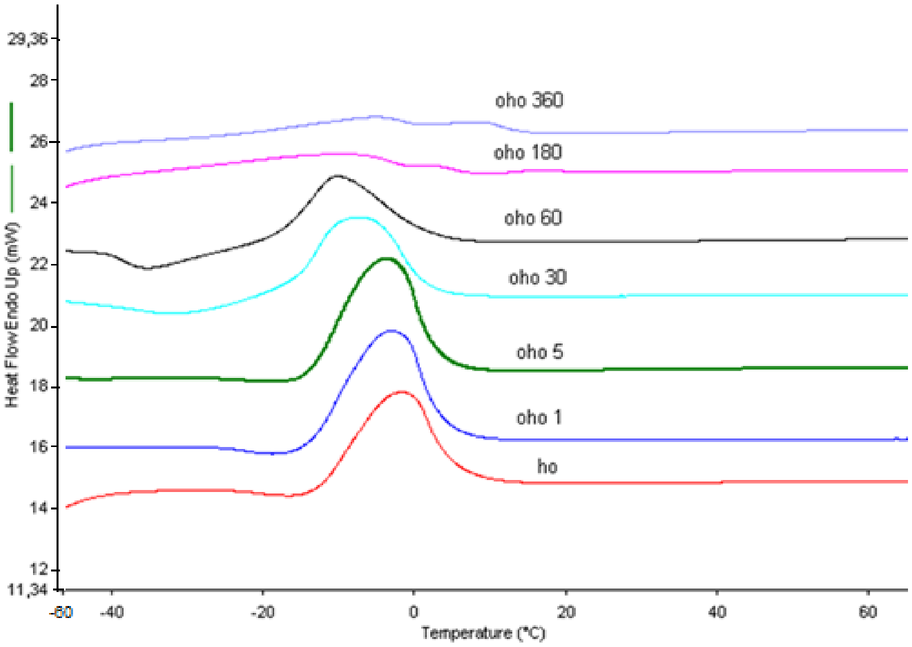

Figure 6. Representative DSC heating thermogram of HO and OHO (Control hazelnut oil; HO, ozone applicated hazelnut oil for 1, 5, 30, 60, 180 and 360 min; OHO1, OHO5, OHO30, OHO60, OHO180, OHO360).

Table 1. DSC data obtained from the heating thermograms of control and OHO^{a}

Ozone treatment time (min)	T_{on} (°C)	T_{off} (°C)	ΔH (J/g)	Peak height (mW)	Range (°C)
control	-12.45± 0.3^{a}	5.14±0.5^{a}	58.28±0.1^{a}	3.16±0.3^{a}	17.59±0.2^{a}
1	-13.22±0.4^{b}	3.60±0.4^{b}	57.79±0.3^{b}	3.68±0.2^{b}	16.82±0.4^{b}
5	-13.51±0.3^{b}	2.91±0.4^{c}	56.83±0.8^{c}	3.79±0.2^{b}	16.42±0.5^{b}
30	-16.67±0.1^{c}	1.94±0.3^{d}	50.31±1.2^{d}	2.74±0.4^{c}	18.61±0.9^{c}
60	-18.72±0.5^{d}	2.02±0.4^{d}	42.49±0.7^{e}	2.34±0.5^{c}	20.74±0.7^{d}
180	-20.82±0.2^{e}	0.90±0.2^{e}	42.90±0.5^{e}	1.03±0.7^{d}	31.72±0.5^{e}
360	-24.16±0.1^{f}	0.65±0.1^{f}	33.48±0.7^{f}	0.66±0.3^{e}	37.81±0.8^{f}

$^{a:}$Same letters within each column do not significantly different (p<0.05). T_{on}: onset temperature of melting, T_{off}:offset temperature of melting, ΔH: enthalpy of melting.

Table 1 presented the thermal properties of control and OHO samples. With an increase in the duration of ozone application, the enthalpy of the heating transition decreased. After 360 min of ozone application, the smallest enthalpy of transition was observed (Fig. 6). This phenomenon is likely a result of the heterogeneous structure of triacylglycerol (TAG) crystals forming due to a remarkable increase in oxidative products during the ozone application procedure. As the ozone application duration increased, the onset (T_{on}) and offset (T_{off}) temperatures moved towards lower values. Additionally, the main endothermic peak widened, leading to an increase in the range of transition temperatures for 180 and 360 min of ozone application.

During the cooling phase, hazelnut oil crystallized, resulting in an exothermic phase change observed in Figure 6 for both control and OHO. The cooling thermogram of control resembled those from earlier studies on the oils [25]. An exothermic peak at approximately -37°C, associated with the triunsaturated TAG crystallization, and a small peak at approximately -15°C, linked to the disaturated TAG crytallization, were evident in the cooling profile of control [25]; [27]. Ozone application altered the cooling profiles related with the exothermic peaks, especially the main peak, which broadened over a wider temperature interval and exhibited a decrease in height of peak with increasing ozone application durations. The small peak had less distinct as the ozone application period increased. Previous research on the cooling profiles of vegetable oils after deep frying, conventional, and microwave heating methods reported a reduction in the height peak related to crystallization, broadening, and a shift to lower temperatures [29]. These changes were associated with an increase in lipid oxidative products and molecules from TAG lysis, such as free fatty acids, monoacylglycerols, and diacylglycerols, potentially hindering TAG molecule alignment for crystal formation [25, 29].

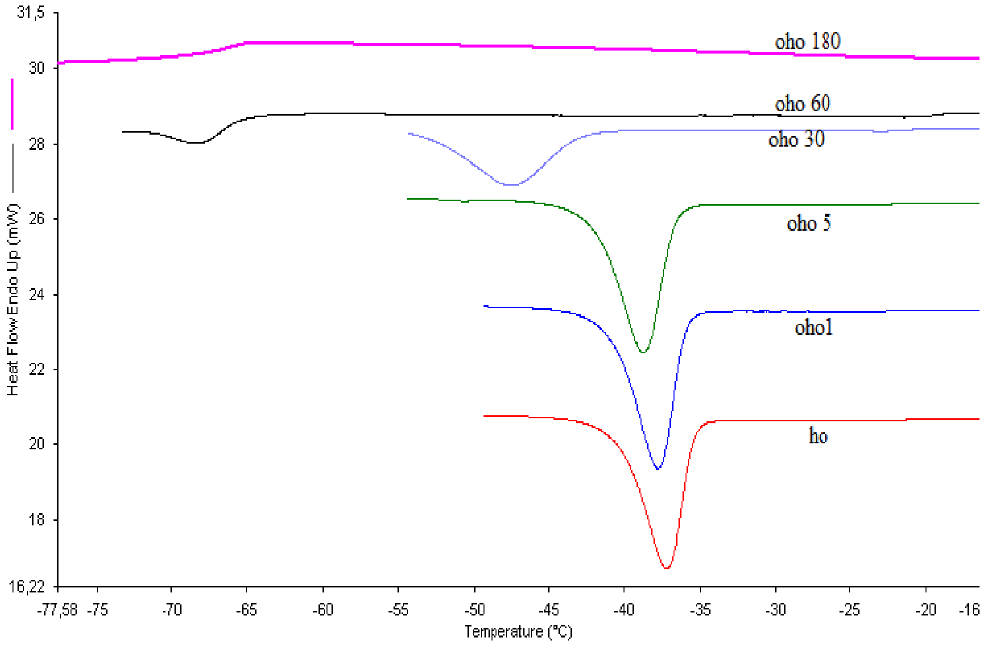

Figure 7. Representative DSC cooling thermogram of HO and OHO. (HO: control hazelnut oil; OHO, ozone applicated hazelnut oil for 1, 5, 30, 60, 180 and 360 min; OHO1, OHO5, OHO30, OHO60, OHO180, OHO360).

Figure 7 illustrates a prominent exothermic peak of control at -37°C, with a smaller distinct shoulder peak at -22°C. With ozone application, the strong exothermic crystallization shifted to lower temperatures, becoming wider and lower as application time increased. Upon completion of the ozone application, the tiny peak became less visible and eventually disappeared.

Table 2. DSC data obtained from the cooling thermograms of control and OHO^{a}.

Ozone treatment time (min)	T_{on} (°C)	T_{off} (°C)	ΔH (J/g)	Peak height (mW)	Range (°C)
control	-35.40± 0.3^{a}	-40.14±0.5^{a}	-48.28±0.1^{a}	-3.91±0.3^{a}	4.74±0.2^{a}
1	-35.95±0.4^{b}	-40.74±0.4^{b}	-47.43±0.3^{b}	-4.16±0.2^{b}	4.79±0.4^{b}
5	-36.68±0.3^{b}	-41.83±0.4^{c}	-42.98±0.8^{c}	-3.88±0.2^{b}	5.15±0.5^{b}
30	-43.06±0.1^{c}	-52.64±0.3^{d}	-25.99±1.2^{d}	-1.42±0.4^{c}	9.58±0.9^{c}
60	--65.65±0.5^{d}	-71.34±0.4^{d}	-11.18±0.7^{e}	-0.59±0.5^{c}	5.69±0.7^{d}
180	-----	-----	-----	-----	-----

^{a} Same letters within each column do not significantly different (p<0.05). T_{on}: onset temperature of crystallization, T_{off}:offset temperature of crystallization, ΔH: enthalpy of crystallization.

Table 2 presents the thermal parameters of control and OHO, including peak enthalpy, beginning and final crystallization temperatures, transition range, and height of peak obtained by thermogram related with cooling. Following ozone application, oxidative products (dimers, polymers, hydroperoxides, and aldehydes) are generated in the hazelnut oil. The melting range of the oil containing free fatty acids and oxidation products tends to shift to a lower temperature, as previously reported [28]. The crystallization enthalpy of hazelnut oil decreased as the ozone application period increased. Due to longer ozone application times, a significant drop in peak enthalpy of OHO30 and OHO60 was seen. Additionally, the crystallization peaks of OHO180 and OHO360 could not be observed, and the crystallization enthalpy could not be determined. In contrast, a minor drop in enthalpy of OHO1 and OHO5 revealed related with the crystallization indicating that less time ozone application could not significantly alter the formation of the crystallization. Previous research on cooling thermograms of thermooxidized oils also found a reduction in crystallization enthalpy, potentially related to triacylglycerol (TAG) partial lysis and the generation of breakdown products (e.g., free fatty acids, monoacylglycerols, diacylglycerols, oxidized TAG) [25]. Crystallization onset (T_{on}) and offset (T_{off}) temperatures moved significantly lower after 1min ozone application, while the moving in temperatures of the crystallization happened more noticeable after 30 min. The change in crystallization peak temperature and decrease in enthalpy related with crystallization might be attributed to TAG depletion, an increase in amount of free fatty acid, and an increment in viscosity.

CONCLUSION

The using of ozonated hazelnut oil in the emulsion preparation affects the functional and rheological properties of emulsions. The EAI of the emulsion containing control was higher than that of the ozone applicated emulsions. The application of ozone had a negative impact on EAI. Furthermore, as the ozone application time increased, ESI suffered more significantly. Additionally, the creaming ratios of the emulsions made with less time ozone applicated hazelnut oils were delayed compared to the creaming ratio of the emulsion prepared with control. The optical microscope results clearly indicated substantial droplet flocculation in emulsions containing OHO with an extended application time. Rheological research revealed that emulsions including OHO showed more solid like behaviour with ozone application time prolonged. The strong exothermic peak related with the crystallization shifted to lower temperatures during the ozone application process, and happened broader and smaller as the application duration extended. The melting curves became less visible or disappeared after 180 and 360 min of ozone application, respectively. Our findings obtained from the study represent valuable results related with the effects of ozone on food components and on functional and rheological properties of food components.

Author Contributions: H.U.K.: Formal analysis, investigation, methodology, writing-original draft. E.İ.: Supervision, conceptualization, methodology, writing-reviewing and editing, modelling. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

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

Ethics statement: No human or animal subjects were used in this research.

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