e-ISSN: 2757-8836
ORIGINAL ARTICLE
J. Raw Mater. Process. Foods vol.6(2), (2025), 79-88

Recieved: 08/10/2025 Revised: 10/11/2025 Accepted article published: 15/10/2025 Published online: 30/12/2025

Effects of Ozone Treatment on the Structural and Thermal Properties of Whey Protein Isolate and Egg White Proteins

Hicran UZUN KARKA [1*], Esra IBANOGLU[2]

[1]Gaziantep University, Naci Topçuoğlu Vocational School, 27310 Gaziantep, Türkiye

[2]Gaziantep University, Department of Food Engineering, 27310 Gaziantep, Türkiye

*Correspondence;
H. UZUN KARKA

E-mail adress:
hicranuzun@gantep.edu.tr

ORCID No: 0000-0003-1098-3197

DOI: https://doi.org/10.57252/jrpfoods.2025.7

Abstract

This study investigates the reaction of ozone with food proteins, focusing on whey protein isolate (WPI) and egg white (EW) proteins, which are widely used in food products due to their functional and sensory properties. Despite their extensive application, information regarding the structural effects of ozone on these proteins remains limited. In this study, proteins were subjected to ozone treatment using two different approaches: aqueous ozonation, in which protein solutions were treated with dissolved ozone at a concentration of 4.5 ppm, and gas-phase ozonation, in which dry protein powders were exposed to ozone gas for 5, 15, and 30 min prior to dissolution. Structural and thermal changes induced by ozone treatment were evaluated using differential scanning calorimetry (DSC), optical activity measurements, and high-performance liquid chromatography (HPLC). DSC analysis showed that ozone treatment increased the denaturation temperature of WPI while decreasing its denaturation enthalpy, indicating partial structural destabilization, whereas the denaturation temperature of EW proteins was less affected. Optical activity measurements revealed an increase in laevorotation of WPI solutions following ozone exposure. HPLC analysis demonstrated a 42–45% reduction in peak areas for both whey and EW proteins, suggesting significant ozone-induced structural modifications. Overall, the results indicate that ozone treatment induces measurable conformational and thermal changes in food proteins, with more pronounced effects observed in WPI.

Keywords: Ozone, protein, optical activity, DSC, HPLC.

1. INTRODUCTION

The U.S. Food and Drug Administration (FDA) has recognized ozone as generally recognized as safe (GRAS), and it is utilized in both aqueous and gaseous forms during food processing (Guo et al., 2020; Zhang et al., 2022). This ability to eradicate harmful bacteria without leaving residues has made it an attractive alternative to traditional chemical sanitizers (Brodowska et al., 2017). Ozone is also used for the postharvest treatment of fruits and vegetables, effectively extending their shelf life while maintaining their quality. Its application helps to reduce fungal decay and other spoilage mechanisms, thereby improving the overall safety and durability of fresh produce (Sarron et al., 2021). Studies have demonstrated that ozonated water treatments can effectively remove pesticide residues from produce, making it an effective tool for ensuring the safety of food products consumed by the public (Heleno et al., 2016).

Furthermore, ozone finds application in wastewater treatment processes associated with food manufacturing. It aids in lowering biological

oxygen demand (BOD) and chemical oxygen demand (COD) of food plant waste, contributing to improved environmental sustainability (Fröhling & Schlüter, 2015). This application not only maximizes food safety but also ensures compliance with environmental regulations (Wulansarie & Bismo, 2015).

In the realm of food packaging and storage, ozone treatment enhances the microbiological quality of stored food products. Its use in washing and sanitizing food contact surfaces aids in preventing cross-contamination and spoilage during food preparation (Panigrahi et al., 2025). The rapid decomposition of ozone back into oxygen also eliminates concerns about chemical residues, which can be a limitation of other disinfecting agents (Brodowska et al., 2017).

Recent advances have focused on improving the effectiveness of ozone applications in various food sectors, ensuring its integration into existing food processing technologies. Its multifunctional role in enhancing food safety, preserving quality, and reducing environmental impacts positions ozone technology as a promising solution in the modern food industry (Dubey et al., 2022).

Chemically, ozone reacts with proteins by oxidizing or cleaving specific amino acid residues through ozonolysis. These reactions result in alterations to the protein's conformation, including changes in folding patterns and binding capabilities, ultimately leading to protein denaturation (Perna et al., 2022; Uzun et al., 2012).

In the current study, the structural impact of ozone treatment on two food-grade proteins—WPI and EW proteins—was systematically investigated. These proteins were selected due to their widespread use in food formulations, where they contribute significantly to functional properties such as emulsification, foaming, and gelation. Ozone, a powerful oxidizing agent, is known to interact with proteins through mechanisms such as oxidation and ozonolysis of susceptible amino acid residues, particularly cysteine, methionine, tryptophan, and tyrosine. These oxidative modifications can induce conformational alterations in the protein's secondary and tertiary structures, thereby affecting its physicochemical and functional properties (Uzun et al., 2012).

One observable consequence of such structural alterations is a change in the optical activity of protein solutions. As proteins unfold or undergo partial denaturation, variations in chiral center arrangements and molecular symmetry can lead to measurable shifts in optical rotation. Furthermore, High-Performance Liquid Chromatography (HPLC) has been widely employed to monitor oxidative degradation and fragmentation patterns of proteins, offering insight into molecular integrity post-treatment. In this context, HPLC was utilized to detect changes in chromatographic profiles indicative of structural disruption or peptide bond cleavage following ozonation.

To complement the structural analyses, the thermal behaviour of ozone-treated proteins was evaluated using differential scanning calorimetry (DSC), which provides quantitative information on protein stability, denaturation temperature, and enthalpy changes. These thermal parameters serve as important indicators of conformational alterations and partial loss of native structure induced by ozone exposure. Therefore, the primary objective of this study was to clearly and systematically investigate the effects of ozone treatment on the structural and thermal properties of WPI and EW proteins by comparing different ozonation approaches (aqueous and gaseous) and treatment conditions. Changes in protein conformation and stability were assessed using optical activity measurements, high-performance liquid chromatography (HPLC), and DSC analysis, in order to elucidate the extent and nature of ozone-induced modifications in these widely used food proteins and to provide a comprehensive understanding of ozone's potential role as a protein-modifying agent in food systems.

2. MATERIAL AND METHODS

2.1 Materials

Powdered WPI (WPI, 98%) (BIOPRO, Lot No. JE 030-3-420) was obtained from Davisco Foods International (Le Sueur, MN, USA). Hen egg albumen powder (Sample No. 1392), representing EW, was kindly supplied by NIVE (Nederlandse Industrie van Eiproducten, Holland). All buffer salts, including potassium dihydrogen phosphate, disodium hydrogen phosphate, and sodium dodecyl sulfate (SDS), were purchased from BDH Chemical Ltd. (Analar analytical grade). Sodium chloride was obtained from JT Baker, while sodium hydroxide was sourced from Riedel-de-Haën. All chemicals used were of analytical grade. All experiments were conducted in triplicate, and results are presented as mean ± standard deviation.

*2.2. Ozonation of Protein Sample*s

Ozone gas was generated using an ozone generator (Ozone Marine, OMS Model, İzmir, Turkey), operating via the corona discharge method. The system maintained a constant flow rate of 1 L/min and an ozone concentration of 40 mg/L. Protein samples were ozonated using two different procedures: Ozone gas was dissolved in double-distilled water or phosphate buffer (pH 7.0), and this ozonated solution was subsequently used to prepare aqueous protein solutions (aqueous ozonation method). Alternatively, dry protein powders were exposed directly to ozone gas in a sealed glass gas-washing bottle. After ozonation, the proteins were dissolved in solution for experimental analyses (gas ozonation method).

In some cases, double-distilled water was used instead of buffer solutions to assess the maximum possible impact of ozone under laboratory conditions. Preliminary tests showed that the presence of buffer salts reduced the amount of dissolved ozone, likely due to chemical interactions between ozone and the buffering components.

Differential scanning calorimetry (DSC) was performed using protein solutions prepared at a concentration of 10% (w/v). Ozone treatment for DSC samples was applied using both aqueous and gas-phase ozonation; aqueous ozonation was conducted at an ozone concentration of 4.5 ppm, whereas gas-phase ozonation involved exposure of dry protein powders to ozone gas for 5, 15, and 30 min prior to dissolution. Optical activity measurements were carried out using WPIsolutions prepared at concentrations of 0.1% and 0.2% (w/w) and treated exclusively by gas-phase ozonation at 8 °C for 15 min; optical activity measurements for EW proteins were not conducted due to solubility and turbidity limitations. High-performance liquid chromatography (HPLC) analyses were performed using protein solutions prepared at a concentration of 0.1% (w/w), including non-ozonated controls and samples treated by aqueous ozonation (4.5 ppm).

2.3. Preparation of Stock Protein Solutions

Phosphate buffer (pH 7.0, ionic strength 0.05 M) was prepared by dissolving 3.76 g of potassium dihydrogen phosphate and 3.44 g of disodium hydrogen phosphate in 2 L of distilled water. The pH was adjusted using 0.1 M NaOH and 0.1 M HCl as needed. WPI was dissolved in either phosphate buffer or double-distilled water at room temperature under continuous magnetic stirring for 1 hour. Egg white proteins were dissolved in phosphate buffer (pH 7.0) containing 0.17 M sodium chloride in order to ensure complete solubilization and to maintain appropriate ionic strength. Egg white proteins are known to exhibit lower solubility and a higher tendency toward aggregation compared to whey protein isolate, particularly under low ionic strength conditions, due to their heterogeneous protein composition and strong intermolecular interactions (Donovan et al., 1975; Mine et al., 1990). The presence of sodium chloride helps to stabilize protein–protein interactions and improves solubility by reducing electrostatic repulsion and aggregation (Cheftel et al., 1989). In contrast, whey protein isolate demonstrates high solubility over a wide range of aqueous conditions; therefore, it was dissolved in either phosphate buffer or distilled water without the addition of salt (Hambling et al., 1992).

2.4. Differential Scanning Calorimetry (DSC)

Stock solutions of WPI and EW (10% w/v) were prepared and used for thermal analysis. Aliquots of the protein solutions were sealed in aluminum

DSC pans and analyzed using a Perkin-Elmer DSC 6 instrument equipped with Pyris software (Perkin-Elmer Inc., Wellesley, MA, USA). Samples were scanned from 15°C to 110°C at a constant heating rate of 5°C/min. An empty pan was used as the reference. The enthalpy of denaturation (ΔH) and the denaturation temperature (*Td*) were calculated from the resulting thermograms, following methodologies reported by Relkin et al. (1999) and Relkin and Sourdet (2005).

The concentration of all protein solutions was 10% (w/v). Sample A represents the untreated control protein solution (without ozone exposure). Sample B corresponds to the protein solution treated with 4.5 ppm ozone using the aqueous ozonation method. Samples C, D, and E represent proteins treated by gas-phase ozonation for 5, 15, and 30 minutes, respectively.

2.5. Optical Activity

Optical activity measurements were performed using a Polaar 3000 polarimeter. Samples were prepared by dissolving proteins (0.1% and 0.2% w/w) in distilled water (pH 7.0), followed by treatment with ozone gas at 8°C for 15 minutes. The ozonated solutions were then stirred magnetically for approximately 1 hour and filtered through Whatman No. 2 filter paper.

The specific optical rotation;

$$[\alpha\ ]_D = \alpha V L^{-1} m^{-1} \qquad (1)$$

where α is the observed rotation (degrees), L is the path length of the sample cell (dm), V is the volume of the solution (mL), and m is the mass of the dissolved solute (g).

2.6. High-Performance Liquid Chromatography (HPLC) Analysis

Ozonated WPI and EW samples were analyzed using reversed-phase HPLC. The chromatographic system consisted of a Shimadzu LC-10ADVP quaternary pump equipped with a Supelcosil LC-318 column and a matching guard column (40 mm × 3 mm i.d.), and detection was performed using a Hewlett Packard Series 1100 UV detector. Elution was monitored at 220 nm. The mobile phase consisted of solvent A: 0.1% (v/v) trifluoroacetic acid (TFA) and solvent B: 0.1% (v/v) TFA in 99.9% (v/v) acetonitrile. The flow rate was maintained at 0.8 mL/min, and the column temperature was held constant at 25°C using an Eppendorf CH-30 column heater. Protein samples (0.1% w/w) were filtered through 0.45 µm filters before injection, and the injection volume was 25 µL. Data acquisition and integration were conducted using ChemStation software (Agilent Technologies, Revision A.09.03). Results are expressed as percentage peak areas. Peak areas were calculated using ChemStation software by automatic integration of the chromatographic peaks. Relative peak area values were obtained by normalizing individual peak areas to the total integrated area of the corresponding chromatogram. Peak area reductions following ozone treatment were calculated as the percentage decrease relative to the non-ozonated control samples. All chromatograms were evaluated based on triplicate analyses, and newly observed peaks were consistently detected across all replicates.

Absolute quantification of individual protein fractions was not performed due to the lack of purified standards for all native proteins and their potential oxidation products; therefore, chromatographic results were expressed as relative peak area percentages, which is a widely accepted approach for assessing structural modifications and relative compositional changes in proteins subjected to oxidative and denaturing treatments (Cataldo, 2003; Almécija et al., 2007; Kiokias et al., 2007).

2.7. Statistical Analysis

Statistical analyses were conducted using SPSS software (version 22.0, SPSS Inc., Chicago, IL, USA). Differences among samples were evaluated using one-way analysis of variance (ANOVA). Statistical significance was accepted at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Thermal stability of WPI and EW Proteins

This study investigated the thermal stability of WPI and EW proteins post-treatment with ozone, employing differential scanning calorimetry (DSC) to analyze thermal denaturation. DSC is recognized for its sensitivity in detecting conformational transitions and thermodynamic properties of proteins, providing quantitative data

on enthalpy changes associated with thermal denaturation (Qian et al., 2017; Jiménez-Flores et al., 2005). The thermal denaturation temperature (T_d) was characterized by peaks in the DSC thermograms, with the initial T_d for untreated WPI recorded at approximately 78.73 °C (Table 1), aligning with previous studies that noted significant thermal transitions in whey proteins (Qian et al., 2017; (Čurlej et al., 2022).

As seen in Table 1, subsequent ozonation treatments yielded a marked increase in the T_d of WPI solutions, reaching 85.08 °C after 15 minutes of ozone exposure. This indicates that ozone treatment enhances the thermal stability of WPI by possibly facilitating interactions that reinforce protein structure (Carrillo et al., 2017; Zanabria et al., 2014). Statistical analysis confirmed these observations, evidencing ozone's significant impact on T_d and enthalpy (ΔH) of WPI solutions ($p < 0.05$). These results are consistent with evidence indicating that ozone interacts with specific amino acid residues, causing modifications that may reinforce native protein conformation and increase thermal resistance (Carrillo et al., 2017; Rosmalia et al., 2023).

In contrast, EW proteins exhibited denaturation at two distinct temperatures: 64.03 °C and 84.74 °C. The latter peak is predominantly attributed to ovalbumin, while the former reflects a conglomeration of other proteins, including conalbumin and lysozyme (Mine et al., 1990; Tarhan et al., 2020). Notably, treatment with ozone markedly influenced the denaturation peaks, with the first transition disappearing after a 30-minute ozone exposure, although the overall thermal stability change was less pronounced compared to WPI. These findings mirror previous observations suggesting that different protein classes react variably to oxidative conditions due to the distinct structural properties of their constituent amino acids (Li, 1983).

When examining enthalpy changes (ΔH), it was observed that both WPI and EW proteins experienced a reduction in ΔH with increasing ozone concentration. This decrease in enthalpy reflects alterations in intramolecular interactions; the disruption of hydrophobic interactions and subsequent aggregation processes were linked to the thermal behaviour of these proteins (Čurlej et al., 2022; Carrillo et al., 2017). This corroborates findings where heat treatments led to the exposure of sulfhydryl groups and resulting changes in protein structural dynamics, which affect both their functional properties and stability (Doiron et al., 2009; Zanabria et al., 2014). Ozone induces oxidation or ozonolysis of amino acids, altering protein folding and binding behaviour (Cataldo, 2003). Thus, although ozone enhances protein stability thermally, it simultaneously reduces the energetic contribution of intramolecular interactions, reflected in lower enthalpy values.

The differential chemical reactivity of amino acid residues with ozone was found to influence the denaturation behaviour, with reports suggesting that redox reactions predominantly affect tryptophan, methionine, and cysteine residues (Li, 1983). Collectively, the results suggest that ozone treatment can significantly alter the thermal stability and denaturation behaviour of both whey and EW proteins. The nature and extent of these changes are closely linked to protein structure, amino acid composition, and the oxidative potential of ozone.

Table 1. The denaturation temperature (*Td*) and enthalpy changes (*ΔH*) for heat denaturation of WPI and EW proteins.

Protein type	Sample	Td_1 (^{0}C)	Td_2 (^{0}C)	ΔH (J/g)
WPI	A	78.73±0.01^a		15.06±0.05^a
	B	80.65±0.02^b		12.68±0.02^b
	C	81.84±0.21^c		11.64±0.02^c
	D	85.08±0.20^d		4.39±0.03^d
EW	A	64.03±0.00^a	84.74±0.02^a	12.75±0.44^a
	B	65.51±0.04^b	84.51±0.04^b	12.15±0.05^b
	C	64.59±0.40^c	84.42±0.07^c	10.62±0.03^c
	D	65.77±0.02^d	83.61±0.02^d	8.61±0.07^d
	E	64.03±0.05^a	83.67±0.08^d	6.46±0.03^e

Concentration of protein solutions was %10. A; pure protein solution (non-treated with ozone), B; Protein solution treated with 4,5ppm ozone (aqueous ozonation method), C; Protein treated with ozone gas 5min (gas ozonation method), D; Protein treated with ozone gas 15min (gas ozonation method), E; Protein treated with ozone gas 30 min (gas ozonation method).

Each value in the table represents the mean ± standard deviation of triple analyses. Means with different letters in each column are significantly (p <0.05) different.

3.2. Optical Activity

Protein optical activity plays a crucial role in characterizing protein structure and behavior. It is significantly influenced by the chirality of the constituent amino acids and the helicity of their polypeptide chains. Native proteins composed primarily of L-amino acids are inherently laevorotatory, exhibiting specific optical rotations that can vary, but in general, globular proteins can display values that fall within a range typically reported around -30^0 to -60^0, while fibrous proteins often present less negative values (Zhu et al., 2024).

In this study, the optical activity of whey proteins was assessed following ozonation using both gas and aqueous methods. Figure 1(a) shows that optical activity values of WPI solutions prepared at the different concentration (0.1 and 0.2% (w/w)). As seen in the Figure 1(a), gas ozonation had strong effect on optical activity of these protein solutions.

Notably, previous experiments with EW proteins encountered challenges due to low solubility and turbidity, which affected optical activity measurements (Guzy & Heath, 1993). In the present study, similar solubility and turbidity issues were observed following ozonation, which interfered with reliable polarimetric measurements. Consequently, optical activity measurements for EW proteins could not be obtained with sufficient accuracy and reproducibility and were therefore not included in the results.

It was found that ozonation leads to significant electrophysical changes, transforming the optical activity of treated proteins toward a laevorotatory form. Specifically, with the gas ozonation technique employed herein, optical activity

increased by 17.2% for the 0.1% (w/w) WPI solution and by as much as 29.0% for the 0.2% (w/w) WPI solution, demonstrating that higher concentrations precipitate more substantial optical changes (Guzy & Heath, 1993).

Figure 1(b) shows that optical activities of WPI solutions ozonated for different periods of time, where the dark-coloured bar shows solution containing 0.1%w/w WPI, while the light-coloured bar shows solution containing 0.2% WPI. As seen in the Figure, the laevorotatory forms of the protein solutions were increased with ozonation time. The change in the laevorotatory form was higher in the 0.2% (w/w) protein solution than 0.1% (w/w) solution. The changes in the optical activities were 17.2 % and 29.0 % for 0.1 and 0.2 % (w/w) solutions, respectively. The observed enhancements in optical activity with prolonged exposure to ozone suggest that the gaseous phase facilitates greater molecular interactions compared to the aqueous phase, thereby influencing the protein structure at a deeper level (Zhu et al., 2024). As ozone interacts with aromatic amino acids such as tryptophan, tyrosine, and phenylalanine, it is hypothesized that these interactions induce structural changes that alter the secondary and tertiary conformations of proteins (Guzy & Heath, 1993). Such changes can lead to denaturation processes, which can occur without necessitating a decrease in molecular weight, as evidenced by earlier studies (Zhu et al., 2024).

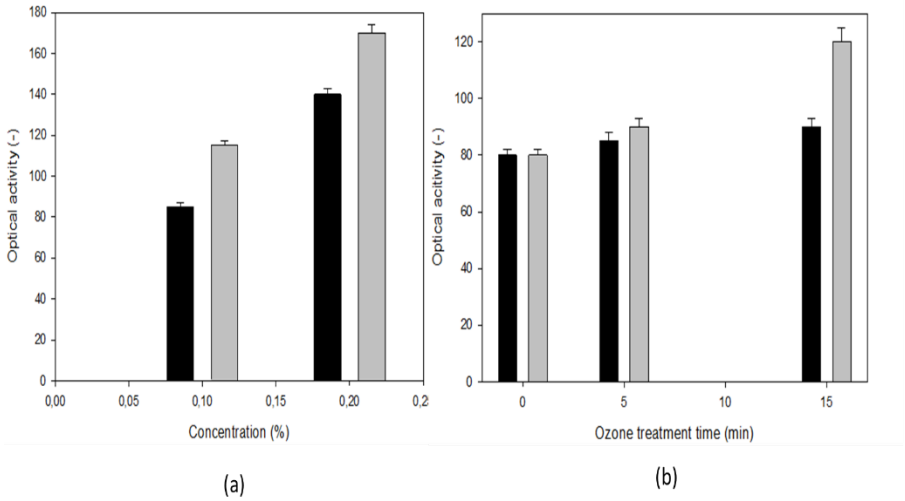

Figure 1. Effects of ozone treatment on the optical activity of WPI. (a) Changes in optical activity at different WPI concentrations (0.1 and 0.2% w/w). Dark bars represent the aqueous ozonation method, while light bars represent the gas ozonation method. (b) Changes in optical activity of WPI as a function of ozonation time. Dark bars indicate WPI solution (0.1% w/w), and light bars indicate WPI solution (0.2% w/w). Error bars represent the 95% confidence interval of the mean (n = 3)

Moreover, the interaction of ozone with thiol groups results in the formation of disulfide bonds, which alters the protein structure. This crosslinking has been reported to correlate with structural modifications of proteins and their optical properties, suggesting that ozone treatment modifies allergenic properties potentially due to structural occlusion of binding sites (Guzy & Heath, 1993). Specifically, ozonation-induced denaturation may account for the increased laevorotation, interpreted as a shift in protein configuration that aligns with observations in similar protein studies (Zhu et al., 2024).

3.3. High Performance Liquid Chromatography (HPLC) Analysis

The analysis of both ozonated (aqueous ozonation method (4.5 ppm)) and non-ozonated solutions (0.1% w/w) of WPI and EW proteins was performed using reverse phase high-performance liquid chromatography (HPLC). The chromatograms obtained for ozonated WPI were compared to those of native WPI, focusing on the

implications of ozone treatment on protein denaturation and structural changes. As noted by Xiong et al. (Xiong et al., 2024), oxidation of sensitive amino acid residues can induce alterations in the secondary and tertiary structures of proteins, potentially leading to increased local flexibility or rigidity within the protein chains.

Figure 2(a) shows the reverse phase HPLC chromatograms for WPI solutions which represents the marked peaks of α-lactalbumin, lactoferrin, bovine serum albumin (BSA), β-lactoglobulin B and A and immunoglobulin, respectively. β-lactoglobulin (50%) and α-lactalbumin (20%) are regarded as major proteins with the percentages of 50 and 20 %, respectively (Hambling et al., 1992), as seen in the chromatograms. Similar chromatograms for WPI have been obtained in previous studies (Almecija et al., 2007; Elgar et al., 2000).

The chromatogram of WPI solution treated with ozone is given in Figure 2(b). The figure reflects a similarity with HPLC chromatogram of denaturation of whey proteins with temperature (Kiokias et al., 2007) which exhibited a decrease in peak area for native whey proteins with increasing temperature. A decrease in peak area of native WPI after ozone treatment was observed from Figure 2(b). The area under the peaks of α-lactalbumin, β-lactoglobulin A and B were reduced by 45 % and the peaks of lactoferrin, bovine serum albumin (BSA) were observed to disappear. In addition, a new peak appeared at of the protein that become exposed upon heating, leading to intermolecular reactions (Cheftel et al., 1989).

23.8 min. This new peak may be explained by the cross binding of some protein molecules. Native β-lactoglobulin has two disulphide bonds and one free thiol group which is buried within the protein structure. α-Lactalbumin is a small compact globular protein stabilized by four disulphide bonds and it does not contain a free thiol group (Brew and Grobler, 1992). We have seen that ozone treatment caused precipitation in protein solutions which may be caused by the formation of crosslinks between adjacent protein chains with a consequent drop in solubility (Cataldo 2003). Thus, ozone treatment may result in the oxidation of thiol groups (-SH) of cysteine with consequent formation of disulfide bonds (crosslinks).

Reverse phase chromatography for native EW proteins (non-treated with ozone) were shown in Figure 3(a). The marked peaks on the chromatogram belong to lysozyme, ovotrasferrin and ovalbumin, respectively. A similar result of reverse phase HPLC chromatogram for EW proteins was obtained in a previous study (Guerin-Dubiard, 2005). Among as many as 40 different proteins contained in the EW, the major proteins involved are ovalbumin (54%), conalbumin (12%), ovomucoide (11%) and lysozyme (3.5%) (Donovan et al., 1975). The higher stability of lysozyme is regarded due to the compactness of this protein, since it has four disulfide cross-linkages and no free thiol group. Ovalbumin has four thiol groups which are buried within the core

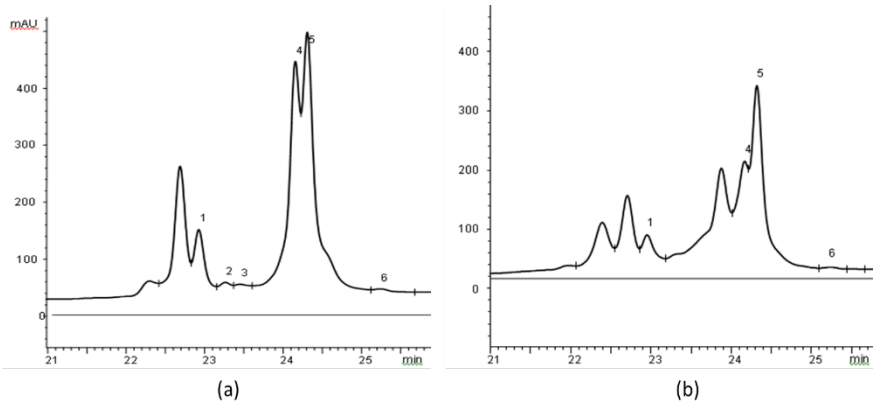

Figure 2. Reverse phase HPLC chromatography. (a) for native WPI solution (non-treated with ozone), 1; α-lactalbumin, 2;lactoferrin, 3; bovine serum albumin (BSA), 4,5; β-lactoglobulin B and A, 6; immunoglobulin. (b) for WPI solution (0.1% w/w) treated with ozone (aqueous ozonation method (4.5 ppm ozone)), 1; α-lactalbumin, 2;lactoferrin, 3; bovine serum albumin (BSA),4,5; β-lactoglobulin B and A, 6; immunoglobulin.

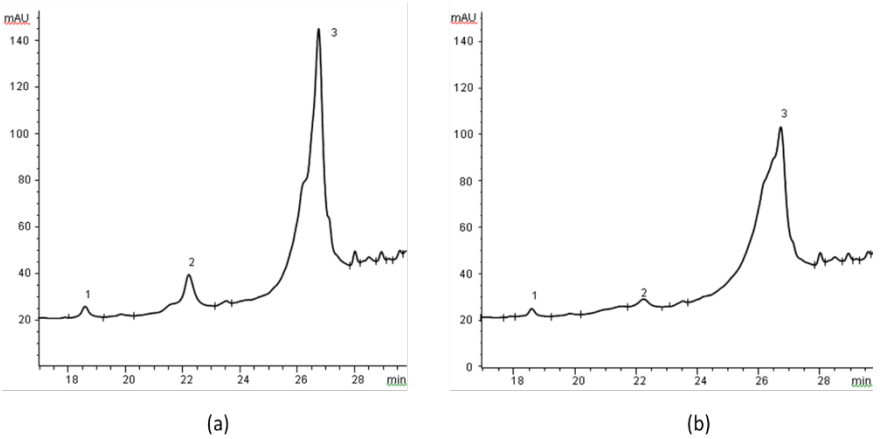

Figure 3. Reverse phase HPLC chromatography (a) for native EW protein (none treated with ozone), 1; lysozyme, 2; ovotransferrin, 3; ovalbumin. (b) for EW protein (0.1% w/w) treated with ozone (aqueous ozonation method (4.5 ppm ozone), 1; lysozyme, 2; ovotransferrin, 3; ovalbumin.

Figure 3(b) showed chromatogram of EW proteins after oxidation by ozone. According to comparison of chromatograms in Figure 3(a) and 3(b), a 42 % decrease in the area under the peaks was determined. In addition, a new peak was observed on the left hand side of ovalbumin peak. The new peak widens the bottom of ovalbumin peak. As a result of ozone oxidation, protein molecule may undergo various changes which is likely to be in aromatic amino acid residues (Cataldo, 2003). In addition, oxidation of thiol groups, products formation from protein oxidation and conversion of some amino acid residues to carbonyl derivatives might be possible changes. Also, cleavage of polypeptide chain and formation of cross-linked protein aggregates may result from oxidation (Kayalı and Çakatay 2004). Some alterations may occur in protein conformation and lead to increased aggregation, fragmentation, distortion of secondary and tertiary structure, susceptibility to proteolysis, and diminution of normal function (Cataldo, 2006).

4. CONCLUSION

This study demonstrated that ozone treatment induces notable structural changes in both WPI and EW proteins. DSC analysis showed increased denaturation temperatures for WPI, suggesting enhanced thermal stability, while enthalpy values decreased for both protein types, indicating partial structural disruption. Optical

activity measurements revealed that ozonation increased the laevorotation of WPI, reflecting alterations in its secondary and tertiary structure. HPLC analysis confirmed significant degradation of major protein components, with the appearance of new peaks suggesting the formation of oxidation products. Overall, ozone significantly affects protein conformation and stability, with a more pronounced impact observed in WPI compared to EW proteins. Collectively, the results confirm that ozone treatment induces both conformational and compositional changes in food proteins. These structural alterations may have implications for the functional and nutritional properties of proteins in food systems, highlighting the need for controlled application of ozone in food processing environments.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

None.

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