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Synergistic Effect of Polyphenols and Ultrasonic on Functional Properties and Structural Composition of Egg White Proteins

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ABSTRACT

Background: One of the useful factors in research and application in food industry is the functional properties of proteins, as egg protein contains a variety of functional properties that can be improved using multiple modification methods, including ultrasonic modification and phenolic compounds known for their antioxidant effect, giving flavor to the product, and their chemical interaction with proteins. Therefore, this study investigated the synergistic effect of polyphenols and ultrasonic on functional properties and structural composition of egg white proteins.

Methods: The synergistic effect of ultrasound for 5 minutes and phenols provided from aqueous plant extract of black seed (*Nigella sativa*) at 0.1% (T1) and 0.2% (T2) concentrations and cardamom (*Elettaria cardamomumate*) at 0.2% (T3) and 0.4% (T4) concentrations, as well as a control treatment (T5) was evaluated on egg white proteins. The chemical contents of egg whites were estimated, and the functional properties (solubility, foaming, emulsification and water binding) of the modified proteins were studied. For structural changes resulting from the modification, they were assessed by Fourier Transforms Infrared Spectrometer (FTIR), Fluorescence Measurement techniques and Scanning Electron Microscopy (SEM).

Results: A significant difference was noticed between the treatments ($p \leq 0.05$) revealing an improved functional properties. The treatment illustrated the highest digestibility in T4, while the digestibility decreased in T1 and the lowest digestibility in T5.

Conclusion: Ultrasonic treatment for 5 minutes and the phenolic compounds of the black seed at 0.2% showed the best results in improving functional properties, digestibility, and change in the secondary structure of egg white protein. The α-helix content decreased and the β-turn increased when treated with phenolic compounds at different concentrations when compared to the control group.

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Introduction

Egg is one of the most popular foods and has a high nutritional value as it contains essential nutritional elements such as protein with all essential amino acids, various minerals and vitamins, phospholipids, carotenoids with antioxidant properties and other biologically active compounds; while having high digestibility too (1). The white part of egg was shown to have various functions including an increase in muscle mass and enhancing immunity as well as reducing fat and lowering the cholesterol. Egg consumption can also help prevent physical weakness, as eating egg can reduce the nutrient deficiencies and metabolic syndromes (2). Lysozime, ovomucoid, ovoinhibitor, and cystain proteins are biologically active proteins in egg white, and their activity can extend the shelf life of table eggs (3). Egg white is widely used in many food products, and its use is not limited to being only a nutritional substance, while it is used for sensory and functional properties too, including foaming, emulsification, gelation, solubility, etc. (4).

Egg white is a common ingredient in cake and sweet industry; because it has an excellent foaming property (5). Modification of protein structure has the potential to significantly improve functional properties. Esterification, phosphorylation, and acetylation have been utilized, as well as physical methods such as heat purification, thermal conditioning, wet and non-thermal treatments (ultrasonic, multi-stage, pressure ultrafiltration), irradiation, and microwaves, together with modification of phenolic compounds in food modifications (6). Phenolic compounds have gained increasing interest as one of the ways to modify proteins, as these compounds can form a bond through non-covalent physical interaction or a covalent bond with the protein (7). Non-covalent interaction is carried out by reversible forces such as hydrogen and ionic bonds and hydrophobic interactions, while covalent interaction is carried out by irreversible bonds, and the mechanisms of interaction between proteins and phenols can affect the chemical composition of proteins, which can lead to changes in nutritional and functional elements; as well as the product quality (8).

The physical, chemical and functional properties of several types of proteins modified with phenolic compounds have been studied. Modification in egg white protein is undertaken by use of phenolic compounds of tea extract at different concentrations, and its effect on physical properties of the gelatinous network of egg white. The modified egg white is used in preparation of surimi gel, which is a fish protein product too (9). Green coffee has been applied in modification of egg white proteins, as it improves the functional properties and nutritional value together with an increase in antioxidant activities (10). So the aim of this research was to determine the synergistic effect of ultrasonic treatment and the phenols extract of black seed and cardamom at different concentrations on improvement of the functional properties of egg white proteins and to evaluate the changes in the structural composition resulting from binding by Fourier transforms infrared spectrometer (FTIR) and scanning electron microscopy (SEM) methods to develop a food system with distinctive properties.

Materials and Methods

Fresh red eggs were collected from Rumaila poultry fields in Safwan/Basrah region. Dry plant parts, the fruits of cardamom plant (*Elettaria cardamomum*) and black seeds (*Nigella sativa*) were used in preparing plant extracts from the local market in Basrah. All chemicals were obtained from Sigma Aldrich (USA). The chemical content of egg white (protein, moisture and ash) was estimated as described before (11). The percentage of fat was determined based on previous researches (12). In preparation of plant extract, (cardamom and black seed), distilled water was used as a solvent in the extraction process at a ratio of 1:10 g/mL. The mixture was placed in tightly sealed containers and mixed with a magnetic mixer and placed in a water bath at a temperature of 45°C for 2 hours. The samples were later removed from the water bath and left at the laboratory temperature to complete the extraction process for 24 hours. The extracts were filtered with Whatman No. 1 filter papers and the filtrate was concentrated with a Vaccum Rotary Evaporator at a temperature of 45°C. Finally, the extracts were dried and stored at a refrigerator temperature until use (13).

For modification with phenol extract and ultrasound, the egg whites were mixed for 5 min in a magnetic mixer. The extract of black seed (*Nigella sativa*, 0.1%) as T1 and (*N. sativa*, 0.2%) as T2 and cardamom (*Elettaria cardamomumate*, 0.2%) as T3 and (*E. cardamomumate*, 0.4%) as T4 were added. The product was mixed again for 2 min, incubated at laboratory temperature for 90 min, and treated with ultrasound for 5 min. After the procedure was completed, the samples were removed and freeze-dried (6, 14). The foam capacity and stability of the modified egg white powder were assessed according to the method mentioned previously (15). A total of 2% of the egg white was homogenized by an electric mixer for 5 minutes in a graduated cylinder at a rotation speed of 7 per minute and the total volume was recorded at 0, 10, 30, and 60 minutes.

The percent of foaming capacity was calculated by the equation of B–A/B×100. A denoted to volume of foam after whipping (mL) and B revealed volume of foam before whipping (mL). The percent of foam stability was evaluated based on the equation (16).

$$\frac{Volume\,of\,remaining\,foam}{Total\,foam\,volume} \times 100$$

To determine the solubility of egg white proteins, 0.25 g of egg white proteins were mixed with 2.25 mL of 0.05 M buffer phosphate solution at pH=7.4. The mixture was stirred for 2 minutes and then was centrifuged at 3000 rpm for 10 min. The protein concentration in the supernatant was measured employing the Biuret method by using Kit Biuret. The solubility percent was estimated according to the equation of protein in supernatant fraction (mg)/ Total protein (g) (17). The sample was prepared at a concentration of 1%; while 6 mL of it were added to 2 mL of sunflower oil. The mixture was later stirred for two minutes with Vortex. Totally, 50 mL of the emulsion were withdrawn at 0 and 10 minutes. The mixture was further diluted with 5 mL of 0.1% SDS. The absorbance was measured at 500 nm by a spectrophotometer. The emulsion capacity was calculated according to the equation of (2×2.303×A×DF)/lØC; while A=absorbance measured at 500 nm, l=cuvette path length (mm), DF=Dilution factor (100), Ø=Oil volume, and C=protein concentration (g/m^3). The emulsion stability was determined according to the equation of $A_0/(A_0 - A_{10})\times\Delta t$; Where A_0 was the absorbance after homogenization, A10 indicated absorbance at 10 minutes and Delta t was 10 minutes (18, 19).

To determine the water binding capacity, 0.25 g of modified egg white powder was dissolved in 10 mL of distilled water, shaked for 30 seconds, stored overnight at 4°C, and centrifuged at 3000 rpm for 30 min. The supernatant fraction was filtered with a filter paper, and the volume was recorded. The water binding capacity was calculated by the equation of

$$WBC = \frac{Volume\,of\,supernatant\,fraction\,(mL)}{Initial\,volume\,(mL)} = \times 100$$

(20, 21). In assessing the digestibility of proteins, a homogeneous sample was prepared by dissolving 10 mg/mL of distilled water in the laboratory. After the initial step of digestion, the pH sample was lowered to 1.5 using 0.1 M HCL. Then it was placed in a water bath at 37°C and 20 µl of 10% of enzyme pepsin was added. The sample was incubated for 2 hours to perform the digestion process. After that, the enzyme was inhibited by adding NaHCO3 (1 molar) to adjust the pH to 7.5 and to stop the digestion process. Totally, 2 mL of each sample was provided for to measure the protein and to determine the degree of decomposition (22). The second digestion stage was carried out using 100 µL of the prepared pancreatin enzyme at a concentration of 2.5%. The samples were placed in a water bath for two hours at 37°C. The enzyme was inactivated at 90°C for 10 minutes. Then, the protein was estimated to determine the degree of decomposition (22).

The FTIR method was used for spectroscopic analysis by mixing 4 mg of egg white powder with 400 mg of potassium bromide (KBr). The mixture was compressed under a pressure of 2500 kg/cm^2 to obtain a tablet with a thickness of 1-2 mm and a diameter of 1 cm. The examination was carried out using a device of infrared radiation at a wavelength of 400-4000 cm^1 by the Japanese company JASCO in Polymer Research Centre at the University of Basrah (23). The fluorescence intensity was determined as described before (24, 25). The prepared protein solution was filtered at a concentration of 0.1 mg/ mL using a filter paper with a pore size of 0.45 mL. The filtrate was collected, and a fluorescence spectrophotometer was applied as -LS-3-Perkin Elmer with an emission intensity of 300-550 nm and an excitation wavelength of 290 nm in the Photo Physics Research Laboratory, College of Science, University of Basrah, Iraq (24, 25). The surface morphology of the samples was evaluated using a scanning electron microscope (Germany, ACE600 EM Leica QUANTA 200) as demonstrated before (26).

Results

Table 1 shows the chemical content of chicken egg white proteins revealing that the percentages of moisture, protein, fat and ash were 88.05%, 10.15%, 0.03% and 0.56%, respectively. Figure 1a shows the effect of egg white proteins treated with ultrasonic waves for 5 minutes, the black seed extract phenols (0.1%, T1 and 0.2%, T2), and cardamom (0.2%, T3 and 0.4%, T4) on foam formation capacity and stability. The results showed significant differences ($p \leq 0.05$) in the ability for foam production and its stability between the treatments. The treatment T2 demonstrated a clear effect on increasing the volume of the foam and its stability, which was recorded as the highest ability to form foam; while its stability reached 650.05 and 100%, respectively.

Table 1: Chemical ingredients of chicken egg white proteins.

Content	%
Moisture	88.05
Ash	0.56
Fat	0.03
Protein	10.15

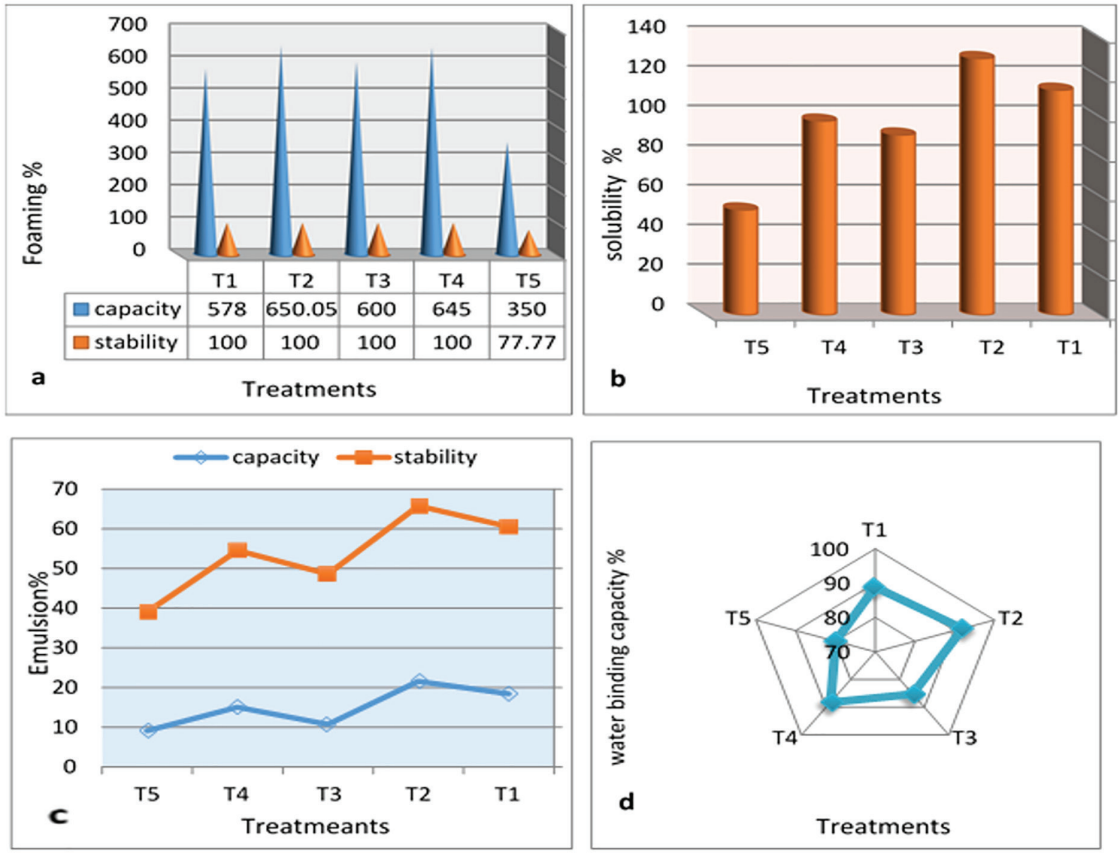

Figure 1: The effect of egg white proteins treated with ultrasound waves and plant derived-phenolic extract on functional properties.

These findings were close in value to the foam capacity and stability for T4 as 645% and 100%, while the foam capacity and stability decreased at T1 and T3 after 60 minutes of whipping. The T5 treatment illustrated the lowest foam capacity and stability reaching to 350% and 77.77%, respectively for the same whipping period.

Figure 1b displays significant differences ($p \leq 0.05$) between the treatments for solubility rate of egg white proteins treated with ultrasonic waves for 5 minutes and the phenols of the aqueous extract, as well as the control treatment. The T2 treatment exhibited the highest percentage in solubility as 129.56% followed by T1 and T4 and T3 and the lowest solubility was for T5 as 53.19% when compared to the rest of the treatments. Figure 1c indicates the emulsion capacity and stability of the egg white proteins treated with ultrasonic waves for 5 minutes and the phenols of the aqueous extract of black seed and cardamom, in addition to the control treatment. It was noted that treatment T1 and T2 led to an increase in the emulsifying capacity to reach 18.46% and 21.63%, respectively. The stability of the emulsion was 60.55% and 65.77% for the two treatments, respectively. The values of emulsification capacity and stability for treatment T3 were lower than the rest of the treatments, reaching 10.73% and 48.65%, respectively and treatment T4 recorded 15.11% and 54.59% for both emulsification capacity and emulsion stability. The T5 treatment showed emulsification capacity and stability of 9.17% and 39.09%, respectively. The results of the statistical analysis indicated significant differences ($p \leq 0.05$) between treatments of emulsion capacity and stability. Figure 1d reveals the highest value for water-binding capacity as 92.01% for treatment T2, which increased with a significant difference from the rest of the treatments as well as the control treatment. Treatment T1 and T4 converged in terms of water- binding capacity and were 88.83% and 88.15%, respectively. The treatment T5 was less able to hold 80% water.

Treatment with ultrasonic and phenols in Figure 2 demonstrated a significant effect ($p \leq 0.05$) on digestibility of egg white proteins, as treatment T4 was found to have the highest digestibility value for egg white proteins (88.69%), followed by treatment T2, which was 87.05%, and treatment T3 that showed a digestibility of 84.54%. The digestibility at T1 decreased to 79.35%, while the digestibility of the control treatment (T5) was 75.55% that was lower than the rest of the treatments. FTIR technology was used to study the effect of ultrasonic treatment (5 minutes) and phenolic compounds at concentrations of 0.1 and 0.2% for black seed and 0.2 and 0.4% for cardamom, as well as a control treatment on the secondary composition of egg white proteins.

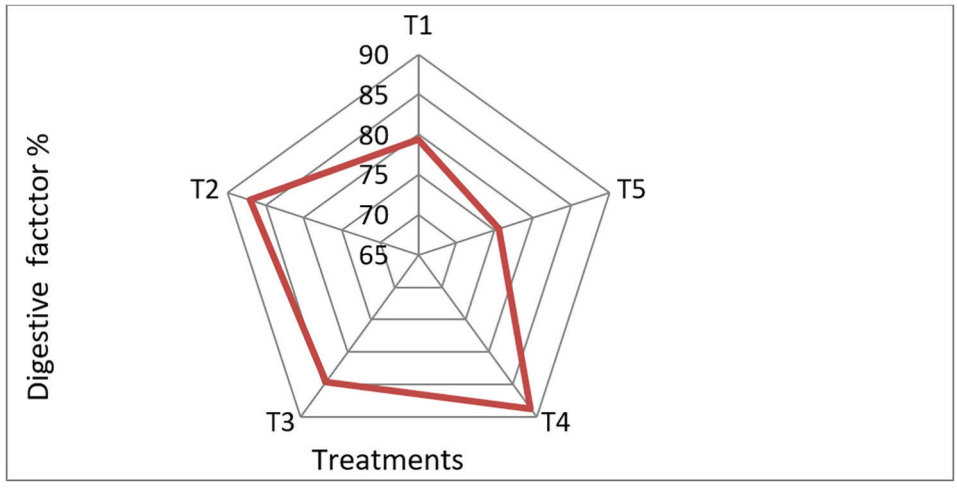

Figure 2: Effect of egg white proteins treated with ultrasound waves and plant derived phenolic extract on digestion factors.

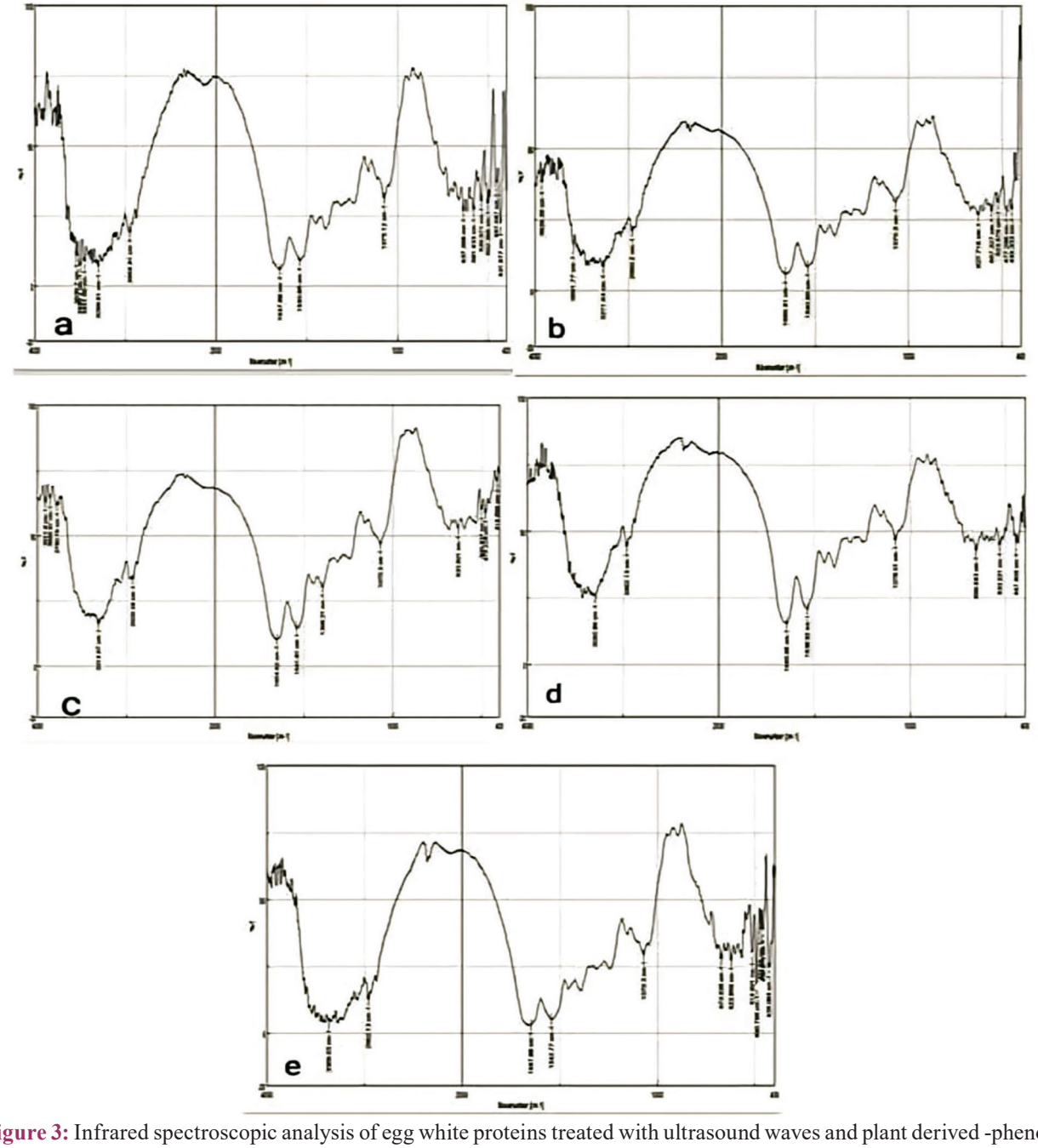

Figure 3: Infrared spectroscopic analysis of egg white proteins treated with ultrasound waves and plant derived -phenolic extract (b-e) in comparison to the control (a).

Figures 3 and Table 2 show the wavenumbers of the vibration frequency of the egg white proteins treated with ultrasound and the phenolic compounds. Figure 3a illustrates the wavenumbers of the vibration frequency of the control treatment proteins, as a wide wavenumber range was observed between the wavenumbers of 3926.36 cm^{-1}-3271.64 cm^{-1} located within the amides A and B indicating the stretching vibration of the O-H and N-H bonds. Also, the wave number of 2960.2 cm^{-1} appeared due to the stretching vibration of the single bond between C-N and C-H, located within amide III. The appearance of the wave number 1540.85cm^{-1} which fell within amide III was due to the vibration of the N-H and C-N bond, while the wave number 1070.3cm^{-1} indicated the stretching vibration of the C-N and C-O bond within amide amide II.

Figure 3b displays the vibration zones of egg white proteins treated with ultrasound waves for 5 minutes and black seed at a concentration of 0.1%. The wave number appeared at 3292.86cm^{-1} indicating stretching vibration of the O-H and N-H bonds, which fell within amides A and B due to the frequency vibration of 2962.13 cm^{-1} revealing the expansion of the bond between C-N and C-H. The frequency vibration of 1645.95 cm^{-1} fell within amide I due to the expansion of the C=O bond, and the wave number 1538.92 cm^{-1} denotes to bending of the N-H bond and expansion of the C-N bond within the amide II band. The wave number was 1078.01 cm^{-1} that was due to the expansion of the N-H bond and the expansion of the C-N bond that was within amide I.

The vibration areas of egg white proteins treated with ultrasound waves for 5 minutes and black seed (0.2%) were shown in Figure 3c. The wavenumbers of vibration frequency from 3539.7 cm^{-1} to 3451.96 cm^{-1} revealed wide bands that represented the presence of O-H and N-H bonds. The wave number of 2954.41 cm^{-1} indicated the expansion of the individual bonds of C-N and C-H, and the wave number 1647.88 cm^{-1} fell within amide I due to the expansion vibration of the C=O bond. The wave number of 1540.85 cm^{-1} fell within amide II due to the expansion vibration of the N-H and C-N bonds. The wave number 1075.12 cm^{-1} demonstrated the stretching vibration of the N-H and C-N bonds within the amide III region.

Figure 3d shows the absorption spectrum of egg white proteins treated with ultrasound waves for 5 minutes and cardamom (0.2%). It was noted that the wave number of the vibration frequency of 3369.3 cm^{-1} represented the stretching vibration of the O-H and N-H bonds that lied within amides A and B. The wave number 2962.13 cm^{-1} was the stretching vibration of the C-N and C-H bonds.

Table 2: Description of the vibrational frequency wavenumbers for egg white proteins treated with ultrasound waves and phenolic compounds.

Concentration %	Frequency (cm^{-1})	Designation	Description
Control	3271.64- 3926.36	Amide A and B	O-H and N-H stretching
	2960.2	Amide III	C-N and C-H stretching
	1540.85	Amide II	N-H and C-N stretching
	1660.41	Amide I	C=O stretching
	1070.3	Amide III	N-H and C-N stretching
Ultrasonic+0.1 black seed	3292.86	A and B	O-H and N-H stretching
	2962.13	Amide III	C-N and C-H
	1645.95	Amide I	C=O stretching
	1078.01	Amide III	C-N and NH
Ultrasonic+ 0.2 black seed	3539.7- 3451.96	Amide A and B	N-H stretching O-H and
	2954.41	Amide III	C-N and C-H stretching
	1647.88	Amide I	C=O stretching
	1540.85	Amide II	C-N and N-H stretching
	1075.12	Amide III	C-N and N-H stretching
Ultrasonic+0.2 cardamom	3369.3	Amide A and B	N-H stretching O-H and
	2962.13	Amide III	C-N and C-H stretching
	1647.88	Amide I	C=O stretching
	1542.77	Amide II	C-N and N-H stretching
	1070.3	Amide III	N-H and C-N stretching
Ultrasonic+ 0.4 cardamom	3911.9 -3314.07	Amide A and B	C-H stretching C-N and
	2929.34	Amide A and B	C-N and NH stretching
	1654.62	Amide I	C=O stretching
	1541.81	Amide II	C-N and N-H stretching
	1396.21	Amide II	C-N and N-H stretching
	1070.3	Amide III	N-H and C-N stretching

The vibration range of the wave number 1647.88 cm^{-1} was due to the expansion of the C=O bond within amide I, while the wave number 1542.77 cm^{-1} was within amide II due to the stretching vibration of the C-N bond and its bending. The N-H bond and the wave number 1070.3 cm^{-1} fell within amide III which was due to the stretching vibration of the N-H and C-N bonds.

The absorption spectrum of egg white proteins treated with ultrasound waves for 5 minutes and cardamom (0.4%) was illustrated in Figure 3e. It exhibits the vibration range between 3911.9 cm^{-1} and 3314.07 cm^{-1} due to the amplitude vibration of the O-H and N-H bonds that fell within amide A and B. The wave number 2929.34 cm^{-1} was located within amide A and B due to the stretching vibration of the bonds C-N and C-H. The absorption at the wave number 1654.62 cm^{-1} was due to the stretching vibration of the binary bond C=O which was located within amide I. The wave number 1541.81 cm^{-1} was located within amide II which indicates the stretching vibration of the C-N bond and N-H bending. The wave number 1396.21 cm^{-1} was located within amide II which indicates the stretching vibration of the C-N bond and N-H bending. The wave number 1070.3 cm^{-1} was located within amide III due to vibration stretching of the N-H and C-N bonds.

Figure 4 shows the effect of treating egg white proteins with ultrasonic waves for 5 minutes and phenolic compounds at different concentrations (black seed 0.1% and 0.2%, cardamom 0.2 and 0.4%), as well as the control treatment on the content of the amino acid tryptophan. It was observed that the emission intensity increased for the T5 treatment to obtain a maximum emission of 21.1 nm. The T3 displayed an emission intensity of 15.9 nm, followed by T1, which revealed an emission intensity of 14.5 nm. The lowest emission intensity was for T4 as it demonstrated an emission intensity of 12.5 nm, and T2 exhibited an emission intensity of 14.5 nm.

Figures 5b-5e illustrate the microscopic structure of enlarged sections of egg white proteins treated with ultrasound waves, phenolic compound extracted from an aqueous black seed (0.1% and 0.2%) and cardamom (0.2% and 0.4%); as well as the control treatment. All samples treated with ultrasound waves and plant-derived phenolic extracts exhibited a network-like structure with a pore size varying according to the concentration of the extract, as well as the dispersion of air bubbles. Figure 5a showed that the control treatment had a rather full protein structure, with bigger protein molecules and a higher level of aggregation.

Discussion

The chemical ingredients of a product are of pivotal importance and can affect the health status of people (27). One study revealed the moisture content of 86.49% and protein content of 10.96% in chicken egg white proteins that are consistent with our findings (28). The ash content of 0.26% was reported in another study that is lower than our findings. A fat content of 0.03%, moisture of 89.46% and protein of 9.69% contents were demonstrated that are lower than our study (29). The difference in the chemical content of egg whites may be due to the diet, age of the animal, season, genetic characteristics, and other environmental factors.

In our study, the combined treatment of ultrasound waves and phenolic compounds showed a significant effect on enhancing the functional properties of egg white proteins such as solubility, emulsifying

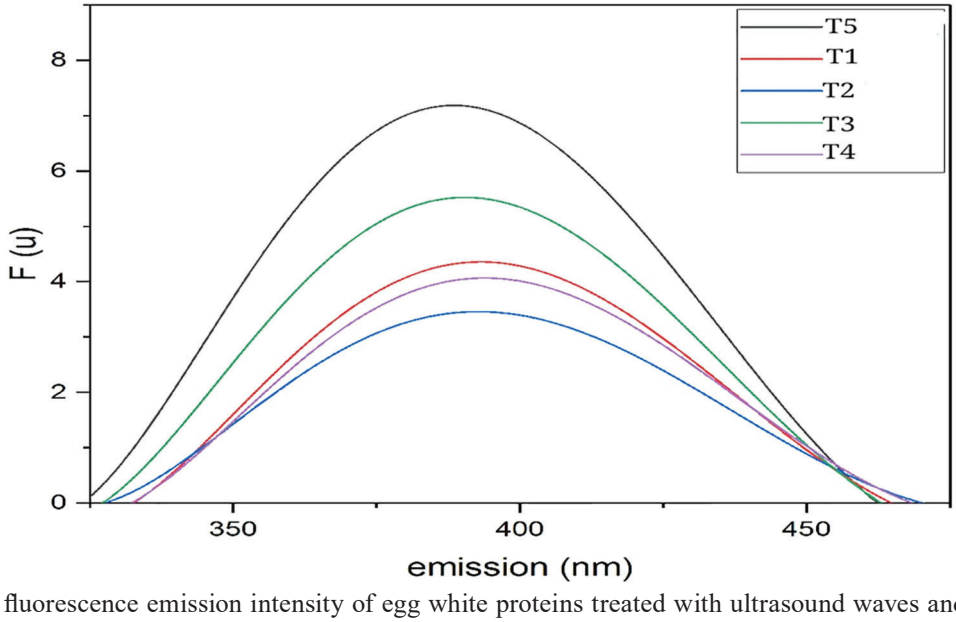

Figure 4: The fluorescence emission intensity of egg white proteins treated with ultrasound waves and plant derived -phenolic extract in comparison to control treatment.

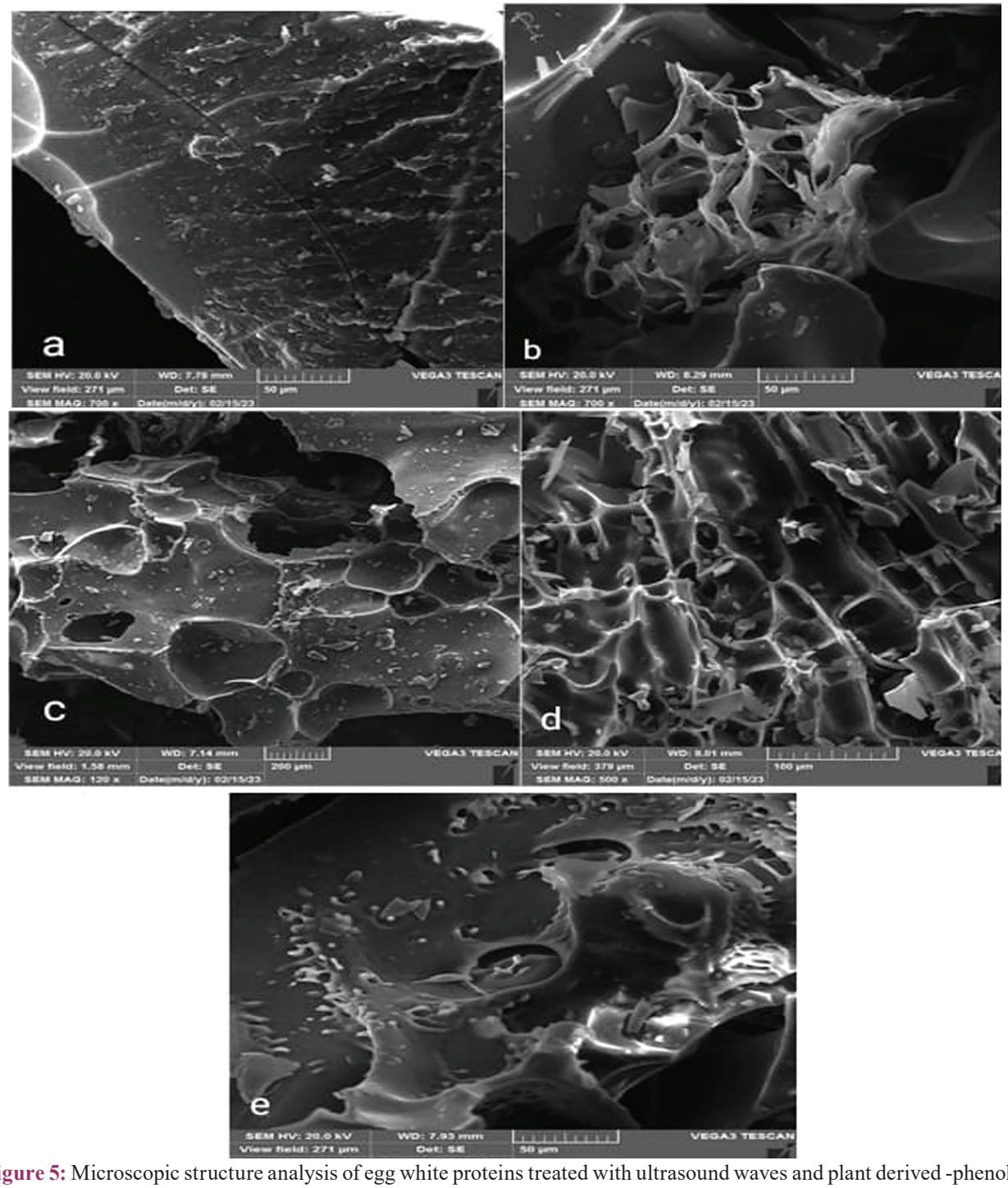

Figure 5: Microscopic structure analysis of egg white proteins treated with ultrasound waves and plant derived -phenolic extract (b-e) in comparison to the control treatment (a).

ability and foaming capacity. We demonstrated the treated proteins to improve foaming formation and stability when compared to the control treatment. These findings are in agreement with those who used phenolic compounds of ferulic acid, tannic acid and gallic acid to modify whey proteins (30). Our results are also in consistent with the study investigated the effect of ultrasonic treatment on the foaming properties and stability of millet proteins (6). The reason for the change in the rate of foam formation and stability may be due to the solubility of the proteins in water and the ability to form a film at the air-water interface. Also, proteins were absorbed faster at the air-liquid interface during bubble formation and were subject to molecular rearrangement at the interface with a superior foaming ability than proteins (30). The modified proteins and oxidized phenolic compounds could increase the foam stability. The stability of the foam increased with the rise in the oxidized phenolic compounds due to an increase in exposure to hydrophobic regions and exposure of protein molecules during treatment (6).

The treatment with ultrasonic and the phenols of the aqueous extract resulted in the highest solubility

values when compared to the control. This is due to the fact that adding the extract can reduce the surface hydrophobic groups and thus increases the solubility (31). It is attributed to the improvement in solubility after ultrasound treatment based on breaking of hydrogen bonds and hydrophobic groups which can lead to a decrease in the molecular weight of the protein and thus an increase in the interaction between the protein and water (31). The increase in protein solubility after ultrasonic treatment can be due to the decrease in molecular weight that increases protein-water interactions. The combined effect of ultrasonic treatment and phenolic compounds can significantly affect both emulsifying capacity and stability too. These findings are identical to our results (6, 31).

Ultrasonic treatment was shown to induce structural modification in proteins, including a reduction in molecular weight, an increase in sulfhydryl and hydrophobic groups, a decrease in α-helix content and an increase in β-sheet, β-turn and random coil structures confirmed by FTIR. The structural changes are attributed to the role of phenolic compounds to increase the surface hydrophobic groups, which is closely associated with improved emulsification properties. The enhanced surface hydrophobic groups were shown to reduce the surface tension, thereby contributing to increase the emulsifying capacity (30). These findings were noticed in T1 and T2 when compared to T3 and T4 in our study. Phenol-modified proteins tended to accumulate at the oil-water interface based on the increase in hydrophobic groups, which reduced the surface tension during homogenization and prevented creaming and coalescence during storage in order to enhance both the activity and stability of the emulsion.

Our results are similar to those reported the water-binding capacity to increase from 74.2% to 82.8% (32). The increase in water-binding capacity of egg white proteins treated with both ultrasonic and phenolic compounds can be attributed to the increase in β-sheets and α-helix content, as well as a significant improvement in protein relaxation and structural conformation (32). The interaction between protein and polyphenolic compounds can change in the secondary, tertiary and quaternary structures of proteins. Moreover, the treatment can lead to an increase in the water-binding capacity. The interactions between polyphenols-modified protein and water molecules were tightly wrapped by the protein network structure and resulted in an increase in water-binding capacity (32).

Regarding digestion factor, our findings are in agreement with those reported before who indicated that ultrasound treatment enhances the digestibility of egg white proteins (33). This improvement is attributed to microscopic and compositional modifications of ultrasound waves to promote molecular interactions and consequently to have a positive effect on protein digestibility (33). This finding was further supported by those who reported a significant increase in the digestibility of ultrasound-treated egg white proteins in comparison to the control (34). They confirmed that covalent bonding between proteins and phenolic extracts can enhance protein digestibility (34). Phenolic compounds were illustrated to influence the activity of protease enzyme which is closely related to their binding affinity. Factors such as amino acids composition, molecular size and protein structure played crucial role in this interaction. The binding of phenolic compounds to enzymes led to either structure destabilization or stabilization, thereby modified the enzyme activity and improved access to protein substrates and ultimately enhanced the protein digestion (35).

Regarding the effect of ultrasonic modification and phenolic compounds on the structural composition of egg white proteins, FTIR spectroscopy revealed the presence of a wavenumber at 1660cm^{-1} that corresponds to the stretching vibration of the C=O bond, which falls within amide I region. This region is located at 1600-1700cm^{-1} and is considered the most sensitive to the changes in secondary structure of proteins and reflects elements such as α-helix, β-sheet, β-tuyns and random coil structures (36). The absorption in the range of 1610–1640 cm^{-1} belongs to the β-sheets, 1640–1650 cm^{-1} to β-turns, 1650–1662 cm^{-1} to α-helices, and 1662–1695 cm^{-1} returns to random coils (36). FTIR analysis could show the decrease in the α-helix content of egg white proteins after the addition of phenolic compounds, while the β-turn content increased. These changes varied based on the concentration of added phenolic compounds indicating that phenolic compounds could influence the secondary structure of egg white proteins through either non-covalent (reversible) or covalent interactions (36).

The decrease in α-helical structure happened due to structural changes in proteins treated with polyphenols. As phenolic compounds contain highly reactive hydroxyl groups, they are capable of oxidizing the protein side chains to quinones and to promote cross-linking with lysyl, tyrosyl, and cysteinyl residues in the protein structure (37). The effect of ultrasonic treatment has been investigated before on the functional and structural properties of millet proteins using FTIR spectroscopy. They found that the spectral regions between 1600 to 1700

cm^{-1} were attributed to the amide I band and from C=O stretching vibration, which is more sensitive to changes in protein secondary structure when compared to the amide II region (1480-1575)cm^{-1} (6). Our comparison of the FTIR revealed differences in the structural and chemical composition of ultrasound treated millet protein in comparison to the control, which can be attributed to the shear forces resulting from the cavitation phenomenon to increase the exposure to more negatively charged amino acids on the protein surface (6).

Fluorescence spectroscopy has been used to evaluate the effect of structural modification on tryptophan emission intensity, where a noticeable decrease was observed in fluorescence intensity in the samples treated with ultrasound waves and phenolic compounds. These results are consistent with those reported before who found that the treatment of soybean proteins with ultrasound and tea polyphenols could affect fluorescence emission intensity (38). Similar findings were reported revealing the effect of phenolic compounds on development of antifungal nanocomposite films based on egg white proteins, while the addition of phenolic compounds led to a reduction in emission intensity (39). The addition of phenolic compounds caused a decrease in the intensity of emission due to the association of molecules of phenolic compounds with the amino acid tryptophan. Thus, the intensity of emission reduced gradually and affected the protein complex formation (39). Fluorescence spectroscopic analysis showed the changes occurring in the protein structure as the emission intensity decreased with increasing concentration used. This emission intensity in proteins can be attributed to the tryptophan amino acid as ovalbumin, which is the main part of egg white proteins, contains three terminal tryptophan groups that can contribute to the intensity emission (39).

SEM analysis revealed that ultrasonic treatment exposed the structural composition of the proteins and led to a reduction in particle size. This suggests that ultrasonic waves can enhance the protein surface area and its ability to bind with phenolic compounds from plant extract and result in formation of a three-dimensional network structure. Additionally, it increased the concentration of soluble protein and hydrophobic groups (40). The ultrasonic treatment combined with plant extract phenolic compound was demonstrated to improve several properties of egg white proteins such as their physicochemical characteristics, functional performance, sensory attributes and the storage stability. These findings are consistent with those who observed similar change in the structural and functional properties of egg white proteins treated with ultrasound and tea polyphenols (32).

Conclusion

The functional properties and structural characteristics of egg white proteins can be improved by ultrasonic treatment and the phenolic compounds extracted from black seed (0.1% and 0.2%) and cardamom (0.2% and 0.4%). The ultrasonic treatment for 5 minutes and the phenolic compounds of the 0.2% black seed showed the best results to improve functional properties, digestibility, and changes in the secondary structure of egg white proteins. The α-helix content decreased and the β-turn increased when treated with phenolic compounds that were verified with FT-IR spectroscopy, fluorescence measurement and SEM.

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Authors' Contribution

R.H.M conducted the laboratory work ,preformed data collection and analysis and participated in the interpretation of the result. A.Z.H was responsible for conceptualizing the research idea, designing the experimental plan and supervising stages of the study**.** Both authors contributed equally to writing reviewing and appoving the final version of the manuscript .

Conflict of Interest

The authors declare no conflict of interest.

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