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ORIGINAL ARTICLE

Functional Properties of Oats and Soybeans Proteins When Treated with *Lavandula Angustifolia* Extract

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ABSTRACT

Background: *Lavandula angustifolia* (lavender), a perennial herb of the Lamiaceae family, is valued for its medicinal and economical importance. Similarly, plant-derived proteins from oats and soybeans provide nutritional, health, and sustainability benefits. This study investigated the functional properties of oats and soybeans proteins when treated with lavender *(Lavandula angustifolia*) extract.

Methods: Oat and soy proteins were studied through chemical, physical, or enzymatic methods. Formulations containing different concentrations of lavender extract (0%, 0.025%, 0.05%, and 0.1% w/w) named as M0, M1, M2, and M3 were prepared to assess their effects on the proteins' physicochemical and functional characteristics of oat and soy. The physicochemical composition (moisture, protein, lipid, and ash) of the protein isolates and lavender material were analyzed. Additionally, the lavender extract was examined for phenolic compounds, minerals, and bioactive components using advanced techniques such as gas chromatography, mass spectrometry, and Fourier transform infrared (FT-IR).

Results: It was shown that mixing the extract with the isolate had an enhancing effect on the functional properties and could affect the pH values, which decreased with increasing the concentration of lavender. The addition of the extract also improved the antioxidant activity during the extraction of 1,1-diphenyl-2-picrylhydrazyl (DPPH)-2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, binding of ferrous ions, and increase in the reductive power. Mixing with the extract at 0.5% concentration demonstrated antibacterial activities against several kinds of bacteria.

Conclusion: Functional properties and antioxidant activities of proteins isolated from oats and soybeans were modified based on concentration of lavender.

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Introduction

The demand for protein-enriched foods has increased during the past few years. This demand can be catered as the proteins which play an essential role in the body such that they contain enough amounts of amino acids (1). Protiens hydroxyls, carboxyls and amino groups as proteins strcture are used as active ingredients of various foods (2). Cereal crops

can provide essential proteins which are critical in human nutrition; while animal proteins are invaluable in food production due to their protein content, but excessive intake of red meats can be associated with possible risk of chronic diseases over time (3). Therefore, there is an increasing shift across the globe from animal-protein sources to vegetable protein sources. The protein molecules which are provided by plant sources are from a variety of foods including whole grains, legumes and nuts. Oats are distinct grains because of their protein level (13-20%) and antioxidants properties (4). Amino acids in oat proteins are rather balanced because the main protein of oats is globulin. Oats are categorized into seeds, fodder, hay and silage. Oatmeal can be incorporated into breakfast cereals, baked goods or used as a meal for obese individuals. It is helpful in reducing LDL level too (5).

The extraction of soy protein from soybean is one of the most crucial sources for meeting the demand for human protein requirements. Owing to these reasons, soy protein industries have growth up recently all around the world specially in Europe and USA and people now prefer to consume fresh food that has a high nutritional value. Soy protein products hav been considered as one of the stable alternatives to animal meat within vegetarian diets because of the high content of proteins with health benefits (6).

Functionality is one of the key properties to assess the proteins that are found in foods. Water soluble proteins were shown to enance the interacial properties of food applications that are of great importance. The functional properties of proteins are dependent on their amino acid sequence and tertiary structure and charge distribution, and also on the manner of interaction between proteins and carbohydrates, fats, proteins, and small food components. Solubility, gel formation, emulsifying, and foaming are considered as important parts of functionality of proteins in in several kinds of foods. However, the grain proteins are sensitive to external factors and treatment conditions such as heat, acids, etc., so several methods were used to improve the functional properties (7).

Improving the protein functionality can be possible upon application of extracts from various plants. Among the medicinal plants that are popular worldwide, lavender as a perennial plant with high nutritional value from the family Lamiaceae has been introduced (7). It has been utilized in treatment of many diseases and has antibacterial, antifungal, and antioxidant propertie (7). So this paper aimed to enhance the functional properties of proteins from oats and soybeans by treating with lavender extract. The enhanced process has also aimed to improve the nutritional value, antioxidant activity and extended storage life proteins from oats and soybeans by treating with lavender extract.

Materials and Methods

All samples of oats and soybeans used in this work were collected randomly considering the saving temperature and other conditions that keeps samples functional. Samples were provided from the Field Crops Department at the College of Agriculture, University of Basrah, Iraq. The chemical composition of lavender leaf powder, oat protein isolate, and soybean protein isolate including moisture, fat, ash, carbohydrates, pH, and protein content was determined according to the standard methods described by (8, 9). An atomic absorption spectrometer of Type 2380 was employed for measurement purposes (10). Parts per million (ppm) were used to indicate the results of the identification of macro- and micro-elements in lavender leaf powder. In addition to acid, one gram of sample ash was utilized too. After combining sulfuric, nitric, and hydrogen peroxide, the mixture was filtered via filter paper and put into a standard 50 mL atomic absorption flask. An atomic absorption apparatus was then used to measure the combination by a Perkin Elmer spectrophotometer**.**

The preparation of an aqueous extract of lavender leaf ground was conducted as described before (11). In the flask, 300 g of raw material were weighed and mixed with distilled water (2000 mL, temperature 60-65°C) and heated for an hour in a water bath. The mixture was kept for about 12 hours at room temperature. The mixture was then passed through a kapron cloth and afterward, distilled water (1000 mL) was added. This was heated to be between 60 and 65°C that was left for an hour in a water bath under conditions of constant stirring, and was further filtered again. The obtained filtrates were used for further analyses following filtration. Then, the filtrate was concentrated at 45°C in a rotary vacuum evaporator. The dried extract was packed in containers for use. Analysis of lavender extract was conducted by gas chromatography (GC, Agilent Company, Model N5973, America, Veromed Laboratory, Tehran, Iran).

The estimation of total phenols was determined according to the Folin-Ciocalteu technique (12). The phenols content in the extracts at a wavelength of 725 nm was measured by Gallic acid standard solution. The range of concentration was between 0 and 300 mg/mL (Figure 1). The inhibitory activity of aqueous lavender extract at concentrations of 0.2%, 0.3%, and 0.5% was evaluated using the well diffusion method.

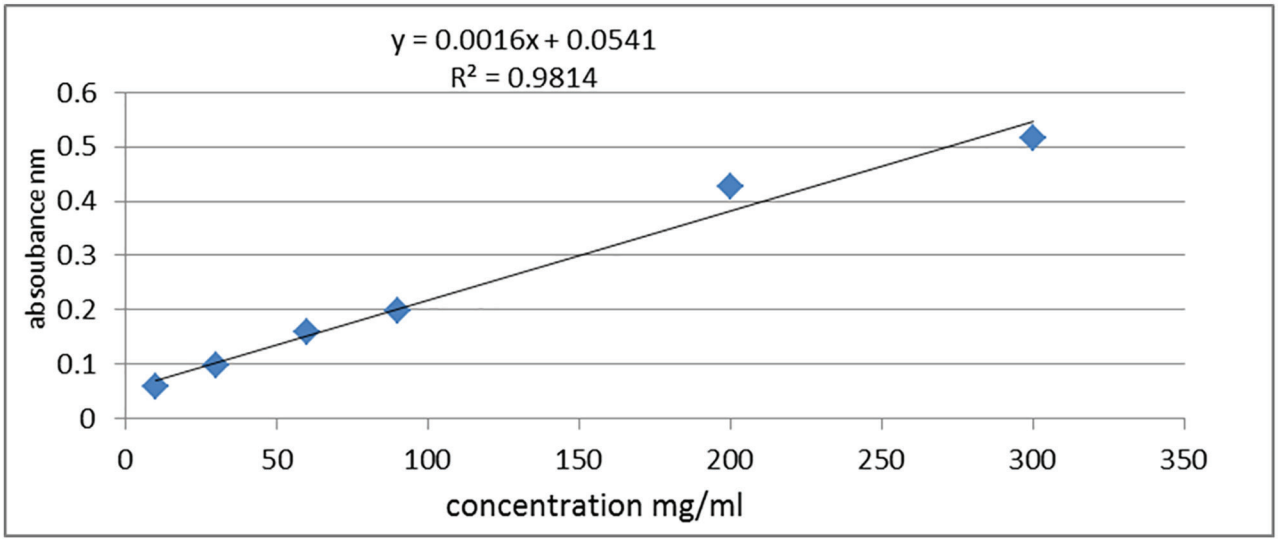

Figure 1: Standard curve for gallic acid.

Standard McFarland solution was utilized to assess turbidity level at a density of 1.5×10^8 cells per unit volume and measurements at 600 nm (13). Bacterial strains were activated by nutrient broth and the tubes at 37°C for 18 to 24 h. Dilutions were then prepared and the growth density was compared to a standard McFarland solution. The inhibitory activity of extract against the bacterial strains was determined by creating 6 mm-diameter wells in Mueller-Hinton agar. Atotal of 100 µL of the solution was introduced into the well, with distilled water as control treatment. The plates were incubated at 37°C for 24 h.

The protein isolate was obtained when the fat from the powder of ground grain was soaked in hexane at a weight-to-volume ratio of 2:1 for 12 h. After oil removal, this operation was repeated three times (14). The fat-removed material after that was dried, ground, and then further filtered using a sieve of 60 mesh holes. The protein isolate was assessed using a modified method. Distilled water was added to the samples at a ratio of 1:20 (w/v), stirred for 1 h and the pH was adjusted to 9.0 by adding 1.0 M NaOH. Filteration of the solution was carried out by a white cheese cloth centrifuge for 20 min at 10,000 rpm for protein precipitation. The supernatant was poured into a beaker and stirred once for 20 minutes. Then, the pH was adjusted to 4.5 using 0.1 M HCl and was left overnight. The pH was adjusted to 7.0 and centrifugation of protein slurry was undertaken for10 minutes at 10,000 rpm, while finally, the protein precipitate was collected and freeze- dried (15).

Protein lavender polyphenol complexes were used for analysis. In brief, 1.0 g of protein isolate was dissolved in 100 mL of deionized water and stirred at 150 rpm for 30 minutes to aid in protein solubilization. The solution was combined with lavender extract at different concentrations (0.025, 0.05, and 0.1% w/w), symboled as M0, M1, M2, and M3, respectively. A soulotion of 0.1 M NaOH was used to adjust the pH to 9.0. The solution was then dialyzed for 48 hours against water in a dialysis tube (8 kDa MWCO) to eliminate free polyphenols and their derivatives after being incubated at room temperature for 24 hours; while continuously stirred at 150 rpm. We then dried the resulting solution in a freeze dryer to produce a porous solid. The control group was produced using identical conditions, but without the addition of lavender extract; while the inhibitory zone's size was conducted in millimeters (mm) (16).

Fourier transform infrared (FT-IR) spectra was performed using FT-IR spectrometer (Jasco, Japan) in the Polymer Research and University of Basrah Center. The measurements were done in infrared region of 400-4000 cm^{-1}. To determine functional properties, the water binding method was used (18). To do so, one gram of each mixture was weighed and placed in test tubes with a known weight. After adding 10 mL of distilled water and stirring for two minutes, it was left for half hour. Centrifugation was performed at 3000 rpm for 20 minutes. The tube containing the precipitate was then weighed after emptying the supernatant. The water binding capacity was calculated according to the formula of $\text{water binding \%} = \frac{\text{weight of sample} - \text{weight of separated liquid}}{\text{weight of sample}} \times 100$ (17).

Estimated foaming of the protein isolate and the extract complex were conducted by foam formation method (19) using 1% mixture of each samples for two minutes with an electric mixer, and then transferring directly into a graduated cylinder and calculating the foam size using the formula of $\text{Foaming \%} = \frac{A-B}{B} \times 100$; while A=volume after whipping (mL) and B=volume before whipping (mL).

Emulsification method was used by centrifuging at 3000 rpm for 15 minutes and addition of 5 mL of sunflower oil and 50 mL of 1% sample; while was blended for two minutes. The stability of the emulation was tracked for 10, 30, and 60 minutes utilizing the equation of

$$\text{Emulsification\%} = \frac{\text{total volume(ml)} - \text{water volume(ml)}}{\text{total volume(ml)}} \times 100 \ (20).$$

For fat binding, one gram of each sample was added to 10 mL of sunflower oil. The complex was then vortexed for 30 seconds and centrifuged at 2500 rpm for 25 minutes. The weight difference of the oil-holding capacity was determined (21). The solubility of the samples was estimated as described before (22). The total protein content was also determined as previously mentioned (9). In brief, 1 gram of the sample was mixed with 0.05 M Tris-HCl buffer solutions at pH 6.5. It was centrifuged at 10,000 rpm for 20 minutes and then, 2 mL of supernatant solution was diluted twentyfold and allowed to rest. Biuret method was performed to measure the amount of soluble protein. The supernatant was checked for absorbance at 550 nm to calculate the solubility employing the equation of

$$\text{protein solubility\%} = \frac{\text{protein in supernatant (mg)}}{\text{protein in samples}} \times 100.$$

To assess the antioxidant activity, the estimated ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH)-2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was done by addition of different amounts of aqueous lavender extract to 2.9 mL of 0.004% (w/v) ethanol solution. Absorbance was measured after incubation at room temperature for 30 min at 517 nm wavelength. For free radical inhibition, 1% of DPPH was computed using the equation of $1\% = \frac{\text{A blank} - \text{A sample}}{\text{A blank}} \times 100$; while A0=Absorption of comparison sample and As=Absorption of sample (23). To conduct ferrous ion binding test, the capacity of mixture to bind the ferrous ion at 562 nm was calculated and the ability of mixture to bind the ferrous ion was estimated using the equation of

$$\text{Ferrous ion\%} = \frac{\textit{absorbance control} - \textit{absorbance of the sample}}{\textit{absorbance control}} \times 100 \ (24).$$

For assay of reductive power, a wavelength of 700 nm was used to measure absorbance. The reducing power was determined using the formula of

$$\text{Reductive power} = \frac{100 - A\ sample}{A\ control} \times 100 \ (25);$$ while A=absorbance. For statistical analysis, Genesta 2011 software was used. Data was obtained randomly and *p* value was set at less than 0.05.

Results

Table 1 demonstrates the chemical composition of proteins isolated from soybeans and oats. The moisture, protein, fat, ash, and carbohydrates percents were different at $p \leq 0.05$. Other values did not show any difference for fat percent (0.83% and 0.84%) in oat and soybean isolates that can be due to source and type of seeds. The approximate composition of lavender leaves is shown in (Table 2). The leaves contained 8.42% moisture, 9.10% protein, 8.03% fat, and 5.13% ash. The concentration of the total phenolic component of the leaves was calculated to be 125 mg/g. High concentrations of K and Ca, and low concentrations of Na and P in mineral analysis results indicated that lavender leaves contained macro-elements. On the contrary, the concentration of microelements for copper and zinc was the lowest, and the largest was for magnesium and iron (Table 2).

Table 3 displays the pH values of the lavender extract as well as the complex made from the protein isolates of oat and soybean with varying concentrations of lavender extract. The lavender extract recorded a pH value ranging from 4.96 to 5.02, indicating that the concentration used may have had a major impact on the pH value.

Table 1: Proximate composition of isolated proteins.

	Composition%				
Sample	**Moisture**	**Protein**	**Fat**	**Ash**	**Carbohydrate**
Oat	4. 25	87.78	0. 83	1.89	5.23
Soybean	3.38	85.49	0. 84	5.33	4.96

Table 2: The composition of lavandula leaves.

Composition%				
Carbohydrate	**Ash**	**Fat**	**Protein**	**Moisture**
69.31	5.13	8.03	9.10	8.42
Total phenols mg/g	125			

Macro- and Micro-minerals							
%			ppm				
P	**Ca**	**K**	**Na**	**Cu**	**Fe**	**Mg**	**Zn**
0.20	1.30	1.81	0.059	9.25	355.75	2645.50	20.50

Table 3: The pH value of Lavandula leaves extract at different concentrations and the effect of adding the extract to protein isolation on the pH value.

Lavandula extract			
pH		**Concentration g/mL**	
5.02		0.025	
4.97		0.05	
4.96		0.1	
Protein isolation			
pH			**Isolate (g)**
Soy		**Oat**	
6.79		5.98	0.975
6.77		6.01	0.95
6.80		6.06	0.9
The pH of Lavandula extract and protein isolate complex			
pH		**Lavandula extract (g)**	**Isolate (g)**
soy+extract	**oat+extract**		
6.58	5.85	0.025	0.975
6.44	5.68	0.05	0.95
6.20	5.52	0.1	0.9

It was also noted that the pH values decreased as the extract concentration increased. The pH values of the soybean isolate ranged from 6.79 to 6.80; whereas the oat isolate, at varying concentrations, ranged from 5.98 to 6.06. Additionally, pH values changed when the complex containing the lavender extract and each of the isolates of soybeans and oats were made at varying concentrations. The addition revealed significant differences ($p \leq 0.05$).

Figure 2 and Table 4 present the compounds in lavender aqueous extract identified by GC-MS. Some groups of chemical compounds were present; among them, the highest percentages were p-coumaric acid and ferulic acid, at 106.2 µg and 1287 µg, respectively, and rutin at 96.9 µg, while chlorogenic acid was present at 43.2 µg.

Table 5 shows the inhibition effect of lavender aqueous extract on three bacterial strains. The inhibitory effect of the lavender aqueous extract in various concentrations on bacterial types resulted in the halo diameter at a 0.5% concentration of 2, 1.5, and 1 mm, respectively for *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*. The minimum diameter of the areola was 0.5 mm at a concentration of 0.2%.

The changes in protein isolates taken from oats

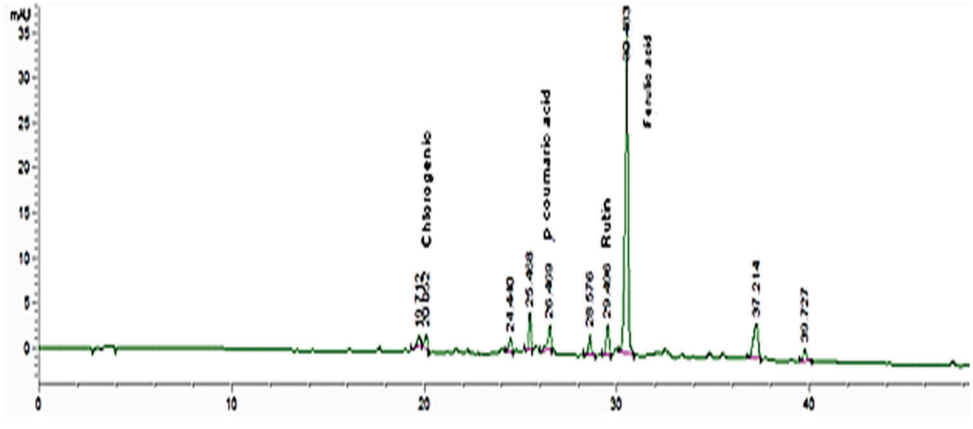

Figure 2: Chart for the active compounds identified by (GC-Mass) technology in the extract of the lavender plant.

Table 4: The active compounds identified by GC-Mass technology in the extract of the lavender leaves.

Sample	Area	Concentration ppm (Ug/g)
Chlorogenic acid	14.4	43.2
Caffeic acid	-	-
P-coumaric acid	35.4	106.2
Ferulic acid	429	1287
Rutin	32.3	96.9
Quercetin	-	-

Table 5: Antibacterial activity of lavender aqueous extract at different concentrations.

Concentration %	Inhibitory zone (mm)		
	Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*
0.2	0.5	1	1
0.3	1	1	1.5
0.5	1	1.5	2
0	-	-	-

(-): Non inhibition.

and soybeans due to treatment with Lavandula extract at different concentrations were evaluated by FT-IR spectroscopy. Figure 3 shows the protein isolate of soybeans treated with extracts at concentrations of 0.1, 0.05, and 0.025 g/g, respectively (Figure 3a-c). The wave numbers in these figures were between 3311 and 3906 cm^{-1}, but the wave numbers for the control treatment (Figure 3d) were 3806.76-3485.69 cm-1, where the O-H and N-H bond-stretching vibrations were present. Wave numbers ranging from 2927.4 to 2360.44 cm^{-1} and from 2925.48 to 2359.48 cm^{-1} were represented for C-H and C-N bond stretching vibrations by treatment with the extract and the control, respectively. For the I amide, the C=O bond was observable at wave numbers of 1653.66 cm^{-1} and 1648.84 cm^{-1} following the extract treatment, while the numbers for the control treatment were at 1650.77 cm^{-1}, since there was a slight difference in the treatment wave numbers from those of the control treatment. The wave numbers of 1546-1020.16 cm^{-1} present in amide II and amide III depicted the bending characteristics of C-O, C-C, C-H, and N-H. Figure 3 (e-g) illustrates Lavandula extract when added to oat protein isolate at the rate of 0.1, 0.05, and 0.025 g/g, respectively. Wave number for the extract was from 3385.42 to 3963 cm^{-1}; while wave number for control treatment was from 3418.21 to 37.84.37 cm^{-1} (Figure 3h). O-H and N-H bond-stretching vibrations were also shown in the figure. The stretching vibrations of the C-N and C-H bonds were displayed by the wave numbers of 2362.37-2927.11 cm^{-1} when treated with the extract and wave numbers from 2361.41 cm^{-1} to 2928.38 cm^{-1} for the control treatment.

For the treatments with the extract, the C=O bond in amide I was present at wave numbers of 1655.59-1658.55 cm^{-1}, while the wavenumber for the control treatment was 1654.62 cm^{-1}. First and second amide bonds were the most obvious vibration bonds in the protein backbone. The spectral region between 1700-1600 cm^{-1} was the most sensitive in secondary structural components of the two proteins, most of which were associated with C=O stretching vibrations. Wave numbers were 1546 cm^{-1} through 1020 cm^{-1}. At 16 cm^{-1}, the bending characteristics of C-O, C-C, C-H, and N-H were present in Amide II and Amide III.

Figure 4a demonstrates that adding lavender extract to the soybean and oat's proteins at varying concentrations could significantly affect the isolate solubility ($p \leq 0.05$). For oat, the solubility of M1 was 24.12% that was higher than M2 and M3, while solubility decreased as concentrations declined. However, M0 solubility was 14.65% and with an increase in extract concentration, soybean isolate M3 had the highest solubility at 33.4%, followed by M2, and then M1. M0 solubility was 15.75% and the addition of the extract increased the complex solubility when compared to the control treatment, showing its impact on the soybean protein solubility.

Figure 4b. shows the foam values of complex made from proteins isolated from soybean or oat and the lavender extract preparation at varying concentrations were significantly different ($p \leq 0.05$), while treatment M2 exhibited the highest foam value that was 41.66% and 45% for isolates of oat and soybean, respectively. The foam value was the lowest for treatment M3, but treatment M1 recorded identical foam values for both isolates. The isolates of soybean and oat had foam values of 41.66% and 30.83%, respectively, for the control treatment M0.

Figure 4c. illustrates that the concentration of lavender extract applied to oat and soybean proteins impacted on the proteins' capacity to bind water. M2 of oats had the highest water binding capacity (1.83%), followed by M3 and M1, while M0 revealed a water binding capacity of 1.88% and significant variations ($p \leq 0.05$) in the water binding values across treatments were noticed. The ability of M3 (2.79%) to bind water to soybean isolate was the highest, followed by M2 and M1. However, M0 (2.94%) water-binding capacity was greater than other treatments.

Emulsification capacity evaluated how well protein-based emulsifiers worked by assessing the absorption of protein at the interface. Figures 4d and 3e demonstrate that adding lavender extract to the protein isolate of soybean and oat at varying concentrations did not significantly alter the protein's ability to emulsify ($p > 0.05$). M0 registered the highest emulsification values for the complex at 0 minutes (54% and 53%), reaching 52% after 60 minutes.

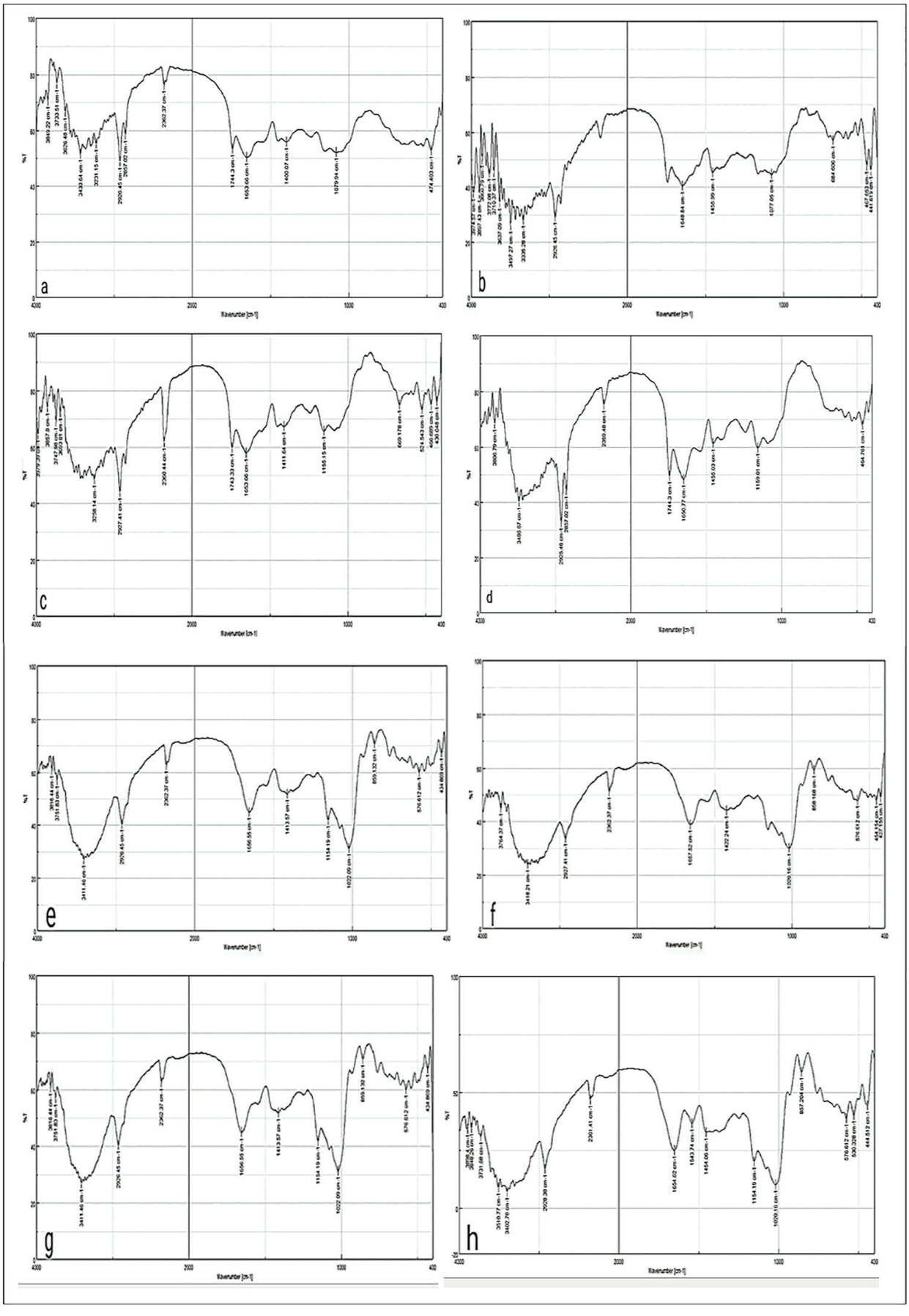

Figure 3: FT-IR spectrum of protein isolates with and without lavender extract. a-c: They represent soybean protein isolate modified with lavender extract at concentration of 0.1, 0.05 0.025%, respectively. e-g: They correspond to oat protein isolate modified with the same concentration of lavender extract. d and h: Theyshow the FT-IR spectrum of soybean and oat protein isolates (control), respectively.

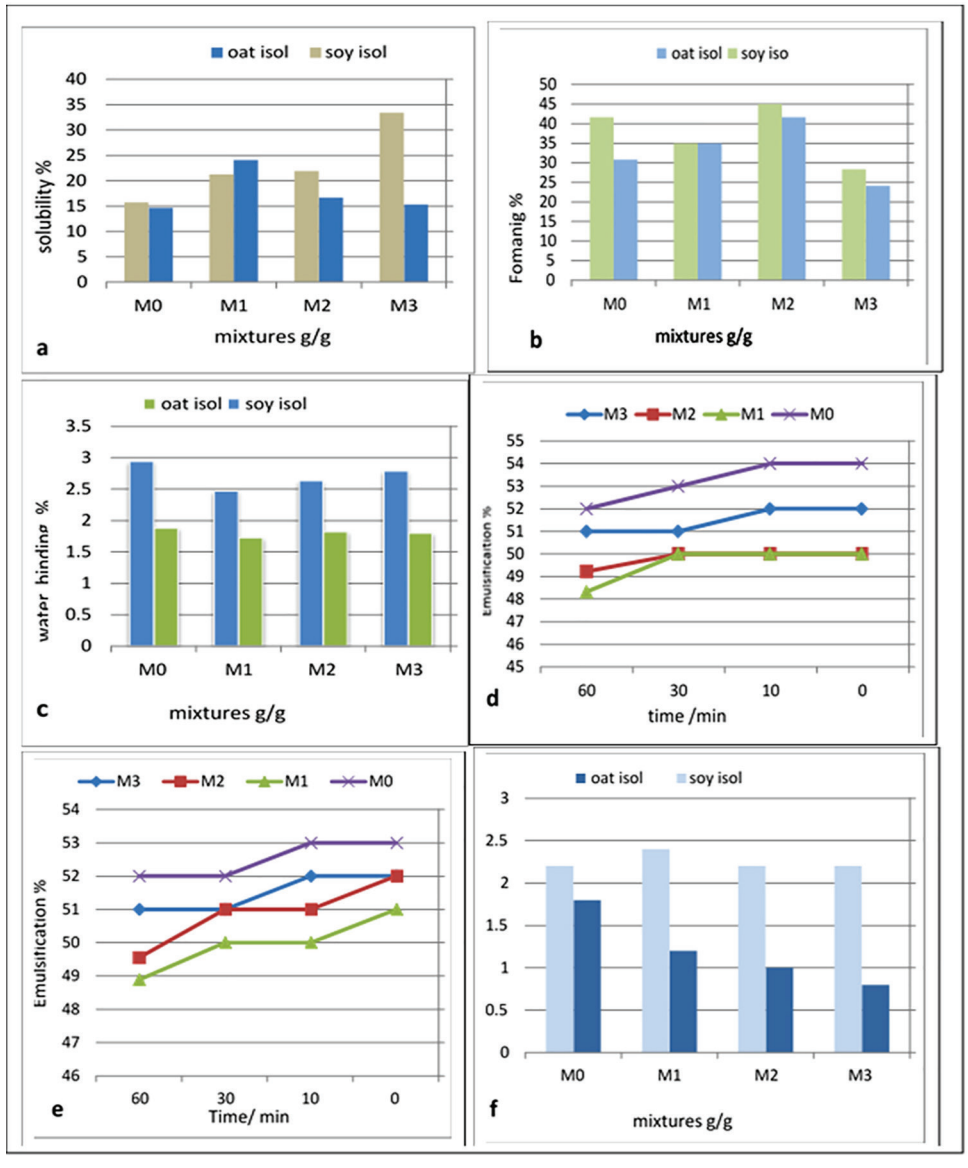

Figure 4: The funactional properties of soybean and oat protein isolates affected by treating with varing concentrations of lavender extract. A: solubility, b: foaming capacity, c: water-binding capacity, d: emulsifying ability, and e: fat-binding capacity.

Compared to complex oat or soybean protein isolates with lavender extract, it displayed lower emulsification values for both oat and soybean isolate, respectively.

Figure 4f exhibits no significant variation in the fat-binding values (p>0.05). M0 treatment outperformed all treatments, with the maximum capacity to bind fat occurring at M1 reaching 1.20 and 2.40 mL/g for the mixture of lavender extract with each isolated oats and soybeans, respectively. The fat-binding capacity of oats was 1-80 mL/g for M2 and M3. M2 and M3 values for soybeans were also found to be similar, matching M0 in terms of their capacity to bind fat.

The ability of the protein isolates treated with various Lavandula extract concentrations to scavenge the DPPH radical was shown in Figure 5a. Based on the concentration of the complex, the values of antioxidant activity showed significant variations (p≤0.05), with M2 exhibiting the highest antioxidant activity for oat at 43.74%, followed by M1 and then M3, while M0 treatment had the lowest antioxidant activity at 23.41%. As the extract content increased, the antioxidant activity of the soybean and lavender mixture also increased. Among the three, M0 had the lowest antioxidant activity at 51.97%, followed by M2 and M3, with M1 showing the highest antioxidant activity at 64.72%. We found that M1 had the highest antioxidant activity for soybean compared to oat and soybean combinations. Antioxidant efficiency was noted for all combinations.

Figure 5b. presents the reducing power of the mixture of protein isolates and lavender extract. The results indicated that the reducing power values varied significantly. M1 of the mixture of oat and an extract of lavender exhibited a higher reducing power of 94.55% when compared to M2 and M3, and treatment M0 had a decreasing power of 36.36%.

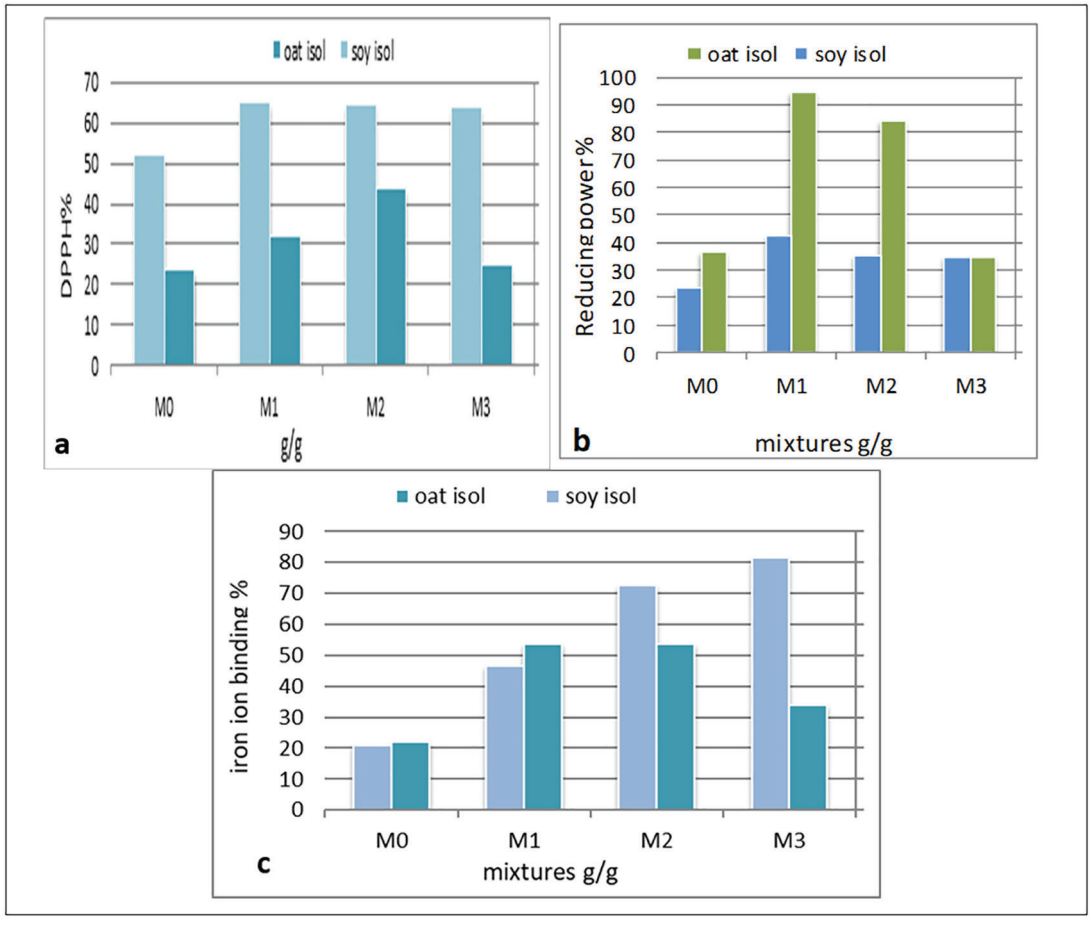

Figure 5: a-c: They illustrate the effect of treating oat and soybean protein isolates with different concentrations of lavender extract on their antioxidant properties, including (a) DPPH radicals scavenging, (b) reducing power and (c) ferrous ion chelating ability.

The control treatment M0 recorded the lowest reductive power of 23.72%, whereas the mixture of soybean and lavender extract at M1 denoted to a reductive power of 42.21%. When oat and soybean mixture was contrasted, we discoverred that M1 in oats had the highest reductive power in comparison to M1 of soybeans and the rest of the complex.

Figure 5c reveals the ability of mixtures of oats and soybeans with the extract prepared at different concentrations to bind the ferrous ion. The results showed significant differences ($p \leq 0.05$) in the ability of the mixtures to bind the ferrous ion, as M0 showed less ability to bind the ferrous ion for each of the oat protein isolates, and soybeans reached 22.1% and 21.05%, respectively, compared to the highest binding capacity for M1 oats, which reached 53.64%. The highest binding capacity for ferrous ions in soybeans was at M3, which reached 81.71%, followed by M2 and M1.

Discussion

Our results showed the chemical composition of isolated proteins affected the type of grain used in this study. It was shown that lavender leaves contained a fair quantity of protein and phenolic compounds besides mineral components, which contribute significantly to its nutritional value and antioxidant efficiency (26). Other nutritional values of the lavender leaves were 6.80% for moisture, 7.49% for ash, 6.13% for protein, 6.52% for fat, and 73.06% for carbohydrates. The trace mineral concentration was 9 ppm for Cu, 480 ppm for Fe, 219 ppm for Mg, and 23 ppm for Zn. With the addition of different amounts of extract, pH values of the isolated protein changed. The study revealed that pH values of lavender extract was dependent on the concentration. The antioxidant extract performance was influenced by the concentration of the extracted solution, as well as the type and amount of the active compounds found in the extract, since lavender extract contains phenolic compounds, tannins, flavonoids, and organic acids that are responsible for its antioxidant action with hydrogen and electron donation, that are later converted to more stable radicals (27).

Since they have an inhibitive function on the generation of free radicals and self-oxidation processes, the phenolic compounds are one of the most powerful antioxidants in diets. They contain one or more O-H group; hence, their effect on oxidative

stability presented characteristics of antioxidants. It suppresses oxygen, chelates metal ions, inhibits self-oxidative enzymes, and activates antioxidant enzymes (28). Previous studies have shown that the addition of phenolic compounds in food can change the food nutritional value and function. Other studies have suggested that the change might occur through interaction of phenols with other ingredients, such as proteins, carbohydrates, and fats (29). Dietary protein-phenol interaction indicate that two pathways are major ways to mediate such an interaction. There are four kinds of binding forces, including hydrofobic, van der Waals, electrostatic, and hydrogen bonding, to form a reversible covalent connection between dietary protein and phenol revealing that phenols are hydrogen donor groups which through interactions between phenol hydroxyls can form hydrogen bonds with the protein amine groups (30, 31).

Other examples of additional forces are van der Waals forces coming from the interaction of atoms affected by the solvent environment, and electrostatic bonding sourcing from the interaction of charged groups within or on the surface of proteins. Covalent bonding involves the covalent interactions developed between reaction molecules in enzymatic or non-enzymatic reactions between proteins and phenolic compounds. Semiquinones and quinones are highly reactive and could react with some of these intermediate products, which include terminal amino acids like cysteine, tryptophan, lysine, and methionine, among several peptide chains in establishing covalent linkages between proteins and phenolic compounds (32).

The formation of covalent bonds takes place between proteins and phenolic compounds. GC-MS identified an extract from leaves of the lavender plant, known to have an immense number of active compounds within, especially in the form of phenolic compounds such as ferulic acid, phenolic acid, rutin, and chlorogenic acid (32). The effect of ultrasonic treatment (5 minutes) and the addition of phenolic compounds at concentrations of 0.1% and 0.2% for black seed extract, and 0.2% and 0.4% for cardamom extract on the functional properties of proteins have been investigated (33). The findings revealed significant improvements in these properties compared to the control group. The authors attributed the observed enhancements in foam formation and stability to increased protein solubility in water and the proteins' ability to form stable films at the air-water interface. During bubble formation, proteins were rapidly adsorbed at the air-liquid interface and underwent molecular rearrangements, leading to superior foaming properties. Additionally, the incorporation of phenolic extracts was found to reduce surface hydrophobicity, which in turn increased protein solubility (33).

The improved surface activity contributed to a reduction in surface tension, ultimately enhancing the emulsifying capacity of the proteins; while the higher foaming capacity due to increases in the net charge of the protein molecules weakened hydrophobic interactions and also increased protein flexibility (34). Rutin was shown to have a wide array of beneficial activities concerning blood pressure, blood sugar, cardio-related, anti-inflammatory and anti-carcinogenic actions, and also enhancing antioxidant activities (35). p-CA is a phenolic molecule providing many beneficial benefits as an antioxidant. Chlorogenic acid, being widely distributed in plants, is one of the major sources of dietary polyphenols and has several health benefits (36). Chlorogenic acid has been found to be an antioxidant too. Our findings are in agreement with a previous study explaining how different concentrations of tea polyphenols altered the impacts of egg white on the microstructure and physicochemical properties of surimi gel (37). Compared to the control, it was perceived by the researcher that the treated sample highly reduced its solubility (37).

The formation compounds resulting from protein-phenolic interactions; whether covalent or non-covalent, were shown to significantly enhance antioxidant activities. This enhancement occurs through the introduction of reactive hydroxyl groups or by protecting phenolic compounds from degradation. Such complexes have potential applications in the food industry, including films, emulsions, and delivery systems (38). The functional properties of certain compounds can be significantly enhanced through various modification techniques. Among these, ultrasonic treatment and the incorporation of phenolic compounds, known for their potent antioxidant activity, were particularly effective in improving the bioactivity and stability of the modified systems. This effect can be explained by antioxidant synergism and forming of protein-phenolic complexes (33).

Furthermore, supplementation of pepsin-treated-digested egg whites with coffee extract improved its antioxidant activity that was assayed as DPPH because the extract of coffee contains chlorogenic acid, which possesses antioxidant property (39). It was blended with oats and soybeans protein isolates too. The extract incorporated on the functional properties along antioxidant characteristics to protein isolates by covalent attachments. FT-IR spectroscopy was used for analyzing changes in the molecular structure of proteins influenced by

interactions with phenolic proteins. It was shown that variations in FT-IR spectra were a pointer to the changes of the conformation in the secondary structure since the functional groups of the proteins taking part in their production had different infrared absorption frequencies. Even though the structure-frequency relationship was more pronounced for amide I modes, both amide I and II vibrational modes were appropriate for secondary structure analysis, since they were mainly dependent on the secondary structure of the proteins and were essentially unaffected by the structure of their side chains (39). Hydrogen bonding between peptides has been highly recognized to conserve the secondary structure of proteins. Amino acid functional groups include C=O and N-H that restore balance to the circumstances surrounding one another (39).

The formation of hydrogen bonds within the protein structure plays a critical role in directly influencing the vibrational energy of amide groups, particularly within the Amide I region (1700–1600 cm^{-1}), which primarily corresponds to the C=O stretching vibrations of the protein backbone (40). These vibrational shifts serve as sensitive indicators of changes in the protein's secondary structure, such as transitions between α-helices and β-sheets, or protein aggregation. Accordingly, FT-IR spectroscopy is considered a precise analytical tool for monitoring these structural transitions and assessing the conformational stability of protein-based systems (40).

The phenolic chemicals can re-model the hydrogen bond network and eventually the protein structure via interactions with amino acid residues. In this work, it was observed that the phenolic chemicals had a significant effect on the proteins, inducing changes in their secondary structures by reducing β-sheet and helix and enhancing random helix and spinning the structure. Another approach had focused on the impact of polyphenols on sunflower proteins structure by amino acids' functional groups (C=O and N-H). The shifting of amide vibration modes had previously indicated that when the protein molecule hydrogen bonding network is examined, the amount of phenolic compounds increased in the disordered structure at the expense of the organized structures. (33)

In our study, FT-IR spectroscopy analysis revealed a decrease in the α-helix content of egg white proteins following the addition of phenolic compounds, accompanied by an increase in β-turn structures. These structural modifications were dependent on the concentration of the added phenolic compounds, suggesting that such compounds can modulate the secondary structure of egg white proteins. The interaction is likely mediated through non-covalent (reversible) or covalent bonds, indicating the potential of phenolic compounds to influence protein conformation and functionality. The observed decrease in α-helical structures in proteins treated with polyphenols is attributed to structural modifications induced by these compounds (40). Phenolic compounds possess highly reactive hydroxyl groups, which can undergo oxidation to form quinones. These quinones, in turn, are capable of forming covalent cross-links with amino acid residues such as lysine, tyrosine, and cysteine, leading to conformational changes in the protein's secondary structure (40).

This study demonstrated the potential of lavender extract to improve the functional properties of oat and soy protein isolates. The study also focused on a limited range of protein sources and concentrations of lavender extract, while other plant proteins or higher levels of supplementation were not evaluated. Future investigations should include larger experimental designs, diverse protein sources, and real-food applications to confirm and extend the present findings.

Conclusion

Our findings revealed that lavender plant extract enhanced some functional properties of oats and soybeans protein isolates and antioxidant activity, which was concentration-dependent, and it might also have had something to do with the type of active compounds in the extract. Functional properties and antioxidant activities of proteins isolated from oats and soybeans were modified, depending on the concentration used and the active compound profile in the extract. Overall, the use of *L. angustifolia* extract represented a promising strategy to expand the industrial applications of plant-based proteins in functional foods and nutraceuticals.

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Authors Contribution

All authors contributed significantly to the conception and design of the study. Dr. Faleeha Hasan Hussein conducted the experiments and collected the data. Dr. Alia Zyara Hashim analyzed the data and contributed to data interpretation. Dr. Anwaar Yasser Hussein drafted the manuscript and revised it critically for important intellectual content.

Dr. Seyed Hadi Razavi performed statistical analysis and contributed to the discussion section. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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