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Physicochemical and Functional Properties of *Moringa* Seed Protein Treated with Ultrasound

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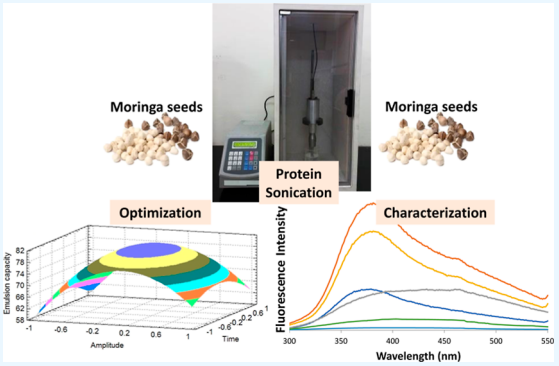
ABSTRACT: Functional and structural properties of Moringa protein concentrate (MPC), obtained from defatted *Moringa oleifera* seed, were investigated after treating it with an ultrasonic technique. For this purpose, dried *M. oleifera* seed powder was defatted and subjected to a simple protein precipitation method to generate a MPC with 73.2% protein contents. Then, a Box−Behnken design was applied to optimize the sonication treatment of MPC where ultrasound amplitude (20−80%), treatment time (5−25 min), and solute-to-solvent ratio (0.1−0.3 g/mL) were studied as factors that influence the protein solubility (PS), emulsion capacity (EC), and foaming capacity (FC) of MPC. The optimal conditions were amplitude of 58%, time of 18 min, and solute to solvent ratio of 0.18 g/mL. At these conditions, PS, EC, and FC were increased to 42, 33, and 73%, respectively, in comparison to untreated one. The structural modification by ultrasound was further confirmed by using Fourier transform infrared spectroscopy which illustrated the MPC modification through the changes in the peak width of amide-I band. Similarly, the intrinsic fluorescence spectral signature also showed a significant increase in the amino residues of MPC. In conclusion, the exposure of hydrophilic groups and the alteration of secondary and tertiary structures induced by ultrasonic treatment improved the functional characteristics of MPC.



1. INTRODUCTION

Protein-energy inadequacy is a significant issue in developing and underdeveloped countries caused by a lack of protein sources, which is aggravated by other socioeconomic factors. Furthermore, the accessibility of animal proteins is limited by a lack of supply and is associated with a higher cost. On the other hand, plant proteins might be a superior choice due to their health-oriented composition, environmental sustainability, dependable origin, and reasonable prices.[1] Native to southern and northern subtropical parts of Pakistan, *Moringa oleifera* (Lam.), also known as the "drumstick tree", is a popular plant. *M. oleifera* seed (MOS) is known as the "plant diamond" because it is exceptionally nutrient-dense.[2] MOS comprises a substantial amount of protein (37−40%) including the essential amino acids, making it a possible source of the functional protein concentrate.[3] Plant proteins have shown tremendous potential in recent years because of their high nutritive quality as well as their functional properties, such as protein solubility (PS), foaming, gelation, and emulsification which make them suitable for their applications as food ingredients.[4]

Regarding their biological activities and functional properties, plant proteins might be enhanced and varied by changing their physical and chemical characteristics. Numerous processes can modify proteins, but the majority of them are carried out by enzymes that specifically target certain areas of proteins.[5] Due to the demand for clean label products and the difficulty of toxicity associated with scaling up this technology, the food industry is now moving away from chemical modification of proteins; therefore, novel physical techniques are widely being used to alter and enhance a functional ingredient.[6] These techniques, whether used individually or in combination with other techniques, include the application of shear and/or electromagnetic forces to modify the protein structure. These approaches typically result in unfolding, disaggregation, reduction in protein size and distribution, and persistent denaturation of the protein structure.[7] Among the various novel physical approaches for protein modification, ultrasound qualify for being easy, energy-saving, environmentally friendly along with cost-effectiveness.[8] Temperature, attenuation velocity, energy, and intensity are all factors that

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determine the effectiveness of ultrasonic treatment effectiveness. It can be classified as high or low energy ultrasound, depending on its frequency and amplitude. Several studies have investigated how ultrasound affects the various proteins' functionality, for instance, oat protein,[9] rapeseed protein,[10] pea protein,[11] and particularly soy protein.[12] Throughout this scenario, the researchers discovered that ultrasonic treatment had a significant impact on the emulsifying, solubility, foaming, and several other functional aspects of these proteins.[13]

This research was designed to measure the impact of ultrasonic amplitude on the functional characteristics such as PS, emulsification, and foaming properties of the Moringa protein concentrate (MPC). In order to determine the modifications resulting from the sonication treatment, the changes in the molecular structure were also examined using Fourier transform infrared (FT-IR) spectroscopy and fluorescence spectroscopies.

2. MATERIALS AND METHODS

2.1. Procurement of Raw Material. The MOSs were procured from Ayub Agriculture Research Institute, Faisalabad, Pakistan. All chemicals were of analytical grade.

2.2. Preparation of MPC. The MOSs were dried in a hot air drying oven and then ground to powder. The seed powder was defatted in Soxhlet apparatus using *n*-hexane as a solvent. The laboratory procedure for preparing the MPC from *M. oleifera* defatted seed flour was based on the isoelectric precipitation method.[14] Using a laboratory stirrer at 123*g* and room temperature, the defatted flour was fully suspended in water in a ratio of 1:20 (w/v). With the help of a 1 M NaOH solution, the pH of the suspension was brought down to 10.0 while being agitated nonstop for 30 min. After that, Whatman filter paper with 11 μm porosity was used to filter the solution in order to get rid of any residual particles. After being centrifuged at 3075*g* for 10 min, the supernatant's pH was brought down to 4.5 with 1 M HCl. After discarding the supernatant, the protein-rich precipitate was redispersed in distilled water that had been pH-adjusted using 1 M NaOH to 7.5. HCl and NaOH were used to maintain the extraction flow as NaOH leads to a higher content of tryptophan and protein concentrates that are less soluble at low pH. The mixture was centrifuged again at 3075*g* for 10 min to collect the protein-curd precipitate which was actually MPC. The extraction procedure was repeated twice to obtain the maximum protein in the solvent, and all the supernatants were pooled together. Then, the supernatant contents were freeze-dried and stored at 4 °C in airtight bags. By using the Kjeldahl method, the MPC protein content (on a total weight basis) was found 73.2%. The yield was found better than a previously reported study[15] and generally depends on the process factors. In MPC, other components like carbohydrates and minerals were ignored, although they may affect the functional properties.

2.3. Sonication Treatment of MPC. The equipment VCX750 (Sonics and Materials, Inc. Newtown, CT, USA) instrument was used to sonicate MPC powder in distilled water. It is equipped with a titanium sonotrode probe with a 3 mm diameter that produces a constant frequency of 20 kHz. MPC dispersions in distilled water at different ratios (0.1, 0.2, and 0.3 g/mL) were sonicated for 5, 15, and 25 min at amplitudes of 20, 50, and 80% with constant pulse duration on the sample after it had been immersed in ultrasound to a depth of 10 mm. The sample was put in an ice container while undergoing ultrasonic treatment to keep its temperature at the room level. All samples were freeze-dried after being subjected to ultrasonic treatment, and they were all kept in airtight containers at 4 °C until analysis. The purpose of the sonication treatment was to study the functional and structural changes between treated and untreated *Moringa* seed protein samples.

2.4. Functional Properties of MPC. *2.4.1. Protein Solubility.* The PS was examined using Ling, Ouyang, and Wang[16] methodology with slight modifications. In a nutshell, deionized water was used to create a 1 g/100 mL MPC solution, which was then agitated for 30 min at room temperature before having its pH changed to 2.0−12.0 with either 0.1 M HCl or NaOH. The mixture was subsequently centrifuged for 15 min at 492*g*. The Bradford technique was used to measure the supernatant protein concentration, and PS was calculated as follows.[17]

$$\text{PS (\%)} = \frac{\text{Protein content of supernatant}}{\text{Total protein content of the sample}} \times 100\%$$

2.4.2. Emulsifying Capacity. The emulsion capacity (EC) was determined using method proposed by Ling, Ouyang, and Wang.[16] Briefly, 15 mL of 2% (w/v) MPC solution and 15 mL of soybean oil were homogenized for 2 min at 10,000 rpm (FSH-2A Homogenizer, China) in a 50 mL graduated centrifuge tube. The emulsion was centrifuged for 2 min at a speed of 1968*g*, and the volume of the emulsion was measured in mL. The EC was calculated using the formula below.

$$\text{EC (\%)} = \frac{\text{Emulsion volume}}{\text{Soybean oil in MPC}} \times 100$$

2.4.3. Foaming Capacity. At pH 7.0, the foaming capacity (FC) was determined using the method of Arte, Huang, Nordlund, and Katina[18] with some alterations. 30 mL of the MPC solution (1 mg/mL) was put into a 100 mL cylinder. At 10,000 rpm, the protein solution was homogenized for 2 min (FSH-2A Homogenizer, Shanghai, China), and the volume of the solution was recorded as V1. The FC was calculated as follows

$$\text{FC (\%)} = \frac{\text{volume of the solution after homogenization}}{\text{30 mL of MPC solution}} \times 100$$

2.5. Optimization of the MPC Modification Process. PS, EC, and FC were evaluated in relation to the impact of three factors, namely, amplitude (*A*), time (*B*), and solute-to-solvent ratio (*C*). For this purpose, a Box−Behnken design (BBD) was applied. This is a statistical tool which does not require the replicates, and the experimental error is calculated on the basis of central points which are replicated twice. The 15 trials including three central points were run to increase the process accuracy. A detailed experimental design describing the actual values of the independent variables is shown in Table 1.

The software program STATGRAPHICS PLUS (Version 5.1, Statistical Graphics Corporation, Rockville, USA, 2000)

Table 1. Experimental Factor Codes and Levels

independent variable	symbol	coded levels		
		−1	0	1
amplitude (%)	*A*	20	50	80
time (min)	*B*	5	15	25
solute-to-solvent ratio (g/mL)	*C*	0.1	0.2	0.3

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Table 2. Central Composite Design with the Effect of Ultrasound Conditions on the Response Parameters[a]

run	amplitude (%)	time (Min)	solute-to-solvent ratio (g/mL)	PS (%)		EC (%)		FC (%)	
				observed value	predicted value	observed value	predicted value	observed value	predicted value
1	80(+1)	5(−1)	0.2(0)	6.02	6.01	65.28	65.42	15.88	15.71
2	50(0)	5(−1)	0.3(+1)	6.35	6.50	72.25	72.95	20.05	20.06
3 (C1)	50(0)	15(0)	0.2(0)	7.80	7.84	81.18	81.32	21.07	21.19
4	50(0)	25(+1)	0.3(+1)	6.98	7.09	70.83	71.47	19.38	19.47
5	20(−1)	5(−1)	0.2(0)	2.94	2.91	58.38	58.17	13.43	13.6
6	50(0)	25(+1)	0.1(−1)	7.55	7.39	75.84	75.14	20.98	20.96
7	80(+1)	15(0)	0.3(+1)	5.78	5.63	66.57	65.72	16.90	17.05
8	20(−1)	15(0)	0.1(−1)	3.08	3.22	61.88	62.72	16.18	16.02
9	20(−1)	15(0)	0.3(+1)	3.88	3.75	60.81	60.31	15.02	14.75
10	50(0)	5(−1)	0.1(−1)	6.78	6.66	75.26	74.61	20.65	20.55
11 (C2)	50(0)	15(0)	0.2(0)	7.78	7.84	81.32	81.32	21.15	21.19
12 (C3)	50(0)	15(0)	0.2(0)	7.95	7.84	81.48	81.32	21.37	21.19
13	80(+1)	15(0)	0.1(−1)	6.50	6.62	68.15	68.64	17.50	17.76
14	20(−1)	25(+1)	0.2(0)	4.02	4.03	59.42	59.27	14.65	14.9
15	80(+1)	25(+1)	0.2(0)	6.18	6.21	63.17	63.37	17.08	16.83

[a]C1, C2, and C3 are central points.

Table 3. Analysis of Variance (ANOVA) of the Predicted Second-Order Polynomial Model for Ultrasound Conditions' Impact on Response Parameters[a]

source	DF	PS (%)		EC (%)		FC (%)	
		MS	*p*-value	MS	*p*-value	MS	*p*-value
A = amplitude	1	13.939[b]	0.0000	64.297[b]	0.0003	8.160[b]	0.0002
B = time	1	0.871[b]	0.0038	0.456[NS]	0.4787	0.016[NS]	0.6851
C = solute-to-solvent ratio	1	0.105[NS]	0.1353	14.231[b]	0.0079	1.960[b]	0.0052
AA	1	24.561[b]	0.0000	773.989[b]	0.0000	88.682[b]	0.0000
AB	1	0.211[NS]	0.0534	2.480[NS]	0.1344	1.464[b]	0.0095
AC	1	0.577[b]	0.0088	0.065[NS]	0.7842	0.078[NS]	0.3876
BB	1	0.830[b]	0.0042	103.163[b]	0.0001	3.961[b]	0.0011
BC	1	0.004[NS]	0.7175	1.0[NS]	0.3086	0.25[NS]	0.1520
CC	1	0.761[b]	0.0050	23.000[b]	0.0029	0.040[NS]	0.5289
total error	5	0.033		0.778		0.087	
cor. total	14						
R^2		99.5918		99.581		99.5776	
adj-R^2		98.8571		98.8268		98.8172	

[a]Significant at 0.05 level. [b]Significant at 0.01 level; NS = nonsignificant; DF = degree of freedom; and MS = mean square.

was used to evaluate the experimental data in accordance with Montgomery.[19] Furthermore, a sample analysis was performed, and the significant deviation among means was identified at a confidence level of 5%.

2.6. FT-IR Spectroscopy. The most efficient method for determining the secondary structure of proteins is FT-IR. An infrared spectrometer (Alpha II FT-IR, Bruker, USA) with an attenuated total reflection (ATR) accessory was used in this research to collect spectra without the use of a thermostat. The ZnSe ATR crystal was equally covered with a small quantity of the samples to produce a spectrum in the transmission mode with a range of 4000−400 cm^{-1}. Software named Opus 7.3.139.1294 (Bruker Optic GmbH, Hamburg, Germany) was used to process the data.

2.7. Determination of Intrinsic Fluorescence. As previously indicated, MPC solutions (10 mg/mL each) were thoroughly mixed in 0.1 M sodium phosphate buffer pH 7.2, which was then centrifuged, and the supernatant's protein concentration was obtained.[20] The supernatants were diluted to 0.002% (w/v) protein concentration and scanned with the fluorescence spectrofluorometer (DW-F96 pro Drawell, China) by exciting solution at 220, 260, and 280 nm excitation wavelengths for tyrosine, phenylalanine, and tryptophan, respectively, and taking spectra in the emission range of 300−550 nm with slit width of 1 nm.[21] The spectra presented are prepared from the average values of three spectra taken for each sample.

3. RESULTS AND DISCUSSION

3.1. Optimization and Validation of the Modification Process. *3.1.1. Fitting the Proposed Model.* A complete BBD comprising 15 runs from three independent variables: amplitude (%) (*A*), time (min) (*B*), and solute-to-solvent ratio (g/mL) (*C*) is shown in Table 2. The treatment efficiency of the sonication was optimized using PS, EC, and FC as response factors.

In order to determine the reliability and validity of second-order polynomial responses, these readings were compared with the predicted values. The predicted values for the response variable were shown to be within the same range as

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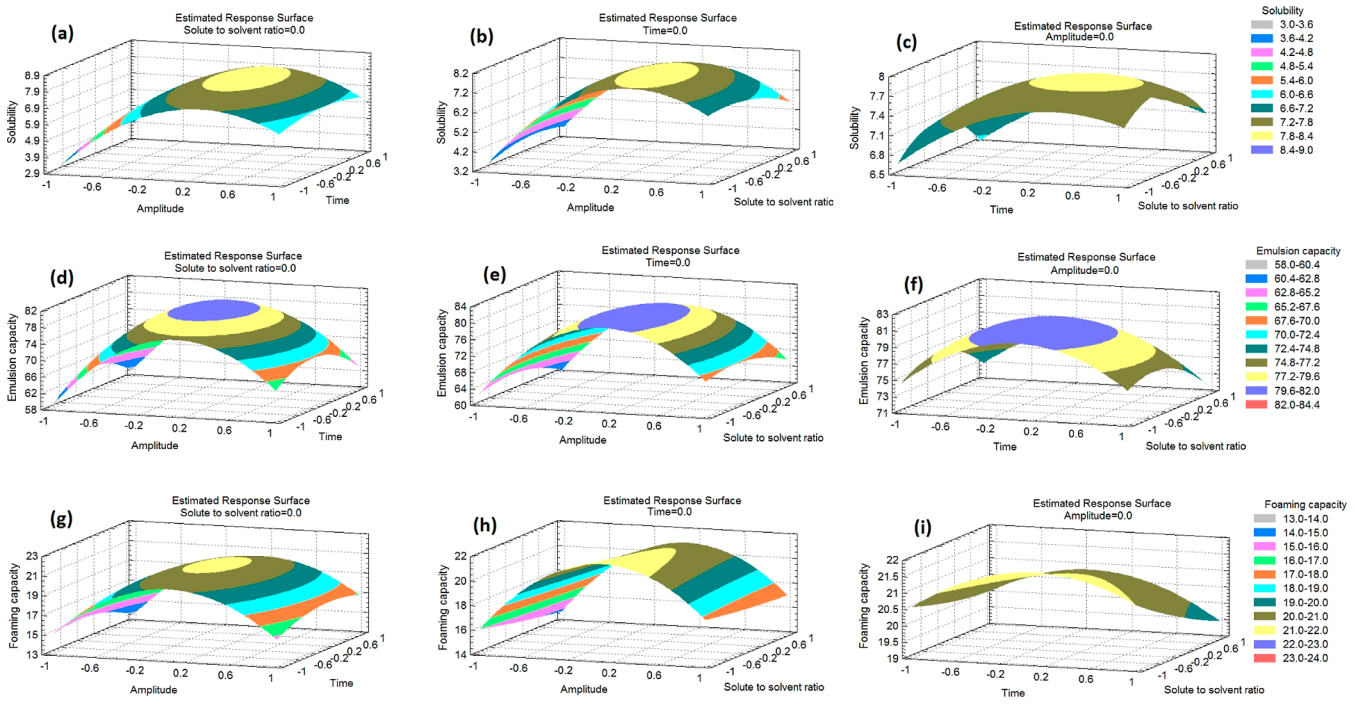

Figure 1. Response surface plots representing the effect of mutual interactions of studied parameters on protein modification: amplitude and time on solubility (a); amplitude and solute-to-solvent ratio on solubility (b); time and solute-to-solvent ratio on solubility (c); amplitude and time on EC (d); amplitude and solute-to-solvent ratio on EC (e); time and solute-to-solvent ratio on EC (f); amplitude and time on FC (g); amplitude and solute-to-solvent ratio on FC (h); and time and solute-to-solvent ratio on FC (i).

Table 4. Coded and Actual Regression Equations for Response Parameters after the Sonication Process[a]

response parameter	regression form	regression equation
PS	coded	$R1 = 7.84333 + 1.32 \times A + 0.33 \times B - 0.115 \times C - 2.57917 \times A^2 - 0.23A \times B - 0.38 \times A \times C - 0.474167 \times B^2 - 0.035 \times B \times C - 0.454167C^2$
	actual	$R1 = 7.84333 + 1.32 \times \text{amplitude} + 0.33 \times \text{time} - 0.115 \times \text{solute-to solvent ratio} - 2.57917 \times \text{amplitude}^2 - 0.23 \times \text{amplitude} \times \text{time} - 0.38 \times \text{amplitude} \times \text{solute-to-solvent ratio} - 0.474167 \times \text{time}^2 - 0.035 \times \text{time} \times \text{solute-to-solvent ratio} - 0.454167 \times \text{solute-to-solvent ratio}^2$
EC	coded	$R2 = 81.3267 + 2.835 \times A - 0.23875 \times B - 1.33375 \times C - 14.4783 \times A^2 - 0.7875 \times A \times B - 0.1275 \times A \times C - 5.28583 \times B^2 - 0.5 \times B \times C - 2.49583 \times C^2$
	actual	$R2 = 81.3267 + 2.835 \times \text{amplitude} - 0.23875 \times \text{time} - 1.33375 \times \text{solute-to-solvent ratio} - 14.4783 \times \text{amplitude}^2 - 0.7875 \times \text{amplitude} \times \text{time} - 0.1275 \times \text{amplitude} \times \text{solute-to-solvent ratio} - 5.28583 \times \text{time}^2 - 0.5 \times \text{time} \times \text{solute-to-solvent ratio} - 2.49583 \times \text{solute-to-solvent ratio}^2$
FC	coded	$R3 = 21.1967 + 1.01 \times A - 0.045 \times B - 0.495 \times C - 4.90083 \times A^2 + 0.605 \times A \times B + 0.14 \times A \times C - 1.03583 \times B^2 - 0.25 \times B \times C + 0.104167 \times C^2$
	actual	$R3 = 21.1967 + 1.01 \times \text{amplitude} - 0.045 \times \text{time} - 0.49 \times \text{solute-to-solvent ratio} - 4.90083 \times \text{amplitude}^2 + 0.605 \text{ amplitude} \times \text{time} + 0.14 \times \text{amplitude} \times \text{solute-to-solvent ratio} - 1.03583 \text{ time}^2 - 0.25 \times \text{time} \times \text{solute-to-solvent ratio} + 0.104167 \times \text{solute-to-solvent ratio}^2$

[a]PS = protein solubility; EC = emulsion capacity; FC = foaming capacity; A = amplitude B = time; and C = solute-to-solvent ratio.

the experimental findings, demonstrating the validity of the measured values. The effects of ultrasound treatment on MPC were investigated with three responses using 15 different trials. The experimental run 5 has shown the lowest effect in all responses with an amplitude of 20%, a time of 5 min, and a solute-to-solvent ratio of 0.2 g/mL, while the experimental run 12 has shown the highest effect in terms of selected responses with an amplitude of 50%, a time of 15 min, and a solute-to-solvent ratio of 0.2 (g/mL). The significance and appropriateness of the response were assessed using analysis of variance (ANOVA) on the acquired values with a 95% confidence level (Table 3). According to the results, the p-value of the overall model is highly significant and indicates the suitability of the model. With a significant p-value ($p < 0.05$) and an acceptable R^2 of PS ($R^2 > 99.59$), EC ($R^2 > 99.58$), and FC ($R^2 > 99.57$), the statistical analysis revealed that the provided models were adequate to reflect the observed values. In addition, the respective adj-R^2 values of 98.85, 98.82, and 98.81 were nearly to R^2, showing a significant and positive relationship among observed as well as predicted data from the regression model (Table 3).

3.1.2. Single Factor Analysis for Protein Modification. According to the findings, the independent variables amplitude (A), time (B), and solute-to-solvent ratio (C) significantly affect MPC alteration as p values from the ANOVA table show significant impact. The solubility of the ultrasonically treated MPC sample significantly increased, which was caused by structural changes under specific conditions. In order to improve protein−water interactions, solubility, and hydrophobicity, it is suggested that a mild ultrasonic treatment can expose the hydrophobic groups in the proteins. Furthermore, strong ultrasound treatment can promote protein denaturation

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and aggregation, resulting in PS loss.[22] Ultrasonic-treated MPC has shown increase in EC with increase in amplitude and decrease after 60% amplitude, and the same trend was observed in FC after ultrasound treatment.[23] In fact, the surface tension at the water−air contact is reduced by dispersed protein, thus resulting in an increased FC.

3.1.3. Effect of Mutual Interactions on Protein Modification. Interactions between amplitude and time (AB) and amplitude and solute-to-solvent ratio (AC) showed a significant change in protein modification while time and solute-to-solvent ratio (BC) showed least influence on protein modification ($p > 0.05$). Response surface 3D plots have also been used to demonstrate the interaction between independent factors (Figure 1). According to the assessment of acquired findings, the maximum solubility was attained by the mutual interaction of amplitude and time (Figure 1a). These findings also demonstrate that when the interaction between the amplitude and solute-to-solvent ratio was made with the central value of time, the PS considerably increased and then reduced at the peak amplitude (Figure 1b). PS, on the other end, decreased as time and solute/solvent ratio were treated as interaction parameters by using the central fixed amplitude (Figure 1c). Similarly, graphical chart depicted response surface studies of correlations between amplitude and time (Figure 1d), amplitude and solute-to-solvent ratio (Figure 1e), and time and solute-to-solvent ratio (Figure 1f). These graphs demonstrated that the chosen factor values were reasonable and had a positive effect on the protein EC. Similar trend could be seen in case of FC (Figure 1g−i). According to the figures, the interactions between the parameters had a significant impact on protein modifications, which was also assessed using ANOVA.

Using the response surface methodology, the model regression equations have also been recorded and contain both coded and actual levels (Table 4).

From the equations in Table 4, the optimal ultrasonic process parameters for the maximum value of each response were calculated, and the findings are reported in Table 5. Processes 1, 2, and 3 were identified as the best ultrasonic processes for modifying MPCs with high PS, EC, and FC, respectively.

Table 5. Influence Coefficient of Ultrasonic Processes Based on PS, EC, and FC for *Moringa* Protein[a]

ultrasonic process-optimized conditions	optimized responses		
	PS (%)	EC (%)	FC (%)
process 1: A = 57.83%; B = 17.94 min; C = 0.175	8.078	81.648	21.850
process 2: A = 52.99%; B = 14.83 min; C = 0.173	7.743	81.326	20.995
process 3: A = 53.53%; B = 12.69 min; C = 0.402	7.752	81.402	21.010
influence coefficient	0.043	0.003	0.040

[a]PS = protein solubility; EC = emulsion capacity; FC = foaming capacity; A = amplitude; B = time; and C = solute-to-solvent ratio.

3.1.4. Optimization and Validation. The optimum conditions obtained from each response were considered as one process. For instance, FC and EC were statistically treated to the optimal circumstances identified through PS optimization. These calculations collectively were referred to as process 1. In a similar vein, depending on FC and EC ideal conditions, reactions were statistically estimated. To create ultrasonic conditions for the modified protein with functional qualities, processes 1, 2, and 3 were compared. Table 5 shows that the PS obtained using processes 1, 2, and 3 was 8.078, 7.743, and 7.752%, respectively. PS has a higher influence coefficient than FC and EC, as computed by (maximum − minimum)/minimum (Table 5). As a result, process 1 with highest coefficient can be selected as the best ultrasonic process.

The experimental design addressed the expected modifications of protein within optimum parameters. As per BBD, the optimum protein alteration seen in terms of solubility (7.84%) was estimated with optimal parameters of amplitude of 58%, time of 18 min, and solute-to-solvent ratio of 0.18 g/mL. Replicating the experimental run with the most suitable parameters confirmed the statistical approach and the regression equation. The observed solubility for modified protein (7.95%) at optimum parameters (amplitude 58%, time 18 min, and solute-to-solvent ratio 0.18 g/mL) verified the model's reliability. The experimental values for EC and FC were also quite close to the estimated ones. Generally, the results indicated that the model for MPC modification by ultrasonication was suitable and appropriate. Additionally, the alteration of the protein was compared across ultrasound-treated and untreated samples. The MPC modified with ultrasound has shown better PS, EC, and FC in comparison to those of untreated MPC.

Ultrasonication is known to examine the idea of cavitation, which results in turbulence, shear stresses, heating, and dynamic agitation. Through cyclic formation and collapse of cavities, it may result in chemical and physical changes that produce aggregates through covalent and noncovalent bonding.[24] The ultrasonic amplitude showed the potential for high MPC modification even though the solute-to-solvent ratio had no impact on protein modification. Lifting the ultrasonic amplitude led to more robust protein denaturation and higher protein modification.[25] The increased solute-to-solvent ratio led to a lower ultrasonic energy density per unit volume, which in turn resulted in a lower level of protein modification. Significant changes that occur in protein functional properties due to sonication include size reduction, electrical conductivity, and zeta (ζ) potential.[26] Even though the solute-to-solvent ratio had a minimal influence on MPC modification, a ratio of 0.18 g/mL was determined to be optimal for the highest MPC modification. The solute-to-solvent ratio frequently fluctuates based on the proteins origin along with its structure.[27] Various ultrasonic intervals (5, 15, and 25 min) were applied for maximal MPC modification at optimal parameters, and the highest modification was found at 18 min. A modest decline in MPC modification was noted as the time was increased further. The structural breakdown of proteins, which typically form aggregates by folding to endure harsh conditions, may be responsible for it. Additionally, proteins may fail to solubilize and end up as the centrifugation residue.[28]

3.2. Functional Properties of Modified MPC. MPC modified by ultrasound at optimized conditions (see process 1 of Table 4) was further compared with untreated samples for functional properties like PS, EC, and FC. These functional properties of ultrasonic-treated MPC were 7.95, 81.18, and 21.37%, respectively, hence increased by 42, 33, and 73% compared to untreated one (Table 6). Plant proteins are easier to obtain and offer cheaper manufacturing costs for industries in many parts of the world. However, plant proteins

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Table 6. Functional Properties of Modified *Moringa* Seed Protein by Ultrasound[a]

MPC	functional properties		
	PS (%)	EC (%)	FC (%)
untreated	5.60 ± 0.17	60.90 ± 1.46	12.35 ± 0.27
ultrasonic treated	7.95 ± 0.11	81.18 ± 1.29	21.37 ± 0.57

[a]PS = protein solubility; EC = emulsion capacity; and FC = foaming capacity.

comparatively have poor functional properties as compared to animal proteins, which can be improved by sonication treatment at different conditions.

3.2.1. Protein Solubility. The interaction of proteins with water provides the basis for the functional property known as PS. PS is complicated and may be influenced by various factors including hydrophobic interactions, electrostatic interactions, and hydrogen bonding which have positive impact on protein−protein and protein−solvent interactions.[29] One of the most fundamental functional properties of each protein is its solubility. The solubility of untreated MPC at neutral pH was 5.60 ± 0.17% while the ultrasonically treated MPC samples' solubility increased significantly by adjusting different pH (2 to 11), reaching to 7.95 ± 0.11%.

Altering both the conformation and structure of the protein using higher intensity ultrasound enhances PS which causes the interior opening of hydrophilic amino acid and enhances the water attraction.[30] The larger area of protein was covered with water due to the high ultrasonic amplitude as the treated proteins molecular weight dropped.[31] The three-dimensional structure of the globular protein may alter as a result of increasing PS, and this could result in a significant increase in electrostatic interactions with high conductivity in comparison to the untreated sample.[32] Under these conditions, better PS resulted from stronger interlinkages between water and protein as more water interacted with proteins and electrostatic interaction increased.[33]

3.2.2. Emulsion Capacity. Proteins emulsifying aspect relates to its ability to generate an emulsion and retain the newly formed emulsion.[34] It is a major element in the development of a variety of processed food products. The EC of untreated MPC was 60.90 ± 1.46% (Table 6), and this capacity increased significantly after ultrasonic treatment to 81.18 ± 1.29%. To determine the EC, the prepared emulsions were left at room temperature. The results showed that the treated samples' EC was a bit greater than the untreated sample.[35] Furthermore, it has been shown that proteins' highest emulsifying power resulted from their improved solubility. MPC improved capacity to emulsify may be the result of changes in aggregation, solubility, and secondary structure.[36]

3.2.3. Foaming Capacity. Proteins are an excellent foaming agent, even though they immediately disperse to the air−water interface and partially unfold to produce a strong, cohesive, and flexible film. The number of hydrophobic amino acids accessible at the protein molecule's surface is related to foaming capabilities.[37] The surface tension at the water−air contact is reduced by dispersed protein, resulting in increased FC.[38] The FC of the untreated sample of MPC was 12.35 ± 0.27% (Table 6). However, after being exposed to ultrasonic treatment, the treated samples FC rose to 21.37 ± 0.57%. Proteins are partially enlarged and denaturized as a result of these alterations, resulting in the claimed improvement in foaming capabilities.[22] Recent research on the impact of high amplitude ultrasonic treatment on dietary proteins reported a similar trend in protein FC.[39]

3.3. Structural Study of MPC. *3.3.1. FT-IR Analysis.* FT-IR was used to investigate the secondary structures of untreated and ultrasound-treated MPC samples. MPC FT-IR spectra may be divided into three distinct wavelength bands (Figure 2). The amide-III zone, which consists of N−H bending and C−N stretching, spreads over a wavelength range of 3350−3475 cm^{-1}. The amide-I zone, which represents C═O bonds, spreads over a wavelength range of 1500−1600 cm^{-1}. The amide-II zone, which represents N−H bonds, spreads over a wavelength range of 1720−1830 cm^{-1}. The amide-I band is the one that responds chemically to changes in the protein's secondary structure the most out of all of these

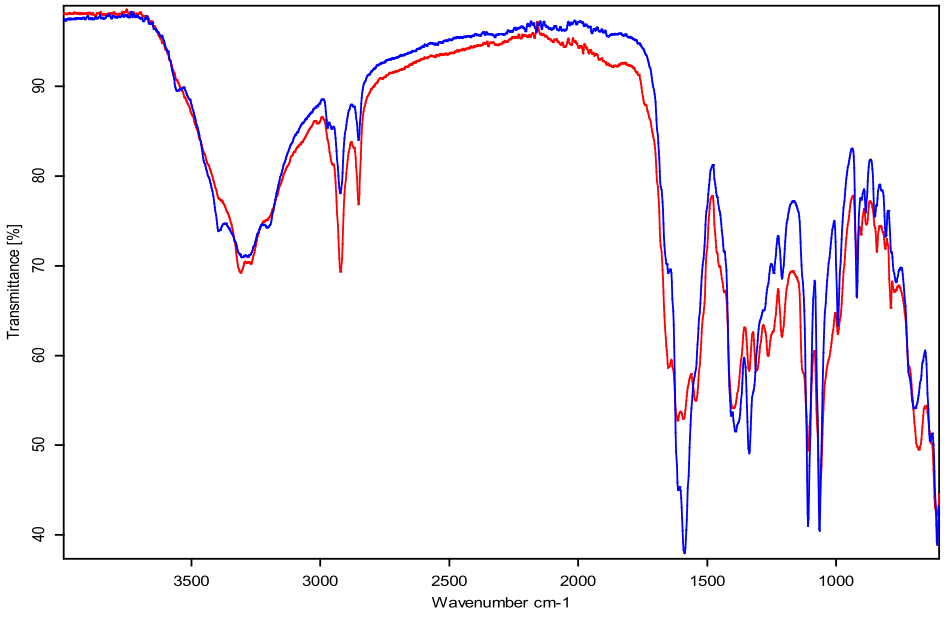

Figure 2. FT-IR spectra of the MPC concentrate of untreated (blue) and sonication treated (red).

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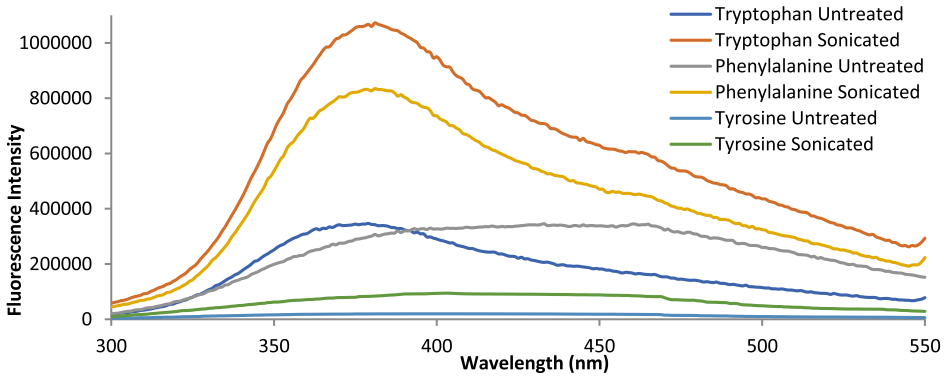

Figure 3. Fluorescence spectra of tryptophan, phenylalanine, and tyrosine residues.

bands. The identification of various secondary protein structures typically relies on the conformationally sensitive amide I band, which appears in the range of 1700−1600 cm^{-1} and is principally caused by the carbonyl stretching vibrations of the protein backbone.[40] Our findings demonstrate that both the amide-II as well as amide-III band regions of the FTIR spectra of untreated MPC did not significantly alter, whereas based on C═O stretching, sonication significantly changed the amide-I area, whereas other regions just experienced a change in peak intensity. In fact, the cavitation mechanism of ultrasound treatment changed the protein secondary structure of MPC by breaking the intermolecular hydrogen bond and increasing the protein flexibility. The same pattern was seen with soy seed protein isolates[41] and sonicated protein isolates from MOSs.[42]

3.3.2. Intrinsic Fluorescence. Intrinsic fluorescence was used to evaluate the tertiary structure of MPC after ultrasound treatment, as shown in Figure 3. A shift in the maximum peak values and the intensity of amino acid residues of the samples were altered when they were subjected to ultrasonic treatment. The significant increase for tryptophan, phenylalanine, and tyrosine in the case of MPC for treated sample was detected at 380 nm. A similar pattern was seen in the case of soy protein isolates[43] and hemp seed protein isolates.[44] Therefore, by following fluorescence intensity and maximum wavelength (λ_{max}), changes in the protein tertiary structure can be identified.[45] The crest peak shift suggested that the MPC tertiary structure had been altered during the ultrasonic treatment. Between untreated and ultrasonically treated MPC, there were considerable variations in the intrinsic fluorescence change. Other proteins such as plum seed protein isolate[46] and chicken bone protein[39] also demonstrated substantial changes when processed by ultrasonic treatment. This technique is applied in order to demonstrate how changes in the protein tertiary structure result from the protein amino acid residues being sensitive to the polarity of the microenvironment.[47] The fluorescence spectrum of a protein shows the amino acid residues in the tertiary structure, such as phenylalanine, tyrosine, and tryptophan. Variations in tryptophan intensity are used as the basis for several intrinsic fluorescence-based tertiary structure studies.[48]

The results, however, contradicted to the findings of Zhu, Zhu, Yi, Liu, Cao, Lu, Decker, and McClements[49] who observed a decrease in intensity by using an ultrasound technique. This might be because of the multiple reasons like amplitude of the ultrasonic treatment applied, the amplitude of the treatment, or the concentration of the fluid that was treated. However, this decrease in intensity might be explained by how certain amino acid residues respond to various stresses on the protein as a function of the structure of the amino acid residues.

4. CONCLUSIONS

The study demonstrates a significant improvement in the functional properties of sonicated MPC with increases of 42, 33, and 73% in comparison to those of the group that did not receive treatment. The MPC was efficiently aggregated by the sonication process without resulting in an appreciable degradation. As the ultrasonic amplitude was raised, sonicated MPC solubility, emulsifying characteristics, and foaming abilities first increased and subsequently declined. The exposure of hydrophilic groups and modifications to the secondary and tertiary structures were ascribed to the functional properties of the sonicated MPC. The acquired results offer enough insights into the fundamental mechanisms driving the structural and functional alterations observed following the MPC sonication treatment. A rational and useful outcome of such findings is that they can be applied as alternatives to animal proteins to address environmental sustainability.

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