Review Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.509

Use of *Moringa oleifera* Lam. seeds and their technological functions: a review

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Abstract

Moringa oleifera Lam. is a plant known worldwide for its wide-ranging technological and medicinal uses, as well as for its high nutritional value found in various parts of the plant. This study aimed to compile information regarding the characteristics of proteins extracted from Moringa seeds through various methodologies and their applications. A considerable amount of protein was observed in the seeds, which can be extracted in different ways and shows potential for use in water treatment and important technological functions. Therefore, it can be inferred that the protein extracted from Moringa seeds has the potential to be further studied across different industrial sectors, with noteworthy potential for innovation in food industry processes and products.

Keywords: proteins; structure; extraction; emerging technologies; water treatment.

Practical Application: *Moringa oleifera* seed cake is a promising source of protein with diverse technological applications. This study focuses on compiling extraction methods and functional properties of Moringa seed proteins, highlighting their potential for applications in water treatment and the food industry. The proteins exhibit coagulation, emulsification, and stabilization functions, which show their potential in the food industry. Given their significant potential, further exploration of Moringa seed proteins can drive innovation in sustainable and environmentally friendly processes, especially in food production and environmental management.

1 INTRODUCTION

Moringa oleifera Lam. is a prominent species within the *Moringaceae* family, and its economic importance is justified by its widespread technological and medicinal applications. The plant is well adapted to hot and dry climates (Shahzad et al., 2018) and is therefore commonly found in tropical and subtropical regions (Ayerza, 2020). Interest in research related to its potential applications is steadily growing.

Moringa exhibits notable characteristics, with its seeds containing between 19 and 47% oil, making them attractive to researchers. Additionally, the cake resulting from the oil extraction process is a protein-rich by-product (Anwar et al., 2007; Dzuvor et al., 2022).

Several studies, such as those by Balbinoti et al. (2024), Baptista et al. (2015), Baptista et al. (2017), Bongiovani et al. (2014), Conceição et al. (2015), García-Fayos et al. (2016), Mateus et al. (2018), Moreti et al. (2019), Paixão et al. (2021), Pavankumar and Singh (2014), Resende et al. (2024), Santos et al. (2016), and Sengupta et al. (2012) have utilized proteins extracted from *M. oleifera* Lam. seeds through various methodologies, primarily in water treatment, yielding promising results.

Proteins are highly versatile macromolecules capable of performing various technological functions, such as foaming, emulsifying, gelling, and thickening. In this regard, several authors, including Aderinola et al. (2020), Cardines et al. (2018), Du et al. (2021), Gautier et al. (2022), Jain et al. (2019), Ogunsina et al. (2010), Paz et al. (2021), Resende et al. (2024), Whang et al. (2023), and have highlighted the technological potential of different extracts in their studies.

This work is a systematic review aimed at analyzing the protein content present in Moringa seeds, as well as their uses and potentialities according to the current state of the literature.

1.1 Relevance of the Work

Moringa oleifera Lam. seed cake is a promising protein source with potential for various technological applications. This study explores the extraction methods and functional properties of Moringa seed cake proteins. These proteins have demonstrated versatility, showing promise in water treatment and other industrial applications. Given their substantial potential, further research into Moringa seed proteins could drive innovation in food industry processes and products. This review highlights the importance of continued exploration to fully harness the industrial potential of this underutilized resource.

Received: May 19, 2025.
Accepted: June 12, 2025.
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Conflict of interest: nothing to declare.
Funding: Coordination for the Improvement of Higher Education Personnel.

2 DATA COLLECTION AND ANALYSIS

The search was conducted on June 9, 2024, using the Web of Science database. The search query included the keywords "*Moringa oleifera*" AND "protein" AND "food," with no restrictions on publication year. The selected indexes were Science Citation Index Expanded (SCIE), Social Sciences Citation Index (SSCI), Emerging Sources Citation Index (ESCI), and Likely Conference Proceedings Citation Index-Science (SPCI-S), and only articles were considered. The systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) methodology for article selection, initially yielding a total of 413 articles. These were subjected to two screening phases, by title and by abstract, resulting in 69 and 48 articles, respectively. Following the full-text review, only 24 articles were included in the final review. Bibliometric analysis was performed using VOSviewer v.1.6.20 and RStudio to generate a scientific mapping through bibliometric network analysis and word cloud visualization.

3 BIBLIOMETRIC ANALYSIS RESULTS

Cameroon is the country with the highest concentration of authors working on the subject of this research (Figure 1A). The articles from this region are primarily associated with the keywords "*Moringa oleifera*," "functional properties," and "protein isolate." Figure 1B presents the most frequently occurring keywords among the analyzed articles, with "antioxidant" being the most common. Additionally, there is a significant presence of protein-related terms such as "foaming properties," "coagulant protein," "protein," and "globulin," highlighting the relevance of Moringa in studies involving protein-based ingredients.

Figure 1C illustrates the relationship between keywords and the publication year. In the period from 2017 to 2019, represented in blue, the keywords reflect the initial rise of interest in Moringa-related studies, including terms such as "nutritional value", "digestibility", "chemical composition", "protein", "components", "products", "human health", and "polyphenol".

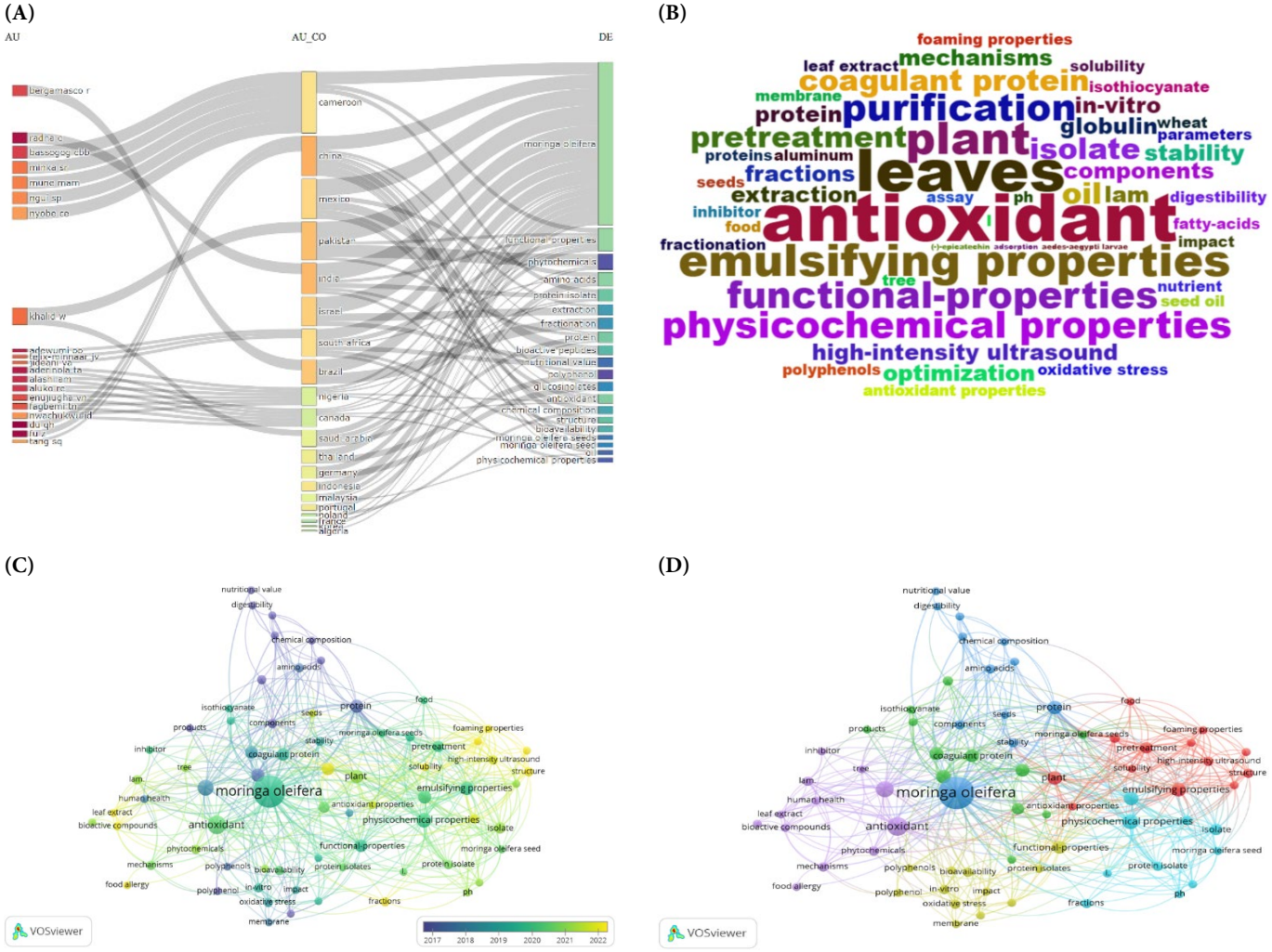

Figure 1. Co-occurrence bibliometric analysis of publications with keywords "*Moringa oleifera*" and "protein" and "food": (A) author, country of publication, and keywords; (B) keywords; (C) visualization of keyword clustering heatmap by year; and (D) visualization of keyword clustering heatmap.

From 2020 to 2022, other research interests began to emerge, such as "antioxidant," "bioavailable," and "phytochemical." There was also a growing focus on protein-related characteristics and a likely link between the keyword "plant" and the increasing demand for alternative protein sources. Terms such as "protein isolates" and interest in Moringa seed properties like emulsification and foaming also became more prominent, along with the use of emerging technologies, such as ultrasound, in more recent studies.

In regard to Figure 1D, which displays keyword co-occurrence through clusters, it is evident that the publication year is directly associated with the sets of keywords used together.

4 PROTEIN CHARACTERIZATION OF *MORINGA OLEIFERA* LAM. SEED

Following protein extraction from Moringa using a saline solution (NaCl), Du et al. (2021) demonstrated through Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis that the proteins exhibited low molecular weights. Jain et al. (2019) showed that molecular weights of 29, 14.2, and 6.5 kDa were the most prominent bands under reducing conditions.

Thus, the amino acid (AA) composition of a protein is of great importance when evaluating its quality. The AA profile of *M. oleifera* Lam. seeds revealed 17 AAs, of which seven are considered essential and 10 non-essential. Among the non-essential AAs, glutamine and arginine were the most abundant, while the sulfur-containing AAs (cysteine and methionine) and aromatic AAs (phenylalanine and tyrosine) were the most prevalent among the essential ones (Gu et al., 2020).

Furthermore, Du et al. (2021) analyzed the secondary structure of Moringa protein using different concentrations of NaCl during extraction, concluding that changes in salt concentration resulted in variations in the secondary structure of the extracted protein through the salting-out process.

The protein composition of Moringa seed fractions was found to be 44.00% albumins (water-soluble), 53.00% globulins I and II (salt-soluble), 0.56% prolamins (alcohol-soluble), and 0.39% glutelins (NaOH-soluble), with albumins and globulins being the predominant fractions (Baptista et al., 2017; Bassogog et al., 2022). An important factor strongly associated with these protein fractions is solubility. The lowest nitrogen solubility of Moringa protein isolate was observed in the pH range of 7–10, which may correspond to the isoelectric point of the material (Jain et al., 2019).

Table 1 presents data showing how variations in pH and temperature cause changes in the protein's secondary structure. Studies by Tang et al. (2024) confirm that pH significantly impacts the structure and functional properties of *M. oleifera* protein at various levels.

Table 1. Changes in the secondary structure of *Moringa oleifera* according to the increase in temperature and pH.

Fraction	Factor	B-Turn (%)	α-Helix (%)	B-sheet (%)	Random coil structure (%)
Protein Isolate	pH 3.0	19.95 ± 0.49	20.15 ± 6.72	4.03 ± 0.25	—
	25°C	24.82	26.81	35.48	12.88
Albumin	pH 3.0	11.45 ± 0.49	10.13 ± 0.11	2.53 ± 0.78	—
	25°C	29.71	14.91	48.98	6.4
Globulin	pH 3.0	14.85 ± 0.49	8.95 ± 0.85	6.13 ± 0.78	—
	25°C	19.68	24.96	51.61	3.75
Protein Isolate	pH 5.0	18.75 ± 0.49	7.43 ± 0.67	14.75 ± 2.06	—
	50°C	16.87	23.77	31.73	27.63
Albumin	pH 5.0	22.20 ± 0.49	4.65 ± 1.45	13.10 ± 0.78	–
	50°C	30.21	16.4	46.87	6.52
Globulin	pH 5.0	17.95 ± 0.49	4.90 ± 2.19	14.68 ± 1.06	—
	50°C	16.92	23.51	44.52	15.04
Protein Isolate	pH 7.0	19.0 ± 0.49	10.13 ± 0.11	2.53 ± 0.76	—
	70°C	24.01	12.49	43.12	20.38
Albumin	pH 7.0	13.55 ± 0.19	8.23 ± 0.71	5.08 ± 0.79	—
	70°C	15.61	33.08	37.66	13.65
Globulin	pH 7.0	17.05 ± 0.49	8.30 ± 0.43	13.50 ± 1.45	—
	70°C	29.92	42.73	23.51	3.84
Protein Isolate	pH 9.0	14.50 ± 0.49	2.63 ± 0.67	12.68 ± 2.14	—
	80°C	24.44	12.29	42.79	20.47
Albumin	pH 9.0	18.05 ± 0.49	8.27 ± 0.78	11.40 ± 1.02	—
	80°C	15.6	16.0	56.88	11.5
Globulin	pH 9.0	18.30 ± 0.49	1.55 ± 0.57	17.78 ± 1.24	—
	80°C	24.95	3.57	51.59	19.88

Source: Aderinola et al. (2020) and Bassogog et al. (2022).

5 USE OF MORINGA IN WATER TREATMENT

Several studies have shown that Moringa acts as a water clarifying agent (Table 2), containing high molecular weight cationic proteins that destabilize particles suspended in water (Baptista et al., 2015).

Based on the findings presented in Table 2, Moringa indeed demonstrates proven water clarification capacity. This raises questions regarding its potential application in similar food products, such as juices and their derivatives, as well as other beverages.

Table 2. Use of Moringa as a source of clarifying agents in water treatment.

Source	Title	Coagulant extraction	Results
Sengupta et al. 2012	Use of *M. oleifera* seed extracts to reduce helminth egg numbers and turbidity in irrigation water	The suspension will have a concentration of 3 or 5% weight per volume (water/powdered seed). Stirring is followed by filtration.	Turbidity decreases more rapidly when the *M. oleifera* extract is added, with a reduction of 85–96%.
Baptista et al. 2017	Protein fractionation of seeds of *M. oleifera* Lam. and its application in superficial water treatment	Defatting, extraction with water, mechanical stirring, and centrifugation. The supernatant was dialyzed with water until the precipitate became visible, followed by centrifugation and separation of the fractions, which, depending on their composition, were extracted with 0.5 M NaCl, ethanol, or NaOH, stirred, centrifuged, and subjected to dialysis.	According to the coagulation/flocculation test, it was found that only the coagulants albumin and globulin (I) had coagulation potential. Removal rates reached 93 and 96% for albumin and globulin, respectively.
Baptista et al. 2015	Coagulation–flocculation process with ultrafiltered saline extract of *M. oleifera* for the treatment of surface water	The defatted material was extracted in a blender through turbolysis with water, followed by agitation using a magnetic stirrer and two consecutive filtrations. Another extract was obtained using a 1 M NaCl saline solution	The NaCl extract performed better than the aqueous extract in water treatment for the removal of color and turbidity.
Mateus et al. 2018	Obtaining drinking water using a magnetic coagulant composed of magnetite nanoparticles functionalized with *M. oleifera* seed extract	The extract was obtained using 1 M NaCl, resulting in concentrations of 0.5, 1.0, and 2.0%. The solution was stirred with a magnetic stirrer, followed by intense agitation. After stirring, the extract was filtered using qualitative filter paper.	*M. oleifera* seed extract used with magnetic nanoparticles showed, after just 10 min of magnetic sedimentation, reductions of 96.8% in turbidity, 97.1% in apparent color, and 58.3% in UV254nm.
Balbinoti et al. 2024	Treatment of low-turbidity water by coagulation combining *M. oleifera* Lam. and polyaluminium chloride (PAC)	The seeds were peeled and crushed. The sieved material was mixed under agitation with a 1 M $CaCl_2$ saline solution. Subsequently, the mixture was filtered using filter paper and a fiberglass membrane.	The extract used is a viable alternative coagulant in the pursuit of more environmentally friendly and safer processes for treating low-turbidity water, as it minimizes the risks associated with aluminum-based coagulants.
Conceição et al. 2015	Removal of excess fluoride from groundwater using natural coagulant *Moringa oleifera* Lam. and microfiltration	"The peeled seeds were crushed with distilled water to produce a standard concentration of 5% (w/v). Subsequently, the solution was subjected to magnetic stirring and vacuum filtration. Various concentrations of this solution were used for coagulation tests.	The results of the tests with the coagulant demonstrated its effectiveness in the removal of fluoride, color, and turbidity from water containing residual levels. The combined process, conducted under conditions of 5 mg F/L and 5 g/L of coagulant, was efficient and met the recommended standards for residual fluoride.
Moreti et al. 2019	The use of *Moringa oleifera* seeds and their fractionated proteins for *Microcystis aeruginosa* and Microcystin-LR Removal from Water	The saline extract was prepared with 1 M NaCl using turbolysis, followed by stirring with a magnetic stirrer and consecutive filtrations. The defatted seed powder was dispersed in water, subjected to mechanical stirring, and centrifuged. The supernatant, after extensive dialysis against distilled water using membranes, was centrifuged again, resulting in a protein fraction with albumin-like characteristics (supernatant) and partial globulin-like characteristics (precipitate).	It can be observed that the protein fractions of albumin and globulin, at ideal dosages, showed efficiencies similar to those obtained with the saline extract and whole seed powder. The ideal dosage for globulin and albumin was considered to be 1.5 mg, while for the saline extract, the ideal dosage was 8 mg/L^{-1}.
Paixão et al. 2021	Discoloration of contaminated water with textile dye through a combined coagulation/ flocculation and membrane separation process with different natural coagulants extracted from *M. oleifera* Lam. Seeds	The aqueous extraction of the protein was carried out under mechanical stirring, followed by centrifugation. The supernatant was dialyzed. A subsequent centrifugation step was performed to separate the protein fractions. The albumin fraction was lyophilized, and the globulin fraction was oven-dried. The saline extraction was performed using 1 M NaCl under magnetic stirring, followed by vacuum filtration.	The coagulant from the albumin protein fraction, which showed a more heterogeneous structure in SEM analysis and a zeta potential of 10.57 ± 0.42, was able to remove nearly all of the RB5 dye present in the aqueous solution.

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Table 2. Use of Moringa as a source of clarifying agents in water treatment.

Source	Title	Coagulant extraction	Results
Resende et al. 2024	Synthesis of hydrogels from fractionated proteins of *M. oleifera* Lam. seeds for the treatment of water contaminated with black reactive dye 5	An amount of 0.5 g of albumin and 0.05 g of maleic anhydride was dissolved in 5 mL of phosphate buffer with a concentration of 0.1 mol/L and pH 7. The mixture was kept under stirring at room temperature. The resulting product was stored for use in hydrogel formation. The same procedure was used for globulin, but at 303 K.	The highest RB5 removal percentages were obtained at pH 2 for the albumin hydrogel and at pH 7 for the globulin hydrogel.
Bongiovani et al. 2014	Improvement of the coagulation/flocculation process using a combination of *M. oleifera* Lam. with anionic polymer in water treatment	Dehulled seeds were ground, defatted, centrifuged, and dried.. The dried sample was mixed with a saline solution and then vacuum filtered. For solvent extraction using the Soxhlet method, *M. oleifera* seeds were milled, dried, wrapped in filter paper, and placed in cellulose extraction cartridges, which were then inserted into Soxhlet extractors. The resulting dried cake was also used to prepare a saline solution.	*M. oleifera* can be used as a potential coagulant in combination with a polyelectrolyte, especially for low-turbidity water.
García-Fayos et al. 2016	*M. oleifera* for drinking water treatment: influence of the solvent and method used in oil extraction on the coagulant efficiency of the seed extract	A protein extract was prepared using raw or defatted seeds and river surface water in a 5% (w/v) suspension, which was mixed with a magnetic stirrer and left to stand. The crude *M. oleifera* extract was then filtered.	It was determined that all extracts have the same protein concentration. However, the protein mass required to achieve maximum coagulation efficiency with the ethanolic extract is 5–33 times lower than that required for extracts from defatted seeds using acetone or hexane. This demonstrates the higher efficiency of the protein in the extract obtained with ethanol as the solvent.
Pavankumar and Singh, 2014	Identification of *M. oleifera* protein responsible for the decolorization and pesticide removal from drinking water and industrial effluent–an in silico and in situ evaluation	The crude seed extract was prepared by grinding the seeds, followed by solvent treatments, and the fractions were purified.	The extract used is responsible for multiple activities and has been suggested for prospective use in large-scale treatment of drinking water and industrial effluents.
Santos et al. 2016	Removal of tetracycline from contaminated water by *M. oleifera* seed preparations	Seed flour was obtained using 0.15 M NaCl, resulting in a saline protein extract. The proteins in the extract were precipitated with ammonium sulfate, and a protein fraction was obtained. This fraction was isolated using a guar gel column previously equilibrated with 0.15 M NaCl. The lectin was eluted with 1.0 M NaCl.	Preparations of **Moringa oleifera** seeds interacted with tetracycline, and may be the lectin responsible for this interaction. The removal of tetracycline by the extract at all tested concentrations and by the seed flour (5 mm particles) indicates that *Moringa* preparations could be used to remove this antibiotic from contaminated water through a simple, natural, and environmentally friendly technology.

LR: Leucine and Arginine.

6 USE OF MORINGA PROTEIN AND ITS TECHNOLOGICAL FUNCTIONS WITH POTENTIAL FOR FOOD APPLICATION

First, different methodologies can be applied to obtain protein isolates for the valorization of Moringa seed cake, including alkaline extraction, isoelectric precipitation, or salting-out methods (Illingworth et al., 2024). Moreover, the protein fraction from *M. oleifera* (MO) identified in Aderinola et al. (2020) exhibited better foaming performance in the pH range of 7–9. Similarly, the protein isolate obtained with an optimal salt concentration was found to act as a foam stabilizer (Du et al., 2021).

On the other hand, Moringa protein isolate showed 36% greater foaming capacity and 29% higher foam stability than commercial soy protein isolate (Jain et al., 2019).

In the study by Ogunsina et al. (2010), defatted Moringa flour demonstrated superior foaming capacity compared to full-fat Moringa flour. It can also be stated that variations in ionic strength and pH can influence solubility and enhance the functional properties of *M. oleifera* protein isolates (Illingworth et al., 2024).

According to Aderinola et al. (2020), among the isolates studied, the isoelectric precipitation method was the most effective for emulsion formation. In Wang et al. (2023), the nanoemulsions developed showed good stability under changes in pH, temperature (80 to 100°C), and sodium ion concentration. Additionally, the protein fraction studied by Du et al. (2022) revealed that changes in salt concentration during extraction affect emulsifying capacity, and that the proper concentration can result in a suitable stabilizer for food emulsions. Jain et al. (2019) found that Moringa protein isolate has lower emulsifying

capacity than commercial soy protein isolate, but similar values to soy protein concentrate. Ogunsina et al. (2010) demonstrated that defatted Moringa seed flour formed better emulsions than its full-fat counterpart.

The surface structure of pure albumin is porous and heterogeneous, whereas in hydrogel form, it is laminar and homogeneous (Resende et al., 2024). Gautier et al. (2022) formulated a yogurt from a Moringa-based beverage and observed that the gel structure was similar to that of traditional milk yogurt. Additionally, ultrafiltered Moringa samples performed well as thickening agents, further improving the technological characteristics of the studied systems (Cardines et al., 2018). Paz et al. (2021) observed that doughs formulated with Moringa displayed pseudoplastic and thixotropic behavior—the latter referring to the fluid requiring a certain time to reach viscosity equilibrium after a sudden change in shear rate.

7 APPLICATION OF EMERGING TECHNOLOGIES

The use of emerging technologies can be employed to investigate possible modifications in protein properties. According to Asif et al. (2024), Moringa protein concentrates subjected to ultrasound treatment showed improvements in functional properties by 42, 33, and 73%, respectively, when compared to untreated groups. In Du et al. (2021), ultrasound treatment resulted in a reduction of approximately 46.3% in emulsifying activity between 0 and 10 min, and a 56% reduction between 0 and 40 min of exposure; however, solubility increased by 44% after 10 min, and foaming capacity increased by 61% after 20 min. Fatima et al. (2023) observed that the solubility, emulsification, and foaming properties of the proteins studied increased by 436, 30, and 83%, respectively, when compared to conventional extraction versus ultrasound-assisted extraction. Tang et al. (2021) reported a 449% increase in solubility from power level 0 to 60; foaming capacity did not show a significant increase, and emulsifying properties increased by approximately 30% from power level 0 to 40, but were reduced with higher ultrasound intensity.

In line with these changes, Asif et al. (2024), Du et al. (2021), Fatima et al. (2023), and Tang et al. (2021) explain that ultrasound treatment did not cause significant degradation. They further report that the observed modifications were caused by the exposure of hydrophilic groups and changes in secondary (Tables 3 and 4) and tertiary protein structures. Additionally, Du et al. (2021) noted that ultrasound treatment disrupted the spherical structure of the protein, resulting in irregular fragments and microstructural changes. The systematic review methodology used in this study did not identify reports of other emerging technologies applied to Moringa proteins in the current literature.

8 CONCLUSION

It is noteworthy that each extraction method results in a different composition, as chemical and physical processes applied can modify the secondary structure of the protein. Moringa protein shows strong potential for application in food systems with technological functions and has already been extensively studied for its use in water treatment. It is also important to emphasize the need for further studies focusing on the application of emerging technologies on the protein structure of *M. oleifera* seed-derived protein isolates.

Table 3. Studies on the technological functions of *Moringa oleifera*.

Source	Foaming stability (FE)	Emulsifying	Gelifying agent	Thickener
Aderinola et al. 2020	X	x	—	—
Wang et al. 2024	—	x	—	—
Cardines et al. 2018	—	—	—	x
Du et al. 2021	X	x	—	—
Jain et al. 2019	X	x	—	—
Paz et al. 2021	—	—	—	x
Resende et al. 2024	—	—	x	—
Gautier et al. 2022	—	—	x	—
Ogunsina et al. 2010	X	x	—	—

Table 4. Changes in the secondary structure of *Moringa oleifera* according to the increase in ultrasound treatment time.

Time (min)	B-Turn (%)	α-Helix (%)	B-sheet (%)	Random structure (%)
0	34.33 ± 1.14b	8.51 ± 1.23b	47.94 ± 0,33a	9.22 ± 1.08b
10	39.78 ± 0.52a	11.80 ± 0.43a	37.68 ± 0.69bc	10.74 ± 0.49a
20	39.51 ± 0.27a	11.68 ± 0.96a	38.19 ± 0.93b	10.62 ± 0.71a
30	40.05 ± 0.34a	12.16 ± 0.23a	36.69 ± 0.24c	11.10 ± 0.04a
40	40.06 ± 0.71a	12.40 ± 0.11a	36.58 ± 0.37c	10.96 ± 0.25a

Different lowercase letters in the same row indicate statistical difference with a significance level of 5%.Source: Du et al (2021).

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