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وحماية المستهلك

FUNCTIONAL PROPERTIES OF ISOLATED AND HYDROLYZED PROTEIN POWDER OF MORINGA LEAVES (*MORINGA OLEIFERA* LAM.)

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ABSTRACT

The study aimed to estimate the functional properties of *Moringa oleifera* Lam. leaves powder protein isolate and its hydrolysates using pepsin. Water Holding Capacity (WHC) and Oil Holding Capacity (OHC) of protein isolate were 3.02 mL /g protein and 2.62 mL /g protein respectively, superior to the oil reducing capacity of the pepsin enzyme hydrolysates. at times 30 and 60 min. They were characterized by high emulsifying activity, reaching 48.08 m^2/g and 53.06 m^2/g, respectively, and outperforming the protein isolate, which reached 41.45 m^2/g. The least emulsifying activity was for pepsin after 120 min of hydrolysis (A_4) and reached 31.50 m^2/g, and the highest emulsion stability was 63.77% for protein hydrolysates using pepsin enzyme at (37 °C) after 60 min of hydrolysis (A_2), while the least emulsifying stability was for protein hydrolysates using pepsin enzyme pepsin after 120 min (A_4) it amounted to 25.10%. The foaming ability of protein hydrolysates using pepsin after 60 min (A_2) was 41.17%, foaming stability at the beginning of hydrolysates was 39.02%, then it gradually decreased with the passage of time.

Keywords: Enzyme hydrolysates, Functional properties, Moringa protein isolate, Pepsin,.

الخصائص الوظيفية للمعزول والمتحلل البروتيني لمسحوق اوراق (*Moringa oleifera* Lam.)

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الخلاصة

هدفت الدراسة الى تقدير الخصائص الوظيفية للمعزول البروتيني لمسحوق اوراق نبات البان (المورينجا) *Moringa oleifera* Lam. ومتحللاته باستعمال انزيم الببسين, اذ بلغت قدرة الاحتفاظ بالماء والاحتفاظ بالزيت للمعزول البروتيني 3.02 مل/غم و 2.62 مل /غم على التوالي متفوقة على قدرة الاحتفاظ بالزيت لمتحللات انزيم الببسين, وتميزت المتحللات البروتينية لانزيم الببسين في الاوقات 30 و 60 دقيقة بنشاط استحلابي مرتفع وبلغت 48.08 غم/$م^2$ و 53.06 غم/$م^2$على التوالي وتفوقت على المعزول البروتيني الذي بلغ 41.45 غم/$م^2$. اما اقل نشاط استحلابي كان لمتحلل الببسين بعد 120 دقيقة من التحلل (A_4) وبلغ 31.50 غم/$م^2$. وبلغت اعلى ثباتية للمستحلب63.77% للمتحلل البروتيني باستعمال انزيم الببسين على درجة حرارة (37) مْ بعد 60 دقيقة من التحلل (A_2), اما اقل ثباتية للاستحلاب كانت لمتحلل الببسين بعد 120 دقيقة (A_4) وكانت 25.10%, وكانت قابلية تكوين الرغوة للمتحلل البروتيني بوساطة انزيم الببسين بعد 60 دقيقة من التحلل (A_2) وكانت 41.17%، وكانت ثباتية الرغوة في بداية التحلل 39.02%، بعدها انخفضت

* **The research is extracted from a doctoral thesis of the first researcher.**

تدريجيا مع زيادة فترة التحلل, وبينت الدراسة اهمية استعمال المعزول البروتيني لمسحوق اوراق المورينجا وبعض متحللاته كعامل استحلاب وعامل رغوة في تحسين جودة المنتجات الغذائية.

الكلمات المفتاحية: متحللات انزيمية، الخواص الوظيفية، معزول بروتين المورينجا، البيبسين.

INTRODUCTION

Moringa leaves have a multipurpose which used as natural medicine, food, feed, natural stimulants for fertilizers, forage and migration of bees **(Al-Taweel & Al-Anbari, 2019)**. Moringa leaves contain all of the essential amino acids in a good proportion, which are the building blocks of proteins **(Al-jubouri *et al*., 2022; Mishra *et al*., 2012)**. Proteins are considered important nutrients for the development of the human body and maintaining its health, as a person needs a sufficient amount of protein to maintain the vital functions of the body, growth, maturity, pregnancy, breastfeeding, and recovery from diseases **(Aziz, 2023; Abdul Rahman *et al*., 2023; Hamdia & Ahamed, 2023)**. Moringa oleifera is a type of fast-growing perennial plant native to India, where it is currently grown in many regions of the world and is considered one of the most useful plants in the world because almost all of its parts can be used as food and in traditional medicines **(Chalob & Abdul-Rahman, 2018; Alwan & Jawad, 2015)** as all parts of Moringa have long been used to treat diseases it is also used in water purification and in the manufacture of supplementary food for children to increase its protein content, as the use of vegetable proteins is a good source of amino acids because of its good functional properties such as solubility, emulsification, foaming, and oil-water binding **(Nasser & Hammood, 2019; Gorissen *et al*., 2018 ; Karim & Shaker, 2016).** The use of vegetable proteins is a good source of amino acids, as it possesses good properties of Moringa protein isolate, which indicates its ability to work as functional components in diets, as Moringa leaf protein isolate can be incorporated into diets for the manufacture of functional foods and the treatment of malnutrition **(Famuwagun *et al*., 2020 ; Khalaf, 2014).** The study aimed to estimate the functional properties of Moringa oleifera leaves powder protein isolate and its hydrolysates using pepsin.

MATERIALS AND METHODS

Preparing research samples

Moringa leaves under study were obtained from the University of Baghdad / College of Agricultural Engineering Sciences/ Medicinal Plants Unit for the season (2021-2022).

Preparing the forms for the study

Moringa leaves (*Moringa oleifera* Lam.) were cleaned and isolated, then dried in an electric vacuum oven at a temperature of 50°C, then ground and sifted with an 80 mash sieve, and kept in polyethylene bags at a refrigerator temperature of 4 ˚C until use.

Preparing of defatted leaf powder

The defatted leaf powder was prepared according to **(Fontanari *et al*., 2012)** by mixing the powder with hexane in a ratio of 1/5 (w/v), then placed on the magnetic stirrer for 3 h, after that the filtrate was separated and then dried at room temperature 25 ˚C for 24 h, grinding the powder and keeping it by freezing at -18 ˚C until use.

Preparing of the protein isolate

The protein isolate of the defatted Moringa leaf powder was prepared according to **(Estela, 2014)** where the defatted leaf powder was mixed with water in a ratio of 1:20 (w/v) with stirring for 2 h, after that the pH was adjusted to 9 and mixed for 2 h, followed by a refrigerated centrifugation process At a speed of 10,000 rpm for 30 min, then the leachate was separated from the precipitate, then the leachate was taken and the pH was adjusted to 4 then a refrigerated centrifugation process was carried out at a speed of 10,000 rpm for 30 min, then the precipitate was separated from the leachate, the precipitate was taken, washed with distilled water several times, and then dissolved the precipitate in a small amount of distilled water, then the pH was adjusted to 7, then the sample was dried and stored by freezing at -18°C until use.

Preparation of protein isolate hydrolysates

Enzymatic hydrolysis by pepsin

The reaction mixture was prepared according to the method of **(Popovic *et al*., 2013)** by mixing 5 g of protein isolate with 100 mL of Glycin-HCl buffer solution (0.1 M) at pH 3, then the enzyme pepsin was added at a concentration of 1%, and the mixture was placed in a shaking incubator At a speed of 200 rpm min at 37 °C, samples were drawn at different times after (30, 60, 90 and 120) min and their code (A_1, A_2, A_3, A_4), respectively. After the expiration of each time, the reaction was stopped by boiling at 100 °C for 5 min, then the samples were centrifuged at a speed of 14500 rpm/ for 5 min, then the clear liquid was separated from the precipitate, the clear liquid was taken and kept in refrigeration until use.

Study of the functional properties of protein isolate and its hydrolysates

Water Holding Capacity

Following a method previosnly described **(Mao & Hua ,2012)** was adopted, where 1 g of the sample was taken and placed in a 15 mL test tube, then 10 mL of distilled water was gradually added to it with stirring by means of the electric mixer and left for 30 min at room temperature, then a procedure was carried out. Centrifugation at a speed of 2000 rpm for 20 min, the filtrate was removed, the tube was weighed with the sample, and the percentage of bound water was calculated as follows.

$$\mathbf{WHC} = \frac{W_2 - W_1}{W_0}$$

Where W_0 = dry weight of the sample. W_1 = tube weight + dry sample before adding water.
W_2 = tube weight + weight of sediment after adding water.

Oil Holding Capacity

The method of **(Mao & Hua ,2012)** was adopted, where 1 g of the sample was taken in a 15 mL tube, the sample was mixed with 10 mL of sunflower oil, and the mixture was left at room temperature 25°C for 30 min, then a cooled centrifugation was carried out at 5000 rpm for 30 min at 25°C, after which the filtrate was carefully removed, then the tube was weighed with the sample, and the fat absorption capacity of the samples was estimated according to the following equation.

$$\mathbf{OHC} = \frac{F_2 - F_1}{F_0}$$

Where F_0 = sample dry weight. F_1 = tube weight + dry sample weight before adding oil.
F_2 = tube weight + sediment weight after adding oil

Emulsifying Activity and Stability Index (EAI , ESI)

The emulsifying properties of all samples were estimated according to the method of **(Popovic *et al*., 2013)** where 90 µL of the sample were mixed with 90 mL of sodium phosphate buffer (M 0.01) at pH 7 Then 30 mL of commercial sunflower oil was added to the mixture and mixed using an electromixer at a speed of 6500 rpm for 5 min, then 50 µL were taken from the bottom of the mixture at a time (0 and 10) min and placed in test tubes, then diluted with 5 mL of sodium dodecyl sulfate solution 0.1% prepared by dissolving it in sodium phosphate buffer (M 0.01) At a pH of 7, then mixed with a slight stirring of the shift, and then read the absorbance of the diluted solution at a wavelength of 500 nm in time (0, 10) min, then estimated the capacity and stability of the emulsion based on the following equation

EAI (m^2/g) = (2.303 ×2×100×A0)/ (C×0.25×10.000)

Where EAI(m^2/g) = emulsifying activity, A = absorbance at 500 nm, C = protein concentration (g/mL) 0.001.

ESI = $A_0 \times t/(A_0 - A_{10})$

Where ESI = stability of the emulsion. A_0 = absorbance at time zero.

t = time after naturalization. A_{10} = absorbance after 10 min.

Foaming Capacity and Stability (FC, FS)

The volume and foam constant of the samples were estimated according to the method of **(Popovic *et al*., 2013)** by whipping 0.5 g of the sample with 50 mL sodium phosphate buffer 0.01M at pH 7 by means of a homogenizer at a speed of 5000 rpm for 2 min, then transferring the mixture to A graduated cylinder and record the volume of foam before and after whipping, then the samples were left after conducting a foam capacity check for (1, 10, 30, 60 and 90) min, then the foam stability was estimated at all times, then the foam capacity was estimated according to the following equation:

$$\textbf{FC} = \frac{\textbf{Foam volume}}{\textbf{total volume}} \times 100$$

Statistical Analysis

The Statistical Analysis System (GenStat 12th Edition) was used to analyze the data to study the effect of different coefficients on the studied traits according to a complete random design (CRD), and the significant differences between the means were compared with the Least Significant Difference-LSD test **(Al-Rawi & Khalaf Allah, 2000)**.

RESULTS AND DISCUSSION

Functional properties

Water holding Capacity (WHC)

The results in (table 1) show ability of the protein isolate and the protein hydrolysates of the leaf powder to bind water. It was found that the highest ability to bind water was for the protein isolate (PI), reaching 3.02 mL/g, and the lowest ability to bind water was for the hydrolysate after 90 min which was1.48 mL/g. and the difference was significant with the rest of the treatments at the level ($p \leq 0.05$). The reason for this can be attributed to the high percentage of protein in the isolate, which was followed by an increase in water binding, as the ability of the protein to bind water is due to its ability to form hydrogen bonds between water molecules and polar groups of peptide chains in the protein, and this leads to an increase in the

ability of the protein to integrate with water due to its content of Hydrophilic, polar amino acids that form hydrogen bonds with water.

The results show that the water absorption capacity of the hydrolysates is low compared to the protein isolate, and this may be explained by the great ability of the protein isolate to open up and swell the peptide chain and then new sites for water binding may be due to the low concentration of polar amino acids of the hydrolysates which led to a decrease in susceptibility Carrying water. Where it was found **(Jasim & AI-Obaidi, 2022 ; Rawdkuen, 2020)** that the ability of protein to absorb water amounted to 2.31 mL/g which is among the results obtained in the study and the ability of protein to absorb water is an important characteristic in viscous foods such as soup broth and baked products, especially when its value ranges from 1.49 to 4.72 mL/g **(Nasser *et al*., 2019 ; Kareem & Shakir, 2016)**.

Table (1): The water and fat holding capacity of the leaf powder protein isolate and its hydrolysates

Samples			Water holding capacity mL/g	Oil holding capacity mL/g
T_1			3.02	2.62
T_2	30 min	A_1	2.28	1.93
	60 min	A_2	2.81	2.30
	90 min	A_3	1.48	1.41
	120 min	A_4	1.65	0.82
LSD			0.1478	0.3308

The results represent an average of three replicates T_1: represents the protein isolate. T_2: represents pepsin hydrolysis at different times. LSD value at a significant level ($p \leq 0.05$).
These results are higher than those obtained by **(Devisetti *et al*., 2016)** for Moringa seed flour and the reason may be due to the different variety as well as the different working conditions for the production of protein isolate, such as drying conditions for example as these conditions are important and have an impact on the result. As indicated by **(Stone *et al*., 2015 ; Rahman, 2018)** the water absorption capacity of the Moringa protein isolate was twice the water absorption capacity of the pea seed isolate where these differences are related to the nature and type of proteins, amino acid composition, protein formation, surface polarity in addition to the number and type of polar aggregates.

Oil holding capacity (OHC)

We note from (Table 1) that the ability of the protein isolate and the protein hydrolysates to bind the fat was good. It was noted that the highest ability to bind the fat was for the protein isolate and amounted to 2.62 mL /g, while it was the least capable of binding the fat to the enzymatic hydrolysate by the action of the enzyme pepsin for 120 min (A_4). It amounted to 0.82 mL /g, and the difference was significant with the rest of the treatments at the level ($p \leq 0.05$). As the lipid binding process is due to the non-polar side chains of the protein, which are associated with the hydrocarbon chains, and thus work to bind the lipid, as the attachment to the lipid is attributed to the presence of hydrophobic groups, and this in turn helps to form hydrophobic bonds with the lipid and increases the amount of bound lipid **(Al-Taweel *et al*., 2022 ; Jasim & Nasser, 2020 ; Jain *et al*., 2019).**

The ability of the Moringa leaves protein isolate to retain oil was 1.94 mL / g protein which is 30% higher than the Moringa seed protein isolate which was 1.9 mL /g and these

results are somewhat similar to the results obtained in this study **(Jain *et al.*, 2019 ; Chalob & Abdul-Rahman, 2018)**. As **(Kandasamy *et al.*, 2012)** indicated that the fat absorption capacity of the protein isolate of Moringa species was between 1.08- 1.34 mL /g of protein and this is less than the results obtained in this study, and the reason may be due to the variety of Moringa used in the production of the isolate. The researcher found that Moringa protein isolate has a good oil absorption capacity, as the fat absorption capacity is an important functional property that increases or improves the flavor and taste retention of different food products as the high oil absorption rate of Moringa protein isolate makes it a good ingredient in the manufacture of chilled meat especially for sausages where the protein usually by preventing the loss of fat and water to get good products.

Emulsificaation Properties

As show in (table 2) emulsifying activity and stability of the emulsion of the protein isolate and the protein hydrolysates of Moringa, where it was noted that the highest emulsifying activity was for the enzyme degraded pepsin for 60 min of hydrolysis (A_2) and it was 53.06 m^2 /g and the lowest emulsifying activity was for the pepsin hydrolyzate for 120 min (A_4) Where it was 31.50 m^2/ g and the difference was significant with the rest of the treatments at the level ($p \leq 0.05$). While the highest stability of the emulsion was for pepsin hydrolysis after 60 min of hydrolysis (A_2) and was 63.77%, while the least stability was for pepsin hydrolysis after 120 min (A_4) and it was 25.10%. Through the results, Through the results, we notice a decrease in the emulsifying activity of the protein isolate compared to the rest of the samples, and the reason for this is attributed to the high concentration of protein, which impedes the process of migration and diffusion of protein around the surface of water and oil, unlike the low concentrations of protein, which leads to the speed of its spread easily around the surface of water and oil and then leads to an increase Emulsification capacity **(Tawfeeq & Ahmaed, 2023 ; Abadi & Naser, 2019)**.

Table (2): Emulsifying activity and stability of Moringa protein isolate and its hydrolysates

Samples			Emulsion activity (m^2/g)	Emulsion stability (%)
T_1			41.45	34.52
T_2	30 min	A_1	48.08	56.73
	60 min	A_2	53.06	63.77
	90 min	A_3	34.26	33.73
	120 min	A_4	31.50	25.10
LSD			0.2329	0.3902

The results represent an average of three replicates T_1: represents the protein isolate. T_2: represents pepsin hydrolysis at different times. LSD value at a significant level ($p \leq 0.05$).

The low protein concentration works to increase the emulsification of the protein due to the increase in the area exposed to the surface, unlike the high concentration of protein that works to reduce the emulsification, which leads to improving the emulsification due to the increase in the tendency to interact with the hydrophobic lipid phase. **(Nashmi and Naser, 2022; Al-Aubadi & Al-Jobouri, 2013)**.

The results agreed with **(Adewumi *et al.*, 2022; Al–samarraie *et al.*, 2013)** when studying the functional properties and amino acid appearance of Moringa oleifera protein isolate, where it

was found that the emulsifying activity was 45.83%, while the stability of the emulsion was 47.28%. These results were higher than the results obtained by **(Bocarando-Guzman *et al*., 2022; Al-Anbari *et al*., 2019; Aziz, 2015)** when studying and comparing the physical, chemical and functional properties of flour and protein isolated from Moringa leaves, where the lowest value for emulsifying activity was found at pH 4.5- 5.5 compared to pH values While the highest value of the emulsion thiol was found at pH8 and was 31.67%, followed by 21.67% at pH 10.

Foaming Properties

As show in (table 3) the results of the foam capacity and its stability for the protein isolate and its decomposers, where the highest value of the foam capacity of the decomposer resulting from the use of the pepsin enzyme after 60 min of the reaction (A_2) was 41.17%, while the lowest value of the foam capacity was for the decomposing of the pepsin enzyme after 120 min of the reaction (A_4) and reached 13.79%, and the difference was significant with the rest of the treatments at the level of ($p \leq 0.05$). The reason for this may be due to the proximity of the pH to the point of electrical equilibrium, as the repulsion decreases and a thin and coherent film is formed as the best foam film is formed at the point of electrical equilibrium.

Table (3): Foam capacity and stability of Moringa leaves protein isolate and its hydrolysates

Samples			Foam capacity (%)	Foaming stability (%)				
				min 1	min 10	min 30	min 60	min 90
T_1			23.07	21.87	18.03	12.28	7.40	3.84
T_2	30 min	A_1	32.43	30.55	24.24	13.79	0	0
	60 min	A_2	41.17	39.02	33.33	25.37	16.66	13.79
	90 min	A_3	38.27	37.50	34.21	28.57	20.63	10.71
	120 min	A_4	13.79	12.28	7.40	0	0	0
LSD			3.404	0.121	0.120	0.129	0.070	0.076

The results represent an average of three replicates T_1: represents the protein isolate. T_2: represents pepsin hydrolysis at different times. LSD value at a significant level ($p \leq 0.05$).

As for the highest stability of the foam in the first minute of the hydrolyzed enzyme pepsin after 60 min of hydrolysis (A_2) by 39.02%, but after that it decreased to (33.33, 25.37, 16.66, and 13.79)% at each of the times (10, 30, 60, and 90) min respectively. The reason may be due to the large size of the peptide and then the formation of flexible membranes around the air bubbles and the possibility of the presence of hydrophobic amino acids, as their presence increases the stability of the foam.

The foam capacity of the enzymatic hydrolysates increased by using (alcalase, flavourzyme, protamex and neutrase) enzymes, reaching (63.9, 60.3, 61.65 and 53.1)% compared to the foam capacity of the protein before hydrolysis which amounted to 47.24%. As for the stability of the foam it was for enzyme hydrolysates alcalase has high stability during the first 10 min but the flavourzyme hydrolysates showed high stability compared to the rest of the hydrolysates after 120 min **(Khalaf & Rahman, 2015; Muhamyankaka *et al*., 2013).**

The results agreed with **(Patil *et al*., 2022; Al-Anbari et al., 2019; Khafaji & Azeez, 2008)** when studying the protein of Moringa leaves and seeds, as it showed that the stability of the protein decreased over time for those studied models. It also indicated that the low charge had an effect on the foam stability, and it was higher than the results obtained by when studying the

chemical composition and some functional properties of Moringa leaves meal, Leucina and Glyricidia, who reported a foam capacity ratio of 10% and a foam stability ratio of 2% for Moringa leaves flour **(Al-Anbari *et al*., 2019 ; Aye & Adegun, 2013)**.

CONCLUSIONS

The study showed the importance of using Moringa leaves powder protein isolate and some of its hydrolysates as an emulsifying and foaming agent in improving the quality of food products. It can be concluded from this study that the protein of Moringa leaves is a good and important source of protein and may be a viable alternative for use in food applications as a functional food due to its good functional properties.

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