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Production of protein isolate and its enzymatic hydrolysates from local pumpkin seeds and studying their functional properties

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ABSTRACT

Background: Pumpkin seeds are a valuable source of high-quality protein and can be utilized as functional food ingredients due to their properties, such as solubility, foam formation, and stability. This study aims to produce protein isolate and its enzymatic hydrolysates from local pumpkin seeds to study their properties. **Methodology:** Preparing defatted pumpkin seeds for protein extraction, followed by the enzymes' hydrolysis using Trypsin and Pepsin enzymes separately and together in two methods. The determination of amino acids and the degree of hydrolysis was conducted; moreover, protein properties were studied, including solubility, emulsifying activity, stability index, foaming capacity, and stability. **Results:** A protein sample was successfully produced from local, peeled, non-soaked pumpkin seeds, yielding a protein percentage of 53.15%. Enzymatic protein hydrolysates were produced at different times. Essential and non-essential amino acids were determined. The functional properties of protein samples and protein hydrolysates were studied, including solubility at various pH levels. The higher emulsifying capacity was observed for the enzymatic hydrolysate using pepsin, while the protein sample dissolved in pH 8 had higher emulsifying stability. The enzymatic hydrolysate produced using pepsin exhibited the highest foaming capacity, while a mixed enzyme (Ma) (pepsin + trypsin) showed a higher foaming capacity and stability. Conclusion: Pumpkin seed protein has numerous nutritional values that can be utilized in the preparation of protein-rich foods for athletes and as a source of Nitrogen in microbial culturing media.

Keywords: pumpkin seeds, protein isolate, protein hydro-lysate, emulsifying capacity, foaming capacity.

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INTRODUCTION

Pumpkin seeds are a rich source of high-quality protein, as they contain essential amino acids. Additionally, enzymatic hydrolysis can improve the biological and functional properties of proteins (1,2). Therefore, decomposers of pumpkin protein can be a vital alternative to foods known as nutritional supplements, as well as a source of potential new bioactive peptides (1,3,4). Pumpkin seed protein is a storage protein, comprising 59% of the protein, which is made up of two main parts. The predominant part is 12S globulins, specifically cucurbitins, and the second part is 2S protein albumins (5).

The isolated protein possesses some bioactive and functional properties even before any hydrolysate process is carried out, making it a perfect point of comparison for the seed protein decomposers themselves. After the extraction process, the protein sample or the defatted seeds can be analyzed to obtain proteolytic proteins from the seeds (4,6). After the extraction process of Cucurbitin, the isolated protein or the defatted seeds themselves can be hydrolyzed to obtain the Cucurbitaceae seed protein hydrolysates.

It was reported that the most abundant amino acids in the seeds of the Cucurbita maxima family are Glutamate and Arginine, in addition to the species belonging to the family Cucurbita maxima possessing an unusual amino acid

known as Cucurbitin, known chemically (-)-3-amino-3-carboxipirrolidine is attributed with anti-inflammatory and anti-parasitic functional properties (7). Solubility can influence the other functions and bioactive properties of protein isolates and their hydrolysates; thus, it is one of the most essential properties of protein (4).

Some researchers have reported that the solubility of pumpkin seeds is very low (less than 20%) at a pH level below 5. The functional properties of particular interest include improving solubility, especially at a pH close to the isoelectric point (pI) (8). Enzymatic hydrolysis is an effective tool for obtaining highly soluble pumpkin seed proteins. The same researcher also found that the protein isolate from pumpkin seeds was less soluble at pH 5, which is the isoelectric point of most dietary proteins. However, its solubility increases when the pH of the solution rises towards more basic conditions or decreases towards more acidic conditions. In addition to improving solubility, it is necessary to know whether the protein isolates and hydrolysates have suitable emulsifying and foaming properties. These functional properties are closely related to the size of the suitable peptides for the hydrolysate. Tiny peptides negatively affect the function of the hydrolysate. Another factor affecting the emulsifying and foaming properties of protein hydrolysates from the cucurbit family is the degree of hydrolysis (DH). The researcher stated that the capacity and stability of emulsification of enzymatic hydrolysates of pumpkin protein, using Alcalase, Flavourzyme, and Pepsin, were improved at pH 7 and a degree of hydrolysis (DH) of 15%. In contrast, a DH of 29-30% had a negative impact on the emulsifying properties (4). Given the scarcity of local studies on protein isolates from Iraqi pumpkin seeds, this study aims to evaluate their functional properties and compare them with their enzyme hydrolysates.

METHODOLOGY

Sample collection: In this research, local pumpkin seeds were used, obtained from local markets.

Preparing the sample for the study: Pumpkin seeds were cleaned, washed with distilled water, and dried with air at room temperature, then a specific weight of seeds was soaked with 1/5 distilled water (seeds/water) for 8 hours, and thereafter the seeds were dried with air at room temperature (9). Finally, it was ground in a laboratory mill and sifted through a 200-mesh sieve, then stored at refrigerator temperature until use. The chemical composition of soaked and unsoaked pumpkin seeds, including moisture, protein, fat, ash, fiber, and carbohydrates, was estimated according to the method described in (10).

Prepare defatted pumpkin seed powder: The powder was prepared according to (11) by treating soaked ground pumpkin seed powder, not soaked, and peeled (pulp only) with two types of solvents separately, one of which is Petroleum ether 40-60° C, the ratio of the seed powder / solvent used is 1: 10 (weight/volume), and the second is a mixture of chloroform/methanol in a ratio (3: 1 volume/volume). The ratio of the seed powder/solvent used is 1: 10 (weight / volume) with continuous stirring by the magnetic mixing device for 24 hours, then the solvent was separated using a central centrifuge and the sediment was taken and dried at room temperature, and then stored in an airtight bottle at the refrigerator temperature until Usage. A protein measurement test was performed for all the defatted samples (soaked, not soaked, peeled pulp only) to select the best solvent and form for use later in the study, using the standard Microkjeldal method (10) and the protein percentage was extracted by multiplying the percentage of nitrogen in the sample at a conversion factor of 6.25.

Preparation of protein isolate: The protein isolate of defatted pumpkin seed powder (peeled) was prepared according to the method mentioned by (12), then lyophilized the sample and stored until use. The total weight of pumpkin seed powder for protein extraction is classified as W_1 (g). After the sample was extracted and freeze-dried, the weight was classified as W_2 (g). The extraction yield was calculated according to the following equation: (13)

$$\text{Extraction yield of pumpkin seed protein isolate}(\%) = W_2/W_1 \times 100\%.$$

Production of protein hydrolysate

Enzymatic hydrolysis by pepsin enzyme: Pepsin was obtained from the German company Merck and the enzyme solution was prepared according to the method reported by (14) with some modification, by mixing 5 g of protein isolate with 100 ml of buffer solution Glycin-HCl (0.1 mol / l) at pH 3, then pepsin enzyme was added at a concentration of 1%. Where the enzyme was added: substrate (E: S) in a ratio of 2: 100 (volume/volume), then the mixture was placed in a shaking incubator at a temperature of 37°C at different times (½ h, 1h,1 ½ h, 2 h) and its symbols are (A1, A2, A3, A4) respectively. After each time has elapsed, the reaction is stopped by boiling at 100°C for 5 minutes. The samples are then centrifuged at 14500 rpm for 5 minutes at 4 °C, separating the filtrate from the precipitate. The filtrate is collected and kept on ice until use.

Enzymatic hydrolysis by trypsin enzyme: Trypsin was obtained from the American company Sigma, following the method described by (15) with some modifications. 5 g of protein isolate was dissolved in 100 mL of Tris-HCl buffer solution (0.1 mol/L) at pH 8. Then, the Trypsin enzyme was added at a concentration of 1%. Where the enzyme was added: substrate (E: S) in a ratio of 2 100 (volume/volume). Then, the mixture was placed in a shaking incubator of 200 rpm/minute at a temperature of 35°C at different times (2 h, 3 h, 4 h, 5 h) and its symbols are (B1, B2, B3, B4) respectively, and after each time has elapsed, the reaction is stopped by boiling at 100°C for 5 minutes, then the centrifugation process was carried out at a speed of 4000g for 30 minutes, at 4°C separating the filtrate from the precipitate, taking the filtrate and preserving in cooling until use.

Enzymatic hydrolysis by a mixture of two enzymes: Pepsin, then trypsin, symbol as Ma: Five g of protein isolates were mixed with 100 ml of buffer solution Glycin-HCl (0.1 mol / L) at pH 3, then the pepsin enzyme at a concentration of 1% was added: substrate E:S in a ratio of 2: 100 (volume/volume). Then, the mixture was placed in a shaking incubator at 37 °C for ½ hour. The pH of the mixture was adjusted to 8, and trypsin enzyme was added at a concentration of 1%. Then, the mixture is incubated again in the shaking incubator for 2 hours. The reaction is stopped by boiling at 100°C for 5 minutes, and then the centrifugation process is carried out at 4000g for 30 minutes, separating the filtrate from the precipitate. The filtrate is refrigerated until use.

Trypsin, then pepsin, symbol as Mb: 5 g of protein isolate was dissolved in 100 mL of Tris-HCl buffer solution (0.1 mol/L) at pH 8. Afterward, the Trypsin enzyme was added at a concentration of 1%. Where the enzyme was added: substrate (E: S) in a ratio of 2:100 (volume/volume). Then, the mixture was placed in a shaking incubator at 37°C for 2 hours. The pH of the mixture was adjusted to 3, and pepsin enzyme was added at a concentration of 1%. Then, the reaction was incubated again in the shaking incubator for ½ hour. The reaction was stopped by boiling at 100 °C for 5 minutes, and the samples were centrifuged at 14500 rpm for 5 minutes to separate the filtrate from the precipitate. The samples were then kept in the cold until use.

Determination of Amino acid analysis for protein isolate and hydrolysates: The percentage of amino acids was estimated using an Amino acid analyzer for each sample after each time of enzymatic hydrolysis by an enzyme (pepsin, trypsin and mixtures of enzymes) as well as the proportion of amino acids to the protein isolate without adding enzymes (as the protein isolate was dissolved 5 g in 100 ml of buffer solution Tris-HCl (0.1 mol / L) at pH 8, and again with 100 ml of buffer solution Glycin-HCl (0.1 mol / L) at pH 3 (as samples for comparison), as 10 µL were taken from each sample and injected into the device. According to the method used in the Environment and Water Department / Ministry of Science and Technology, by using the Sykam/Germany device, the conditions for injection into the device were as follows:

“Mobile phase: mix buffer (methanol+acitonitral).”
“Flow Rate: 2 ml/min”
“Temperature: room temperature.”
“Detection: OPA +FMOC (340nm, 450nm).”
“The dimensions of the column used were 4.6 x 150 mm.”

Determination of Total Protein for Protein Isolate and Hydrolysates: The total protein was determined according to the Bradford method (16) using a standard curve for a BSA (bovine serum albumin) solution with different concentrations (0.031, 0.062, 0.125, 0.25, 0.5) mg/mL, prepared with Coomassie brilliant blue G-250.

Determination of the Degree of hydrolysis for protein isolate and hydrolysates: The degree of hydrolysis of the samples was determined according to the method described by (14) with some modification, as 0.5 ml of the supernatant obtained after the hydrolysis process and then 0.5 ml of TCA (0.5 mol/liter) was added to it, and the mixture was incubated for 30 minutes at 4°C, then a centrifugation was carried out at a speed of 14500 rpm/min. The filtrate was taken, then the protein concentration was determined by (16), and the degree of hydrolysis was calculated according to the following equation:

$$\textbf{DH (\%)} = \frac{\textbf{Proteins TCA}}{\textbf{Proteins total}} \times \mathbf{100}$$

Study of the functional properties of enzymatic hydrolysates and protein isolate: The functional properties of all the above hydrolysates were studied in addition to the protein isolate whose symbol was IP, and two samples were added to it, one of which was a protein isolate dissolved in a Glycin-HCl buffer solution at pH 3, which was symbolized as IP3, and the other sample was a protein isolate dissolved in a buffer solution Tris-HCl at pH eight which is symbolized as IP8.

Solubility: The solubility was determined according to what was stated in (14) with some modification, by using different levels of pH, where the weight of 10 mg of the sample was dissolved in 1 ml of each of the following, respectively:

Glycine/HCl buffer (0.1 Mol/L) (pH 3).
Acetate buffer (0.1 Mol/L) (pH 4, pH 5).
Phosphate buffer (0.1 Mol/L) (pH6, pH7, pH8).
Carbonate buffer (0.1 Mol/L) (pH 9).

The samples were then mixed with a magnetic stirrer for 1 hour. The solutions were centrifuged at 14500 rpm for 10 minutes at 4 °C. The supernatant was taken, and the soluble protein was determined by (16), and the solubility was calculated according to the equation:

$$\textbf{solubility}(\%) = \frac{\textbf{Dissolved protein concentration}}{\textbf{The concentration of the Original protein (10 mg/ml)}} \times \mathbf{100}$$

Emulsifying Activity and Stability Index (EAI, ESI): The emulsifying properties of all samples were determined according to the method described in (14). Then the Emulsifying Activity and Stability Index were determined based on the equation:

EAI(m2/g) = (2.303 ×2×100×A) / (C×0.25×10.000)

Where EAI(m2/g) = Emulsifying Activity, A= Absorbance at 500nm, C= the protein concentration 0.001 (g/ml).

ESI = A0 ×t/(A0 –A10)

Where ESI = Emulsifying Stability Index, A0= Absorbance at 0min, t= after homogenization, A10 = Absorbance at 10 min.

Foaming Capacity and stability (FC, FS): Foam capacity is the percentage increase by volume as a result of whisking. Capacity and foam stability were determined for the samples according to the method described in (14). Then the stability of the foam was determined at all times. The capacity of the foam was calculated according to the following formula:

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

RESULTS

Prepare defatted pumpkin seed powder: Table (1) shows the chemical composition of soaked and unsoaked pumpkin seeds including moisture (4.30,4.43)%, protein (20.78, 24.5)%, fat (36.0, 32.8)%, ash (3.33)%, fibers (11.35,12.0) and carbohydrates (24.24,22.94)%, respectively.

Table (1): The chemical composition of soaked and unsoaked pumpkin seeds

Contents%	Soaked pumpkin seeds %	Unsoaked pumpkin seeds %
Moisture	4.30	4.43
Protein	20.78	24.5
Fat	36.0	32.8
Ash	3.33	3.33
Fibers	11.35	12.0
Carbohydrates	24.24	22.94

Table (2) shows the percentage of protein in defatted pumpkin seeds (whole, soaked and unsoaked) using two types of solvents, one of which is a mixture of chloroform/methanol (1:3 volume/volume) and the other is petroleum ether 40-60°C as the seeds exceeded (whole un soaked)) by the percentage of protein on the seeds (whole soaked) when using petroleum ether solvent, as the protein content reached 37.12% and 34.12%, respectively, Whereas, the protein ratio for the two types of seeds above, but using chloroform/methanol mixture (1: 3 volume/volume) instead of petroleum ether was 33.25 and 32.15% respectively. Whereas the peeled, defatted, and not-soaked seeds, using petroleum ether, outperformed the rest of the test sample in terms of protein content, which was 53.15% for this reason, this type of seed was used in the preparation of protein isolate later, as it is a rich source of protein.

Table (2): Determination of protein in defatted pumpkin seeds, soaked, unsoaked, and peeled

Seed treatment	Type of solvent	Protein(%)
whole soaked	chloroform / methanol(1:3v/v)	32.15
Whole unsoaked	chloroform / methanol(1:3v/v)	33.25
whole soaked	petroleum ether (40-60° C)	34.12
Whole unsoaked	petroleum ether (40-60° C)	37.12
Peeled unsoaked	petroleum ether (40-60° C)	53.15

Preparation of protein isolate: The protein isolate prepared from the defatted peeled pumpkin seeds is shown in (Figure 1) where it was found that every 100 grams of dried peeled seeds gives approximately 59 grams dry weight of defatted seed powder, this weight of the powder when used to prepare the protein isolate gives approximately (18 grams dry weight) protein isolate, meaning that every 100 grams of peeled raw seeds provides 18 grams of protein isolate, and it was found that the percentage of protein in this protein isolate is 72.406%.

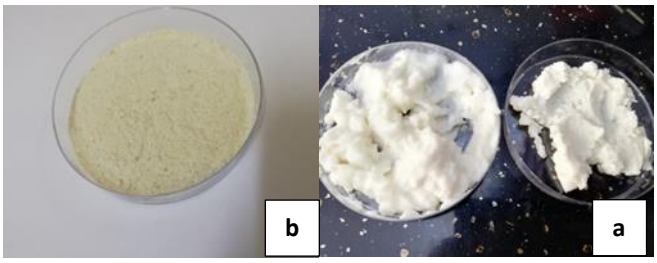

Figure (1) a: the protein isolate before drying, b: the protein isolate after drying

Production of protein hydrolysate: The essential and non- essential amino acids resulting from enzymatic hydrolysis using the enzyme pepsin at pH 3, and trypsin at pH eight both separately and at different times (Table 3), with its comparison with the protein isolate dissolved once in the buffer solution at pH 3, and again in the buffer solution at pH 8. As for the hydrolysates resulting from enzymatic hydrolysis using the pepsin enzyme, the

enzymatic hydrolysate of pepsin after 1/2 hour of decomposition contains the most significant number of essential and non- essential amino acids compared with the other decomposers shown in the same table as 14 amino acids of the total 16 amino acid and met the highest concentration among them, with a concentration of 106.444ppm. The results of the enzymatic hydrolysis procedure of the protein isolate using the mixture of enzymes Ma (pepsin + trypsin) and Mb (trypsin + pepsin) and comparing it with the protein isolate dissolved in pH (3 and 8) separately to show the effect of dissolution in a medium suitable for the enzyme action (Table 4).

Table (3): The essential and non-essential amino acids were obtained from the enzymatic hydrolysis of the protein isolate from pumpkin seeds using the enzymes pepsin and trypsin at varying times.

	A.A.(ppm)	IP pH3 IP_3	Pep ½h A_1	Pep 1h A_2	Pep 1.1/2h A_3	Pep 2h A_4	IP pH8 IP_8	Try two h B_1	Try 3h B_2	Try 4h B_3	Try 5h B_4
Essential amino acid	1. Histidine	38.724	-	9.682	26.308	14.644	13.238	-	-	13.789	-
	2. Isoleucine	0.069	12.666	7.523	43.840	-	4.889	14.260	14.761	51.549	3.303
	3. Leucine	-	7.539	-	-	8.563	-	-	-	3.098	-
	4. Lysine	-	19.621	-	37.489	235.131	0.943	18.337	30.102	3.103	-
	5. Methionine	82.526	106.444	-	-	76.646	11.120	-	22.250	-	-
	6. Phenylalanine	-	-	5.342	4.129	43.549	-	9.276	9.988	12.180	0.633
	7. Threonine	151.916	42.757	66.490	94.800	114.592	16.673	17.004	18.869	-	12.717
	8. Valine	-	35.969	41.269	179.388	66.783	0.022	0.004	10.077	21.878	24.835
Nonessential amino acid	9. Arginine	95.693	58.371	47.320	551.231	264.605	11.752	47.153	-	-	-
	10. Cystine	151.546	53.862	1.839	3.298	-	24.315	4.521	-	10.737	
	11. Glycine	174.976	37.309	13.763	85.126	47.451	53.837	20.406	25.307	16.023	16.550
	12. Tyrosine	0.080	64.070	8.067	-	-	0.455	0.537	0.773	-	-
	13. Alanine	-	24.694	-	-	20.890	-	-	-	-	-
	14. Aspartic acid	-	12.339	-	4.071	-	311.076	1.011	0.747	0.129	-
	15.Glutamic acid	364.024	8.189	33.525	262.773	9.762	105.990	136.593	125.641	194.433	57.305
	16. Serine	25.268	92.025	64.176	105.154	-	-	29.801	31.276	19.651	3.775

* The numbers (1-8) represent the essential amino acids.
* The numbers (9-16) represent the non-essential amino acids.

Table (4): Essential and non-essential amino acids obtained from the enzymatic hydrolysis of the protein isolate of pumpkin seeds using an enzymatic mixture (Ma) (pepsin + trypsin), (Mb) (trypsin + pepsin).

	A.A. (ppm)	IP pH3	IP pH8	Pep+Try M_a	Try+Pep M_b
Essential amino acid	Histidine	38.724	13.238	237.407	359.102
	Isoleucine	0.069	4.889	3206.786	3618.948
	Leucine	-	-	226.159	407.177
	Lysine	-	0.943	525.940	480.354
	Methionine	82.526	11.120	2531.651	357.984
	Phenylalanine	-	-	-	83.512
	Threonine	151.916	16.673	-	2881.240
	Valine	-	0.022	735.914	8857.121
Nonessential amino acid	Arginine	95.693	11.752	8.083	-
	Cystine	151.546	24.315	232.645	185.384
	Glycine	174.976	53.837	84.713	133.152
	Tyrosine	0.080	0.455	11.838	0.394
	Alanine	-	-	8.079	12.940
	Aspartic acid	-	311.076	7.346	52.460
	Glutamic acid	364.024	105.990	126.767	175.543
	Serine	25.268	-	506.425	768.924

Degree of hydrolysis and total protein concentration of protein isolate and enzymatic hydrolysates: The protein isolate dissolved in the buffer solution with pH 3 and 5 had a higher protein concentration than other hydrolysates (Table 5).

Table (5): Total protein concentration and degree of hydrolysis of protein isolates and hydrolysates from pumpkin seeds.

Sample	Total protein concentration mg/ml	Degree of hydrolysis%
IP3	1.06	72.40
A1	0.95	92.50
A2	0.92	94.75
A3	0.96	88.59
A4	0.95	91.71
IP8	1.02	64.37
B1	0.99	89.36
B2	0.98	88.87
B3	0.96	89.21
B4	0.99	87.49
Ma	0.81	87.17
Mb	0.61	99.40

Functional properties of enzymatic hydrolysates and protein isolate: Solubility:

Table (6): Solubility of protein isolates and hydrolysates from pumpkin seeds at different pH levels

sample	Solubility %						
	pH3	pH4	pH5	pH6	pH7	pH8	pH9
IP3	37.35	35.75	35.19	4.70	2.72	3.57	4.51
A1	41.61	41.09	15.72	9.22	9.01	11.84	21.48
A2	47.18	41.69	25.85	13.46	12.28	15.72	17.66
A3	50.02	46.90	18.30	12.06	12.27	17.36	18.51
A4	45.14	43.77	19.68	10.36	8.06	11.52	12.67
IP8	10.04	8.97	4.97	4.09	2.63	3.12	3.51
B1	49.66	49.06	36.92	4.11	4.61	6.52	5.81
B2	48.45	48.05	34.68	4.08	6.83	9.07	9.48
B3	61.10	56.02	42.04	6.11	6.11	6.31	7.35
B4	57.38	57.18	12.10	4.33	4.84	5.44	6.55
Ma	34.82	34.63	16.97	11.19	9.96	11.93	13.40
Mb	29.44	26.69	7.44	6.14	4.36	4.85	7.11

Each number is the rate of three repetitions.

Emulsifying Activity and Stability Index (EAI, ESI):

Table (7): Capacity and stability of the emulsifier of protein isolates and hydrolysates from pumpkin seeds

Sample	EAI(m2/g)	ESI(min)
IP3	146.28	193.65
A1	164.15	125.49
A2	164.89	255.71
A3	161.21	257.35
A4	165.81	300
IP8	159.18	360
B1	163.60	103.25
B2	162.86	157.85
B3	158.07	128.05
B4	156.60	354.16
Ma	11.60	157.5
Mb	14.37	65

Foaming Capacity and stability (FC, FS):

The highest value of the foam capacity of the hydrolysate resulting from the use of the pepsin enzyme was reached after 1 ½ h of the reaction (A3) and it was 54% at the pH used in this experiment, which is 7, followed by the Ma with foam capacity (52%) (Table 8).

Table (8): Capacity and stability of foam for protein isolates and hydrolysates from pumpkin seeds

Sample	Foam capacity (%)	Foaming stability after				
		1 min	*10 min*	*30 min*	*60 min*	*90 min*
IP3	0	-	-	-	-	-
A1	10	10	2	2	2	2
A2	8	8	4	4	4	4
A3	54	46	4	4	4	4
A4	30	24	4	4	4	4
IP8	0	-	-	-	-	-
B1	4	4	4	4	4	4
B2	6	6	6	6	6	6
B3	6	6	6	6	6	6
B4	14	10	0	0	0	0
Ma	52	34	16	14	10	6
Mb	16	6	2	0	0	0

DISCUSSION

Prepare defatted pumpkin seed powder: A researcher indicated that the percentage of protein in defatted pumpkin seeds peeled (Cucurbita maxima) using petroleum ether (40-65° C) was (42.75%), this difference in protein ratios in Table (1 and 2) may be due to the difference in the variety (17) and the method (18), and releasing fats will increase protein ratio.

Preparation of protein isolate and hydrolysates:

The appearance of four essential amino acids in the protein isolate dissolved in the pH 3 (IP3) are (His, Ile, Met and Thr) where Thr was the highest concentration among them, with a concentration of 151.916 ppm in addition to the appearance of six non-essential amino acids in the same isolate (Arg, Cys, Gly, Tyr, Glu, Ser) and the amino

acid Glu with the highest concentration among the total essential and non-essential amino acids in the isolate itself, as its concentration reached 364.024ppm. (Table 3)

The defatted protein meal in the Cucurbita moschata and the hydrolysates resulting from its hydrolysis using enzymes (Alcalase, Flavourzyme, Protamex, Neutrase) contain several essential amino acids ("Isoleucine, Leucine, Lysine, Tryptophan, Histidine, Threonine, Valine, Methionine, cysteine, Tyrosine, phenylalanine") and nonessential ("Aspartic Acid, Glutamic Acid, Serine, Glycine, Arginine, Alanine, Prolamine") with concentrations that vary according to enzymatic hydrolysis (19,20).

As shown in Table (3), the enzymatic hydrolysis of pepsin after (1, 1 ½, 2 hours), many essential and non-essential amino acids were also shown in varying concentrations. The protein isolated dissolved in buffer solution at pH 8 and its comparison with the hydrolysates resulting from the use of trypsin enzyme at (2, 3, 4, 5 hours) as the protein isolate lacks amino acids (Leu, Phe, Al, Ser) while (Phe, Ser) were found in the trypsin hydrolysates at all mentioned hydrolysis times. As for the amino acid Leu, it was detected only after 4 hours of enzymatic hydrolysis at a concentration of 3.098 ppm. It was also found that the concentration of Glu amino acid exceeds the rest of the amino acids appearing at each time of the enzymatic hydrolysis of trypsin, as its concentration (136.593, 125.641, 194.433, 57.305) at (2, 3, 4, 5 hours) respectively.

Table (4) indicates the enzymatic mixture, there was an increase in the concentration of amino acids (Ile, Val, Lys, Ser) in the two enzymatic mixtures Ma and Mb, the concentration (3206.786, 735.914, 525.940, 506.425 ppm), respectively, in the mixture Ma and (3618.948, 8857.121, 480.354, 768.924 ppm), respectively, for the Mb mixture, some of them were not present and the others were present in low concentrations in the protein isolate.

The appearance of the amino acid Phe in the mixture Mb at a concentration of 83.512 ppm was observed; it then disappeared in the mixture Ma. Also, the amino acids (Leu and Al) appear in the two enzymatic mixtures (Ma and Mb), as the concentration of Leu was (226.159, 407.177 ppm) respectively in the two enzymatic mixtures (Ma and Mb), while the concentration of Al was (8.079, 12.940 ppm), respectively in the two mixtures (Ma and Mb). The amino acid Thr also disappeared from the enzymatic mixture Ma. The enzymatic mixture Mb contained all the essential and non-essential amino acids except for the amino acid Arg.

Some researchers used a mixture of (pep + try) (w/w) E: S 1: 100 in analyzing the seeds of Seinat (Cucumis melo var. tibish) belonging to the family Cucurbitaceae and observed the presence of all the essential amino acids (21). The non-essential amino acids mentioned in the current study (Table 3), except for Al, are comparatively consistent with the results of the current research on the mixture of enzymes Mb. However, the latter differs in the presence of the amino acid Al and the disappearance of Arg. The highest concentration in the previous researcher's study was the amino acid Glu (12.00 g/100 g), followed by the amino acid Arg, which had a concentration of 9.68 g/100 g.

Degree of hydrolysis and total protein concentration of protein isolate and enzymatic hydrolysates:

The higher hydrolysis led to a lower protein concentration, depending on the type of hydrolysis and the type of enzyme used, as well as the duration of the hydrolysis. The highest degree of hydrolysis by using the pepsin enzyme was in the sample A2 (after an hour of hydrolysis), as the degree of hydrolysis rate was (94.75%), and the protein concentration (0.92 mg/ml), which is the lowest concentration in this enzyme group, while when using the trypsin enzyme The highest degree of hydrolysis was in the sample B1 and B3 (after 2 and 4 hours of hydrolysis), reaching 89.36 and 89.21%, respectively. The total protein concentration was (0.99 and 0.96 mg/ml), respectively, as the samples B1 and B3 were characterized by a higher concentration of Glu (136.593 and 194.433 ppm), respectively (Table 3).

Using the enzyme mixture, the Mb (Try + pep) had the highest degree of hydrolysis among all the studied samples (99.40%) and total protein concentration (0.61 mg/ml), which is the lowest protein concentration obtained during enzymatic hydrolysis, and this shows us that as the degree of hydrolysis increases the protein concentration decreases, and that this Mb contains a lot of essential and non- essential amino acids and in high concentrations (Table 4), as it has a high percentage of amino acids Ile, Lys, Leu, His, Met, Phe, Thr, Val, Cys, Gly, Glu and Ser and the highest essential amino acid was Val with a concentration (8857.121ppm) and the highest non-essential amino acid was Ser at a concentration (768.924ppm).

Some researchers indicated that the highest values of the degree of hydrolysis were at the end of the reaction for the enzyme pepsin and alcalase (29.0 and 27.1%) respectively, compared to the flavourzyme enzyme, which reached a final degree of hydrolysis (8.5%) at a time (120 minutes) and that the hydrolysis rate using pepsin was stable, which led to a steady increase in hydrolysis within (120 minutes), while using the enzyme alcalase and flavourzyme, the degree of hydrolysis increased rapidly in the first (30 minutes) and remained constant until the end of hydrolysis, due to the difference in time and enzyme (14). It was reported that the protein hydrolysis time of cucurbit seeds influences the degree of hydrolysis, and the degree of hydrolysis value typically increases with increasing hydrolysis time (4).

It was found that the hydrolysis of protein isolate from seint (Cucumis melo var. tibish) using an enzyme (trypsin and pepsin), as after stopping the trypsin activity, the pH was adjusting and pepsin was added, and different times were used to stop the reaction (30, 60, 90, 120 and 160min), consequently, the values of the degree of hydrolysis increased with the increase in time and ranged from (11.27 to 28.23%), the researcher also mentioned that the type of protein and the alkaline protease (such as alcalase) may give higher activity compared to neutral and acidic proteases such as (Trypsin and pepsin) (21,22).

The effect of the type of enzyme used (pepsin, trypsin, and alcalase) in the enzymatic hydrolysis of watermelon seed protein (Citrullus lanatus L) of the cucurbitaceae family where trypsin gave the highest degree of hydrolysis of (26.26%) compared to pepsin and alcalase (19.38 and 13.16%) respectively, and this is attributed to the trypsin affinity with the C-terminal sites that can generate more amino acids than the peptides (23,24).

Functional properties of enzymatic hydrolysates and protein isolate:

Solubility: The highest solubility of enzymatic hydrolysates was at pH numbers (3-4) while their solubility decreased at pH numbers (5-6), and almost was higher at (8-9), where the highest solubility rate for B3 was (61.10%) at the pH 3, while the protein isolate IP3 had the highest solubility at pH 3 (37.35%) (Table 6).

It was reported that the solubility of cucurbitin was high at acidic and alkaline pH values, but very low in the pH range of 5.0 to 7.0. Generally, the hydrolysis of proteins into smaller peptides increases solubility (14). It was indicated that pumpkin seed protein isolate PSPI (*Cucurbita pepo*) at pH values (3-8) has the lowest solubility at pH 5, which is the isoelectric point (pI) for most dietary proteins, the solubility of PSPI isolate increases as the pH of the solution increased towards more alkaline or decreased towards more acidic conditions. In contrast, with the enzymatic hydrolysis of pepsin, the solubility of the protein isolate improved at all the used pH values (8).

Some researchers said that the increase in protein solubility as a result of enzymatic hydrolysis is due to the enzyme that leads to the opening of the protein molecules and thus both polar and non-polar amino acids that were inside the protein become exposed on the surface and therefore the polar amino acids interact with water molecules through hydrogen bonds or electrostatic interference, thus increasing protein solubility (23). The solubility of the protein isolate of bitter melon (Momordica charantia) seeds of the same cucurbita family was carried out at pH (2-10) that the lowest level of solubility was at pH (4.5-5), and the highest solubility of the protein was at pH (2) (more than 80%) and in the alkaline region (7-10) it reached (62-76.5%) (25). The reason for this may be a decrease in the charged amino acids Aspartic and Glutamic in the alkaline region. A slight increase in the solubility of the hydrolysates by Alcalase in the pH range (3-8) was (60-76%). In contrast, the solubility of the hydrolysates of pepsin decreased over the entire pH range, but it remained above 90% at all pH values (4).

Emulsifying Activity and Stability Index (EAI, ESI):

The emulsification capacity was high and nearly stable for the protein isolates dissolved in buffer solutions with pH levels of 3 and 8 (IP3, IP8), as well as for all the hydrolysates produced by pepsin and trypsin. Still, we observe a decrease in their presence in the hydrolysates with the mixture of pepsin and trypsin enzymes (Mb, Ma) (Table 7). As for the hydrolysates using a mixture of enzymes, Ma (pep + try), the degree of hydrolysis was high (99.40%) (Table 4), which led to a decrease in its emulsification capacity to 11.60 (m2/g). In contrast, the highest emulsification stability was observed at 360 minutes for the protein isolate dissolved in a buffer solution with a pH of 8 (IP8), which may be attributed to the effect of pH. The isolate, followed by the stability form B4, which is obtained from the use of trypsin enzyme after 5 hours of hydrolysis, with a stability of 354.16 minutes.

In a study of the functional properties of the pumpkin protein enzyme hydrolysates (*Cucurbita pepo. Cv Olinka*) using enzymes (Alcalase, Flavourzyme, Pepsin) indicated that the emulsifying properties are related to the degree of hydrolysis as the capacity and stability of the emulsifier improved upon PH 7 and degree of hydrolysis 15%, where the highest values of EAI and ESI were (143.28 ± 3.5 m2 / g) and (87.5 minutes) respectively when the degree of hydrolysis was 9.2% with Flavourzyme, while the increase in the degree of hydrolysis affected (29-30%). Negatively, the capacity and stability of the emulsion, and this indicates that the size of the peptide molecule affects the functional properties of the hydrolysates (14). Other research used pumpkin protein isolate nanoparticles and achieved improvement in emulsification performance (27,28).

Foaming Capacity and stability (FC, FS):

The results of the foam capacity of the hydrolysate may be due to the high solubility of this form of hydrolysates at pH 7 (Table 6), as the form (A3) had the highest solubility (1.18%) compared to the solubility of the rest of the sample at the same pH when the solubility for Ma (0.81%).

The highest foam stability in the first minute of the hydrolysate pepsin enzyme A3 was 46%, but it quickly decreased to 4% at each of the time points (10, 30, 60, and 90 minutes). In contrast, the resulting hydrolysate of the enzymatic mixture Ma showed higher stability of foaming at times (10, 30, 60, and 90 minutes) compared to the rest of the study samples (16, 14, 10, and 6%, respectively). The reason may be due to the large size of the peptide, which allows for flexible membranes around the air bubbles. As for the protein isolate (IP3, IP8), it did not show any foam, and the reason may be that the type of proteins is unable to bind quickly and form bonds between the hydrophobic and hydrophilic parts.

The results of this study are somewhat consistent with (29) in his analysis of the characteristics of pumpkin seed protein from the variety Cucurbita Maxima that pumpkin seed extracts showed a weak ability to create foam, which is defined as the ability of proteins to reduce surface tension at a short time when a new surface was formed. He mentioned that the instability of the foam began before the end of the formation of the foam, and also noted that the foam breakdown observed after the formation of the foam with all ionic strength that the pumpkin seed protein extracts need to be modified to improve the stability of the foam and to serve as better functional components in food applications. While (25) indicated that the foam capacity increased for enzymatic hydrolysate using enzymes (alcalase, flavourzyme, protamex, neutrase) as it reached (63.9%, 60.3%, 61.65%, 53.1%) compared to the capacity of protein before hydrolysis (47.24%), as for the stability of the foam, the alcalase hydrolysates had high stability during the first 10 minutes. Still, the hydrolysate of the flavourzyme showed higher stability than the rest of the hydrolysates after 120 minutes, as the small size of the hydrolytic peptides allowed them to rapidly absorb into the interface between air and water, which reduces surface tension. It was reported that there was a significant increase in foam capacity ($p \leq 0.05$) for all enzymatic hydrolysates of pumpkin protein (*Cucurbita pepo. Cv Olinka*) using enzymes (Alcalase, Flavourzyme, Pepsin), where it was mentioned that the solubility of the protein contributes significantly to improving the foam of the protein, as the hydrolysates resulting from the enzyme Alcalase had high foaming properties, which were associated with the highest solubility of these hydrolysates at the used pH for the experiment was (7), where the researcher obtained a foam capacity higher than the foam capacity resulting from the hydrolysate of fish protein and wheat gluten, as for the stability of the foam also, the Alcalase hydrolysates were more stable compared to other samples, as the most stable foam was obtained after 30 minutes of hydrolysis (29.8% DH), and 55% of the initial foam was preserved after 60 minutes of leaving it at static (14,30).

CONCLUSION

The study demonstrated that pumpkin seed protein isolate and its enzymatic hydrolysates possess distinct functional properties, making them suitable for use in the food industry as protein-rich ingredients or as nitrogen sources in microbial culture media. The study recommends that future research analyze the properties of the resulting peptides in terms of their health effects and technological applications.

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انتاج معزول البروتين ومتحللاته الأنزيمية من بذور اليقطين المحلي ودراسة خصائصها الوظيفية

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الخلاصة

خلفية عن الموضوع: تعد بذور اليقطين مصدراً قيماً للبروتينات ذات النوعية الجيدة ويمكن استخدامها كمكونات وظيفية في الغذاء بسبب خواصها مثل الذوبانية، تكوين الرغوة والثباتية. تهدف هذه الدراسة الى انتاج معزول بروتيني ومتحللاته الأنزيمية من بذور اليقطين المحلية لدراسة خصائصها.

الطرق ومواد العمل: تحضير بذور اليقطين منزوعة الدهن لاستخلاص المعزول البروتيني وتحليل هذا البروتين باستعمال انزيمي التربسين والببسين بشكل منفصل ومعاً بطريقتين. جرى تقدير الحوامض الأمينية ودرجة التحلل، وكذلك جرى تقدير خصائص البروتين مثل الذوبانية، فعالية الأستحلاب وعامل ثباتيته، سعة الرغوة وثباتيتها. **النتائج:** تم الحصول على المعزول البروتيني من بذور اليقطين المحلية المقشرة وغير المنقوعة بنسبة بروتين **53.15**%. انتجت متحللات البروتين الأنزيمية من هذا المعزول بأوقات مختلفة. جرى تقدير الحوامض الأمينية الأساسية وغير الأساسية. تم تحليل الخصائص الوظيفية للمعزول ومتحللاته، مثل الذوبانية في قيم **pH** مختلفة.عند قياس سعة الاستحلاب، كان المتحلل الأنزيمي باستخدام الببسين هو الأكثر سعة، بينما كان المعزول البروتيني الذائب في الرقم الهيدروجيني 8 يمتلك اعلى ثباتية استحلاب. المتحلل الأنزيمي باستخدام الببسين اظهر اعلى سعة للرغوة والمتحلل الأنزيمي (**Ma**) باستخدام (الببسين + التربسين) معاً اظهر اعلى سعة رغوة واعلى ثباتية. **الاستنتاجات:** يمتلك المعزول البروتيني من بذور اليقطين قيم تغذوية عديدة والتي يمكن ان يستخدم في تحضير اغذية عالية البروتين للرياضيين ومصدر نتروجيني في الأوساط الغذائية لزراعة الأحياء المجهرية.

الكلمات المفتاحية : بذور اليقطين، معزول بروتيني، متحلل بروتيني، سعة الاستحلاب، سعة الرغوة.