ANTIHYPERTENSIVE AND ANTIOXIDANT FUNCTION OF ENZYMATIC HYDROLYSATE FOR BARLEY PROLAMINE

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ABSTRACT

This study was aimed to identify the role of barley prolamin (BP) hydrolysates which prepared from local barley (class Ibaa 99), prolamins isolate using pepsin and trypsin (individually - synergistically) in inhibiting the ACE 1 and as antioxidant agent. The Amino acids (AAs) content of barley protein were estimated by HPLC technique, and barley prolamin (BP) was isolated from whole barley flour (WBF) using 70% ethyl alcohol, and then purified depending IP and centrifugation. Prolamin molecular weight (M.wt) was determined using the electrophoresis technique. Barley prolamin isolate (BPI) subjected to enzymatic hydrolysis using 20U of pepsin and trypsin separately and synergistically for 8 h. Aliquot of the BPI hydrolysate was taken every 60 min to determine the degree of hydrolysis (DH%), antioxidant and antihypertensive properties. The results showed that barley protein contained 22 amino acids, and the percentage of the essential amino acids (EAA) and polar amino acids (AAs) were 35.08, 45.86 % of the barley protein composition, respectively. Prolamin constituted 24% of the total barley proteins and the BPI contained 90.5% protein. Electrophoresis pattern showed that most of the prolamin bands have M.wt about 34-55 KD. Synergistic hydrolysis of BPI gave the highest values for DH (50.68 %) after 8 h hydrolysis. The antioxidant function included the radical scavenging activity (RSA) and reducing power (RP) of hydrolysates was directly proportional to the DH%. The highest RSA value was 45% in the pepsin hydrolysates sample, and the highest absorbency for RP assay was 0.69 by synergistic hydrolysates sample after 8h. Bitter taste appeared in hydrolysates prepared by Pepsin and synergistic hydrolysis after 7h. The ACE 1 inhibition activity was proportional to the DH%, and the maximum activity reach to 81.44% after 8 h in pepsin hydrolyzed samples.

Key words: protein, amino acids, degree of hydrolysis, ACE1, antioxidant

الفعل الخافض للضغط والمضاد للأكسدة للمتحللات الأنزيمية لبرولامين الشعير

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المستخلص

هدفت الدراسة الحالية الى التعرف على دور متحللات برولامين الشعير المحلي صنف اباء99 (Ibaa 99) المحضرة من التحلل الإنزيمي لمعزول بروتين برولامين الشعير باستعمال أنزيمات البيبسين والتربسين بشكل (فردي – تآزري) في تثبيط أنزيم ACE1 وكعامل مضاد للأكسدة. تم تقدير محتوى الأحماض الأمينية في بروتين الشعير باستخدام تقنية HPLC وتم فصل وتنقية معزول برولامين الشعيرمن دقيق الشعير باستخدام 70٪ كحول إيثيلي اعتمادا على نقطة التعادل الكهربائي والطرد المركزي. قدر الوزن الجزيئي للمعزول باستخدام تقنية الترحيل الكهربائي ثم عُرض المعزول لعملية تحلل أنزيمي باستخدام 20 وحدة أنزيمية من البيبسين و التربسين لمدة 8 ساعات. سحبت أجزاء من المتحلل بعد كل ساعة وقدرت درجة التحلل والفعل المضاد للأكسدة ونسبة التثبيط لـ ACE1. بينت النتائج أن البرولامين يشكل 24% من مجموع بروتينات الشعير وأن معزول البرولامين يحتوي على 90.5٪ بروتين وكانت معظم الأوزان الجزيئية لـه بين 34–55 كيلو دالتون. أظهرت النتائج تواجد 22 حامض أميني ضمن بروتينات الشعير، وشكلت الحوامض الأمينية الأساسية والقطبية من تركيب البروتين نسبة 35.08، 45.86٪ على التتابع. أعطى التحلل الإنزيمي التآزري للمعزول أعلى درجة بعد 8 ساعات (50.68%) و كان هناك تناسب طردي بين درجة التحلل للمعزول ودورها كمضادات أكسدة (RSA+ RP), أذ كانت أعلى درجة ل RSA- هي 45 % للمعزول المتحلل بالبيبسين و أعلى أمتصاصية لفحص RP هي 0.69 للمعزول المتحلل تأزريا. ظهر الطعم المر في متحللات البيبسين و التآزري بعد 7 ساعات. ارتفعت درجة تثبيط لـ ACE 1 بشكل متوافق مع ارتفاع درجة التحلل وتقدم الوقت لتبلغ ذروته ا بعد 8 ساعات من تحلل البيبسين وكانت.81.44٪ .

الكلمات المفتاحية: بروتين، حوامض امينية، درجة التحلل ، مضادات الاكسدة، الأنزيم المحول للانجوتنسين ACE1.

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INTRODUCTION

A soy and wheat protein is the most common proteins generated from cereal plants. The demand for ancient grains is still rising even if the use of pulses proteins has recently increased rapidly (23). After wheat, rice, and corn, barley is presently the fourth most produced grain in the world. Barley has been domesticated for more than 10,000 years. More than two-thirds of the barley produced worldwide is utilized as animal feed, while 16% is used for human consumption. Due to the many health benefits associated with soluble fiber glucans over 2% and the other phytochemicals it contains, recently barley has gained more popularity (4). Barley prolamin protein makes up the majority of the barley protein (52%) and accounts for 87% of the main storage proteins found in the endosperm. Glutlin is a second major storage protein of the endosperm and makes up 13% of all major storage proteins. Barley also contains more than 10% others proteins. The albumin and globulin that make up the cytoplasmic proteins are secondary storage proteins which constitute for a minor portion of the barley proteins (BP) that are concentrated in the aleurone layer and the embryo (24). Barley is widely used as a raw material in the starch industry, a significant amount of protein is produced as a byproduct that can be used in the food industry (28); mixing barley into diets has been widely studied due to the presence of beta-glucans, phenolic compounds that reduce cholesterol and blood glucose levels (27). Hydrolysis processes play a major role in improving functional protein and antioxidant properties which are important in foods, pharmaceutical and industries (10). Angiotensin- converting enzyme (ACE 1) (EC 3.4.15.1) is a central component of the renin–angiotensin system (RAS), which controls blood pressure by regulating the volume of fluids in the body. It converts the hormone angiotensin I to the active vasoconstrictor angiotensin II. Therefore, ACE 1 indirectly increases blood pressure by causing blood vessels to constrict. Various types of ACE 1 inhibitors are widely used as pharmacological drugs for the treatment of high blood pressure to cardiovascular problems (28). In the absence of information on the role of prolamin proteins in lowering blood pressure and their antioxidant role, this study aimed to investigate the inhibitory role of enzymic hydrolysate of barley prolamine against ACE1 and as natural antioxidant agents.

MATERIALS AND METHODS

Barley flour preparation

Barley (class Ibaa 99) sample was obtained from the Ministry of Agriculture / agricultural research center; they were grown in north Baghdad – Iraq, on 2021. Barley sample was grinded according to Abadi & Naser, (2) with Brabender Laboratory mill after conditioning to 14 % moisture for 30 hours, then stored in a plastic container at 4 ºC for further analysis. The whole barley flour was defatted at low temperature by conventional hexane extraction with ratio of 1:6.

Hippuryl -L- histidyl- Lleucine (HHL), Angiotensin converting enzyme (ACE 1) 2 unit, Hippuric acid (HA) , tri nitro benzene sulfonic acid (TNBS), DPPH, pepsin, trypsin were purchased from Sigma. All other chemicals were of analytical grade. Barley flours' approximate composition was determined using AOAC methods 17(1), using 5.7 as the protein coefficient. Total carbohydrate was calculated by difference.

Amino acid analysis

Amino acids were analyzed according to the Jajić *et al.,* (12) method, using High performance liquid chromatography (HPLC) technology. With multiple pumps, multiple photodetectors, and C18 separating column, the first mobile phase (Na_2HPO_4), the second mobile phase was mixture from acetone nitrate, methanol, and water in a ratio of (10:45:45) v/v., Ophthalaldehyde (OPA) as reagent. The reading Hydroxyl proline and Proline were recorded at a wavelength of 262 nm and the rest of the amino acids were recorded at 338 nm. The concentration of amino acids in the barley flour sample was calculated based on the area of each amino acid peak.

Protein fractionation: Extraction and fractionation of barley proteins were carried out according to Alu'datt *et al* method (5) with some modification. WBF proteins were extracted using five different solvents, including distilled water (D.W), sodium chloride (0.5 M), ethanol (70%), glacial acetic

acid (HAC) (50%), and sodium hydroxide (0.1 M). Figure 1 shows the proteins' extraction steps. The WBF samples were mixed with extraction solutions 1:10 (w: v) for 2 hours, and then centrifuged for 30 minutes at 10,000× g at 24 ºC. The supernatant was concentrated using rotary evaporator and lyophilized. N % in Protein fractions were determined by micro-Kjeldahl method then converted to protein using the conversion factor 5.7.

Defatted barely flour (DBF)

Water-Soluble (Albumin**)**

NaCl- Soluble=

Globulins)

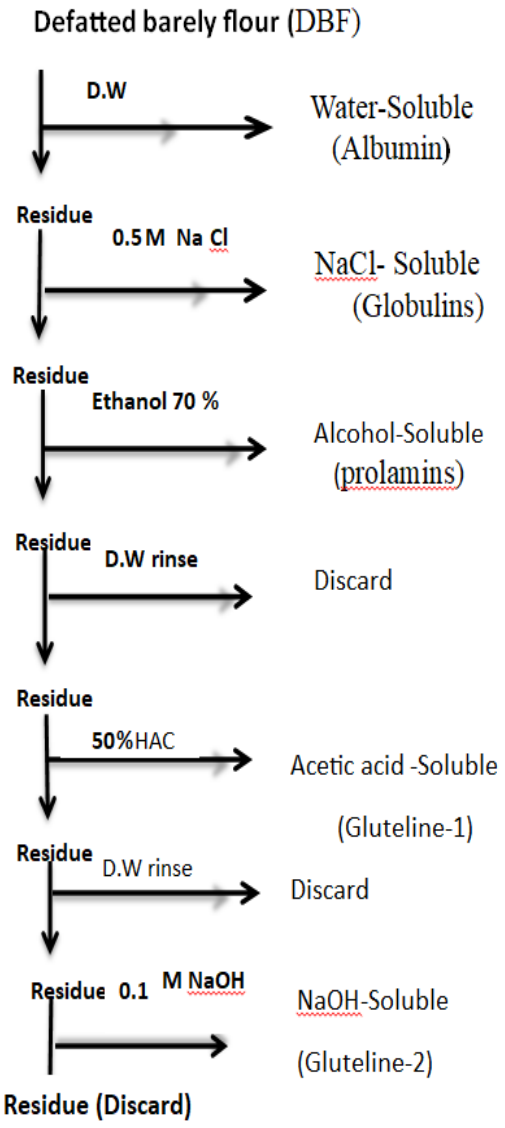


Figure 1. Flow chart for barley proteins fractionation using different solvents.

Barley prolamins Isolation (BPI): The BPI was run as described by Pinciroli *et al.* (20), based on protein isoelectric point (PI) then centrifugation. The optimal pH for prolamin precipitation was determined by mixing 10 g of extracted barley prolamin with 300 ml of distilled water (D.W), and the pH was adjusted to 12 using NaOH (1, 0.1M) solution with continuous stirring for 30 min at room temperature (25 ºC ± 2) then centrifuged at (10000 × g)for 10 min. The supernatant was collected and divided equally into 10 parts, the pH of the supernatant samples were adjusted to (2, 3, 4,5,6,7, 8, 9, 10, and 11) using HCl solution (1, 0.1M). All samples Centrifuged at (10000 × g) for 15 minutes, then the supernatant was collected, lyophilized, weighed, and the percentage of protein was determined by the Micro-Kjeldahl method.

Gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out with 1: 24 (w/v) Bis-Acrylamide/ acrylamide gels according to (21, 26).

Enzymic hydrolysis

Preparation of BPI enzymic hydrolysates using pepsin: Hydrolysis of (BPI) was carried out due to the method described by Garrett *et al.* (8). BPI was suspended in DW at a 1:50 (g/ml) ratio. The mixture's pH was adjusted to 2, and incubated at 40 °C with shaking for 30 minutes. Pepsin {(20 units) (20 mg enzyme/g BPI)} was added for the hydrolysis process, which was carried out in a shaking incubator at 37°C for 8 hours. Every an hour up to 8 hours aliquot of the reaction mixture were taken to determine the DH%, antioxidant and antihypertensive activity. Each time, the enzyme was inactivated by heating the reaction mixture to 95 °C for 8 minutes; the supernatant was collected and stored at -18 °C for additional analysis after centrifugation at (5000× g) for 10 minutes.

Preparation of BPI enzymic hydrolysates using trypsin: Trypsin hydrolysis of BPI was carried out according to Garrett *et al.* (8) method , with some modification. BPI and D.W were mixed at a ratio of 1:50 (W/V), the pH of the mixture was set at 8.5, then incubated for 30 minutes at 40 ºC while being shaken, trypsin {(20 U) (26.5 mg enzyme) /g BPI)} was used to hydrolyze the BPI at 37 °C for 8 hours in a shaking incubator. Every an hour up to 8 hours, aliquot of the reaction mixture were taken to determine the DH%, antioxidant and antihypertensive activity, as mentioned

previously in preparation of pepsin-hydrolyzed BPI.

Preparation of BPI enzymic hydrolysates using Synergistic effect: BPI hydrolysis using pepsin and trypsin synergistically was conducted according to Garrett *et al.* (8) method, with some modification. . BPI was first treated individually with pepsin (as previously described), and after 4 hours of hydrolysis, the pH adjusted to 8.5 for trypsin – assisted hydrolysis. Fractions of the hydrolysate were then collected hourly, and heated to 95°C for 8 minutes to inactivate the enzyme. Each hydrolysate aliquot was centrifuged at (5000× g) for 10 minutes, and the supernatant was collected and kept at -18°C for additional analysis.

Determination of DH (%) of BPI

DH % was estimated as described by Liu, and Chiang. (14). Standard curve of amino acid leucine was used to calculate DH% of samples, L-leucine solution (5-55mM) used to prepare the standard curve. As a part of the procedure, 250µ L of each hydrolysate was mixed with 2 mL of phosphate buffer (0.2 M, pH 8.2) and 2 mL of 0.1% TNBS which attend instantly. The reaction mixture was incubated at 50 °C for 1 hour in dark bottle in a shaking water bath. The reaction was stopped by adding 3 mL of HCL (0.1 N) and maintaining the temperature at 30°C for 30 min. After that the absorbance against water at a wavelength of 340 nm was measured. The DH (%) was calculated using the following equation:

DH (%) = [(Lt-L0) / (Lmax-L0)] ×100 Lt is the conc. of specific amino acid at time. temperature at 30 °C for 30 min. After that, the absorbance against

L0 is the conc. of specific amino acid at zero time

LMAX is the maximum conc, of specific amino acid obtained after hydrolysis the substrate using 6 N HCl at ≥110 ºC for 24 hours

ACE 1 Inhibitory activity assay

ACE 1 inhibitory activity assay of the BPI was run according to Al-Shammary and Dosh (3). Standard curve of Hippuric acid (HA) was used to estimate HA concentrates in ACE1 reaction mixture. HA solution (0.03 -0.3µ mol) used to prepare the standard curve. An aliquot (100 µL) of BPI hydrolysates (20mg/ml) was mixed with 200 µL substrate, (5 mM of HHL dissolved in 0.1 M Sodium phosphate buffer pH 8.3, containing 0.3 M (NaCl). The reaction started by adding 20 µL of ACE 1 solution (0.2 U) to the mixture at 37 ºC for 30 mints. The reaction was stopped by adding 0.250 ml of HCl (1 M), and the mixture was mixed by vortex for 20 seconds, then 2 ml of ethyl acetate was added, vortexed then centrifuged at (2000×g) for 5 min, 2mL of top layer (containing HA extracted into ethyl acetate) was taken after centrifugation, then ethyl acetate was evaporated off using vacuum oven. The residual HA was re-dissolved in 2 mL distilled deionized water before reading the absorbance at 228 nm. The amount of HA released in the absence of BPI samples was 5.6 µM in 30 mints, which was considered as total enzyme activity (100%); the following equation was used to calculate the enzyme remaining activity.

ACE 1 inhibition %= A-B/A ×100

A= concentration of released HA in the absence of inhibitors.

B= concentration of released HA in the presence of inhibitors

Anti-oxidant activity of BPI hydrolysate

The DPPH radical scavenging activity (RSA) was carried out according to Perera *et al.* (19) method. One ml of DPPH (0.1mM in 95 % ethanol) was added to 1ml of sample solution (20 mg/mL). The mixture was mixed vigorously, placed in dark place for 30 min at room temperature then centrifuged (8000×g) for 10 min. Absorbance for mixture was measured at 517nm. Butylated Hydroxyl Anisole (BHA) was used for comparison in this test. The DPPH of RSA (%) was calculated as following:

%(RSA) = [(C – (B- A)/C] × 100

A= (sample) is the absorbance reading of 1 mL of sample solution + 1 mL of DPPH (0.1-mM)

B= (blank) is the absorbance reading of 1 mL of sample solution + 1 mL of ethanol (95%).

C= (control) is the absorbance reading of 1 mL of distilled water + 1 mL of DPPH (0.1Mm).

Reducing power activity (RPA)

Reducing power (RP) experiment was carried out according to method described by Li *et al.* (13). A series of sample solutions (20 mg/mL) dilutions were prepared. Aliquot of 1mL was mixed with 2.5 mL phosphate buffer (0.2M,

pH 6.6) and 2.5ml of (1%) potassium ferricyanide. The mixtures were mixed strongly and incubated in a water bath at 50 °C± 1 for 30 mints, then 2.5mL of TCA (10%) was added to the mixture and centrifuged (5000 ×g) for 5min, 2.5 mL of supernatant was mixed with 2.5mL of D.W, then mixed with 0.3ml ferric chloride (0.1%). The absorbance of the mixture was read at 700 nm. BHT (3mg/ml) was used as comparison sample.

RESULTS AND DISCUSSION

Chemical compositions of whole barley flour (WBF): The chemical composition of WBF (class Ibaa 99) is illustrated in Table 1. The percentages of protein (14.28%) and fat (2%) in this variety were higher than that obtained by Abadi & Naser (2), the percentages of carbohydrates, moisture, ash, and fiber where 69.4%, 12%, 5.1%, and 6.15% respectively which were lower as compared to that reported by (2) for same class. This could be attributed to storage conditions, crop servicing during the cultivation stages, and the influence of environmental factors.

Table1. The chemical composition of the experimental WBF (class Ibaa 99) and barley prolamin isolates BPI

chemical composition	WPF	BPI
Fiber	5	-
Ash	4.1	-
Carbohydrate	64.32	4.36
Fat	2	-
Protein	14.28	90.54
Moisture	9.3	5.1

The chemical composition of BPI is also shown in the same table. The obtained isolate contains a significant amount of protein (more than 90%); this indicates the effectiveness of the method used to isolate the prolamine based on sedimentation and the isoelectric point (PI) at pH 4.

Fractionation of WBP

Table 2 shows the percentage of protein fractions of WBF (class Ibaa 99). The total protein (TP) content of the defatted WBF (class Ibaa 99) was 14.28 % as determined by the micro-Kjeldahl method. The total amount of proteins fractions extracted from WBF was 10.97 % which constitutes 76.82 % (w/w) of the total protein content (14.28%) and the percentage of un extracted protein was 17.5%. This means that 5.7% of the total protein content was lost during the sequential extraction processes. This is consistent with that reported by Houde *et al*. (9) who mentioned that the amount of proteins extracted from WBF or barley flour from which the outer shell has been removed, is always less than the actual amount of protein present in the whole barley flour sample because most of the protein is in the cell, and may be due to the short extraction time or the strong binding of proteins to starch. The same table explained that Glutelin-2 and Prolamine fractions were the major protein fractions, accounting for 27.3 %, 24 % of TP, respectively, while the acetic acid-soluble Glutelin-1 and Albumins percentages 7.7% &14 % of the TP, respectively; While the lowest percentage was the globulin (3.85% of TP). According to the Ciccocioppo *et al.* (6) the classification and fractionation of plant proteins on the basis of solubility is only an approximation of the actual protein composition.

Table 2. Percentage of proteins fractions extracted from WBF (class Ibaa 99) using different solutions

Fraction	Extraction solvents	Extracted protein fraction (%)	protein fraction (%) from total protein
Albumins	D.W	1.99	13.93
Globulins	Na Cl 0.5 M	0.55	3.85
Prolamin	Ethanol alcohol 70%	3.43	24.01
Glutelins1\acid	Acetic acid 50%	1.1	7.7
Glutelins2\ base	Na OH 0.1 M	3.9	27.31
Un extractable protein	-------	2.5	17.5
Loss of protein	-------	0.81	5.67
Total Extracted protein	-------	10.97	76.82
Total protein	-------	14.28	

Chemical composition of BPI

The precipitation and dissolution of prolamin at various pH levels are shows in Figure2. The solubility increased at pH values above and below 4, pH 4 was the pH where prolamine precipitated most effectively. When the pH is below the protein's PI, it is positively charged; when the pH is above the protein's PI, it is negatively charged. The values of the Protein's PI range from 4 to 12, with values between severely acidic and strongly alkaline. Along with the values of the protein's dissociation constant (pKa), this mostly depends on the type of amino acids that exist in the protein (11).

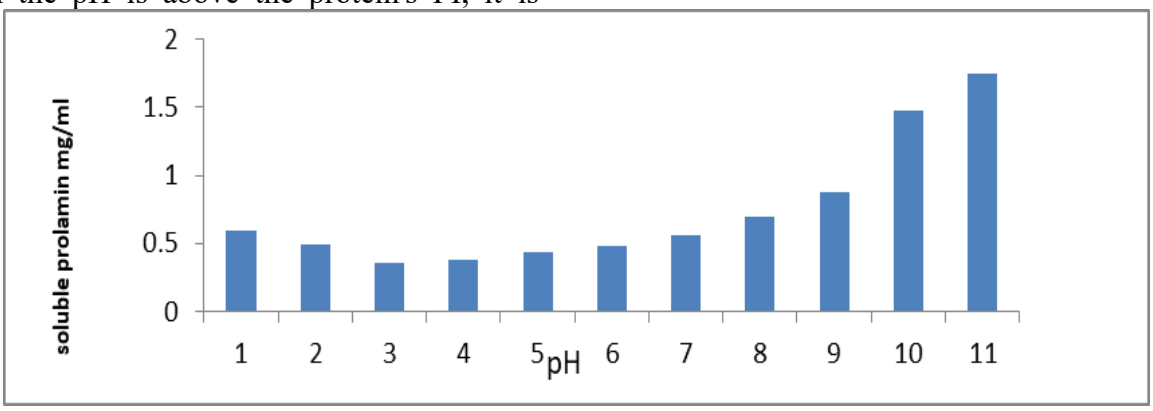


Figure 2. Optimization of pH for BPI preparation

Amino acids content of barley protein (class Ibaa 99): The percentage of amino acids (AAs) in BP is shows in Table 3. The findings indicate that the barley protein contained 22 amino acids. Glutamic acid and Proline recorded the largest percentages among the other A.AS (300 and 131.6 mg/g respectively). The proportion of essential and polar AAs in BP is 35.08 and 39.68%, respectively. According to the FAO (7), the EAA (Histidine, Tyrosine, Valine, Methionine, Tryptophan, Leucine Phenylalanine, Isoleucine, Lysine, Cystine) found in WBF protein have provided (79.3, 38,112, 95.2, 44.4, 104.4, 95.2, 121, 104.4,92.8 %) respectively, from requirement percentage of A.As per gram of the body's need from protein.

SDS-PAGE of the BPI : Figure 3 illustrates the electrophoresis assay pattern of the BPI. The electrophoresis method was used to prove the purity of the extracted prolamin, as well as to estimate its molecular weight. It has been noticed that the majority fractions of the protein has a M.wt between 34-55 kDa, which is similar to the result obtained by Mickowska *et al.* (18) when they studied M.wt of prolamin from different barley varieties were determined. Vaccino *et al.* (25) stated that barley prolamin is composed of three components: S-rich prolamin, which accounts for about 75% of the prolamin fractions with M.wt of 30-55 kDa, and S- poor prolamin, which accounts for less than 12% of total prolamin with M.wt ranging from 30-80 kDa. The proportion of heavy molecular weight prolamin to total is under 10%.

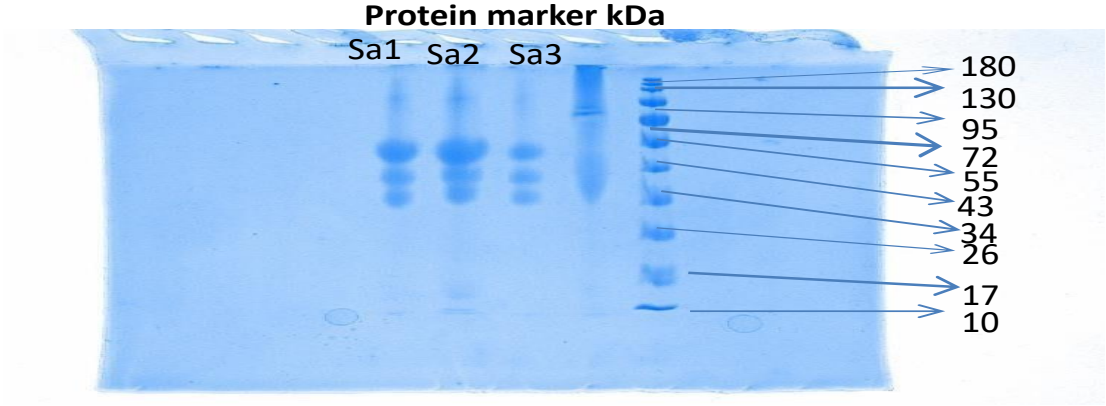


Figure 3. The electrophoresis pattern SDS-PAGE of the BPI; Sa1, Sa2 and Sa3 are three samples of extracted prolamin

Table 3. The amino acids of WBF and the percentages of EAA recommended according to FAO and WHO

Amino Acid	barely flour %	A.As in total protein mg/g	Recommendations EEA of FAO/WHO mg\g protein	overing percentage of A.As from BP to FAO requirement
Aspartic acid	0.96	67		
Glutamic Acid	4.282	300		
Asparagine	0.052	3.6		
Serine	0.41	28.7		
Glycine	0.56	39.2		
Histidine	0.182	12.7	16	79.3
Citrulline	0.053	3.7		
Threonine	0.5	35	25	14.8
Arginine	0.452	31.6		
Alanine	0.482	33.7		
Tyrosine	0.223	15.6	41	38
Valine	0.64	44.8	40	112
Methionine	0.34	23.8	25	95.2
Tryptophan	0.26	18.2	41	44.4
Phenylalanine	0.56	39.2	41	95.2
Isoleucine	0.52	36.4	30	121
Leucine	0.91	63.7	61	104.4
Lysine	0.4	28	48	58.3
Cysteine	0.2	12		
Cystine	0.332	23.2	25	92.8
Proline	1.88	131		
Hydroxy Proline	0.09	6.3		
EAA	35.08			
NEAA	64.91			
P	39.68			
NP	43.08			

EAA = Essential amino acid. NEAA = Non-essential amino acid. P= Polar amino acids. NP=Non polar amino acids. FAO (7)=

Hydrolysis of barley prolamin isolates (BPI)

Figure 4 displays the degree of hydrolysis (DH %) of BPI by two proteolytic enzymes (pepsin, trypsin, and pepsin + trypsin). Pepsin as well trypsin was used individually & synergistically to obtain an extensively hydrolyzed BPI hydrolysate. The final BPI hydrolysate obtained by hydrolysis using those enzymes represents a pool of peptides resembling those generated during the BPI digestion in the human digestive duct. It has been noticed that DH% was proportional to the time of hydrolysis and it ranged from 0.15% - 50.68% during 8 hours hydrolysis. After 8 hours of synergistic hydrolysis the highest DH was (50.68 %) and in pepsin assisted hydrolysis was (40.98%), while the lowest degree of hydrolysis was observed with trypsin assisted hydrolysis (28.17%). Bitter taste was recognized after 7and 8 hours of hydrolysis with pepsin and synergistic hydrolysis. Liu, X., *et al.* (15) reported that the main reason for the bitterness of soy protein hydrolysate were hydrophobic bitter peptides with molecular weight less than 1 kDa. As stated by to Zeece (30), bitterness in proteins is off-beat, but some amino acids and peptides can have bitter properties. Bitter - tasting amino acids include proline, valine and isoleucine. Amino acid bitterness is connected with the size, shape in addition to hydrophobic character of the R group for each amino acid.

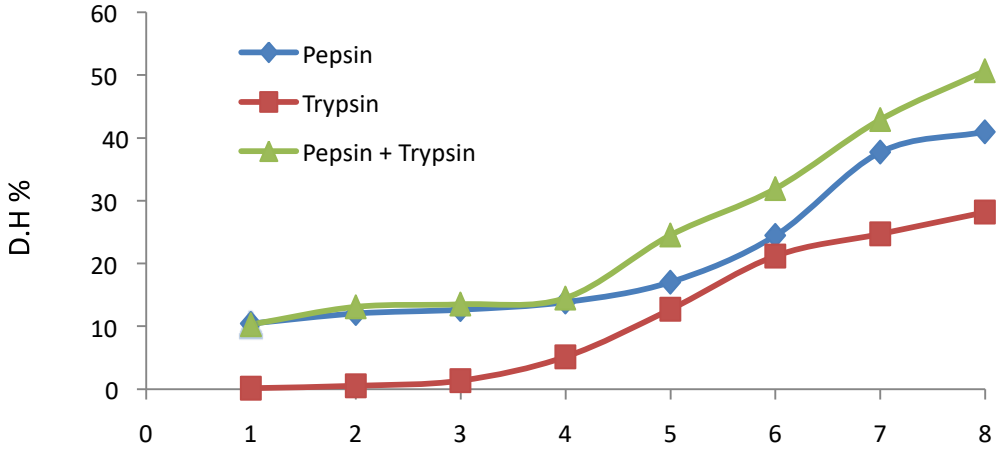
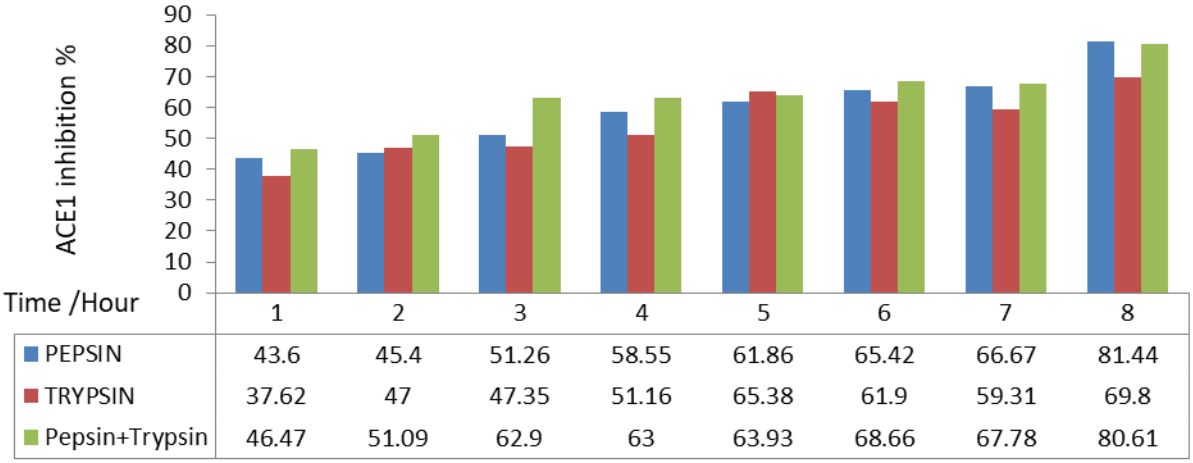


Figure 4. Degree of hydrolysis as time function for BPI by pepsin, trypsin and pepsin +trypsin. The results are the average of three replicates.

ACE 1 Inhibitory Activity of BPI hydrolysate: Figure 5 shows the ability of BPI hydrolysates in inhibit ACE1. It has been noticed that the inhibition activity increased through the hydrolysis period, and reached its maximum value after 8h of hydrolysis. The value of inhibition ranged from 37.6 % after 1 h of trypsin- assisted hydrolysis to 80.6% after 8 h of pepsin- assisted hydrolysis. Although the D.H % in the synergistic hydrolysis was higher than that of the single hydrolysis as shown in Figure 3, the degree of inhibition was higher in the single hydrolysis using pepsin. This is due to the nature of the peptides released by digestion, which are related to the type of enzyme, activity, mechanism, besides the site of action of the enzyme. These results indicate that the BPI peptides generated during pepsin hydrolysis had a greater ACE1 inhibitory activity than these produced by trypsin or synergistic hydrolysis. These results agreed with Lo, & Li. (16) findings in studying the hydrolysis of Soy Protein, where pepsin assisted hydrolysis gave peptides with a greater inhibitory role than trypsin assisted hydrolysis, this inhibitory role is due to peptides with an M.wt less than 3 KDa. Megías, *et al* (17) stated that sunflower globulin is a potential source for ACE 1 inhibitory peptides when hydrolyzed with pepsin and pancreatin after 3 hours of incubation at 37 ºC.

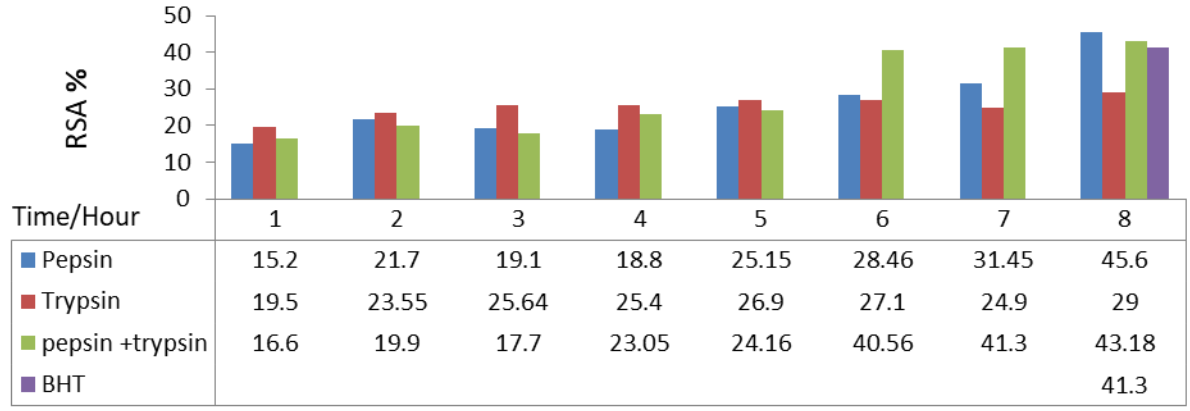


Time /Hour	1	2	3	4	5	6	7	8
PEPSIN	43.6	45.4	51.26	58.55	61.86	65.42	66.67	81.44
TRYPSIN	37.62	47	47.35	51.16	65.38	61.9	59.31	69.8
Pepsin+Trypsin	46.47	51.09	62.9	63	63.93	68.66	67.78	80.61

Figure 5. Barley protein isolates hydrolytes inhibitory activity against ACE1 during the hydrolysis period (1-8 Hours)

Antioxidant activity of the BPI hydrolysate Figure 6 illustrates the antioxidant activity of BPI hydrolysate based on radical scavenging activity (RSA) using DPPH. BHT was used as a comparison model; its free RSA was 41.3 %. The RSA of BPI hydrolysates was ranged between (45.6 %- 15.2 %), and the highest degree was after 8 hours of hydrolysis by pepsin, while the lowest RSA was after 1 hour using pepsin in hydrolysis process. For all treatments (pepsin, trypsin and synergistic digestion), there were generally an increase in

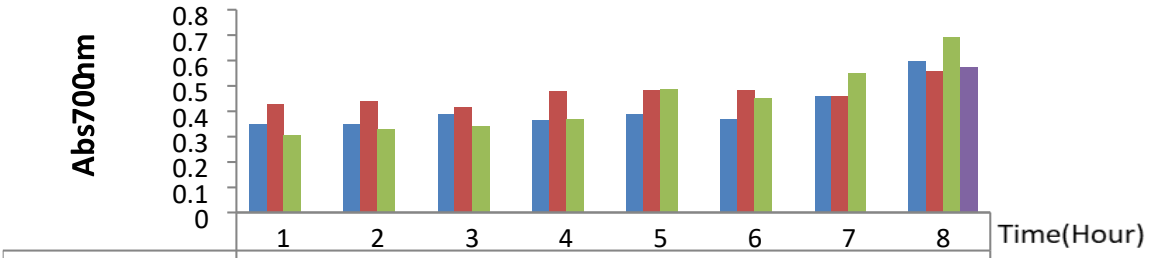
RSA % with the progress of hydrolysis. This could attributed to the variations in peptide M.wt and structure through the enzymic hydrolysis process. According to Liu & Chiang (14), the RSA of the hydrolysates are affected by the type of amino acid in peptides liberated during enzymatic hydrolysis which depends on protease specificities. Based on reducing Power (RP), Figure 7 displays the antioxidant activity of BPI hydrolysate samples.



Time/Hour	1	2	3	4	5	6	7	8
Pepsin	15.2	21.7	19.1	18.8	25.15	28.46	31.45	45.6
Trypsin	19.5	23.55	25.64	25.4	26.9	27.1	24.9	29
pepsin +trypsin	16.6	19.9	17.7	23.05	24.16	40.56	41.3	43.18
BHT								41.3

Figure 6. Radical scavenging activity (RSA) for BPI Hydrolysates. Each experiment was performed in triplicate and the arithmetic mean was used

In all treatments, the increase in the percentage of hydrolysis (% DH) resulted increased in RP. The maximum value was reached after 8 hours of pepsin- assisted hydrolysis, and the lowest value was reached after 1 hour. The absorbency in RP of BPI hydrolysates was ranged from 0.35 to 0.69. The (BHT) RP of the comparative model was 0.57. Zhao (31) claimed that electron donors, which function as reducing agents and can prevent the availability of intermediates for oxidizing fats and so limit the oxidation rate, may be primary or secondary antioxidants. RP values from a comparable test carried out on a variety of sand-roasted and microwave-cooked barley varieties by Sharma and Gujral (22) were lower than the values of this study.



	1	2	3	4	5	6	7	8
Pepsin	0.35	0.35	0.39	0.363	0.388	0.37	0.46	0.6
Trypsin	0.427	0.44	0.415	0.48	0.482	0.483	0.46	0.56
Pepsin +	0.306	0.33	0.341	0.37	0.488	0.453	0.548	0.69
BHT								0.573

Figure 7. Reducing Power (RP) activity of BPI Hydrolysates prepared using enzymes individually & synergistically

CONCLUSION

The outputs of BPI hydrolysate had the antioxidant activity (RSA and RP). This may be because prolamin contains a high percentage of non-polar amino acids (Proline), also the same outputs was effective to inhibit the ACE 1 up to 81% by producing low M.wt peptides.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

REFERENCES

1. A.O.A.C. 1995. Official methods of analysis.16th ed. Washington, DC: Association

of Official Analytical Chemists. DOI: 10.12691/ajfn-3-6-1.

2. Abadi, F. A.and J. M. Naser. 2019. Effect of wet gluten addition on stalin characteristics of barley bread. The Iraqi Journal of Agricultural Science, *50*(1): 390-397. https://doi.org/10.36103/ijas.v50i1.305.

3. Al-Shammary, O. A. and S .K. Dosh. 2020. Isolation and purification of αs2-CN local goat milk and studying its hydrolysates inhibition efficacy towards ace1. Plant Archives, *20*(1): 1917-1925. https://doi.org/10.22034/chemm.2023.366342.1618.

4. Alu'datt, M. H., K. Ereifej, A.AbuZaiton, A. Alrababah, M.Almajwal , A. Rababah, and W. Yang . 2012 .Anti-oxidant, anti-diabetic, and anti-hypertensive effects of extracted phenolics and hydrolyzed peptides from barley protein fractions. International Journal of Food Properties, 15(4):781-795. https://doi.org/10.1080/10942912.2010.503357.

5. Alu'datt, M. H., T. Rababah, K.Ereifej, I. Alli, M. A. Alrababah, A. Almajwal, and M.N.Alhamad. 2012. Effects of barley flour and barley protein isolate on chemical, functional, nutritional and biological properties of Pita bread. Food Hydrocolloids, *26*(1): 135-143. http://dx.doi.org/10.1016%2Fj.foodhyd.2011.04.018.

6. Ciccocioppo, R., D. A. Sabatino, and G.R.Corazza. 2005 .The immune recognition of gluten in coeliac disease. Clinical & Experimental Immunology, 140(3): 408-416. https://doi.org/10.1111%2Fj.1365-2249.2005.02783.x.

7. Consultation, F. E. 2011. Dietary protein quality evaluation in human nutrition. FAO Food Nutr. Pap, 29: 1-66. https://doi.org/10.1111/nbu.1206.

8. Garrett, D. A., L. M. Failla, and J. R. Sarama. 1999. Development of an in vitro hydrolysis method to assess carotenoid bioavailability from meals. Journal of Agricultural and food chemistry, 47(10):4301-4309. https://doi.org/10.1021/jf9903298.

9. Houde, M. N., Khodaei .N, Benkerroum and .S. Karboune. 2018. Barley protein concentrates: Extraction, structural and functional properties. Food Chem; 254:367-376. https://doi.org/10.1016/j.foodchem.2018.01.156.

10. Islam, M., Y. Huang, , S. Islam, , B. Fan, L. Tong, , and F. Wang, 2022. Influence of the degree of hydrolysis on functional properties and antioxidant activity of enzymatic soybean protein hydrolysates. Molecules, 27(18), 6110.

11. Kozlowski, L. P. 2016. IPC–isoelectric point calculator. Biology Directs 11(1):1-16. https://doi.org/10.1186/s13062-016-0159-9.

12. Jajić, I. S. Krstović., D. Glamočić, S. Jakšić, and B. Abramović. 2013. Validation of an HPLC method for the determination of amino acids in feed. Journal of the Serbian Chemical Society, 78(6): 839-850. http://dx.doi.org/10.2298/JSC120712144J

13. Li, X., J. Deng, ,S. Shen, T. Li, M Yuan., R Yang., and C. Ding. 2015. Antioxidant activities and functional properties of enzymatic protein hydrolysates from defatted *Camellia oleifera* seed cake. Journal of Food Science and Technology, 52: 5681-5690.https://doi.org/10.1007/s13197-014-1693-z.

14. Liu, B. L and S. P. Chiang. 2008. Production of hydrolysate with antioxidative activity and functional properties by enzymatic hydrolysis of defatted sesame (*Sesamum indicum L.*). International Journal of Applied Science and Engineering, 6(2): 73-83.

15. Liu, X., D.Jiang, and D. G. Peterson. 2014. Identification of bitter peptides in whey protein hydrolysate. Journal of agricultural and food chemistry, 62(25):5719 - 5725. https://doi.org/10.1021/jf4019728

16. Lo, W. M., and E. C. Li-Chan. 2005. Angiotensin I converting enzyme inhibitory peptides from in vitro pepsin− pancreatin hydrolysis of soy protein. Journal of Agricultural and Food Chemistry, 53(9): 3369-3376. https://doi.org/10.1021/jf048174d

17. Megías, C., M. D. Yust, J. Pedroche, H. Lquari, J. Girón-Calle, M. Alaiz, & J.Vioque. 2004. Purification of an ACE 1 inhibitory peptide after hydrolysis of sunflower (*Helianthus annuus L.*) protein isolates. Journal of Agricultural and Food Chemistry, 52(7): 1928-1932. https://doi.org/10.1021/jf034707r.

18. Mickowska, B., P. Socha, D Urminská, and E.Cieślik. 2012. The comparison of prolamins

extracted from different varieties of wheat, barley, rye and triticale species: amino acid composition, electrophoresis and immune detection. Journal of Microbiology, Biotechnology & Food Sciences, 2021: 742-752.

19. Perera, H. D. S. M., J. Samarasekera, S. M. Handunnetti, and O. V. D. S. J. Weerasena. 2016. In vitro anti-inflammatory and antioxidant activities of Sri Lankan medicinal plants. Industrial Crops and Products, 94: 610-620. https://doi.org/10.1016/j.indcrop.2016.09.009.

20. Pinciroli, M., P. Aphalo, A. Nardo, M. C. Añón, and A.V.Quiroga . 2019. Broken rice as a potential functional ingredient with inhibitory activity of renin and angiotensin converting enzyme (ACE 1). Plant Foods for Human Nutrition, 74: 405-413. https://doi.org/10.1007/s11130-019-00754-6.

21. Schägger, H. 2006. Tricine–sds page. Nature protocols, *1*(1): 16-22. https://doi.org/10.1038/nprot.2006.4.

22. Sharma, P., H. S.Gujral. 2011.Effect of sand roasting and microwave cooking on antioxidant activity of barley. Food Research International, 44(1): 235-240. https://doi.org/10.1016/j.foodres.2010.10.030.

23. Shevkani, K., N. Singh.,Y. Chen., A. Kaur, and L. Yu .2019. Pulse proteins: Secondary structure, functionality and applications. Journal of Food Science and Technology, 56: 2787-2798. https://doi.org/10.1007/s13197-019-03723-8

24. Sullivan, P., E. Arendt, and E. Gallagher. 2013. The increasing uses of barley and barley by-products in the production of healthier baked goods. Trends in Food Science & Technology, 29(2): 124-134. https://doi.org/10.1016/j.tifs.2012.10.005.

25. Vaccino, P., H. A. Becker, A. Brandolini, F. Salamini and B, Kilian. 2009. A catalogue of *Triticum monococcum* genes encoding toxic and immunogenic peptides for celiac disease patients. Molecular Genetics and Genomics, 281: 289-300. https://doi.org/10.1007/s00438-008-0412-8.

26. Wafaa, N., H. A. Elbarbary, E. M. A. Ibrahim, H. A. Mohamed, and H .Jenssen. 2022. Effect of enzyme type and hydrolysis time on antibacterial and antioxidant activity of whey protein hydrolysates. Iraqi Journal of Agricultural Sciences, 53(6): 1340-1357. https :// doi. Org /10.36103 / ijas. V5 3i6 .1650.

27. Wang, W., and E. G. De Mejia. 2005. A new frontier in soy bioactive peptides that may prevent age related chronic diseases. Comprehensive Reviews in Food Science and Food Safety, 4(4): 63-78. https://doi.org/10.1111/j.1541-4337.2005.tb00075.x.

28. Xie, X., C. Zhu, D. Wu, and M. Du. 2022.Autoregressive Modeling and Prediction of the Activity of Antihypertensive Peptides. Frontiers in Genetics, 12, 801728. https://doi.org/10.3389/fgene.2021.801728.

29. Yalçın, E., S. Çelik, and E. İbanoğlu. 2008. Foaming properties of barley protein isolates and hydrolysates. European Food Research and Technology, 226: 967-974. http://dx.doi.org/10.1007/s00217-007-0618-8

30. Zeece, M. 2020. Introduction to the Chemistry of Food. Academic Press, (PP.226-231). https://doi.org/10.1016/C2015-0-04257-5.

31. Zhao, H., W. Fan, J. Dong, J. Lu, J. Chen, L. Shan, and W. Kong.2008. Evaluation of antioxidant activities and total phenolic contants of typical malting barley varieities. Food Chemistry, 107(1): 296-304. https://doi.org/10.1016/j.foodchem.2007.08.018.