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EFFECT OF BARLEY PROLAMIN HYDROLYSATE TO INHIBIT ACE I CAPACITY

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ABSTRACT

The aim of this work was to evaluate the potential of local barley (class Ibaa 99), an underutilized plant, as a potential functional ingredient in blood pressure (BP) treatment and health promotion. For this purpose, the ability barley prolamin isolates hydrolysate (BPIH) to inhibit Angiotensin-converting enzyme I (ACE I) was studied. Prolamin was extracted from barley, then digestion by pepsin and the effect of successive purifications using gel filtration sephadex [GF-(G25)] technology on the efficiency of ACE I inhibition capacity was studied. The results showed the efficiency of the pepsin in hydrolyzing barley prolamins, and the percentage of degree hydrolyses (DH %) increased with time. The ACE I inhibition value increased in parallel with the increase in DH %, reaching the highest value (81.44%) after 8 hours. In addition, inhibition capacity value increased after purification of BPIH using GF-(G25) to reach 88.74% in the first purification then 92.36% after the second 1. Increase inhibition was proportional to rise concentration of low M.Wt peptides of the BPIH, as the M.Wt of the peptides was less than 3.484 kDa in the first step of GF-(G25) and less than 1.470 kDa in the second step of GF-(G25), with an increase in concentration the hydrophobic amino acids (A.As) in these peptides (33 , 52.4%), respectively.

Key words: **ACE I inhibiter, Amino acids, Barley, Degree of hydrolyses, Gel filtration, prolamin**

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INTRODUCTION

High BP is one of the factors that lead to multiple chronic diseases such as atherosclerosis, heart failure, stroke, and others diseases. Vascular diseases linked to the human and nutritional lifestyle, which includes an increase in the intake of table salt and obesity (Feng et al., 2017). Reducing the effectiveness of the ACE I are reducing the release of substances and compounds within specific systems, the low concentration of these substances lead to low BP (Al-Khafaji, 2008). ACE I (EC3.4.15.1) is a metal enzyme that contains zinc in the active site. The enzyme is labeled as a Di - carboxypeptidase because its main function is to cut a dipeptide (His-Leu) consisting from the C-terminus of the angiotensin I (Ang I), which consists of 10 A.A.s to convert it into an octa peptide (Ang II), which is a vasoconstrictor hormone. The action of ACE I is within the Renin-Angiotensin-system (RAS) (Aluko, 2018). Because of the negative effects associated with the formation of the Ang II, which represented by it being a vascular smooth muscle cell constrictor, stimulating hypertrophy, stimulating fibrosis of the lining of blood vessels, encouraging the formation of aldosterone, which increases the concentration of sodium in the blood, and finally causing inflammation (Hussain and Awan, 2018). Guyer and Banerji (2015) stated that more

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than 20 % of people who take chemical ACE I inhibitors are exposed to a group of side effects, such as loss of taste, high levels of potassium in the blood, skin rashes, dilatation of blood vessels, causing dry cough and skin edema. On the other hand, Ishida et al. (2011) mentioned that the use of natural compounds reduces side effects of inhibitors, and the most important of these compounds are vital natural peptides because they are highly specialized and therapeutically efficient and can give in high doses. Exposing different proteins to proteolytic enzymes can produce a group of vital peptides with functional and physiological effects, such as their effect on the nervous system, immune system, endocrine glands, and their role as anti-hypertensive agents (Korhonen and Pihlanto, 2003). The biological role of peptides depends on their type and sequence of A.As as well as their M.Wt. Bioactive peptides are often, contain a number of A.As ranging from 3-20 per peptide (Meisel and FitzGerald, 2003). Peptides resulting from enzymatic hydrolysis of rice prolamin, Sword bean, Sunflower protein, Adzuki bean seeds possess enzyme inhibitory properties to ACE I (Megías, et al., 2004; Durak et al., 2013; Zhang et a., 2018). The results of Hallfrisch et al., (2003) showed that increasing intake of foods made from whole grains for barley

MATERIALS AND METHODS

Barley flour preparation

Local barley (class Ibaa 99) sample obtained from agricultural research center of Baghdad; the barley crop produced in the Abu Ghraib district/ Baghdad for the year 2021. Barley sample was conditioning and grinding according to Kiryluk et al., (2000). Defatted barley flour (DBF) was prepared using cold conventional hexane extraction method with ratio 1:6, and then stored in a plastic container at 4 ± 2◦C for further analysis.

Materials: ACE I, Hippuryl-L-histidyl-L-leucine (HHL), Hippuric acid (H.A), Tri nitro Benzene Sulfonic acid (TNBS), pepsin and sephadex G-25 purchased from Sigma. All other chemicals were from analytical grade. Barley flours' approximate composition was determined using A.O.A.C methods 17(1997), using 5.7 as the protein coefficient. Total carbohydrate calculated by differences.

Fractionation of barley proteins and isolation of prolamin: According to the method of Alu'datt et al. (2012) and Socha et al. (2016) extraction and fractionation of barley proteins from DBF was performed with some modifications. Barley prolamin was extracted using ethanol (70%) after extracting Albumin and Globulins using distilled water (DW) and sodium chloride (0.5 M), respectively. DBF samples were mixed with extraction solutions at a ratio of 1:10 (w: v) for 2 h, then centrifuged (10.000 × g) for 30 min at 25 ± 2 °C. The supernatant was concentrated and dried using a rotary evaporator and lyophilized. Barley prolamin isolate (BPI) was prepared according to Pinciroli et al. (2019) method that based on the isoelectric point (PI) in pH 4 and centrifugation (10.000 × g) for 10 min. The sediment collected, lyophilized, weighed, and the percentage of protein estimated by Micro-Kjeldahl method, using 5.7 as the protein coefficient.

Gel electrophoresis for BPI

M.Wt and Purity of BPI was determined according to Haider et al. (2012) and Abood, and Mohammed (2017) Recommendations using Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with ratio 1:24 (w: v) Bis-Acrylamide/ acrylamide gels.

Enzymatic hydrolysis of BPI using pepsin

BPI hydrolysates (BPIH) were perform according to Chatterjee et al. (2015) described. BPI was suspend in D.W at a ratio 2:100 (g: ml). Firstly, the mixture's pH was adjusted to two, and incubated at 40 ± 2°C with shaking for 30 minutes. Pepsin was added to BPI with ratio 0.02:1 (Pepsin: BPI), the mixture was shaken at 37°C for 8 hours. An aliquot was withdrawn after every hour; the enzyme inactivated by heating the reaction mixture more than 90°C for 8 minutes; then centrifugation (5.000× g) for 10 minutes. The supernatant collected, lyophilized then stored at -18 ± 2 °C for additional analysis. The DH % and antihypertensive capacity of the eight samples performed simultaneously and under the same conditions for each experiment.

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Determination of DH % for BPIH

The DH % was measured by means of the reaction of amino groups (NH3) with TNBS, the percentage of DH % was calculated according to Chatterjee et al.(2015).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.

ACE I **Inhibition capacity assay**

The inhibitory of ACE I capacity was estimated according Al-Shammary and Dosh (2020) method. The amount of H.A calculated according to the Alu'datt et al. (2012) method. The amount of H.A released in the absence of inhibitor (BPIH) was 5.60 µM in 30 minutes, which considered as total enzyme capacity (100%); the following equation used to calculate the enzyme remaining capacity. ACE I inhibition % = A-B/A ×100

A= concentration of emitted H.A in the absence of inhibitor (BPIH).

B= concentration of emitted H.A in the presence of inhibitor (BPIH) = The reaction time that gave the highest ACE I inhibition was taken and the enzymatic hydrolysis process was repeated under the same conditions to obtain a sufficient amount of BPIH for use in subsequent experiments. According to Raymond et al. (1984), employing a 3.5 kDa cutoff bag for dialysis, high M.Wt peptides retained whereas those less than 3.5 kDa filtered out of the dialysis bag. The filtrate collected, lyophilized, and then used in a GF - (G25) experiment.

Column Chromatography GF- (G25) of prolamin hydrolyzates: Gel filtration GF-(G25) steps performed as Hameed (2012) described. A column of sephadex G-25 (57x1.5cm) was employed after being buffered and washed with 0.2 M Sodium phosphate buffer (pH 7.0), with ionic strength (0.2M) of Na Cl. Sephadex G-25 was prepared according to the Pharmacia Company recommendations; degassing process was carried out for 10 minutes before pouring the column. The column equilibrated with the same buffer at a flow rate of 30 mL/h. The column calibrated with blue dextran (~2,000 kDa) to estimate the column void volume (V_O). Blue dextran dye (BDD) and Glucagon Hormone (G.H) (3.484 kDa) were passed in column at a concentration of 1 mg/ml, the tubes were collected and absorbance was determined at wavelength of 620 nm and 214 nm, respectively. A distribution coefficient (Kav) values for fractions estimated according to Risan (2012) equation:

$$\mathbf{Kav = Ve - V_O / Vt - V_O}$$

Where:

Kav = A distribution coefficient

Ve = Elution volume for fractions

V_O = Column void volume (elution volume for BDD)

Vt = Total bed volume

The lyophilized BPIH dissolved in buffer solution (0.1 g/ml) and a volume of 1.0 ml passed into the column. Fraction parts descending from the column collected under the same conditions, and the absorbance measured at a wavelength of 280 nm. Each peak parts were collected separately as shown in the graph (Figure 3), lyophilized, DH % and ACE I Inhibitory capacity were measured separately with 20 mg/ml and 5 mg/ml concentration of protein (BPIH) soluble for each test respectively.

Repeat the gel filtration (G25) experiment

After conducting an ACE I inhibition test for the peaks that appeared in the gel filtration, the part which give the highest inhibition rate were selected, and the gel filtration process was repeated with the same conditions for that part. The absorbance was measured at a wavelength of 214 nm .Each parts were collected separately as shown in the graph (Fig 4), lyophilized, DH % and ACE I Inhibitory capacity were measured separately as previously measured in first GF-(G25).

Determination of M.Wt for fractions using the RP-HPLC technique: The M.Wt of the peptides fractions that gave high inhibition capacity, which separated by GF-(G25) technique and lyophilized estimated according to Alu'datt et al. (2023) method. Dissolve 50 mg of lyophilized sample in 0.250 mL of D.W (pH 7), then filter the mixture using a Millipore filter (0.2 micron). An RP-HPLC column measuring (5 × 4.6 × 150) C_{18} produced by Cecil \ adept U.K Company was used. Separation conditions included a liquid

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phase of 5:95 (v: v) solution A: B which was filtered with a Millipore filter (0.45 micron). Solution [A] was consists of D.W free of ions with 0.1% formic acid, and solution [B] was consists of 0.1% formic acid mixed with 5% acetonitrile v: v. Fifty microliters of the sample was injected into the device after raising the temperature to 50°C at a flow rate of 0.5 ml/min. The readings recorded at a wavelength of 214 nm. Following substances were using to create an M.Wt standard curve H.A (197.8 Dalton), HHL (429.17 Dalton), and vitamin B_{12} (1355.37 Dalton). All substances standard exposed to the same conditions as the analyzed model. The readings recorded at a wavelength of 228, 214, and 363 nm, respectively.

Amino acid analysis

A.As analyzed according to the Lestari et al. (2022) method with some modification; using High-performance liquid chromatography (HPLC) technology. The HPLC device was equipped with multiple pump, column C_{18} ODS (150 × 4.6 Id), 5 µm particles size and photodetector type UV-VIS, and the readings were recorded at 254 n.m. First mobile phase was Sodium acetate (0.14 M, pH 6 with 0.05 % trimethylamine) and the second one was mixing from acetonitrile-water in a ratio (60:40) v: v. Volume Injection was 20 µL at room temperature (25 ± 2 °C), Derivation was methanol: tri ethylamine: water: Phenyl isothiocyanate in ratio 7.5: 1: 1: 0.5. 30 µL of the above derivative were added to each model after the extraction process.

RESULTS AND DISCUSSION

Chemical compositions of barley prolamin isolate (BPI): The chemical composition of BPI was illustrate a high percentage of protein (90.54 %), a low percentage of moisture (5.1) and carbohydrates (4.36), while fat, ash and fiber were not detected (N.D); this indicates the effectiveness of the defatting procures and sedimentation procedure in the potential isoelectric point (PI) at pH 4.

SDS-PAGE of BPI

Figure 1 shows the electrophoresis test pattern for BPI. Electrophoresis technique used to prove the purity of the BPI as well as estimate its M.Wt. The results showed that the majority of protein fractions had M.Wt between 34-55 kDa with little protein fractions having lower M.Wt, which is similar to the result obtained by Mikowska et al. (2012) when they studied the M.Wt of prolamin from different varieties of barley.

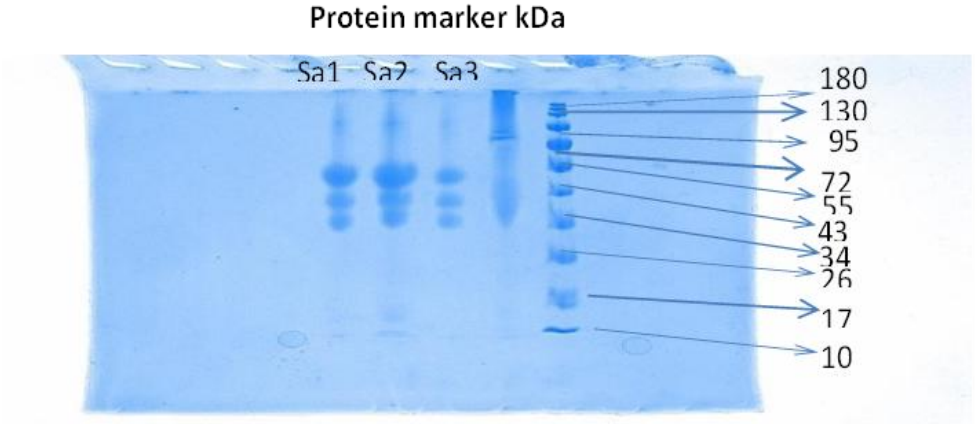

Figure 1. The electrophoresis pattern SDS-PAGE of the BPI; Sa1, Sa2 and Sa3 are three samples of extracted BPI, ST: Standard ladder

Enzymatic hydrolysis

Figure 2 shows the DH % of BPIH with pepsin assisted. The results show an increase in the percentage of DH % with increasing hydrolysis time, reached the highest value (40.98%) after 8 hours from incubation. The increase in the concentration of released amine groups (NH3) to 35.07 mM (estimated based on the straight-line equation for amino acid L-leucin) is a reflection of the increase in DH %. The bitter taste appeared in BPIH after 7 hours from incubation. Liu et al. (2014) mentioned that the main reason for the bitter taste is the increased concentration of peptides with low M.Wt (less than 1 kDa) that rich of hydrophobic A.As.

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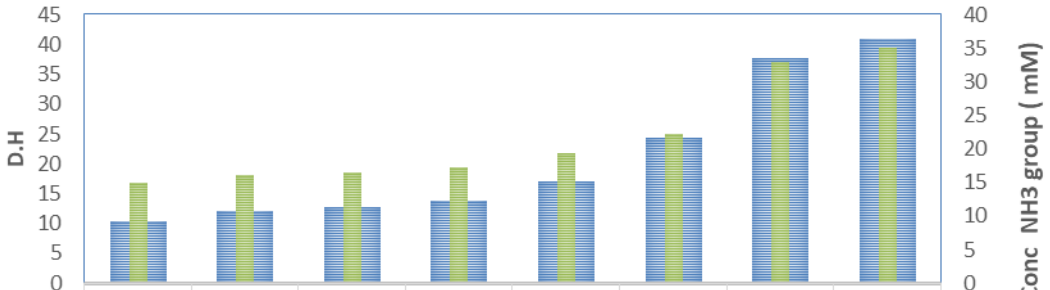
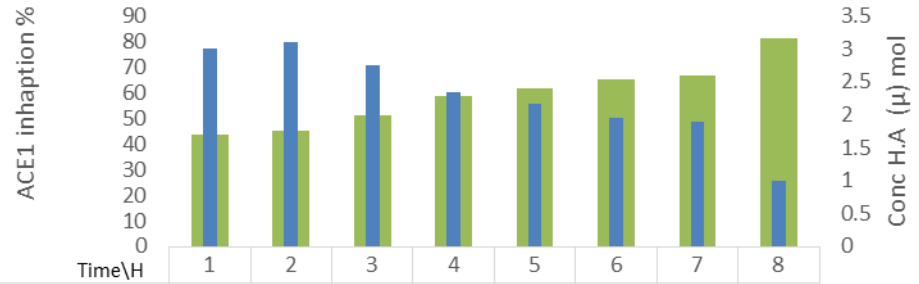


	1	2	3	4	5	6	7	8
Pepsin .DH %	10.44	12.04	12.65	13.86	17.04	24.44	37.72	40.98
Peptides Conc. (terminal NH3 group)	15.02	16.07	16.47	17.26	19.35	22.24	32.93	35.07

Figure 2. Degree of hydrolysis and NH3 concentration (mM) as time function for BPI by pepsin digestion

ACE I Inhibition capacity for BPIH

Figure 3 shows the ability of BPIH to inhibit ACE I capacity. It reveals that the inhibition capacity increased through the hydrolysis period, and reached its maximum value after 8 h of hydrolysis .On the other hand, there is decrease in the amounts of H.A released from the enzyme reaction with the substrate (HHL), noting that the total amount of H.A released in this reaction in the absence of the inhibitory agent (BPIH) was 5.68 µ mol, The value of ACE I inhibition ranged from 43.6 % after 1 hour to 81.4% after 8 hours of pepsin- assisted hydrolysis, while the of ACE I inhibition % in a similar study conducted by Alu'datt et al. (2012) was ranged between 73-87 %. The results of total H.A released reflected the degree of ACE I inhibition which decreased from 3.1µmol in the first hour to go down to1 µmol after 8 hour from incubation.



Time\H	1	2	3	4	5	6	7	8
ACE1 inhaption %	43.6	45.4	51.26	58.55	61.86	65.42	66.67	81.44
Conc HA µ mol	3.01	3.1	2.76	2.35	2.16	1.96	1.89	1

Figure 3. Percentage of ACE I inhibition and total concentration of H.A released

Purification of ACE I inhibitory peptides using Gel filtration chromatography (GFG25): To assess the ACE I inhibitory capacity of the BPIH, purification of peptides from BPIH was undertaken using GF-(G25) column. Fractions F1, F2 and F3 collected then lyophilized as shown in Figure 3. The figure shows that the fractions parts appeared after BDD and G.B appearance that means they have M.Wt less than 3.484 KDa. This reflects the efficiency of the dialysis bags (3.5 kDa cutoff) that used to separate low M.Wt peptides from high one. Risan (2012) stated that gel filtration chromatography appears to be an effective method for proteins separating because it separates proteins by size. Large molecules that cannot enter the Sephadex gel pores excluded to descend at the beginning time, while small molecules that enter pores left to descend relatively late. Therefore, this analytical method may be a useful for discrimination in the separation and fractionation of proteins based on M.wt.

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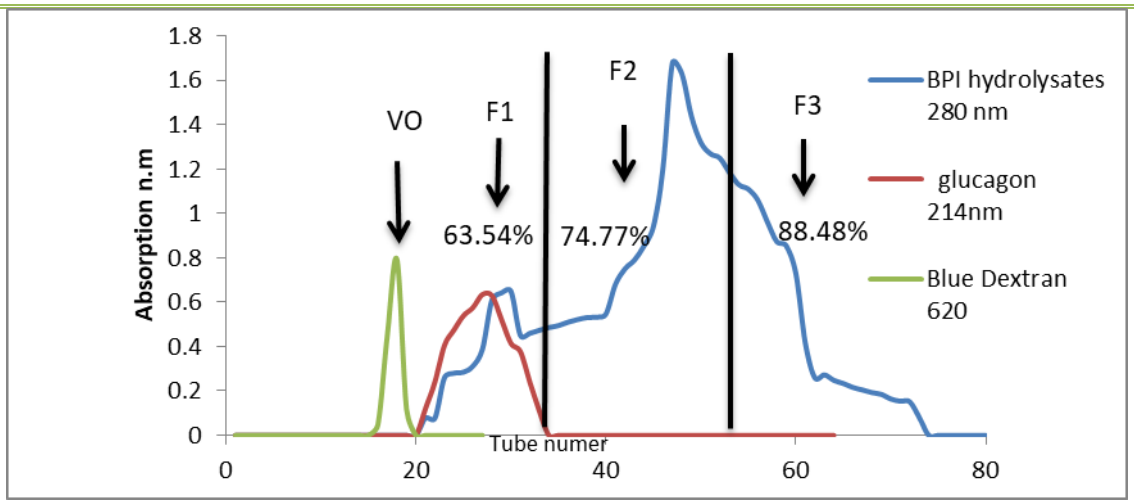

Figure 3. Sephadex (G 25, 57x1.5cm column) gel filtration chromatogram of BPIH prepared by pepsin assisted. Separation was performed at a rate of 30 ml / 60 minutes and collected with 2 ml \ tube, Green line is BDD for Void volume (Vo); red line indicates to Glucagon hormone (G.H), blue line indicates to separated fractions (F1, F2 and F3) for BPIH

Table 1 Indicates the ACE I inhibition rate and the concentration of terminal amino groups (NH3) present in the peptides. All separated fractions have an inhibition rate, and the highest value was at F3 (88.74%), which contains peptide fragments with low M.Wt less than F1 and F2. This is consistent with the concentration of the terminal NH3 group's results, where the concentrations of NH3 increased as the M.Wt decreased, F3 fraction was the highest concentration of the terminal NH3 (56.31Mm).

Table 1. ACE I inhibition capacity and Peptides Conc. (terminal NH_3 group)

Fractions / Type of test	F1	F2	F3
ACE I inhibition capacity %	63.35±1.5	74.2± 1.5	88.74±1.3
Peptides Conc. (terminal NH3 group)	50.9±2.4	54.11±1.7	56.31±1.2
Kav	0.31	0.9	1.33

Purification of ACE I inhibitory peptides using second-time gel filtration chromatography: Figure 4 shows the peptides purification from the F3 fraction using GF-(G25) a second time to evaluate ACE I Inhibition capacity for its parts . Because no clear peaks were visible, the tubes were divided to obtain the F fractions (I, II, III, and IV).

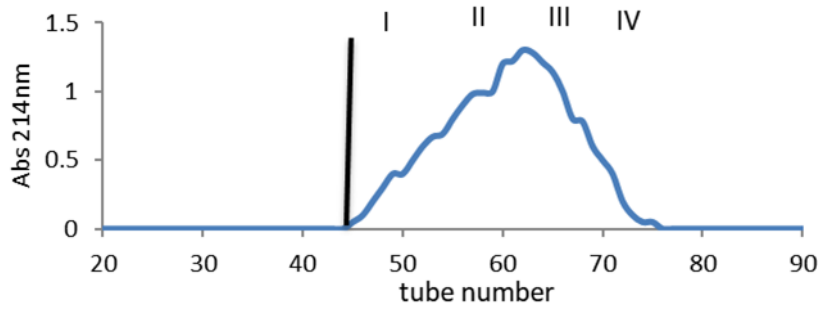

Figure 4 . Sephadex G-25 (57x1.5cm column) gel filtration chromatogram of fractions F3. Separation performed at a rate of 30 ml / 60 minutes and collected at a fraction volume of 2 ml / tube. Separated fractions were (F3 - I, F3 - II, F3 - III and F3 - IV).

Figure 5 represent of ACE I inhibitory capacity for F fractions. It shows increasing of inhibition capacity of ACE I with the decrease of the peptides M.Wt which obtained from

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GFG25 (second time), reached highest value (92.36%) in the last fraction (IV), with significant differences with the other fractions (I, II and III). According to Aluko (2015) small peptides (di-, tri- or tetra peptides) are known to have the highest ACE1 inhibitory capacity. Considering these results, we selected F3- IV fraction for further fractionation by RP-HPLC Technique.

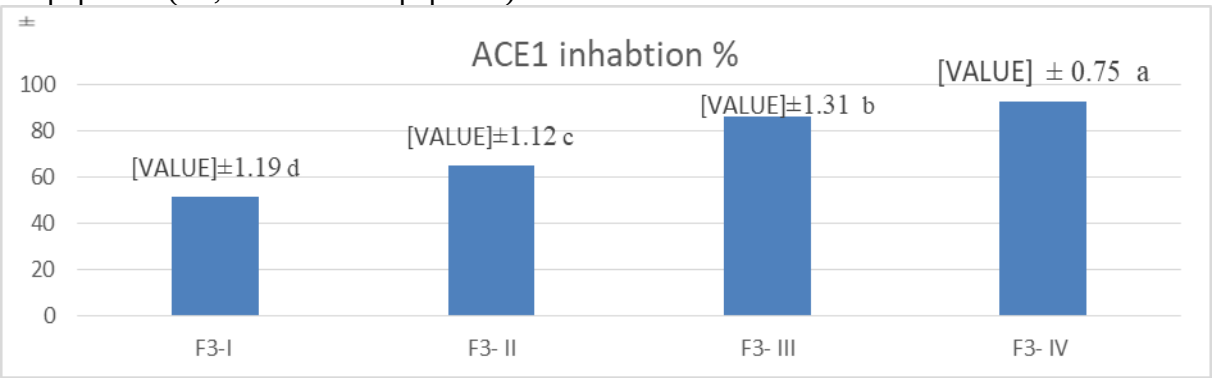
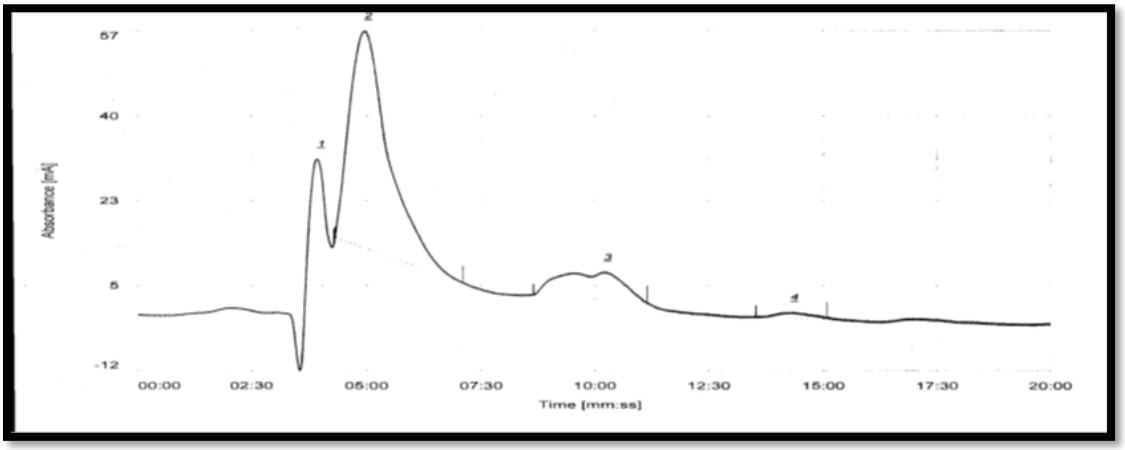


Figure 5. ACE I inhibitory capacity for F fractions

Figure 6 (a, b) shows the chromatogram obtained using RP-HPLC for fraction F3-IV. HA, HHL, and vitamin B12 retention time (R.T) was also appear in the same Figure. It is clear from (Figure 5 a, b), that the F3-IV fraction consists from four main parts and its peptides have M.Wt between 369.46 -1470.30 Dalton. The majority peptides (67.18%) had an M.Wt between 637.1 and 1470.30 Dalton. The ACE I inhibitory capacity of the F3-IV fractions is the result of mixture peptides effect.

a



b

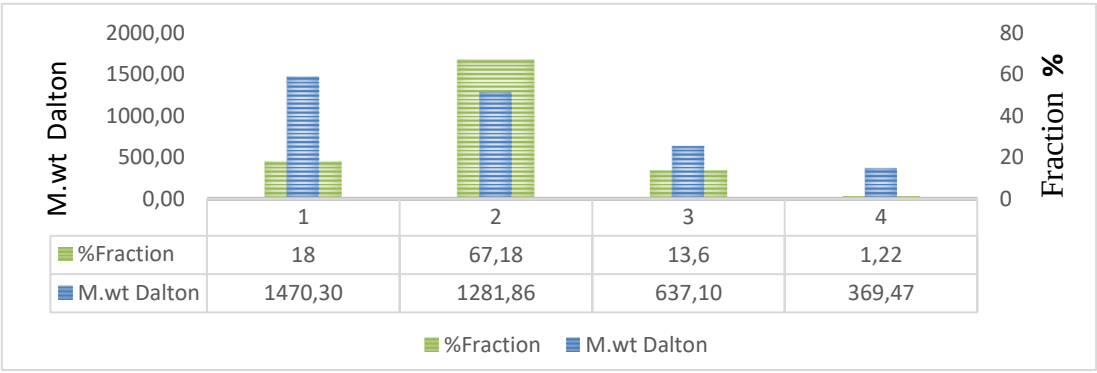


Figure. 6 a: is a RP-HPLC chromatogram for the F3-IV fraction. Arrows indicate to the R.T for; 1: Vitamin B12 (1.355 kDa); 2: HHL (429.17 kDa); 3: Hippuric acid (H.A) (0.179 kDa). Fig. 5 b: is an M.Wt of the fractions shown on the chromatogram and the concentration of each one

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The percentage of amino acids (A.As) in the samples: Table 4 shows the percentage of A.As in whole barley flour (WBF), BPI, F3 fraction and F3-IV fraction. The results showed that hydrophobic A. As was constitute (36.8, 33%) of WBF and BPI, while hydrophilic amino acids constitute (10.31, 12.32%) of them, respectively. Glutamic acid and Proline recorded highest percentages among the other A.As. This is consistent with what Arendt and Zannini (2013); Murkowski et al. (2012) found, when they studied barley protein .The percentage of hydrophobic A.As in F3 and F3-IV fractions increased to 52.4% and 66.88 %. On the other hand, the percentage of hydrophilic A.As for those fractions decreased to reach 9.85% and 6.2%, respectively. Increase and decrease of A.As content in these parts is due to the effect of purification processes to isolate some proteins or peptides with high M.Wt, in addition to the pepsin role in cutting the A.As chains (Xing et al. 2023; Risan (2012). This may be the reason for rise ACE I inhibition value for these parts, which rose to 88.74 then 92.36%, although the concentration of the inhibitory factor was reduced to quarter, which concurrent with the increase in the percentage of hydrophobic acids, especially proline. These results were consistent with Kitts and Weiler (2003) previous results, and also showed that hydrophobic A.As are among the most influential factors on the binding of peptides to the active site of ACE I, especially when they are at the C-terminus. Kohama et al. (1988) and Li et al. (2004) stated that the digestion of plant proteins can enhance the production of antihypertensive peptides especially, low M.Wt peptides, doubles and triples, which contain in their parts a hydrophobic A.As, including Leu-Lys-Pro, Leu-Gly-Pro, and Asp-Leu- Pro.

Table 4. The percentage of amino acids in whole barley flour (WBF), BPI, F fraction and F3-IV fraction.

Amino acid	WBF %	BPI %	F3 fraction%	F3-IV fraction%
Asp	6.722	1.2	2.4	2.7
Glu	30	35.5	16.56	13.6
Asn	0.364	5.3	1	ND
Ser	2.871	3.7	1.636	ND
Gln	1,61	0.9	4.1	0.5
His	1.274	2.57	6.2	2.47
Arg	3.165	2.61	5.3	1.12
Thr	3.5	2.51	3.22	0.08
Ala	3.375	2.51	5.8	7.56
Pro	13.8	14.86	21.01	26.81
Tyr	1.561	4.71	4.1	6.11
Val	4.482	3.8	9.6	10.4
Ile	3.641	3.52	3.11	5.15
Leu	6.372	5.64	3.55	5.5
Phe	3.922	3.09	2.02	3.11
Trp	1.82	4.4	6.2	6.53
Lys	2.8	0.67	1.7	2.4
Gly	3.92	1.31	1.5	4.06
Met	2.38	0.8	1.1	1.82
Cys	2.381	0.4	0.9	ND
Total	100	100	100	100
Hydrophobic amino acids %	36.8	33	52.4	66.88
Hydrophilic amino acids %	10.31	12.32	9.85	6.2
Acidic amino acids%	36.72	36.7	18.96	16.3
Basic amino acids %	7.24	5.85	13.2	16.7

Hydrophobic A.As: Ala, Pro, Val, Met, Ile, Leu, Phe, and Trp. Hydrophilic A.As: Ser, Thr, Cys, and Tyr. Acidic A.As: Asp and Glu. Basic A.As: His, Arg, and Lys. ND = not detected

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CONCLUSION

We conclude that the lower M.Wt in peptides and increased concentration of hydrophobic A. As improves the efficiency of ACE I inhibition capacity. Therefore, BPIH is a promising component of peptides with good biological properties.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

The authors declare that this manuscript is original, has not been published previously, and is not currently under consideration by any other journal. All figures and tables are original and prepared by the authors. Any material obtained from third parties has been included. Author/s signature on Ethical Approval Statement. The present study did not involve human subjects or the use of experimental animals; therefore, no ethical or animal welfare approval was required.

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AUTHOR'S CONTRIBUTION STATEMENT

All authors made equal contributions to the study design, methodology, experimental work, data analysis, and manuscript writing. All authors reviewed and approved the final version of the manuscript.

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تأثير متحللات بروتينات الشعير على تثبيط انزيم ACE I

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المستخلص

كان الهدف من هذا العمل هو تقييم أمكانية الشعير المحلي (صنف إباء 99)، كغذاء وظيفي محتمل في علاج ضغط الدم. تمت دراسة قدرة متحللات معزول بروتينات الشعير (BPIH) على تثبيط الإنزيم المحول للأنجيوتنسين I (ACE I). تم أولا، استخلاص بروتينات الشعير وهضمهِ بواسطة أنزيم البيبسين ودراسة تأثير عمليات التنقية المتعاقبة باستخدام تقنية كروموتكرافي الترشيح الهلامي باستخدام هلام السيفادكس (G25)–GF G25 على كفاءة تثبيط أنزيم ACE I. بينت النتائج كفاءة البيبسين في هضم وتحليل بروتينات البروتينات. ازدادت درجة التحلل المائي(DH %) مع تقدم الوقت و ارتفعت قيمة تثبيط ACE I بالتوازي مع زيادة DH% لتصل إلى أعلى قيمة لها (81.44%) بعد 8 ساعات من الهضم الأنزيمي. أرتفعت كفاءة التثبيط بعد تنقية BPIH باستخدام (G25)–GF لتصل إلى 88.74% في التنقية الأولى ثم 92.36% بعد التنقية الثانية. وكانت زيادة التثبيط متناسبة مع ارتفاع تركيز البتيدات المنخفضة الوزن الجزيئي، حيث كان الوزن الجزيئي البتيدات أقل من3.484 كيلو دالتون في الخطوة الأولى من الترشيح الهلامي وأقل من1.470 كيلو دالتون في الخطوة الثانية من الترشيح ، مع ارتفاع تركيز الأحماض الأمينية الكارهة للماء في هذه البتيدات ليصل ألى 33% و% 52 على التوالي.

الكلمات المفتاحية: مثبطات ACE I، الشعير، الأحماض الامينية، درجة التحلل، الترشيح الهلامي، البروتينات