FUNCTIONAL PROPERTIES OF ENZYMETICALLY MODIFIED WHEAT GLUTEN

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ABSTRACT
This study was aimed to investigate the potentiality of gluten inclusion into functional foods. The effect of controlled enzymatic hydrolysis on the antioxidant properties of Pepsin , Trypsin and Papain-assisted wheat gluten hydrolysates have been studied. Lyophilized and dried gluten from durum wheat, commercial durum gluten and whey proteins were enzymatically hydrolyzed. Based on antioxidant activity of the obtained hydrolysates, papain hydrolysed gluten were selected for this study. Functional properties (water holding capacity, emulsifying capacity and stability, foam formation and stability, protein solubility, and oil binding capacity) were investigated for the selected samples. Results revealed that the enzymatic modification improved the functional properties of all selected proteins significantly (P<0.05), with the superiority of the lyophilized and dried wheat gluten in some functional properties especially in alkaline pH and pH 4. .

Key word: antioxidant properties , pepsin , trypsin , papain, gluten hydrolysates.
Part of M. Sc. Thesis of the 1st author.

الخصائص الوظيفية لكلوتين الحنطة الصلبة المحور إنزيميا"

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المستخلص:
هدفت الدراسة الحالية إلى بحث إمكانية استخدام الكلوتين في الأطعمة الوظيفية ، تمت دراسة تأثير التحلل الأنزيمي للكلوتين بفعل إنزيمات الببسين والتربسين والبابين على خاصية المتحللات الإنزيمية كمضادات أكسدة طبيعية . أستخدم في هذه الدراسة كلوتين القمح الصلب المجفف و كلوتين القمح المجفد بالإضافة إلى الكلوتين التجاري للقمح الصلب واستعملت بروتينات الشرش للمقارنة. تم اختيار متحللات الكلوتين الناتجة من فعل أنزيم البابين لإكمال هذه الدراسة بناءً على نشاطها المضاد للأكسدة، وتم فحص خواصها الوظيفية المتمثلة (قابلية مسك الماء ، قابلية الاستحلاب وثبات المستحلب ، تقدير تكوين وثبات الرغوة ، قابلية الذوبان وقابلية مسك الدهن). أظهرت النتائج أن التحوير الأنزيمي قد حسن من الخصائص الوظيفية لجميع البروتينات المختارة بشكل ملحوظ (P <0.05) ، مع تفوق الكلوتين المجفد والمجفف في بعض الخصائص الوظيفية وخاصة عند الأرقام الهيدروجينية القلوية (9–12) وكذلك عند الرقم الهيدروجيني 4.

الكلمات المفتاحية: الخصائص المضادة للأكسدة ، ببسين ، تربسين ، بابين ، متحللات الكلوتين.
جزء من رسالة الماجستير للباحث الأول.

***Received:27/8/2019, Accepted:7/11/2019**

INTRODUCTION

Wheat is considered one of the most important and essential cereal crops worldwide, in terms of utilization and production. In Mediterranean countries, the durum wheat are used in several bakery industries such as macaroni and bread, and to guarantee the high production of durum kind of wheat, several native and global programs are designed in this line (15). In the later years, cereals and their ingredients accepted as functional foods because they provide proteins, dietary fiber, vitamins , energy, antioxidants, and minerals that required for human health. Wheat products may be the most common functional foods in the future, its total global annual product near 600 million tons (11). Wheat is contain two main types of proteins: gluten proteins (represent (85%) of total wheat proteins) which include simple gliadin (50-55%) and gluteinin polymer (45-50%) the rest of wheat protein are non – gluten proteins which represent 15% of total wheat proteins these are include globulin (40%) and albumin(60%) (7). Gluten is an important protein for many technological food applications especially bakery ones and is also responsible for the viscoelasticity of flour dough. It also plays an essential role in conferring the unique baking quality of wheat by cohesively, improving the capacity of water absorption, ,elasticity and viscosity of dough (24 ; 30). The natural wheat protein can be modified to improve its quality and nutritional value (9). The enzymatic modification which considered the most safety method to get a good functional and nutritional characteristic (25). Hence it has been used to improve the solubility of wheat gluten, methods using several enzymes such as pepsin, trypsin, and papain (11). This study was designed to investigate the functional properties of durum wheat gluten before and after enzymatic medication by trypsin, pepsin, and papain enzymes.

MATERIALS AND METHODS

Wheat samples: The durum wheat (*Triticum durum*)used in this study was native (Smeto) variety, grown at Mosul region in 2018.

Enzymes: Enzymes were used Trypsin (Fluca, Switzerland), pepsin (Sigma, Germany), and papain (BDH, England).

Preparation of wheat gluten: The wet gluten was extracted from durum wheat (*Triticum durum).* Wheat grains were conditioned to 14 % moisture before milling. AACC method No. 10-38 (1) was used for gluten extraction and estimation from flour.

Chemical analysis: Proximate compositions of all wheat and flours were studied using AOAC methods (3). Total carbohydrate was calculated by difference.

Enzymatic treatment of wheat gluten

1. Papain treated wheat gluten:

Gluten hydrolysates were prepared using papain, according to Bandyopadhyay and Ghosh (4) with some modifications. The gluten was mixed with distilled water in ratio of 1:20 and the pH was adjusted to 10 with NaOH (0.1M) and incubated at 50˚C for 1 hour until the protein completely dissolved . The pH re-adjusted to 8 using hydrochloric acid (0.1M), and incubated for 15 minutes at 37˚C. 2000 & 3000 units per 1g of gluten was added individually , and incubated at 50˚C for 7hr. Aliquot of the hydrolysates were taken after (1,2,3,4,5,6,7)hrs., and the reaction terminated by placing the samples in boiled water bath for 5 minutes, centerfugated at 5000xg for 15 minutes. The supernatant collected and stored at (-18 ˚C) until use.

2. Trypsin treated wheat gluten

Gluten protein hydrolysis was carried out using trypsin enzyme, according to Liu and Chiang (19) with some modifications. The gluten was mixed with distilled water in ratio of 1:20 and the pH was adjusted to 8 with NaOH (0.1M). The mixture was incubated at 50˚C for 1hour until the protein completely dissolved, then incubated at 37˚C for 15 minutes .The enzyme was added at different concentrations (4000 & 5000 units per 1g of gluten) and samples were taken after (1, 2, 3, 5, 6, 7)hrs, placed in boiling water bath for 5 minutes for enzyme inactivation and centrifuged at 5,000x g for 15 min. The supernatant collected and kept at (-18˚C) until use

3.Pepsin treated wheat gluten

Gluten hydrolysis was conducted, using pepsin, according to Chatterjee et al. (7) with some modifications. The gluten was mixed with distilled water in ratio of 1:20, pH was adjusted to 2 by HCl (0.1M). The mixture was

incubated at 50˚C for 1hour until the protein completely dissolved and was incubated at 37˚ C for 15 minutes. Different concentrations (4000 & 5000 units per 1g of gluten) of pepsin was used for gluten hydrolysis after (1, 2, 3, 5, 6, 7) hrs samples of hydrolysates were taken and placed in a boiling water bath for 5 minutes for enzyme inactivation then centrifuged at 5,000x g for 15 min. The supernatant was collected and kept at (-18˚ C) until use .

Determination the Degree of Hydrolysis (DH): The degree of hydrolysis was tested according to Liu & Chiang (19). The standard solution of L- Lucien (55Mm) was prepared by dissolving 0.361g L-Lucien in small amount of distilled water and the volume was completed to 50 ml. The required concentrations were prepared as show in Table 1.

Procedure

To 0. 250 ml of each of the above solutions , 2 ml of SDS(1%) and 2 ml sodium phosphate (0.2125 M) at pH 8.2 and 2ml of TNBS solution (0.1%) were added. The mixture was incubated at 50˚C for 1hour at dark place . The reaction was stopped by adding 4 ml of HCl solution (1M) . The samples were kept at room temperature for 30 minutes and the absorbency was read at 340 nm. The standard curve was plotted as the relation between the concentration of the L-Lucien and the absorbance reading at 340 nm.

Table 1. L-Lucien concentrations used in standard curve of the degree of hydrolysis determination

Concentrations (mM)	Stock solution(ul)	D . W (ul)	Final Volume (ul)
0	0	1000	1000
5	50	950	1000
15	150	850	1000
25	250	750	1000
35	350	650	1000
45	450	550	1000
55	550	450	1000

The studied samples (0.250 ml of each) were transferred to a test tube and subjected to the above steps. NH_3 groups were calculated using the standard Lucien amino acid curve and the degree of degradation was calculated according to the following equation (14):

$$DH = [(L_t - L_0) / (L_{max} - L_0)] * 100$$

L_t = concentration of α-NH_3 released in the time $_t$ .L_0 =α-NH_3 found in the original protein sample .

L_{max}= total α- NH3 in the undigested sample , which can be obtained after acidification using HCL (6 M) at 120˚C for 24h

Determination of antioxidant activity:

DPPH Radical-Scavenging Activity (RSA)

The RSA was measured according to Laohakungit *et al*., (18) with some modulations. One ml of the sample (4 mg / ml) was mixed with 1 ml of DPPH solution (0.1 M). The mixture kept at dark place at room temperature for 30 minutes , and then centrifuged at 10,000x g for 5 min. The absorbency was measured at 517 nm , and the percentage of the scavenging activity was calculated according to the following equation :=

$$Radical\ Scavenging\ activity\ A = [C - (B - A) / C] \times 100$$

A= Spectrophotometer reading of the tested sample at517 nm wavelength.

B = the absorption reading of the control sample at 517 nm (prepared by mixing 1 ml of ethyl alcohol with 1 ml of the sample under study).

C = reading of the positive control at 517 nm (obtained from mixing 1 ml of DPPH with 1 ml of distilled water).

Functional properties

1. Solubility determination: Solubility of the protein was determined according to the method suggested by Catterjee *et. al.*, (7) . A sample of gluten (50 mg) was dissolved in 20 ml of distilled water and the pH adjust to different values (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12), and left for 15 minutes under controlled pH, then centrifuged at 10000 * g for 15 minutes. The supernatant was collected and the total nitrogen content was estimated . The percentages of solubility were calculated as follows :

Solubility % = protein content in the supernatant/ protein content in the sample *x* 100 .

2. Water holding capacity determination

Onsaard *et al.* (21) method was followed with some modification, 0.5 g of the experimental sample was mixed with 10ml distilled water, vortexed for 5 minutes . The pH was adjusted to (4, 7, 12) and left at room temperature for 15 minutes, and was centrifuged at 10,000 g for 10 minutes . Water holding capacity was calculated using the following equation :

W.H.C = W2-W1/ W0

W2 = Tube weight + weight of the precipitate after water removal .

W1 = Tube weight + Sample before water addition

W0 = weight of Sample

3.Oil binding capacity determination

Onsaard *et al*. (21) method was followed with some modification, 0.5 g of the sample was mixed with 10ml sun flower oil placed on the vortex for 15 minutes , then the pH adjusted to (4, 7, 12) and left at room temperature for 15 minutes , then centrifuged at 10,000 g for 10 minutes . Oil binding Capacity was calculated by the following equation :

Oil Binding Determination (gm oil /gm sample) = F2-F1 / F0

F0 = Weight of the sample .

F1 = Tube weight + sample weight before adding oil

F2= Weight of the tube + weight of the deposit after removing the oil

4. Estimation the foam formation capacity and stability

Cano – Medina *et al.*,(23) method was adopted with some modification. One gram of the experimental samples was mixed with small amount of distilled water for one minute, and the volume completed to 100 ml , the pH of the experimental samples were adjusted to (4 , 7 , 12), 50 ml of each sample were placed in 150 ml flasks ,then vortexed for one minute at maximum speed and then transferred to a 100 ml graduated cylinder . The volume was measured before and after whipping. The ratio of foam capacity and stability was calculated as follow:

Foam capacity % = volume after whisking– volume before whipping / size before whisking*100

Foam stability % = foam size after a certain time/foam time zero *x* 100

5.Emulsification and emulsion stability

Sharm *et al.*,(23) method was adapted with some modification, 5 ml of the samples (1%) at three different pH values (4, 7, 12) were mixed with 5 ml sunflower oil. The mixture was homogenized, (10,000 cycles / minute). Centrifuged at (3500*g) for 5 minutes and the emulsions layer volume was measured by the included cylinder. The percentage of emulsification capacity was calculated using the following equation:

Emulsification capacity % = Emulsion layer size / Total size * 100

The stability of emulsion was measured by placing the emulsion in a water bath at 85 C for 30 min and then centrifugation (3500*g) for (5) minute and the emulsion layer volume was measured using the inserted cylinder. Emulsion stability was calculated using the following equation

Stability of emulsion = Emulsion layer after heating / Total volume before heating *100

Statistical analysis

Statistical Analysis System (SAS) (22) was used for the analysis of data, to study the effect of different treatments in the studied traits in full randomized design (CRD). The differences between mean were compared with the least significant difference (LSD).

RESULTS AND DISCUSSION

Chemical components of durum wheat, wheat flour, dried and Lyophilized durum wheat gluten and commercial gluten

Table (2) shows the percentages of moisture, protein, fat, ash, fiber and carbohydrates of durum wheat and wheat flour(72-76% extracted), dried Lyophilized durum wheat gluten and commercial gluten. The percentages of moisture were (6.61, 9.6, 4.49, 3.72, 5%) respectively. Moisture content has a significant impact on the quality of wheat storage and is also an important factor in determining the quality of the resulting flour and its water absorption. Due to the wheat conditioning, moisture percentage has increased in the flour. Protein content of wheat and flour were 17.5% and 13%, respectively. Protein is of great importance in determining product quality. The same table also showed that the fat percentage were (2.64 , 1.91%)

respectively. Many studies confirm the importance of flour fat bread manufacturing and the rheological properties of the dough, despite their small quantity. The percentage of ash was 3.07 and 1.9 %, respectively. Ash content is an important measure related to the quality of milling process and also it is a strong indicator of flour color and purity. It is noted that the ratio of fibers does not correspond to the ratios indicated by Zain El-abideen, (29), who pointed out that the percentage of fiber in Iraqi wheat varieties ranged between 2 - 2.7%. Iuliana *et al*., (16) reported that the percentage of carbohydrates for wheat varieties ranged from 65-75%, and this is similar to our finding in the Iraqi wheat strain in this study.

Table 2. Chemical composition of durum wheat and wheat flour(72-76%), dried and Lyophilized durum wheat gluten and commercial gluten (%).

Treatment	Ash	Carbohydrate	Fiber	Protein	Fat	Moisture
Durum Wheat	3.07	67.07	3.11	17.5	2.64	6.61
Flour72-76%	1.9	71.49	2.1	13	1.91	9.6
Dried gluten	2.90	10.04	0.21	80.5	1.86	4.49
Lyophilized gluten	2.35	10.36	0.25	79.6	3.72	3.72
Commercial gluten	1.74	27.0	0.66	65.4	0.2	5

Table 2 also shows the chemical composition of dried and Lyophilized durum wheat gluten and commercial gluten. The percentage of moisture, protein, fat, fiber, ash and carbohydrate for dried durum gluten were (4.49, 80.5, 1.86, 0.21, 2.90, 10.04 %) respectively, and for Lyophilized durum wheat gluten (4.4, 74.36, 0.1, 19.5, 1.5%) respectively, while for commercial gluten were (4.5, 71.4, 0, 21.7, 2.3%) respectively. The difference in the chemical composition of experimental gluten is due to the difference in the source, in method of extraction and in methods of drying the sample (2 ;27).

Enzymatic treatment of wheat gluten

The effect of enzymes concentrations (pepsin, papain and trypsin) on the hydrolysis of dried, lyophilized and commercial gluten and antioxidant properties were studied individually.

1-Pepsin treated wheat gluten and whey protein

Tables 3 show the degrees of hydrolysis(DH) of dried, lyophilized and commercial gluten and whey proteins treated with pepsin (3000, 4000 units / g protein). As it is obvious the degree of hydrolysis increased, as the enzyme concentrations increased with the hydrolysis time. The bitter taste appeared in the hydrolysates after (3) hours of enzymatic hydrolysis when (3000units / g protein) was used, and after (1) hours when (4000units / g protein) was used. Elmalimadi, (11) studied the effect of heat pretreatment for wheat gluten on the enzymatic hydrolysis induced by alcalase. The results indicated that the heat treatment significantly improved susceptibility of WGPs to alcalase and the DH (%) varied from 2 to 30 % over 195 min of hydrolysis.

Table 3. Degrees of hydrolysis(DH) of dried, lyophilized, commercial and whey proteins treated with pepsin (3000 and 4000 U /g protein) at pH 7 and temperature 37°C. B represents bitterness appearance , the values in the table are average of duplicate reading.

Time	DH%							
	Whey protein		Lyophilized gluten		Dried gluten		Commercial gluten	
	3000 U/g	4000 U/g	3000 U/g	4000 U/g	3000 U/g	4000 U/g	3000 U/g	4000 U/g
0	0	0	0	0	0	0	0	0
1	0.08	0.24	2.97	25.07 B	1.65	2.41 B	4.36	15.14 B
2	3.26	2.12	3.18	25.4	3.49	5.11	6.88	15.27
3	3.66	2.28	3.46 B	33.09	7.74 B	9.08	7.29 B	19.34
4	4.51	3.42	5.0	34.11	9.96	12.38	7.37	26.74
5	7.04	8.11	5.25	34.96	10.25	12.74	8.27	29.88
6	7.04	10.87	5.97	39.89	12.64	13.07	10.95	31.79
7	8.72	13.93	13.03	43.59	12.93	13.07	11.64	33.62

2- Trypsin treated wheat gluten and whey protein

Table 4 indicates the degrees of hydrolysis (DH) of dried, lyophilized and commercial gluten and whey proteins treated with trypsin (4000, 5000 units / g protein). The bitter taste was observed after 6 hours of enzymatic hydrolysis of gluten samples under study. It was noted that the trypsin was less effective in gluten hydrolysis as compared to pepsin, in contrast it was more effective in hydrolysis of whey proteins. ECabrera-Chávez *et al.*,(12) found " that the DH of hydrolysis of trypsin treated durum, bred wheat and gluten fractions were 1.16–1.40%. The influence of hydrolysis on the isoelectric point was more evident in durum wheat gluten.

Table 4. Degrees of hydrolysis (DH) of dried, lyophilized, commercial and whey proteins treated with trypsin (4000 and 5000 U /g protein) at pH 7 and pH 6. The results represent a repeat rate. The letter B represents the time of the appearance of Bitter taste in the protein hydrolysates

Time	DH %							
	Whey proteins		lyophilized gluten		lyophilized gluten		commercial gluten	
	4000 U/g	5000 U/g	4000 U/g	5000 U/g	4000 U/g	5000 U/g	4000 U/g	5000 U/g
0	0	0	0	0	0	0	0	0
1	4.35	5.22	0.65	4.54	2.2	0.77	1.81	1.67
2	10.76	9.9	0.86	6.75	3.66	1.09	2.68	1.71
3	11.37	11.33	1.55	8.79	4.31	3.02	3.2	2.32
4	12.43	12.63	3.22	11.99	5	3.44	5.09	2.89
5	14.62	13.69	4.36	12.44	6.84	4.28	4.64	3.99
6	15.4	14.05	4.4 B	13.84 B	7.08 B	8.69 B	7.75 B	15.12 B
7	16.01	20.44	5.24	13.93	9.36	13.62	9.92	16.2

3- Papain treated wheat gluten and whey protein

Tables 5 shows degrees of hydrolysis (DH) of dried, lyophilized and commercial gluten and whey proteins treated with papain (2000, 3000 units / g protein). It has been noticed from tables (3,4,5) that papain was more effective in the hydrolysis of all protein samples under study as compared with pepsin and trypsin. The DH of papain treated samples increased rapidly in the first four hours, then began to slow down. It nate worthily that the whey protein hydrolysates showed no bitter taste through the entire hydrolysis time.

Table 5. Degrees of hydrolysis(DH) of dried, lyophilized, commercial and whey proteins treated with Papain (2000 and 3000 U /g protein) at pH 7 and 50°C. The data represent average of diplicates rate. The letter B represents the time of bitter taste appearance in the protein hydrolysates.

Time	DH %							
	whey proteins		lyophilized gluten		dried gluten.		commercial gluten	
hr.	2000 U/g	3000 U/g	2000 U/g	3000 U/g	2000 U/g	3000 U/g	2000 U/g	3000 U/g
0	0	0	0	0	0	0	0	0
1	5.54	5.9	13.22	22.08	12.74	12.32	12.69	25.85
2	11.48	9.69	21.93	29.28	19.49	19.52	28.89	39.93
3	21.21	14.63	25.96	32.8	23.2	23.68	35	46.36
4	21.49	23.61	27.19	35.36	25.48	28.48	36.22	48.72
5	22.71	26.46	27.19 B	40 B	25.48	35.04	43.71	51.49
6	27.11	28.04	27.19	41.6	25.48 B	37.28 B	45.58 B	52.59 B
7	33.99	39.72	27.19	42.56	25.48	38.24	56.89	55.48

Radical-Scavenging Activity (RSA)

Table 6 shows the Radical-scavenging activity (using DPPH) of pepsin, trypsin and papain treated proteins (dried, lyophilized, commercial and whey proteins). It was observed that radical-scavenging activity of all hydrolysates increased as hydrolysis time increased. The difference in the radical-scavenging activity of the treated proteins can be attributed to the differences in the degrees of enzymatic degradation, the type, molecular weight of product, size and configuration of peptide produced, as well as the type and sequence of amino acids (13 ; 18 ; 25).

Gluten hydrolysates (induced by pepsin enzyme) had a higher radical scavenging activity compared with other hydrolysates, however, these hydrolysates were excluded because the bitter taste was appeared at the first hour of the hydrolysis. The hydrolysates which obtained after four hours papain induced hydrolysis was free of bitter taste and gave higher RSA as compared to trypsin induced hydrolysates. Therefore, this group was selected to complete this study.

Table 6. The Radical-scavenging activity (using DPPH) of pepsin, trypsin and papain treated proteins (dried, lyophilized, commercial and whey proteins).

Time hr.	RSA %							
	whey proteins		dried gluten		lyophilized gluten		commercial gluten.	
	Pepsin							
	3000 U/g	4000 U/g	3000U/g	4000 U/g	3000 U/g	4000 U/g	3000 U/g	4000 U/g
0	0	0	0	0	0	0	0	0
1	4.36	2.71	2.38	8.21	0.3	7.78	11.55	19.85
2	5.07	4.71	4.15	10.48	10.33	18.02	17.31	24.41
3	6.71	4.87	6.95	12.78	13.85	21.51	18.01	25.61
4	7.58	6.71	12.79	18.89	17.22	27.21	25.22	32.32
5	10.05	7.94	21.51	27.34	23.68	33.01	27.49	35.07
6	10.81	11.22	22.49	29.15	25.93	36.39	29.41	37.01
7	16.91	15.73	30.39	36.53	29.69	41.6	44.03	49.57
	Trypsin							
hr.	3000 U/g	4000 U/g	3000U/g	4000 U/g	3000 U/g	4000 U/g	3000 U/g	4000 U/g
0	0	0	0	0	0	0	0	0
1	0.71	10.25	7.31	8.69	6.11	1.86	0	8.27
2	3.38	15.84	13.22	13.99	9.22	14.31	7.75	11.76
3	3.79	19.27	16.6	12.25	10.65	15.74	9.79	18.41
4	6.2	20.71	19.55	18.85	15.22	20.31	16.44	19.09
5	13.12	11.53	20.96	27.11	17.98	23.07	17.12	27.53
6	14.91	21.53	20.96	34.23	20.63	25.67	25.56	33.86
7	15.78	27.93	21.38	40.47	22.95	27.5	31.89	34.19
	Papain							
hr.	2000 U/g	3000 U/g	2000U/g	3000U/g	2000 U/g	3000 U/g	2000 U/g	3000 U/g
0	0	0	0	0	0	0	0	0
1	1.49	10.1	1.343	23.36	15.181	24.7	11.25	23.67
2	1.54	10.87	4.313	24.97	16.89	31.28	16.55	34.14
3	3.33	12.71	5.617	25.91	18.762	32.48	18.23	37.44
4	5.13	13.79	6.96	25.91	22.385	33.16	19.69	39.36
5	7.49	16.15	9.972	26.85	23.158	33.42	19.71	40.36
6	10.15	20.35	11.762	27.79	27.594	33.69	22.84	40.41
7	13.07	24.19	14.408	28.19	31.202	36.65	26.72	45.66

Functional properties

1. Solubility

Table 7 shows the solubility (%) of the experimental proteins before and after enzymatic treatment at different pH values. The solubility of modified whey proteins were (0.52, 0 .56, 0.68, 0.81, 0.75, 0.61, 0.60, 0,78, 1.03, 1.34, 1.48, 1.41) at pH (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12) respectively. While the solubility of dried, lyophilized and commercial gluten after enzymatic treatment were (0.77, 0.75, 0.85, 0.80, 0.86, 0.79, 0.76, 0.95, 1.18, 1.35, 1.40, 1.42) ,(0.27, 0.27, 0.42, 0.80, 0.90, 0.78, 0.84, 1.01, 1.28, 1.45, 1.45, 1.48) and (0.94, 0.93, 0.85, 0.86, 0.89, 0.74, 0.67, 0.78, 1.07, 1.10, 1.39, 1.30) at pH (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12) respectively. Solubility of Papain treated proteins were the best in alkaline pH especially at PH 8 and 10 (Table 5).These results are similar to that of Bomara *et al.* (5) who recorded that the enzymatic treatment improved solubility, and close to results of Olanca and Ozay (20) who noticed a significant increase in gluten solubility at neutral and alkaline pH especially at pH 7, 8, and 10.

Table 7. Percentage of solubility of dried, lyophilized and commercial gluten protein and whey proteins before and after enzymatic treatment (3000 unit /g protein of the Papain enzyme).

	Before enzymatic treatment				After enzymatic treatment			
	Solubility %							
Protein samples / pH	Whey proteins	Commercial gluten	Dried gluten	lyophilized gluten	Whey proteins	Commercial gluten	Dried gluten	lyophilized gluten
1	0.52a	0.29ab	0.05b	0.06b	0.57c	0.94abc	0.77b	0.27c
2	0.45a	0.36ab	0.22b	0.22ab	0.56c	0.93abc	0.75b	0.27c
3	0.44a	0.37ab	0.11b	0.17ab	0.68bc	0.85bc	0.85b	0.42c
4	0.48a	0.28ab	0.04b	0.21ab	0.81bc	0.86bc	0.80b	0.8b
5	0.50a	0.34ab	0.08b	0.19ab	0.75bc	0.89bc	0.86b	0.90b
6	0.49a	0.50a	0.07b	0.20ab	0.61bc	0.74bc	0.79b	0.78b
7	0.52a	0.53a	0.09b	0.19ab	0.60bc	0.675c	0.76b	0.84b
8	0.63a	0.30ab	0.14b	0.12ab	0.78bc	0.78bc	0.95a	1.01ab
9	0.68	0 .19b	0.17b	0.19ab	1.03ab	1.07abc	1.18ab	1.28ab
10	0.72a	0.18b	0.21b	0.18ab	1.34a	1.10ab	1.35a	1.45a
11	0.74a	0.24ab	0.40ab	0.24ab	1.48a	1.39a	1.40a	1.45a
12	0.76a	0.52a	0.73a	0.52a	1.41a	1.3a	1.42a	1.48a
L.S.D	398 NS	0.302 *	0.463*	0.378*	0.461 *	0.407 *	0.487*	0.532 *

The results reveal a significant improvement (P<0.05)in the other functional properties of the tested proteins treated enzymatically with papain (3000 units/ g protein) (Table 8): the statistical analysis indicate that water holding capacity (WHC) of wheat gluten was increased significantly for all tested proteins especially at a pH (12) with insignificant improvement at the other pH values. These results are in agreement with Bomara et el. (5) who found a significant improvement in WHC of wheat gluten. Additionally, Deng *et al.*, (10) found slight increase in water holding capacity of enzymatically modified wheat gluten and an improvement in emulsifying and stability of gluten hydrolysates (using wheat – bug protease)at neutral and alkaline pH , and our results were close to their results except at pH 4.

Table 8. Effect of enzymatic treatment (papain 3000 units/ g protein) on wheat gluten (Triticum durum) water holding capacity at different pH values

	After enzymatic treatment				Before enzymatic treatment				
Protein samples / pH	Whey protein	Comme-rcial gluten	Dried gluten	lyophiliz-ed gluten	Whey protein	Commer-cial gluten	Dried gluten	lyophilized gluten	L.S.D
4	9.24 a	4.46 b	1.5 de	2.9 c	1.33de	2.72 cd	1.22 e	1.175 e	1.284*
7	5.42 a	2.405 b	2.675 b	2.64 b	2.41 b	2.19 b	1.11 c	1.135 c	0.892*
12	6.02 a	2.945 d	4.605 b	4.44 b	2.34de	3.045 c	1.355 e	1.72 e	1.169*

Table 9 indicates that the emulsifying capacity of dried, lyophilized and commercial gluten was improved at pH (4, 7 and 12). Meanwhile the emulsion stability was also significantly improved at pH 12 in all tested proteins except the dried sample. This result was similar to that of several researchers findings (17; 28) who noticed an improvement in capacity with no change in emulsifying stability of wheat gluten modified (using acid protease from

Aspergillus susamii). Bombara et al. (5) found the same increasing in emulsifying capacity of wheat flours modified using protease.mTable 10 illustrates the foaming ability of the experimental proteins. It has been noticed that the best improvement in this property achieved after 60 minutes especially in lyophilized gluten. The same improvement also seen in foam stability, but the best effect was after 30 minutes.

Table 9. Effect of enzymatic treatment of wheat gluten (Triticum durum) with Papain (3000 units/g protein) on emulsion ability (%) and emulsion stability(%) at different pH values

Protein sample / pH	After enzymatic treatment (emulsion ability) Whey protein	Commercial gluten	Dried gluten	lyophilized gluten	Before enzymatic treatment (emulsion stability) Whey protein	Commercial gluten	Dried gluten	Lyophilize-ed gluten	L.S.D.
4	60 bc	82.5 a	62.5 b	65 b	55 c	62.5 b	47.5 d	55 c	7.38*
7	62.5 b	72.5 a	72.5 a	50 cd	45 df	55 c	52.5 c	42.5 f	6.82*
12	75 b	85 a	85 a	75 b	70 b	72.5 b	70 b	62.5 c	7.05*
pH	Post enzymatic treatment (emulsion stability)				Pre enzymatic treatment (emulsion stability)				**L.S.D**
4	55 b	80 a	45 c	55 b	47.5bc	45 c	50 bc	45 c	7.38*
7	57.5 b	67.5 a	52.5 bc	42.5 d	45 cd	55 b	45 cd	40 d	8.03*
12	75 a	80 a	62.5 bc	65 b	45 d	65 b	55 c	55 c	7.66*

Table 10. Effect of enzymatic treatment of wheat gluten (Triticum durum) with Papain (3000 units/g protein) on foam formation capacity at different pH values

Protein samples / Time	pH	After enzymatic treatment Whey proteins	Commerci-al gluten	Dried gluten	lyophilized gluten	Before enzymatic treatment Whey proteins	Commercial gluten	Dried gluten	lyophilized gluten
0.0	4	92.5a	91ab	75ab	92.5a	87.5a	82.5ab	62.5b	82.5a
	7	82.5bc	82.5bc	57.5e	82.5bc	72.5bc	72.5ce	42.5de	72.5b
	12	92.5a	92.5a	82.5a	87.5ab	87.5a	87.5a	82.5a	75b
15 Minute	4	85abc	86.5abc	61ce	72.5ef	82.5a	82.5ab	57.5bc	61cd
	7	82.5bc	72.5	53.5e	70ef	62.5de	67.5ef	42.5de	67.5bc
	12	82.5bc	84abc	72.5ab	75ce	72.5bc	77.5bc	72.5a	65c
30 Minute	4	84bc	82.5bc	75g	65fd	72.5bc	72.5ce	47.5cd	55d
	7	72.5ef	72.5d	53e	62.5d	62.5de	63f	2.5f	52.5de
	12	77.5ce	77.5cd	69bc	62.5d	67.5cd	62.5fg	57.5bc	57.5d
45 Minute	4	72.5ef	72.5d	5g	59d	62.5de	65f	42.5de	45ef
	7	65fg	52.5e	12.5g	58d	52.5f	52.5t	2.5f	40f
	12	65fg	59e	69bc	62.5d	52.5f	55.5gt	51.25bcd	47.5ef
60 Minute	4	58gh	58e	0t	58d	57.5ef	52.5t	0f	0g
	7	52.5h	5t	2.5g	7.5g	40g	37.5r	0f	0g
	12	56h	52.5e	56.5e	56d	55ef	52.5t	37.5e	2.5g
LSD	-	8.22 *	9.31 *	10.05 *	8.73 *	8.92 *	8.61 *	11.45 *	9.52 *

Bombara *et al.*, (5) also found an increase in foam expansion of enzymatic modified wheat gluten. Another researchers recorded a significant increase in both foaming capacity and stability at pH of 6, 7, 8 but they are not study the time of foam stability(20).

Table 11. Effect of enzymatic treatment of wheat gluten (Triticum durum) with Papain (2000 units/ g protein) on foam stability at different times

Protein samples / Time	Ph	After enzymatic treatment				Befor enzymatic treatment			
		Whey proteins	Commercial gluten	Dried gluten	lyophilized gluten	Whey proteins	Commercial gluten	Dried gluten	lyophilized gluten
0.0	4	95a	97.5a	92.5a	95a	92.5a	92.5a	82.5a	72.5a
	7	87.5ab	87.5bc	62.5cd	82.5bc	72.5c	72.5cd	52.5d	57.5bc
	12	92.5a	97.5a	75b	92.5ab	72.5c	85ab	67.5bc	62.5b
15 Minute	4	95a	92.5ab	82.5ab	72.5ce	82.5b	87.5a	72.5b	62.5b
	7	82.5b	82.5ce	53.5d	62.5df	62.5d	62.5e	40ef	52.5cd
	12	87.5ab	92.5ab	65cd	75ce	72.5c	75c	52.5d	57.5bc
30 Minute	4	87.5ab	82.5ce	72.5bc	65edf	62.5d	77.5bc	62.5c	52.5cd
	7	70c	72.5d	42.5	62.5df	47.5f	52.5f	35fg	42.5f
	12	77.5c	77.5ed	65cd	65edf	62.5d	62.5e	37.5ef	42.5f
45 Minute	4	77.5c	72.5dg	62.5cd	61.5f	52.5fe	65de	52.5d	42.5f
	7	62.5d	62.5fh	22.5f	59f	32.5t	40tg	22.5ht	32.5hg
	12	72.5c	67.5gh	45e	57f	52.5fe	52.5f	27.5gh	47df
60 Minute	4	45h	57.5f	37.5e	58f	52.5fe	52.5f	45de	32.5hg
	7	47.5hg	60fh	7.5t	2.5t	27.5t	32.5g	17.5t	25h
	12	52.5g	ce 80	35e	58.5f	45f	45t	25gh	32.5hg
LSD	-	9.61 *	8.33 *	10.93 *	12.48 *	8.92 *	8.37 *	9.63 *	8.51 *

Table (12) shows the effect of enzymatic treatment on the oil holding capacity of dried, lyophilized and commercial gluten and whey proteins with Papain enzyme. The oil holding capacity has been increase significantly ($P<0.05$) in all treatments, it becomes after treated (2.12, 4.87, 1.92, 2.50),for protein under study respectively. This result was similar to that of researchers finding (9 , 4) who reported a significant increase in oil holding capacity of wheat gluten. But we can say that there is no difference in those results when we view to the type of the tested wheat that they use which was the soft wheat.

Table 12. Effect of enzymatic treatment of wheat gluten (Triticum durum) with papain (2000 units/g protein) on oil holding capacity

Protein samples	Before enzymatic treatment	After enzymatic treatment
lyophilized gluten	4.87 a	1.82 a
Dried gluten	2.12 b	1.105 a
Commercial gluten	1.92 b	1.19 a
Whey proteins	2.505 b	1.24 a
L.S.D	1.027 *	0.688 NS

Conclusions

Enzymatic modification of wheat durum gluten by (2000 units/g protein) of Papain reveals a positive effects on different functional properties at different pH values especially alkaline and pH 4

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