FUNCTIONAL PROPERTIES OF ENZYMATICALLY MODIFIED WHEAT GLUTEN

A. S. Jasim
Researcher

J. M. Nasser
Assist. Prof.

Dep. of Food Sci., Coll. Agric. Engin. Sci., University of Baghdad, Baghdad, Iraq
dr.jassim6699@yahoo.com

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.

*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 annual global 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 glutenin 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 mediation 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, centrifugated 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 1 hour 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 5,000xg 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 1 hour 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 1 hour 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. NH₃ groups were calculated using the standard Lucien amino acid curve and the degree of degradation was calculated according to the following equation (14):

$$DH = \left( \frac{L_t - L_0}{L_{max} - L_0} \right) \times 100$$

$L_t$ = concentration of α-NH₃ in the time $t$, $L_0 = \alpha$-NH₃ 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 :

$$A = \frac{C - (B - A)}{C} \times 100$$

A = Spectrophotometer reading of the tested sample at 517 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:

The studied samples (0.250 ml of each) were transferred to a test tube and subjected to the above steps. NH₃ groups were calculated using the standard Lucien amino acid curve and the degree of degradation was calculated according to the following equation (14):
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:

\[ \text{W.H.C} = \frac{W_2 - W_1}{W_0} \]

where:
- \( W_2 \) = Tube weight + weight of the precipitate after water removal.
- \( W_1 \) = Tube weight + Sample before water addition.
- \( W_0 \) = 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 sunflower 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:

\[ \text{Oil Binding Determination ( gm oil/gm sample )} = \frac{F_2 - F_1}{F_0} \]

where:
- \( F_0 \) = Weight of the sample.
- \( F_1 \) = Tube weight + sample weight before adding oil.
- \( F_2 \) = 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:

\[ \text{Foam capacity %} = \frac{\text{volume after whisking} - \text{volume before whipping}}{\text{size before whipping}} \times 100 \]

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:

\[ \text{Emulsification capacity %} = \frac{\text{Emulsion layer size}}{\text{Total size}} \times 100 \]

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:

\[ \text{Stability of emulsion} = \frac{\text{Emulsion layer after heating}}{\text{Total volume before heating}} \times 100 \]

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 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     |
| Flour(72-76%)        | 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.

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 | Whey protein 3000 U/g 4000 U/g | Lyophilized gluten 3000 U/g 4000 U/g | Dried gluten 3000 U/g 4000 U/g | Commercial gluten 3000 U/g 4000 U/g |
|------|-----------------------------|---------------------------------------|--------------------------------|-------------------------------------|
| 0    | 0                           | 0                                     | 0                               | 0                                   |
| 1    | 0.08                        | 0.24                                  | 2.97                            | 25.07 B                             |
| 2    | 3.26                        | 2.12                                  | 3.18                            | 25.4                                |
| 3    | 3.66                        | 2.28                                  | 3.46 B                          | 33.09                               |
| 4    | 4.51                        | 3.42                                  | 5.0                             | 34.11                               |
| 5    | 7.04                        | 8.11                                  | 5.25                            | 34.96                               |
| 6    | 7.04                        | 10.87                                 | 5.97                            | 39.89                               |
| 7    | 8.72                        | 13.93                                 | 13.03                           | 43.59                               |
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 | Whey proteins | DH %       | Lyophilized gluten | Lyophilized gluten | Commercial gluten |
|------|---------------|------------|--------------------|--------------------|-------------------|
|      | 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                 |
| 1    | 4.35          | 5.22       | 0.65               | 4.54               | 2.2               | 0.77              |
| 2    | 10.76         | 9.9        | 0.86               | 6.75               | 3.66              | 1.09              |
| 3    | 11.37         | 11.33      | 1.55               | 8.79               | 4.31              | 3.02              |
| 4    | 12.43         | 12.63      | 3.22               | 11.99              | 5                 | 3.44              |
| 5    | 14.62         | 13.69      | 4.36               | 12.44              | 6.84              | 4.28              |
| 6    | 15.4          | 14.05      | 4.4 B              | 13.84 B            | 7.08 B            | 8.69 B            |
| 7    | 16.01         | 20.44      | 5.24               | 13.93              | 9.36              | 13.62             |

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 worthy 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 duplicates rate. The letter B represents the time of bitter taste appearance in the protein hydrolysates.

| Time | Whey proteins | DH %       | Lyophilized gluten | Dried gluten | Commercial gluten |
|------|---------------|------------|--------------------|--------------|-------------------|
|      | 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                 |
| 1    | 5.54          | 5.9        | 13.22              | 22.08        | 12.74             | 12.32             |
| 2    | 11.48         | 9.69       | 21.93              | 29.28        | 19.49             | 19.52             |
| 3    | 21.21         | 14.63      | 25.96              | 32.8         | 23.2              | 23.68             |
| 4    | 21.49         | 23.61      | 27.19              | 35.36        | 25.48             | 28.48             |
| 5    | 22.71         | 26.46      | 27.19 B            | 40 B         | 25.48             | 35.04             |
| 6    | 27.11         | 28.04      | 27.19              | 41.6         | 25.48 B           | 37.28 B           |
| 7    | 33.99         | 39.72      | 27.19              | 42.56        | 25.48             | 38.24             |

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. | whey proteins | dried gluten | lyophilized gluten | commercial gluten |
|---------|---------------|--------------|--------------------|-------------------|
| 0       | 3000 U/g      | 4000 U/g     | 3000 U/g           | 4000 U/g          |
| 1       | 4.36          | 2.71         | 2.38               | 8.21              |
| 2       | 6.71          | 4.87         | 6.95               | 12.78             |
| 3       | 7.58          | 6.71         | 12.79              | 18.89             |
| 4       | 10.05         | 7.94         | 21.51              | 27.34             |
| 5       | 10.81         | 11.22        | 22.49              | 29.15             |
| 6       | 16.91         | 15.73        | 30.39              | 36.53             |

| Time hr. | whey proteins | dried gluten | lyophilized gluten | commercial gluten |
|---------|---------------|--------------|--------------------|-------------------|
| 0       | 3000 U/g      | 4000 U/g     | 3000 U/g           | 4000 U/g          |
| 1       | 0.71          | 10.25        | 7.31               | 8.69              |
| 2       | 3.38          | 15.84        | 13.22              | 13.99             |
| 3       | 3.79          | 19.27        | 16.6               | 12.25             |
| 4       | 6.2           | 20.71        | 19.55              | 18.85             |
| 5       | 13.12         | 11.53        | 20.96              | 27.11             |
| 6       | 14.91         | 21.53        | 20.96              | 34.23             |
| 7       | 15.78         | 27.93        | 21.38              | 40.47             |

| Time hr. | whey proteins | dried gluten | lyophilized gluten | commercial gluten |
|---------|---------------|--------------|--------------------|-------------------|
| 0       | 3000 U/g      | 4000 U/g     | 3000 U/g           | 4000 U/g          |
| 1       | 1.49          | 10.1         | 1.343              | 23.36             |
| 2       | 1.54          | 10.87        | 4.313              | 24.97             |
| 3       | 5.33          | 12.71        | 5.617              | 25.91             |
| 4       | 5.13          | 13.79        | 6.96               | 25.91             |
| 5       | 7.49          | 16.15        | 9.972              | 26.85             |
| 6       | 10.15         | 20.35        | 11.762             | 27.79             |
| 7       | 13.07         | 24.19        | 14.408             | 28.19             |

| Time hr. | whey proteins | dried gluten | lyophilized gluten | commercial gluten |
|---------|---------------|--------------|--------------------|-------------------|
| 0       | 3000 U/g      | 4000 U/g     | 3000 U/g           | 4000 U/g          |
| 1       | 1.49          | 10.1         | 1.343              | 23.36             |
| 2       | 1.54          | 10.87        | 4.313              | 24.97             |
| 3       | 5.33          | 12.71        | 5.617              | 25.91             |
| 4       | 5.13          | 13.79        | 6.96               | 25.91             |
| 5       | 7.49          | 16.15        | 9.972              | 26.85             |
| 6       | 10.15         | 20.35        | 11.762             | 27.79             |
| 7       | 13.07         | 24.19        | 14.408             | 28.19             |

**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).

| Protein samples | pH | Whey proteins % | Commercial gluten % | Dried gluten % | L.S.D |
|-----------------|----|----------------|---------------------|----------------|------|
|                 |    | Before enzymatic treatment | Solubility % | After enzymatic treatment |    |
| Protein samples | pH | Whey proteins | Commercial gluten | Dried gluten | lyophilized gluten | Whey proteins | Commercial gluten | Dried gluten | lyophilized gluten | L.S.D |
| 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               | 0.68a | 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 al. (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

| Protein samples | pH | Whey protein | Commercial gluten | Dried gluten | lyophilized gluten | Wheat protein | Commercial gluten | Dried gluten | lyophilized gluten | L.S.D |
|-----------------|----|--------------|-------------------|--------------|-------------------|--------------|-------------------|--------------|-------------------|------|
|                 |    | After enzymatic treatment | Before enzymatic treatment | | | | | | | |
| 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. m

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 samples | pH | After enzymatic treatment (emulsion ability) | Before enzymatic treatment (emulsion stability) | L.S.D. (emulsion stability) |
|-----------------|----|---------------------------------------------|-------------------------------------------------|-----------------------------|
| Whey protein    | 4  | 60 bc 82.5 a 62.5 b 65 b                  | 55 b 62.5 b 47.5 d 55 c                           | 7.38*                       |
| Commercial gluten | 7  | 62.5 b 72.5 a 50 cd 45 df                   | 55 c 52.5 c 42.5 f 6.82*                         |
| Dried gluten    | 12 | 75 b 85 a 75 b 70 b                        | 72.5 b 70 b 62.5 c 7.05*                         |
| Lyophilized gluten | 4  | 55 b 80 a 45 c 55 b                        | 47.5 bc 45 c 50 bc 45 c 7.38*                    |
| Whey protein    | 7  | 57.5 b 67.5 a 42.5 d 45 cd                  | 55 b 65 b 65 c 45 d 8.03*                        |
| Commercial gluten | 12 | 75 a 80 a 62.5 bc 45 d                      | 80 a 72.5 a 72.5 a 57.5 bc 65c 7.66*             |
| Dried gluten    | 4  | 92.5a 91ab 75ab                            | 92.5a 87.5a 82.5ab 62.5b 82.5a                   |
| Lyophilized gluten | 7  | 82.5bc 82.5bc 82.5a 87.5ab                  | 72.5bc 72.5ce 42.5de 72.5b                        |
| Whey protein    | 12 | 92.5a 92.5a 82.5a 87.5ab                   | 82.5a 82.5a 57.5bc 61cd                         |
| Commercial gluten | 4  | 85abc 86.5abc 61ce 72.5ef                  | 82.5a 82.5a 42.5de 67.5bc                        |
| Dried gluten    | 7  | 82.5bc 72.5 53.5e 70ef                     | 62.5de 67.5ef 42.5de 67.5bc                      |
| Lyophilized gluten | 12 | 82.5bc 84abc 72.5ab 75ce                   | 72.5bc 72.5a 65c 65c 65a 65b 72.5a 65c 65c 75c |
| Whey protein    | 4  | 84bc 82.5bc 75g 65fd                      | 72.5bc 72.5ce 47.5cd 55d                         |
| Commercial gluten | 7  | 72.5ef 72.5d 53e 62.5d                     | 62.5de 63f 2.5f 52.5de 52.5e 52.5f 72.5e 57.5d 57.5d |
| Dried gluten    | 12 | 77.5ce 77.5cd 69bc 62.5d 67.5cd 62.5fg     | 62.5fg 57.5bc 65c 65f 42.5de 65f 42.5de 45f      |
| Lyophilized gluten | 4  | 84bc 82.5bc 75g 65fd                      | 72.5bc 72.5ce 47.5cd 55d                         |
| Whey protein    | 7  | 72.5ef 72.5d 53e 62.5d                     | 62.5de 63f 2.5f 52.5de 52.5e 52.5f 72.5e 57.5d 57.5d |
| Commercial gluten | 12 | 77.5ce 77.5cd 69bc 62.5d 67.5cd 62.5fg     | 62.5fg 57.5bc 65c 65f 42.5de 65f 42.5de 45f      |
| Dried gluten    | 60 | 58gh 52.5e 62.5d 52.5f 52.5f               | 55.5gt 51.25bdc 47.5ef                           |
| Lyophilized gluten | 4  | 58gh 52.5e 59e 69bc 62.5d 52.5f 52.5f       | 55.5gt 51.25bdc 47.5ef                           |
| Whey protein    | 7  | 52.5h 5t 2.5g 7.5g 40g                     | 37.5r 37.5r 0f 0g 80 a 57.5bc 65c 65f 42.5de 52.5f |
| Commercial gluten | 12 | 56h 52.5e 56.5e 56d 55ef 52.5t 37.5e        | 2.5g 2.5g 2.5g 2.5g 2.5g 2.5g 2.5g 2.5g 2.5g    |
| Dried gluten    | 4  | 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 | Whey proteins | After enzymatic treatment | Befor enzymatic treatment |
|-----------------------|----|---------------|---------------------------|---------------------------|
|                        |    |               | Commercial gluten         | Dried gluten              |
|                        |    |               | lyophilized gluten        | Whey proteins             |
|                        |    |               |                            | Commercial gluten         |
|                        |    |               |                            | Dried gluten              |
|                        |    |               |                            | lyophilized gluten        |
| 0.0                    | 4  | 95a           | 97.5a                     | 92.5a                     |
|                        | 7  | 87.5ab        | 87.5b                     | 62.5cd                    |
|                        | 12 | 92.5a         | 97.5a                     | 75                        |
| 15 Minute              | 4  | 95a           | 92.5ab                    | 82.5ab                    |
|                        | 7  | 82.5b         | 82.5ce                    | 53.5d                     |
|                        | 12 | 87.5ab        | 92.5ab                    | 65cd                      |
| 30 Minute              | 4  | 87.5ab        | 82.5ce                    | 72.5bc                    |
|                        | 7  | 70c           | 72.5d                     | 42.5                      |
|                        | 12 | 77.5c         | 77.5ed                    | 65cd                      |
| 45 Minute              | 4  | 77.5c         | 72.5dg                    | 62.5cd                    |
|                        | 7  | 62.5d         | 62.5fh                    | 22.5f                     |
|                        | 12 | 72.5c         | 67.5gh                    | 45e                       |
| 60 Minute              | 4  | 45h           | 57.5f                     | 37.5e                     |
|                        | 7  | 47.5gh        | 60fh                      | 7.5t                      |
|                        | 12 | 52.5g         | ce                        | 35e                       |
|                       | LSD|               |                            | 8.33*                     |
|                       |    |               |                            | 10.93*                    |
|                       |    |               |                            | 12.48*                    |
|                       |    |               |                            | 8.92*                     |
|                       |    |               |                            | 8.37*                     |

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

REFERENCES
1. AACC.2000. Approved Methods of the American Associacan of Cereal Chemists, 10th ed. AACC, St. Paul, MN, USA
2. Abadi, F.A. and J.M. Nasir. 2019. Effect of wheat gluten addition on stalin characteristics of barley bread. Iraqi Journal of Agricultural Sciences. 50 (1):390-397.
3. A.O.A.C. 1998. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists , Washington,DC,pp:143
4. Bandypadhyay , K . and S. Ghosh .2002. Preparation and characterization of papain-modified sesame (Sesamum indicum L .) Protein isolates . Journal of agricultural and food chemistry , 50 (23): 6854-6857
5. Bombara , N. ; M . C . Anor and A . M. R . Pilosof . 1997. Functional properties of
protease modified wheat flours. Lebensm. – Wiss. U. – Techno. 30: 441 – 447.

6. Cano – Medina, A.; H. Jimenez – Islas ; L. Dendooven; R.P. Herrera; G. Gonzalez – Alatorre and E.M. Escamilla – Silva. 2011. Emulsifying and foaming capacity and emulsion and foam stability of sesame protein concentrates. Food Research International. 44(3): 684-692

7. Chatterjee, R.; T.K. Dey; M. Ghosh and P. Dhar. 2015. Enzymatic modification of sesame seed protein, sourced from waste resource for nutraceutical application. Food and Bioproducts Processing. 94: 70-81

8. Csiró, L. Day. 2011. Wheat gluten: Production, Properties and Application. In Handbook of Food Proteins. Wood Head Publishing. pp:267-288

9. Dalali, B. and K.H. Al-Rekabi. 1989. Food Chemistry. Ministry of Higher Education and Research, University of Mosul, Dar al-Kutub.10-Welsch. t. L.1979. Meat and Dairy Analogs from Vegetable Proteins. JAOCS., 56:404 – 406

10. Deng, L. I.; W.A. Ztaoxia; Y.A. Sheng; S. J. Que and Z.H. Fengqin. 2016. Improvement of functional properties of wheat gluten using acid protease from Aspergillus usamii. Journal. Pone, 10: 1371

11. Elmalimadi, M.B. 2018. Functional and Biological Properties of Enzymatically Modified Wheat Gluten. Ph. D. Dissertation. University of Belgrade, Faculty of Technology and Metallurgy. pp:45-50

12. ECabrera-Chávez, F.; J. M. Ezquerra-Brauer; R. Herrera-Urbina; C. M. Rosell; and O. Rouzaud-Sánchez. 2010. Physicochemical properties of wheat gluten proteins modified by protease from sierra (Scomberomorus sierra) fish. International Journal of Food Properties, 13(6), 1187-1198

13. Hadeel, S.Y. and A.S. Khalida. 2018. Sesame oil extraction and antioxidant activity of Lignans from locally cultivated sesame seeds (Sesame indicum L.). Iraqi Journal of Agricultural Sciences. Vol.47(2).

14. Jamdar, S. N.; V. Rajalakshmi; M. D. Pednekar; F. Juan; V. Yardi and A. Sharma. 2010. Influence of degree of hydrolysis on functional properties, antioxidant activity and ACE inhibitory activity of peanut protein hydrolysates. Food Chemistry, 121(1), 178-184.

15. Impiglia, A.; M. Nachit; D. LaViandra and E. Porceddu. 1995. Effect of Gliadin and Glutenin Components on Gluten Strength in Durum Wheat. CIHEAM – Options Méditerranéennes. pp:167-172

16. Iuliana, B.; S. Georgeta; L. Violeta and A. Iuliania. 2010. Physicochemical and Technological Characteristics. Cereal Chem, 66(6): 456-461

17. Kong, X.; H. Zhou and H. Qian. 2007. Enzymatic preparation and functional properties of wheat gluten hydrolysates. Food Chemistry, 101(2): 615-620

18. Laohakungit, N.; O. Kerchchoechuen; R. Kaprasob and F.B. Matta. 2017. Volatile flavor, antioxidant activity and physicochemical properties of enzymatic defatted sesame hydrolysates. Journal of Food Processing and Preservation, 41(4): 13075

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

20. Olanca, B. and D.S. Ozay. 2015. Preparation and functional properties of gluten hydrolysates with wheat- bug (Eurygaster spp.) protease. Cereal Chemistry, 87(6): 518-523

21. Oonasr, E.; P. Pomsamud and P. Audtum, P. 2010. Functionality properties of sesame protein concentrates from sesame meal. Asian Journal of Food and Agro – Industry, 3(4): 420-431

22. SAS. 2012. Statistical Analysis System, Users Guide. Statistical. Version 9.1 ed. SAS. Inst. Inc. Cary. N.C. USA. pp:520-535

23. Sharma, L.; C. Singh and H.K. Sharma. 2016. Assessment of functionality of sesame meal and sesame protein isolate from Indian cultivar. Journal of Food Measurement and Characterization, 10(3): 520 – 526

24. Sotomayor Grijalva, M.C. 2013. Evaluation of Enzymatic Hydrolysis of Wheat Gluten at Different Protein Concentrate. M.Sc. Thesis. Holland / Faulted: Agrotechnology and Food Sciences – Wageningen University.

25. Shamurad, H.Y.; M.J. Khadom; F.A. Haider and K.A. Shakir. 2019. Evaluation the
antioxidant activity of sesame coat and sesame cake extracts. *Iraqi Journal of Agricultural Sciences*. 50(3):776-782.

26. Vioque , J.; R. Sanchez – Vioque ; A. Clemente ; J. Pedroche . and F. Millano. 2000. Partially hydrolyzed rapeseed protein isolates with improved functional properties. *Journal of the American Oil Chemists Society*, 77(4) : 447-450

27. Wadhawan, C. K. and W. Bushuk.1989. Cereal and technological characteristics’. *Cereal Chem*, 66(6): 456-461

28. Wang,J.; M. zhao ; X. Yang and Y. Jiang. 2006. Improvement of functional properties of wheat gluten by enzymatic hydrolysis and ultra filtration. *Cereal Science*, 44(1): 93-100

29. Zain El-abideen, M. Wageh. 1979. Study of install specification standard flour appropriate to the production of bread and Iraq’s samoon. M. Sc. in Food Science. In The Department of Food Science. Collage of Agriculture. Baghdad University:64-73.

30. Zilic , Saldana . 2013. Wheat gluten : Composition and Health Effect . Nova Science Publishers , Inc . Chapter IV. pp: 525-590.