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Scientific Research

Optimizing production of hydrolyzed protein of sweet almond meal by ultrasound pretreatment and alcalase enzyme

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ABSTRACT

Sweet almonds are a good source of high quality proteins and the amount of protein in almonds is reported to be 16-22% based on dry weight (Sat, 1993). Almond proteins have high digestibility and albumin and globulin constitute 88-99% of its main proteins (Song et al., 2002). In this research, optimization of sweet almond protein enzymatic hydrolysis process was done using alcalase enzyme and ultrasound pretreatment. The investigated variables were hydrolysis time, enzyme concentration and ultrasound application time, and the investigated responses were hydrolysis degree, total antioxidant capacity and iron ion chelating activity. Then the effect of enzyme concentration on iron ion reduction activity and antioxidant capacity of the whole optimal treatment was evaluated. Next, a comparison of functional properties (solubility, foaming properties and emulsifying properties) of optimal hydrolyzed treatment with non-hydrolyzed protein was done at different pH. The results showed the highest amount of antioxidant activity (DPPH radical inhibition activity (62.97%), iron ion chelation (72.85%) and appropriate hydrolysis degree (31.38%) with the use of alcalase enzyme under conditions of application of ultrasound waves. (400 W) 3.59 minutes, the hydrolysis time was 171.50 minutes and the enzyme concentration was 1.95%.The antioxidant activity of the hydrolyzed protein was dependent on the concentration and the hydrolysis significantly increased the functional properties of the sweet almond protein. According to the results obtained in this research, the hydrolyzed protein product can be used in the food industry as a natural preservative and nutrient composition and in pharmaceutical products to increase functional properties and improve nutritional properties.

1.Introduction

Lipid oxidation is considered a destructive reaction during processing and a factor in reducing the shelf life of foods. Antioxidants are compounds that can reduce the rate of oxidation and the formation of free radicals, and thus protect cells from oxidative damage. These antioxidant compounds may be synthetically produced and added to foods. Or they may be naturally present in them (2). Among antioxidants, bioactive peptides have attracted the attention of many researchers. These compounds are able to compete with synthetic antioxidants and maintain the quality of foods by preventing oxidation and microbial spoilage (3). The properties of bioactive peptides depend on their amino acid composition and sequence (4). These properties include immunomodulatory (5), antioxidant (6), anticancer (7), and antihypertensive (8) effects. Many studies have demonstrated the antioxidant properties of hydrolyzed proteins. For example, Longyan et al. (2021) reported that by hydrolyzing flaxseed protein to release various bioactive peptides using three plant proteases, ficin, papain, and bromelain, 20 peptides with significant biological activity were identified among the produced peptides. Finally, they stated that this protein is a potential source of peptides with inhibitory activity of angiotensin-converting enzyme and dipeptidyl peptidase (9). Ido et al. (2019) reported that the type of protease enzyme has a significant effect on the antioxidant properties of the hydrolyzed protein by producing salmon hydrolyzed protein with alcalase and papain enzymes (10). The use of various pretreatments, especially ultrasound waves, has received much attention. Ultrasound waves are defined as sound waves with frequencies above the human hearing threshold (20 to 100 kHz). According to studies, these waves cause changes in the secondary and tertiary structures of proteins, leading to better access of proteins to enzymes (11).

In this regard, Li et al. (2018) studied the effect of different intensities of ultrasound pretreatment (200, 40, and 600 W) on the antioxidant and structural properties of alcalase-hydrolyzed quinoa protein isolate and showed that ultrasound treatment with an intensity of 400 W significantly improved the degree of hydrolysis and antioxidant properties of the hydrolyzed protein. Also, changes in the secondary structure of proteins and surface hydrophobicity were caused by the opening of the protein structure, and scanning electron microscopy also showed a change in the shape of the proteins (12).

In recent years, hydrolyzed proteins and bioactive peptides with health-promoting properties have been extracted from a variety of animal and plant sources, and plant sources have received more attention due to their more affordable price and less allergenicity (13). Meanwhile, sweet almonds, scientifically known as Prunus dulcis, are a nutrient-rich food and a rich source of vitamin E, dietary fiber, B vitamins, and essential minerals such as magnesium, copper, manganese, calcium, and potassium. The protein content of commercial almond varieties is reported to be 16-23%, and these proteins have a high digestibility percentage, with albumin and globulin constituting a major part of the main proteins (14). Therefore, considering the appropriate protein content of almond meal and its health-promoting properties, almond meal is a suitable source for the production of bioactive peptides. (15). In this study, alcalase enzyme was used to produce hydrolyzed proteins with better functional and nutritional properties than the original protein. The aim of this study was to optimize the production of hydrolyzed sweet almond meal protein with antioxidant properties using the enzyme alcalase and to investigate the effect of the degree of hydrolysis on the ability to inhibit DPPH free radicals, ferrous ion chelation, ferric ion reduction, and total antioxidant power

of the resulting peptides compared to the original protein.

2- Materials and Methods

2-1- Materials

Alcalase enzyme (Au/kg) 35, Coomassie Brilliant Blue (G250), DPPH free radical, potassium ferricyanide and ferric chloride were obtained from Sigma and soda, hydrochloric acid from Merck. Sweet almond meal was obtained from a local store. Other materials used, including vitamin C, were obtained from Gorgan Standard Collaborative Laboratories and were of laboratory grade.

2-2- Preparation of sweet almond protein concentrate

First, sweet almond meal was powdered with a grinder; then, to remove fat, the resulting powder was mixed with hexane in a ratio of 1:10 (weight/volume) and stirred for 7 hours using a shaker at 200 rpm at room temperature. After settling of sweet almond powder, hexane was separated and to remove maximum hexane, the defatted powder was placed in an oven at 30°C for 1 hour and passed through a 40 mesh sieve for homogenization. In order to extract sweet almond protein, the defatted powder was mixed with distilled water in a ratio of 1:10 and its pH was adjusted to pH=11 by adding 1N sodium hydroxide and stirred for 1 hour. Then, the resulting solution was centrifuged at 7000×g for 30 minutes at 4°C. In the next step, the pH of the supernatant was adjusted to pH=4 (isoelectric pH of sweet almonds) and to precipitate proteins, the resulting solution was centrifuged at 10000×g for 20 minutes (refrigerated centrifuge made in South Korea, Hanil Company, model Combi-514R). The protein precipitate was then washed twice with 10 ml of distilled water each time and centrifuged at 5000 × g for 5 minutes. The resulting precipitates were then dried using a freeze dryer (made in South Korea, Operon Company, model FDB 5503) and stored at -20°C until testing (14).

2-3- Ultrasonic pretreatment

5 grams of sweet almond protein concentrate was added to 100 ml of phosphate buffer (0.5 M, pH = 7) and stirred for 2 hours. In order to apply ultrasonic pretreatment, the samples were exposed to the influence of an ultrasonic probe with a constant frequency of 20 KHz and a constant power (200) for the times mentioned in Table 1 (11).

2-3- Enzymatic hydrolysis

For the enzymatic hydrolysis process, sweet almond protein concentrate was dissolved at a concentration (w/v) of 5% in 0.2 M phosphate buffer and pH = 8 for the activity of the alkalase enzyme and its complete hydration was allowed by continuous stirring using a magnetic stirrer for 30 minutes at room temperature. After the incubator temperature reached 50°C for alkalase, the samples were placed in the incubator and after the incubator temperature stabilized (enzyme in the proportions mentioned in Table 1), it was added to the solution. The reaction was carried out at the times mentioned in Table 1. After the desired times, in order to inactivate the enzyme, the Erlenmeyer flasks were placed in a 90°C water bath for 10 minutes and then cooled to room temperature using an ice-filled container. Then, the samples were centrifuged at 10,000 × g for 20 minutes and their supernatant was separated and dried using a freeze dryer and stored at -20°C until the tests were performed (16).

Table 1. Enzymatic hydrolysis treatments of sweet almond protein from Design Expert software

Enzyme concentration ((percentage	Hydrolysis time ((minutes	Ultrasonication time ((minutes	
2	120	5	1
2	120	0	2
2	120	10	3
1	210	10	4
3	30	0	5
1	120	5	6
3	30	10	7
3	120	5	8
2	30	5	9
1	30	10	10
3	210	10	11
2	120	5	12
1	210	0	13
2	210	5	14
3	210	0	15
1	30	0	16
2	120	5	17

2-4- Determination of the degree of hydrolysis

Hydrolyzed protein suspension and trichloroacetic acid (M 0.44) were mixed in a volume ratio of 1:1 and incubated for 15 min at 4°C. Then, the mixture was centrifuged at 10000×g for 10 min. The amount of protein in the supernatant containing M 0.22 trichloroacetic acid was determined by the Bradford method (17) (a rapid and accurate spectroscopic analytical method used to measure the concentration of protein in solution. The Bradford method has a standard using bovine serum albumin protein, according to which the amount of protein is determined. This reaction depends on the amino acid composition of the measured protein). Finally, the degree of hydrolysis was determined using equation 1:

Equation 1

DH (%) = { Protein (TCA+ Supernatant)/ Protein (almond hydrolysate suspension) }× 100

TCA: Trichloroacetic acid

2-5-Investigation of antioxidant properties of hydrolyzed protein

2-5-1- DPPH free radical scavenging activity

To determine the percentage of DPPH free radical scavenging activity, first the hydrolyzed protein was dissolved in distilled water at a concentration of (40 mg/ml). Then, 1.5 ml of each sample was mixed with 1.5 ml of ethanolic DPPH solution (0.15 mM) and vortexed for 20 seconds. Then, the resulting mixture was centrifuged at 2500 rpm for 10 minutes and kept in the dark for 30 minutes. The absorbance of the supernatant solution was read at a wavelength of 517 nm. The percentage of DPPH free radical scavenging was calculated using Equation 2 (18).

Equation 2 I (%) = $I = [\frac{Acontrol-Asample}{Acontrol}] \times 100$

Acontrol is the control absorbance (an equal volume of distilled water was mixed with the DPPH solution instead of the sample) and Asample is the sample absorbance.

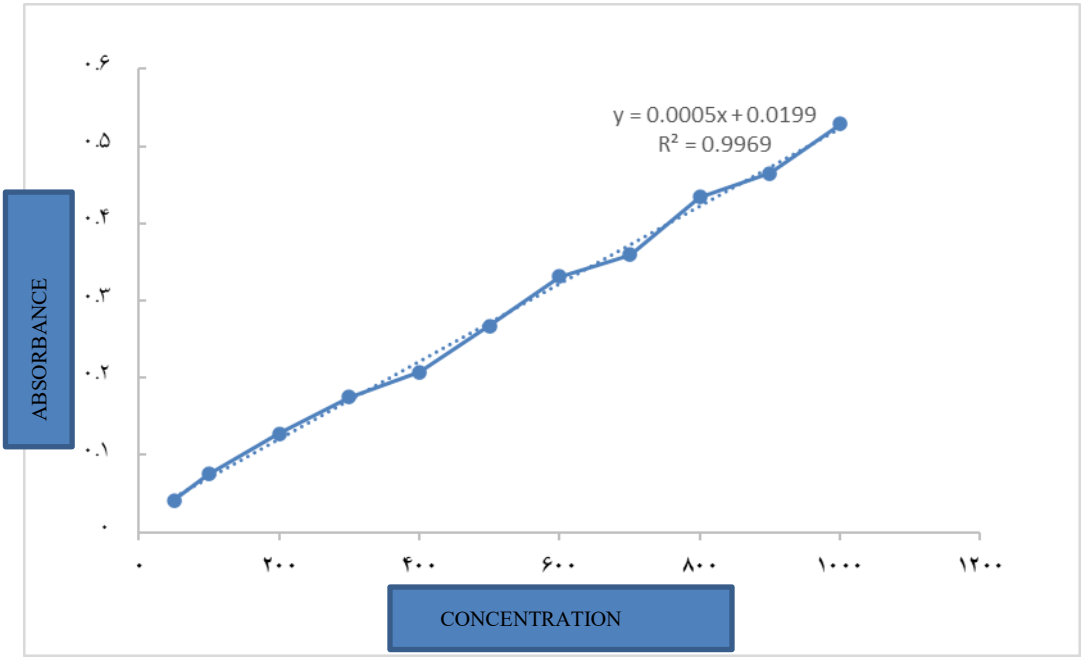

Figure 1. Bradford standard curve

2-5-2- Iron ion chelating activity

To evaluate the iron ion chelating activity, 1 ml of the sample dissolved in distilled water (concentration 40 mg/ml) was mixed with 0.05 ml of ferric chloride solution (mM2) and 1.85 ml of double distilled water. Then, 0.1 ml of ferrozin solution (mM5) was added and the mixture was stirred vigorously (the ferrozin solution was prepared in the mentioned concentration in the laboratory environment and added to the previous solution and stirred with a stirrer). The absorbance was read at 562 nm after 10 minutes of keeping the mixture at room temperature (19). Double distilled water was used as the control sample. The chelating activity of the samples was calculated using equation 3:

Equation 3 Chelating effect (%) =

Acontrol is the control absorbance and Asample is the sample absorbance.

2-5-3- Total antioxidant capacity

To evaluate the total antioxidant capacity, 0.1 ml of the sample dissolved in distilled water at a concentration (mg/ml) of 10-100 was added to 1 ml of the reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in an Eppendorf tube and placed in a 90°C water bath for 90 minutes. After cooling, the absorbance of the samples was read at 695 nm. Double distilled water was used as a control sample. Higher absorbance indicates higher total antioxidant capacity (20).

2-5-4- Iron ion reducing activity

To evaluate the reducing power of iron ion of hydrolyzed samples, 0.5 ml of the sample dissolved in distilled water at a concentration (mg/ml) of 20-100 was mixed with 0.5 ml of 0.2 M phosphate buffer (pH=6.6) and 0.5 ml of 1% potassium ferricyanide (W/V) and incubated at 50°C for 20 minutes. Then, 0.5 ml of 10% trichloroacetic acid solution was added to the mixture and centrifuged at 2500 rpm for 10 minutes. Finally, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.2 ml of 0.1% ferric chloride (W/V). The absorbance of the sample was read at 700 nm after keeping the mixture at room temperature for 10 minutes. The increase in the absorption of the reaction mixture indicates an increase in the reducing power. Finally, the reducing power of the protein is compared with the vitamin C source (21).

2-6- Examination of the functional properties of hydrolyzed protein

2-6-1- Solubility

The method of Jamdar et al. (2010) was used to determine the solubility of protein concentrate and sweet almond hydrolyzed protein. 200 mg of hydrolyzed protein was dissolved in 20 ml of distilled water and the pH of the mixture was adjusted to 1 to 12 using 1 N sodium hydroxide or hydrochloric acid. The solution was centrifuged at 10,000 g for 10 minutes, then the amount of protein in the eluate was determined using the Bradford method and the solubility percentage was determined based on the amount of soluble protein per total protein of the sample (22).

2-6-2-Emulsification

 First, 5 ml of grape seed oil and 15 ml of 1% protein concentrate or sweet almond hydrolyzed protein solution were mixed together and the pH was adjusted to 3, 5, 7 and 9 using 1N sodium hydroxide or hydrochloric acid. The mixture was homogenized using a homogenizer at 20,000 rpm for 1 minute, then, 50 µl of the emulsion sample was removed from the container at 0 and 10 minutes after homogenization and mixed with 5 ml of 0.1% sodium dodecyl sulfate solution. The absorbance of the diluted solution was read using a spectrophotometer (UV-visible spectrophotometer, UK model T80) at 500 nm. The absorbance at the initial time (A0) and 10 minutes after emulsion formation (A10) was used to calculate the emulsifying activity using equation 4 (23).

Equation 4

EAI (m2/g) = (2 × 2.303 × A0)/ 0.25 × protein weight (g)

2-6-3-Foaming

15 ml of a 0.5% solution of the sample was prepared using 1 N sodium hydroxide or hydrochloric acid, and homogenized at 3, 5, 7 and 9 rpm for 2 minutes to introduce air into the solution at room temperature. Then, the whipped sample was quickly transferred to a 25 ml graduated cylinder and the total volume was

recorded after 1 minute. The foaming capacity (FC) was calculated using equation 5 (23).

FC (%) = (A /B) × 100 Equation 5

A is the volume of foam after beating (ml) and B is the initial volume before beating (ml).

2-7- Statistical analysis

Data analysis was evaluated in a completely randomized design using one-way analysis of variance and SPSS version 16 software. All tests were performed in 3 repetitions and means were compared using Duncan's multiple range test to check the significance of the variable at the $p<0.05$ level and graphs were drawn with Excel 2021 software. Model optimization and validation were performed based on the response level.

The response surface has been determined.

3- Results and Discussion

3-1- Effect of hydrolysis time, enzyme to substrate ratio, protease type and ultrasound pretreatment time on the degree of hydrolysis of sweet almond hydrolyzed protein

According to Figure 2, Part A, at fixed times of ultrasound application, increasing the hydrolysis time with alcalase enzyme to 120 minutes significantly increased the degree of hydrolysis to 32.82%, and further increase in time to 210 minutes decreased the degree of hydrolysis of the produced hydrolyzed proteins, and at fixed times of hydrolysis, increasing the time of ultrasound application to 7 minutes increased the degree of hydrolysis, but after this time, the increasing slope of the degree of hydrolysis decreased. The highest degree of hydrolysis (34.062%) was obtained in hydrolysis with alcalase enzyme at 177.25 minutes and the time of ultrasound application was 8.42 minutes. Figure 2, part b, shows the effect of enzyme concentration and ultrasonic application time on the degree of hydrolysis of sweet almond hydrolyzed protein. According to the figure, at constant enzyme concentrations, increasing the ultrasonication time to 8 minutes significantly increased the degree of hydrolysis, but then with further increase in time, the increasing slope of the degree of hydrolysis decreased. At constant ultrasonication times, increasing the enzyme concentration to 2.8% also significantly increased the degree of hydrolysis, and no significant effect was observed with further increase in enzyme concentration. According to Figure 2, part c, at constant hydrolysis times, increasing the enzyme concentration to 2.5% increased the degree of hydrolysis, but further increase had no significant effect. On the other hand, at constant enzyme concentrations, increasing the hydrolysis time to 180 minutes increased the degree of hydrolysis, but further increase had no significant effect. The lack of further increase in the degree of hydrolysis with further increase in hydrolysis time could be due to excessive protein hydrolysis and the inhibitory effect of the produced peptides (24). Regarding the effect of ultrasound on the degree of hydrolysis, it has been reported that ultrasound, by changing the secondary and tertiary structures of proteins, leads to the opening of the structure of proteins, which leads to better performance of protease enzymes (25). These results were in line with the results of Kaveh et al. (2019) and Yasemi et al. (2013) in the hydrolysis of fenugreek seed protein and the intestines and viscera of bighead carp, respectively, who stated that excessive increase in enzymatic hydrolysis time does not have a significant effect on the degree of hydrolysis. Regarding the positive effect of ultrasound on the degree of hydrolysis, similar results have been reported by Ashraf et al. (2020) and Uluko et al. (2015) in the hydrolysis of mung bean and lupin, respectively. (11 and 15).

The results showed that the effect of all the variables studied on the degree of hydrolysis of

the hydrolyzed protein obtained from alcalase had a significant effect, and among them, the effect of hydrolysis time was more significant ($p<0.05$)

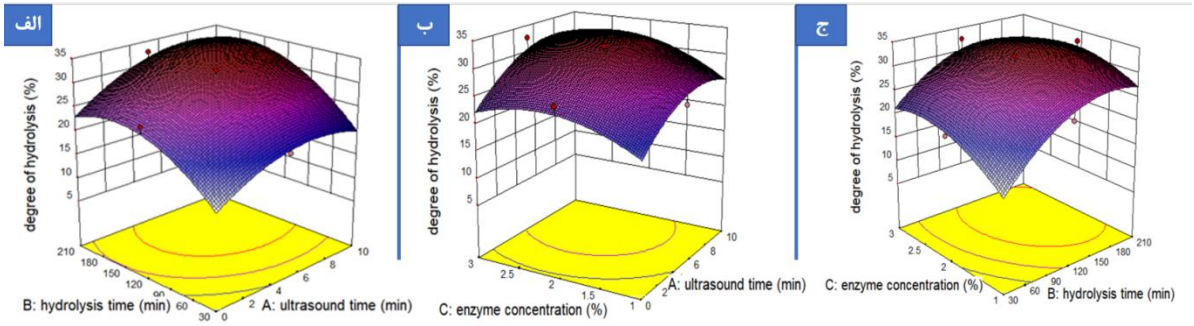

Figure 2- The effect of hydrolysis time, enzyme concentration and ultrasound application time on the degree of hydrolysis of sweet almond hydrolyzed protein with alcalase enzyme.

3-2- The effect of hydrolysis time, enzyme to substrate ratio, protease type and ultrasound pretreatment time on the DPPH radical scavenging activity of sweet almond hydrolyzed protein

Figure 3 shows the effect of hydrolysis time, enzyme concentration and ultrasound application time on the DPPH radical scavenging activity of sweet almond hydrolyzed protein with alcalase enzyme. At fixed hydrolysis times, increasing the enzyme concentration to 2.5% significantly increased the DPPH radical scavenging activity, and it decreased with further increase. On the other hand, at fixed enzyme concentrations, increasing the hydrolysis time to 180 minutes increased the DPPH radical scavenging activity, and further increase had no significant effect. In general, the highest DPPH radical scavenging activity (62.29%) was achieved at a hydrolysis time of 163.23 min and an enzyme concentration of 1.94%. In this regard, it has been reported that hydrolysis with increasing time produces proton-donating peptides that can react with DPPH free radicals, but further hydrolysis causes the decomposition of these peptides, resulting in a decrease in antioxidant power (26). In accordance with these findings, Wen et al. (2019) reported that the application of ultrasound waves with a frequency of 20 kHz and a power of 200 W had a positive effect on the DPPH free radical scavenging activity of hydrolyzed watermelon seed protein (27).

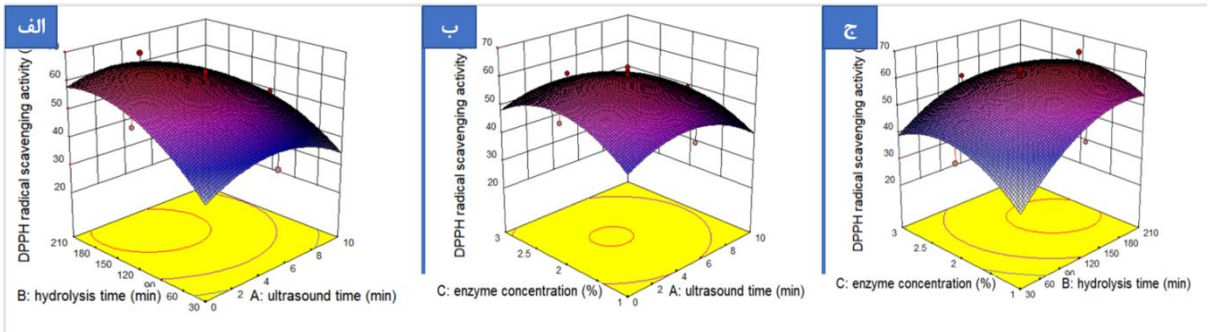

Figure 3- Effect of hydrolysis time, enzyme concentration and ultrasound application time on DPPH radical scavenging activity of hydrolyzed sweet almond protein with alcalase enzyme

3-3- Effect of hydrolysis time, enzyme to substrate ratio, protease type and ultrasound pretreatment time on iron ion chelating activity of hydrolyzed sweet almond protein

The results of variance analysis showed that the effect of all variables studied except enzyme concentration had a significant effect on the iron ion chelating activity of hydrolyzed sweet almond protein obtained with alcalase. In the meantime, the effect of hydrolysis time was significantly greater than other variables. Figure 4 shows the effect of hydrolysis time, enzyme concentration and ultrasound application time on the iron ion chelating activity of hydrolyzed sweet almond protein with alcalase enzyme. According to the results obtained, it was determined that at fixed hydrolysis times, increasing the enzyme concentration to 2.7% significantly increased the iron ion chelating activity, and a further increase had a negative effect. On the other hand, at constant enzyme concentrations, increasing the hydrolysis time to 175 minutes significantly increased the iron ion chelating activity, and with further increase in time, this ability decreased. The highest chelating activity On the other hand, at constant enzyme concentrations, increasing the hydrolysis time to 175 min significantly increased the iron ion chelating activity, and this ability decreased with further increase in time. The highest iron ion chelating activity (68.14%) was obtained at a hydrolysis time of 184.25 min and an enzyme concentration of 2.57%. In general, it has been reported that many factors affect the chelating ability of hydrolyzed protein, including: amino acid composition of the original protein, type of enzyme used, amino acid sequence, and degree of hydrolysis (28). These results are in agreement with the findings of Jamdar et al. (2010) in peanut protein hydrolysis (22).

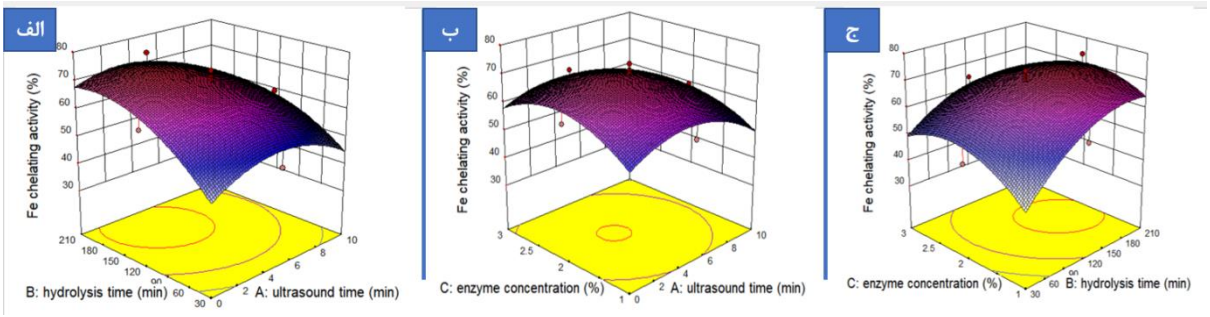

Figure 4- The effect of hydrolysis time, enzyme concentration and ultrasound application time on iron ion chelating activity of hydrolyzed sweet almond protein with alcalase enzyme

3-4- Optimization and evaluation of model validity

After data analysis, the software reported the optimal conditions for obtaining the highest DPPH radical scavenging activity (62.97%), iron ion chelation (72.85%), and appropriate hydrolysis degree (31.38%) with the alcalase enzyme as ultrasonication time of 3.59 minutes, hydrolysis time of 171.50 minutes, and enzyme concentration of 1.95%. Hydrolysis was performed under the mentioned conditions, and iron ion chelating activity of 75.24%, DPPH radical scavenging activity of 57.34%, and hydrolysis degree of 28.56% were calculated.

3-5- Total antioxidant capacity

According to Figure 5, increasing the concentration of hydrolyzed almonds to (mg/ml) 80 significantly increased the antioxidant capacity ($p<0.05$), but further increase in concentration had no significant effect ($p<0.05$). The highest and lowest total antioxidant capacity was related to the hydrolysate with a concentration of 80 (mg/ml) and 20 (mg/ml) at the rate of 0.924 and 0.410 (absorption at 695 nm). From the other container, in all concentrations, the total antioxidant capacity of sweet almond hydrolysate protein was significantly higher than that of non-hydrolysate protein and lower than that of vitamin C at a concentration of 100 (mg/ml) ($p<0.05$). The high total antioxidant capacity of sweet almond hydrolysate protein hydrolysate indicates that the alcalase enzyme is capable of producing hydrolysates with electron-donating ability that can act as an antioxidant compound to inhibit free radicals (29). Similar to these results, Kaveh et al. (2019) reported that the total antioxidant capacity of hydrolyzed fenugreek protein was concentration-dependent, and increasing the hydrolyzed concentration from (mg/mL) 10 to (mg/mL) 40 significantly increased the total antioxidant capacity, and further increase in concentration had no significant effect; they also reported that the hydrolyzed antioxidant capacity produced at all concentrations was less than the total antioxidant power of vitamin C at a concentration of (mg/mL) 50 (30). Also, Bogtaev et al. (2009) reported the concentration-dependent total antioxidant capacity of hydrolyzed shark protein and stated that the total antioxidant capacity was less than the synthetic antioxidant BHA (31).

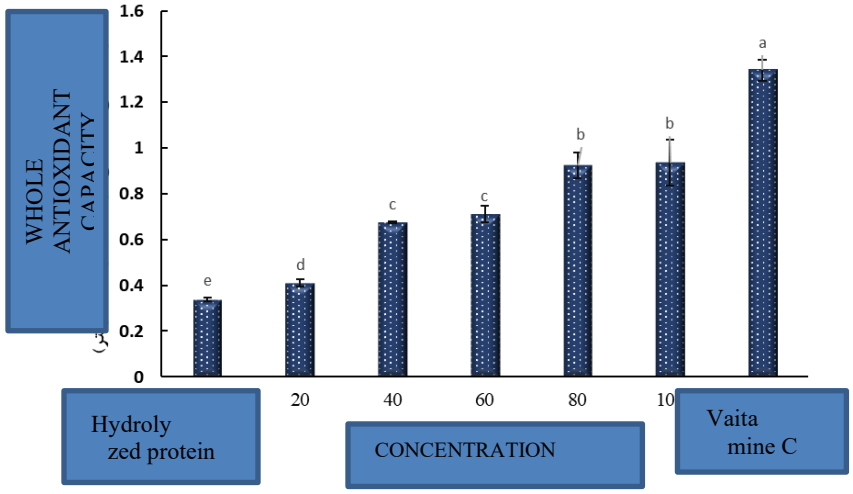

Figure 5- The effect of concentration on the total antioxidant capacity of sweet almond hydrolyzed protein compared to non-hydrolyzed protein and vitamin C at a concentration 100 (mg/ml)

3-6- Iron ion reducing activity

According to Figure 6, the reducing power of sweet almond hydrolyzed proteins was significantly higher than that of non-hydrolyzed proteins. On the other hand, increasing the concentration from (mg/ml) 20 to (mg/ml) 80 increased the reducing power of the hydrolyzed products from 0.452 to 0.968 (absorption at 700 nm); further increasing the concentration to

(mg/ml) 100 significantly decreased the reducing power ($p<0.05$). Comparing the reducing power of the samples with the antioxidant power of vitamin C showed that sweet almond hydrolyzed protein had lower reducing power at all concentrations. In general, the reductive activity of sweet almond hydrolyzed proteins can be attributed to the release of reductive amino acids such as tryptophan, lysine, and methionine (22). These results were in agreement with the findings of Xie et al. (32) and Zhao et al. (33), who investigated the effect of concentration on the reductive power of alfalfa and canola hydrolyzed proteins, respectively.

Hydrolyzed protein

3-6- Iron ion reducing activity

According to Figure 6, the reducing power of sweet almond hydrolyzed proteins was significantly higher than that of non-hydrolyzed proteins. On the other hand, increasing the concentration from (mg/ml) 20 to (mg/ml) 80 increased the reducing power of the hydrolyzed products from 0.452 to 0.968 (absorption at 700 nm); further increasing the concentration to (mg/ml) 100 significantly decreased the reducing power ($p<0.05$). Comparing the reducing power of the samples with the antioxidant power of vitamin C showed that sweet almond hydrolyzed protein had lower reducing power at all concentrations. In general, the reductive activity of sweet almond hydrolyzed proteins can be attributed to the release of reductive amino acids such as tryptophan, lysine, and methionine (22). These results were in agreement with the findings of Xie et al. (32) and Zhao et al. (33), who investigated the effect of concentration on the reductive power of alfalfa and canola hydrolyzed proteins, respectively.

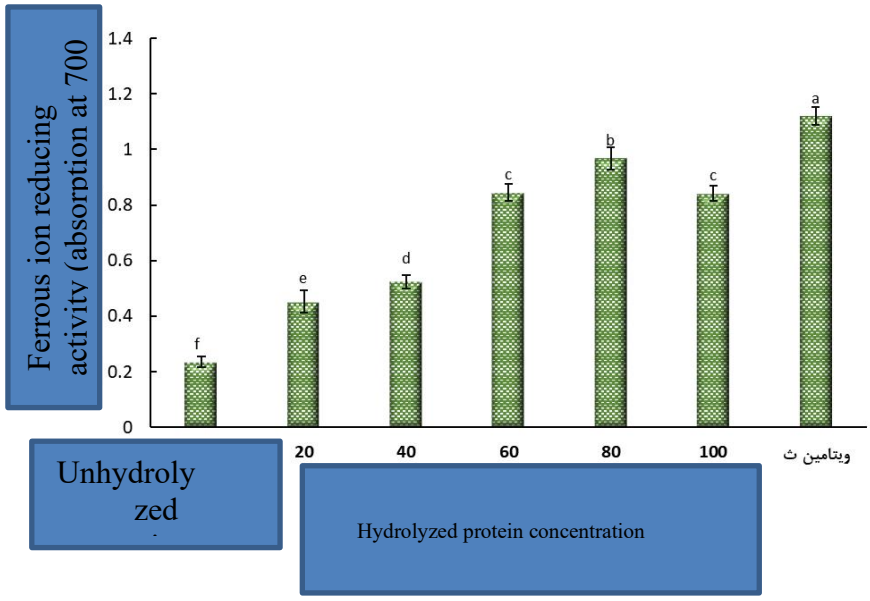

Figure 6- The effect of concentration on iron ion reduction activity of sweet almond hydrolyzed protein compared to non-hydrolyzed protein and vitamin C at a concentration 100 (mg/ml)

3-7- Functional Properties

3-7-1- Solubility

Figure 7 shows the solubility profile of almond protein concentrate and its hydrolyzed protein at pH 1-12. In general, the lowest solubility of

protein concentrate (26.5%) occurred at acidic pHs, especially pH 4 (its isoelectric point). Studies have shown that the decrease in solubility at acidic and isoelectric pHs of a protein is due to the precipitation of high molecular weight proteins and primary peptides. At the isoelectric point, the solubility of the protein is very low and the repulsive force between adjacent protein molecules is minimized, the molecules attract each other and the protein precipitates (23). The solubility of protein concentrate increased significantly with increasing pH, such that the highest solubility (98.68%) was achieved at pH 10. In this regard, it has been reported that by hydrolysis and reduction of molecular weight of peptides and production of amino acids, sensitivity to low pH and solubility are maintained in a wide range of pH. The increase in solubility of proteins at different pHs can be attributed to the positive effect of controlled enzymatic hydrolysis on molecular weight reduction and increase in charged groups. Also, enzymatic hydrolysis increases the solubility of proteins by breaking down insoluble protein aggregates, producing smaller peptides, increasing the accessibility of hydrophilic groups, and facilitating the reaction of hydrophilic amino acids with the aqueous environment (34). Similar to these findings, studies showed that the hydrolyzed protein of pumpkin meal by the enzyme alcalase had a high solubility at alkaline pH, especially pH 11, compared to acidic pHs by 93.40% (35). The positive effect of the hydrolysis process on increasing the solubility of proteins has been reported in various studies, including Lee et al. (22) and Samai et al. (36) who reported an increase in the solubility of the hydrolyzed proteins produced by producing tea and fava bean seeds, respectively, compared to the initial rutin. They performed hydrolysis using trypsin and alcalase enzymes at a concentration of 1.5-3% at an optimum temperature of 37-50°C, pH = 7-8.5, and a duration of 3 hours.

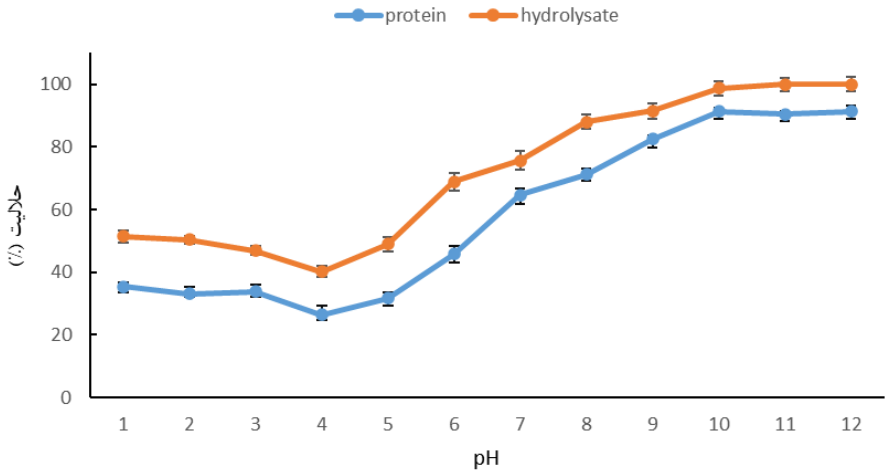

Figure 7- Solubility of hydrolyzed protein and sweet almond protein concentrate at pH 1-12

-7-2- Emulsification

Emulsification capacity is the ability of an emulsifier to form and stabilize small droplets of the dispersed phase during homogenization and during storage of the freshly prepared emulsion (23). In general, hydrolysis significantly increased the emulsification capacity of sweet almond protein, and both hydrolyzed protein and sweet almond protein concentrate had greater emulsification capacity at alkaline pHs. The

lowest (2.50) and highest (78.160) emulsification values were for the hydrolyzed protein at pH=9 and the protein concentrate at pH=3, respectively. The lower emulsifying ability of the samples at acidic pH (close to the isoelectric point) is due to their low solubility, which causes precipitation of proteins and loss of their emulsifying properties (7). These results indicate that sweet almond protein is not suitable for use as an emulsifier in acidic food products. The increase in emulsifying power by enzymatic hydrolysis can be attributed to the maintenance of solubility, improvement of emulsifying activity as a result of changes in peptide composition, and the ability to form a film around oil droplets at acidic pH (37). Similar to these results, Sarabandi et al. (23) and Shariat Alavi et al. (38) reported an increase in the emulsifying ability of casein and tomato seed proteins by enzymatic hydrolysis, respectively. They also stated that all hydrolysates had greater emulsifying ability at alkaline pH. They had higher foaming capacity than the acidic pH.

4-4-3-Foaming

The foaming capacity is another important functional characteristic of proteins and their hydrolysates. Studies have shown that the foaming capacity of proteins is greatly influenced by the type of initial protein, pH and hydrolysis conditions, degree of hydrolysis, and the concentration and type of enzyme used. On the other hand, solubility, surface charge, reactivity of peptide chains, and film formation at the interface of bubbles are other factors affecting the foaming efficiency of hydrolyzed proteins (23). The foaming capacity of sweet almond protein and its hydrolysate is shown in Figure 8. In general, almond hydrolysate had a higher foaming capacity at all pHs compared to its protein concentrate. Also, the foaming capacity of both samples at alkaline pHs was significantly higher than at acidic pHs. ($p<0.05$). The low foaming capacity of the samples at acidic pH could be due to their low solubility. On the other hand, the improvement of foaming ability by enzymatic hydrolysis could be due to the increased flexibility of the peptides, facilitating the formation of interfacial membranes and films around air bubbles and consequently foam production (39). In this regard, Popović et al. (40) reported an increase in the foaming ability of pumpkin protein after enzymatic hydrolysis.

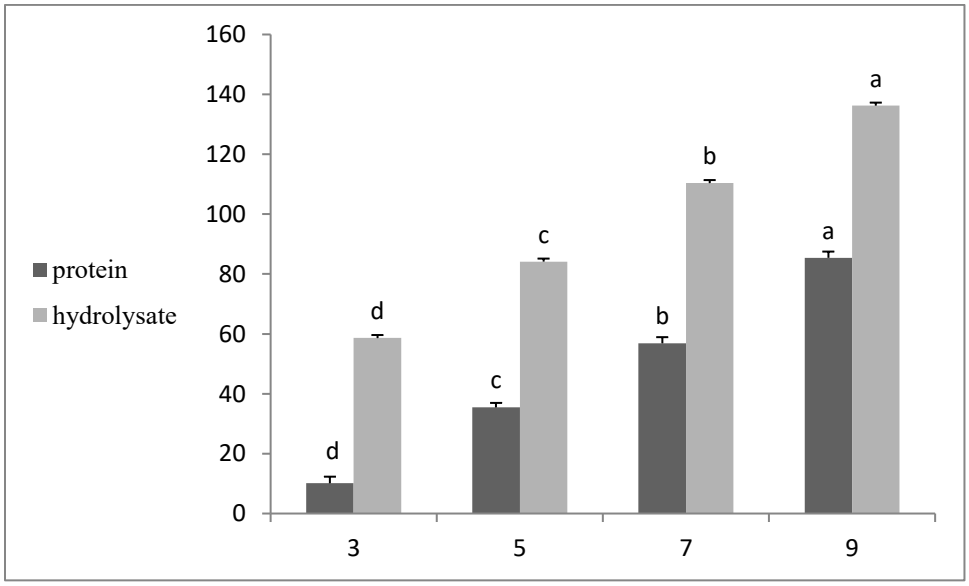

Figure 8- Foaming capacity of hydrolyzed protein and sweet almond protein concentrate

4- Conclusion

The results of optimizing the hydrolysis conditions (enzyme concentration, hydrolysis time) and the time of applying ultrasound on sweet almond hydrolyzed protein showed that the highest antioxidant activity (DPPH radical scavenging activity (62.97%), iron ion chelation (72.85%), and appropriate degree of hydrolysis (31.38%) were achieved with the alcalase enzyme under the conditions of ultrasound application time of 3.59 minutes, hydrolysis time of 171.50 minutes, and enzyme concentration of 1.95%. Investigating the effect of concentration on iron ion reducing activity and total antioxidant capacity of the optimized treatment of hydrolyzed protein showed that its antioxidant potential was significantly dependent on concentration. On the other hand, evaluating the functional properties (solubility, foaming, and emulsification) of hydrolyzed protein compared to non-hydrolyzed protein showed that hydrolysis significantly improved its functional properties.

6- References

[1] Steinbrenner, H., Speckmann, B. and Klotz, L.O., (2016). Selenoproteins: Antioxidant selenoenzymes and beyond. Archives of Biochemistry and Biophysics, 595, pp.113-119.

[2] Parcheta, M., Świsłocka, R., Orzechowska, S., Akimowicz, M., Choińska, R. and Lewandowski, W., (2021). Recent developments in effective antioxidants: The structure and antioxidant properties. Materials, 14(8), p.1984.

[3] Sánchez, A. and Vázquez, A., 2017. Bioactive peptides: A review. Food quality and safety, 1(1), pp.29-46.

[4] Chen, C., Chi, Y.J., Zhao, M.Y. and Xu, W. (2012). Influence of degree of hydrolysis on functional properties, antioxidant and ACE inhibitory activities of egg white protein hydrolysate. Food Science and Biotechnology, 21: 27-34.

[5] Gauthier, SF., Pouliot, Y., and Saint-Sauveur, D. (2006). Immunomodulatory peptides obtained by the enzymatic hydrolysis of whey proteins. International Dairy Journal, 16(11):1315–23.

[6] Jakubczyk, A., Karaś, M., Rybczyńska-Tkaczyk, K., Zielińska, E. and Zieliński, D., (2020). Current trends of bioactive peptides—New sources and therapeutic effect. Foods, 9(7), p.846.

[7] Wang, C., Tian, L.L., Li, S., Li, H.B., Zhou, Y., Wang, H., Yang, Q.Z., Ma, L.J. and Shang, D.J., 2013. Rapid cytotoxicity of antimicrobial peptide tempoprin-1CEa in breast cancer cells through membrane destruction and intracellular calcium mechanism. PloS one, 8(4), p.e60462.

[8] Ganguly, A., Sharma, K. and Majumder, K., (2019). Food-derived bioactive peptides and their role in ameliorating hypertension and associated cardiovascular diseases. Advances in food and nutrition research, 89, pp.165-207.

[9] Langyan, S., Khan, F. N., Yadava, P., Alhazmi, A., Mahmoud, S. F., Saleh, D. I., Zuan, A. T. K. (2021). In silico proteolysis and analysis of bioactive peptides from sequences of fatty acid desaturase 3 (FAD3) of flaxseed protein. Saudi journal of biological sciences, 28(10), 5480-5489.

[10] Idowu, A.T., Benjakul, S., Sinthusamran, S., Sookchoo, P. and Kishimura, H., (2019). Protein hydrolysate from salmon frames: Production, characteristics and antioxidative activity. Journal of Food Biochemistry, 43(2), p.e12734.

[11] Farzanfar, F., Sadeghi Mahoonak, A., Ghorbani, M., Hosseini Qaboos, S.H., Kaveh, S. (2024). The effect of ultrasound pretreatment on the antioxidant properties of hydrolyzed protein from flaxseed meal using alcalase and pancreatin enzymes by response surface methodology. Journal of Food Science and Technology (Iran), 21(147), 187–205.

[12] Li, X., Da, S., Li, C., Xue, F. and Zang, T., (2018). Effects of high-intensity ultrasound pretreatment with different levels of power output on the antioxidant properties of alcalase hydrolyzates from Quinoa (Chenopodium quinoa Willd.) protein isolate. Cereal Chemistry, 95(4), pp.518-526.

[13] Rutherfurd-Markwick, K.J., (2012). Food proteins as a source of bioactive peptides with diverse functions. British Journal of Nutrition, 108(S2), pp.S149-S157.

[14] Udenigwe, C.C., Je, J.Y., Cho, Y.S. and Yada, R.Y., (2013). Almond protein hydrolysate fraction modulates the expression of proinflammatory cytokines and enzymes in activated macrophages. Food & function, 4(5), pp.777-783.

[15] Kaveh, S., Mahoonak, A. S., Ghorbani, M., & Jafari, S. M. (2022). Fenugreek seed (Trigonella foenum graecum) protein hydrolysate loaded in nanosized liposomes: Characteristic, storage stability, controlled release and retention of antioxidant activity. Industrial Crops and Products, 182, 114908.

[16] Sadeghi Mahoonak, A.R., & Kaveh, S. (2022). Assessment of ACE-inhibitory and antioxidant activities of the peptide fragments from

pumpkin seeds. Iranian-Journal Nutrition Science Food Technology, 17(3), 45–56.
[17] Bradford MM. (1976). A rapid and sensitive method for the quantitation of microgram of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248–54.
[18] Kaveh, S., Sadeghi, M.A., Ghorbani, M.,Jafari, M., Sarabandi, K. (2019).Optimization of production of antioxidant peptides using enzymatic hydrolysis of fenugreek seed. Journal of Food Science and Technology, 15 (84), pp. 75-88.
[19] Kaveh, S., Sadeghi Mahoonak, A., Erfanimoghaddam, V., Ghorbani, M., Gholamhossein pour, A.A., & Raeisi, M. (2024). Optimization of the effect of hydrolysis conditions and type of protease on the degree of hydrolysis and antioxidant properties of the protein hydrolysate from the skipjack fish (Katsuwonus pelamis) viscera by the response surface methodology. Journal of Food Science and Technology (Iran), 20(144), 131–152.
[20] Alvand, M., Sadeghi Mahoonak, A., Ghorbani, M., Shahiri Tabarestani, H. and Kaveh, S., (2022). Comparison of the Antioxidant Properties of Hydrolyzed Turkmen Melon Seed Protein by Pancreatin and Alcalase. Food Engineering Research, 21(2), pp.75-90.
[21] Kaveh, S., Sadeghi Mahoonak, A., Erfani Moghadam, V., Ghorbani, M., Gholamhosseinpour, A., Raeisi, M. (2023). Evaluation the antioxidant properties of purified bioactive peptides from the wastes of skipjack fish (Katsuwonus pelamis) processing, by pepsin and trypsin digestive enzymes. Journal of Food Science andTechnology, 20 (141):200-222
[22] Jamdar, S.N., Rajalakshmi, V., Pednekar, M.D., Juan, F., Yardi, V. and Sharma, A., (2010). Influence of degree of hydrolysis on functional properties, antioxidant activity and ACE inhibitory activity of peanut protein hydrolysate. Food chemistry, 121(1), pp.178-184.
[23] Sarabandi K, Sadeghi Mahoonak A, Hamishekar H, Ghorbani M, Jafari M. (2019). Functional and Antioxidant Properties of Casein Hydrolysate Prepared with Pancreatin. Iranian Journal of nutrition science and food technology, 13 (4) :61-74.
[24] Kaveh, S., Sadeghi, M. A., Ghorbani, M., Jafari, M., & Sarabandi, K. (2019). Optimization of factors affecting the antioxidant activity of fenugreek seed's protein hydrolysate by response surface methodology.
[25] Jin, J., Ma, H., Wang, K., Yagoub, A. E. G. A., Owusu, J., Qu, WYe, X. (2015). Effects of multi-frequency power ultrasound on the enzymolysis and structural characteristics of corn gluten meal. Ultrasonics sonochemistry, 24, 55-64.
[26] Batista, I., Ramos, C., Coutinho, J., Bandarra, N. M., & Nunes, M. L. (2010). Characterization of protein hydrolysates and lipids obtained from black scabbardfish (Aphanopus carbo) by-products and antioxidative activity of the hydrolysates produced. Process Biochemistry, 45(1), 18-24.
[27] Wen, C., Zhang, J., Zhang, H., Duan, Y., & Ma, H. (2019). Effects of divergent ultrasound pretreatment on the structure of watermelon seed protein and the antioxidant activity of its hydrolysates. Food chemistry, 299, 125165.
[28] Torres-Fuentes, C., Alaiz, M., & Vioque, J. (2012). Iron-chelating activity of chickpea protein hydrolysate peptides. Food chemistry, 134(3), 1585-1588.
[29] Arabshahi-Delouee, S. and Urooj, A., 2007. Antioxidant properties of various solvent extracts of mulberry (Morus indica L.) leaves. Food chemistry, 102(4), pp.1233-1240.
[30] Kaveh, S., Sadeghi Mahoonak, A., Ghorbani, M., Sarabandi K. (2020). Comparison of antioxidant properties of fenugreek seed protein hydrolyzed with alcalase and pancreatin. Journal of Innovation in Food Science and Technology, 11(4),77-88.
[31] Bougatef, A., Hajji, M., Balti, R., Lassoued, I., Triki-Ellouz, Y. and Nasri, M., 2009. Antioxidant and free radical-scavenging activities of smooth hound (Mustelus mustelus) muscle protein hydrolysates obtained by gastrointestinal proteases. Food chemistry, 114(4), pp.1198-1205.
[32] Xie, Z., Huang, J., Xu, X. and Jin, Z., 2008. Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. Food chemistry, 111(2), pp.370-376.
[33] Zhao, Q., Xiong, H., Selomulya, C., Chen, X.D., Zhong, H., Wang, S., Sun, W. and Zhou, Q., 2012. Enzymatic hydrolysis of rice dreg protein: Effects of enzyme type on the functional properties and antioxidant activities of recovered proteins. Food chemistry, 134(3), pp.1360-1367.
[34] Cui, Q., Sun, Y., Zhou, Z., Cheng, J., & Guo, M. (2021). Effects of enzymatic hydrolysis on physicochemical properties and solubility and bitterness of milk protein hydrolysates. Foods, 10(10), 2462.
[35] Muhamyankaka, V., Shoemaker, C. F., Nalwoga, M., & Zhang, X. M. (2013). Physicochemical properties of hydrolysates from enzymatic hydrolysis of pumpkin (Cucurbita moschata) protein meal. International Food Research Journal, 20(5), 2227.
[36] Samaei, S. P., Ghorbani, M., Sadeghi Mahoonak, A., & Alami, M. (2021). Investigation of functional and antioxidant properties of faba bean protein hydrolysates using combines hydrolysis. Food Processing and Preservation Journal, 12(2), 25-38.

[37] Mohanty, U., Majumdar, R. K., Mohanty, B., Mehta, N. K., & Parhi, J. (2021). Influence of the extent of enzymatic hydrolysis on the functional properties of protein hydrolysates from visceral waste of Labeo rohita. Journal of Food Science and Technology, 58, 4349-4358.

[38] Shariat, A. M., Sadeghi, M. A., Ghorbani, M., & Alami, M. (2020). Evaluation of Functional Properties of bioactive protein hydrolysate derived from tomato seed. Journal of food science and technology, 16(96), 185-197.

[39] Betancur-Ancona, D., Sosa-Espinoza, T., Ruiz-Ruiz, J., Segura-Campos, M. and Chel-Guerrero, L., 2014. Enzymatic hydrolysis of hard-to-cook bean (Phaseolus vulgaris L.) protein concentrates and its effects on biological and functional properties. International journal of food science & technology, 49(1), pp.2-8.

[40] Popović, L., Peričin, D., Vaštag, Ž., Popović, S., Krimer, V., & Torbica, A. (2013). Antioxidative and functional properties of pumpkin oil cake globulin hydrolysates. Journal of the American oil chemists' society, 90, 1157-1165.

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بهینه سازی تولید پروتئین هیدرولیز شده کنجاله بادام شیرین به وسیله پیش تیمار فراصوت و آنزیم آلکالاز

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چکیده

بادام شیرین یک منبع خوبی از پروتئین های با کیفیت بالا می باشد و مقدار پروتئین در بادام بر اساس وزن خشک ۲۲-۱۶٪ گزارش شده است (سات، ۱۹۹۳) .پروتئین های بادام قابلیت هضم بالایی دارند و آلبومین و گلوبولین ۹۹-۸۸٪ از پروتئین های اصلی آن را تشکیل می دهند (سانگ و همکاران، ۲۰۰۲). در این پژوهش بهینه سازی فرآیند هیدرولیز آنزیمی پروتئین بادام شیرین با استفاده از آنزیم آلکالاز و پیش تیمار فراصوت انجام گرفت. متغیرهای مورد بررسی زمان هیدرولیز، غلظت آنزیم و زمان اعمال امواج فراصوت و پاسخ های مورد بررسی درجه هیدرولیز، ظرفیت آنتی اکسیدانی کل و فعالیت شلاته کنندگی یون آهن بود. سپس تاثیر غلظت آنزیم بر فعالیت احیاء کنندگی یون آهن و ظرفیت آنتی اکسیدانی کل تیمار بهینه ارزیابی شد. در ادامه، مقایسه خصوصیات عملکردی (حلالیت، خاصیت کف کنندگی و خاصیت امولسیون کنندگی) تیمار بهینه هیدرولیز شده با پروتئین هیدرولیز نشده در pH های مختلف انجام گرفت. نتایج نشان داد بیشترین میزان فعالیت آنتی اکسیدانی (فعالیت مهار رادیکال DPPH (۶۲/۹۷ درصد)، شلاته کنندگی یون آهن (۷۲/۸۵ درصد) و درجه هیدرولیز مناسب (۳۱/۳۸ درصد) با کاربرد آنزیم آلکالاز در شرایط زمان اعمال امواج فراصوت(۴۰۰ وات) ۳/۵۹ دقیقه، زمان هیدرولیز ۱۷۱/۵۰ دقیقه و غلظت آنزیم ۱/۹۵ درصد حاصل شد. فعالیت آنتی اکسیدانی پروتئین هیدرولیز شده به غلظت وابسته بود و هیدرولیز به‌میزان قابل توجهی سبب افزایش خصوصیات عملکردی پروتئین بادام شیرین شد. با توجه به نتایج به دست آمده در این پژوهش، محصول پروتئین هیدرولیز شده قابلیت استفاده در صنایع غذایی به عنوان یک نگهدارنده طبیعی و ترکیب مغذی و در فراورده های دارویی جهت افزایش خواص عملکردی و بهبود خصوصیات تغذیه ای را دارد.