Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Evaluation of the functional and antioxidant properties of peptides derived from enzymatic hydrolysis of Spirulina algae

Andisheh Tavakoli[1], Reza Farahmandfar[2*], Ali Motamedzadegan[3], Peiman Ariaii[4], Maryam Asnaashari[5]

1- PhD student, Department of Food Science and Technology, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

2- Associate Professor, Department of Food Science and Technology, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

3- Professor, Department of Food Science and Technology, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

4- Associate Professor, Department of Food Science and Technology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran

5- Department of Animal Processing, Animal Science Research Institute of Iran (ASRI), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

ARTICLE INFO

Article History:

Received:20234/5/12

Accepted:2024/12/22

Keywords:

hydrolyzed protein,
Spirulina algae,
antioxidant properties,
functional properties.

DOI: 10.22034/FSCT.22.161.69.

*Corresponding Author E-m.asnaashari@yahoo.com

ABSTRACT

Enzymatic hydrolysis of Spirulina algae protein, commonly practiced in the food industry, leads to increased protein value and the production of biologically active and functional peptides with high digestibility and desirable antioxidant properties. This study aims to investigate the antioxidant and functional properties of bioactive peptides derived from enzymatic hydrolysis of Spirulina algae. In the evaluation process, Spirulina algae proteins were hydrolyzed using alcalase and flavozyme enzymes for different durations of 10, 20, and 30 minutes. The degree of hydrolysis, protein recovery, antioxidant properties of the peptides assessed through DPPH (1,1-diphenyl-2-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) assays, as well as the functional characteristics of the peptides including solubility, emulsifying and foam capacity and stability were evaluated. The results of these evaluations demonstrated that peptides derived from enzymatic hydrolysis of Spirulina algae exhibited high antioxidant properties and could act as scavengers of free radicals. Furthermore, these peptides displayed favorable functional properties, indicating their potential applications in various industries, including the food sector. Additionally, based on the results, alcalase enzyme showed higher capability in producing hydrolyzed proteins with higher degree of hydrolysis, protein content, and recovery, as well as better antioxidant and functional properties compared to flavozyme enzyme. The hydrolysis time also had a positive impact on these parameters. Therefore, this study highlights the antioxidant and functional properties of peptides derived from enzymatic hydrolysis of Spirulina algae, suggesting their potential utility as valuable compounds in food products and other industries.

[DOI: 10.22034/FSCT.22.161.69]

1-Introduction

enters algal proteins, they are broken down into smaller peptides and amino acids [4, 9, 10]. The use of florzyme and alcalase enzymes in the hydrolysis of spirulina algae leads to the formation of smaller and digestible peptides. This process can improve the digestibility and absorption of proteins in the body and can also be used in the production of food products and nutritional supplements.

The purpose of this study is to use spirulina algae as a valuable protein source, and to investigate the antioxidant, functional and health-giving properties of hydrolyzed proteins from this alga. In this research, flowerzyme and alcalase enzymes have been selected as the enzymes used in the hydrolysis of spirulina algae proteins. Based on this selection, the enzymatic and biological properties of peptides and amino acids resulting from hydrolysis are evaluated.

2- Materials and methods

2-1 raw materials

Spirulina algae, in the form of green powder, was obtained from Noor Daru Company in Gonbad-Kavus. Alcalase enzyme (extracted from*Bacillus licheniformis*) and Flowerzyme (extracted from *Aspergillus oryzae*) and amino acids standard were purchased from Sigma Aldridge, USA. Bovine serum albumin, DPPH and ABTS reagents, ascorbic acid, hydrochloric acid and hydrochloric acid were purchased from Merck, Germany.

2-2 Measurement of spirulina protein

Keldahl method was used to measure the amount of protein in spirulina. In this method, the samples were digested and then by titration, the total amount of precipitated protein was calculated in the aqueous phase. This method was based on the AOAC standard of 2005 [11].

2-3 spirulina protein hydrolysis

In this method, 50 grams of spirulina samples were placed in an Erlenmeyer flask with a volume of 250 ml. Then, 100 ml of distilled water was added to Erlenmeyer at a ratio of 2:1 and homogenized using a digital stirrer for 2 minutes. The sample was then placed in a water bath at 85°C for 20 minutes to deactivate the internal enzymes. Then, by adding a sodium hydroxide solution with a normal concentration of 0.2, the optimal pH for the activity of enzymes (alcalase (8.5) and flowerzyme (7)) was adjusted. The samples were placed in a bath with a temperature of 57°C for alcalase enzyme and 55°C for flowerzyme enzyme. Then, with a constant speed of 200 rpm, the enzyme (with 1% protein of the original sample) was added to the sample. Samples were taken at specific times (10 and 20 minutes) and at the end of the experiment (time 30 minutes), it was placed in a bath with a temperature of 95 degrees Celsius to stop the enzymatic reaction. After cooling, the hydrolyzed proteins were centrifuged at a constant speed of 6700

Bioactive peptides have specific protein parts whose molecular mass is less than 6000 daltons and have 2-20 amino acids. These peptides are inactive in the main protein structure and after being released according to their type and amino acid sequence, they have a positive effect on the function and condition of the body and as a result the health of the person. Among these effects, we can mention immunological, antimicrobial, antioxidant, anti-blood pressure and anti-cancer effects [1, 2]. In recent years, hydrolyzed proteins with antioxidant and health-promoting properties have been produced from many animal and plant sources such as milk, soybeans, wheat germ, canola, egg yolk protein, oysters, and fish and shrimp waste [3, 4]. Among plant, marine and animal sources suitable for the production of hydrolyzed protein, marine sources, especially algae, have received more attention due to their reasonable price and less allergenicity [5, 6].

Spirulina is a blue-green algae found in fresh water and seas. This alga has been noticed due to the many nutritious compounds in it. Spirulina contains high protein (60-70%), carbohydrates (20-30%), lipids (20-30%), essential vitamins (such as vitamin C and vitamin B12 and E), minerals (such as iron, calcium and magnesium), omega-3 fatty acids, antioxidants and carotenoids [7, 8]. These nutritious compounds can act as a complete and useful source of nutrition for the human body. Spirulina can also be used as a plant protein source for people following a vegetarian diet due to its high protein production capacity [8]. The proteins in spirulina algae contain essential and non-essential amino acids that are needed for the construction and development of body cells. One of the methods of using spirulina algae protein is to produce hydrolyzed protein using enzymes. Enzymes act as catalysts and start the process of hydrolysis of algae proteins. Hydrolysis of proteins means breaking them down into smaller peptides that are easily digested by the body [2]. One of the most important and decisive factors in the enzymatic hydrolysis process using commercial enzymes is the appropriate choice of protease enzyme. There are different types of commercial enzymes that have been used with real success in the process of hydrolysis of food proteins. This accurate and correct choice of protease enzyme can have a significant effect on improving the activity and efficiency of protein hydrolysis and ultimately lead to improving the quality and properties of food products. Among the enzymes used in enzymatic hydrolysis are flowerzyme and alcalase enzymes [4, 9, 10]. These enzymes act as catalysts and start the process of hydrolysis of algae proteins. Flowerzyme is a proteolytic enzyme that enters proteins and breaks them down into peptides and smaller peptides. This process can increase the digestibility and absorption of proteins. Alkalase is an enzyme capable of working in environments with non-acidic pH. When alcalase

[DOI: 10.22034/FSCT.22.161.69]

speed of 500. The nitrogen content in the sample supernatant was determined using the Keldahl method and the percentage of soluble protein was calculated using equation (4) [16].

Equation 4:

100x (gram weight of prototype / gram weight of water of solid material dissolved in supernate) = Solubility index in water

2-6-2 Capacity and stability of emulsifier

50 milliliters of distilled water and 50 milliliters of rapeseed oil were added to 3 grams of the sample and homogenized for 30 seconds with a homogenizer (APU500b, Dilkofnavar, Iran), then divided into equal amounts in 4 test tubes and centrifuged with a g2000 spin for 5 minutes (Behdad, Iran). EC was reported according to equation (5) below [17].

Equation 5:

Total volume / volume of emulsified part = EC (%)

10 milliliters of rapeseed oil was mixed with 30 milliliters of 1% protein solution and their pH was adjusted to five different pHs of 2, 4, 6, 8, 10, then it was homogenized with a homogenizer at a speed of 2000 rpm for one minute, then 50 microliters was taken from the liquid part of the bottom of the tube with a microsampler, which was done at times t=0' and t=10'. Then, the obtained samples were mixed with 5 ml of 1% sodium dodecyl sulfate at zero and ten minutes, and the absorbance of the diluted solution was read with a spectrophotometer at a wavelength of 500 nm [17].

Equation 6:

AD/tD A0 × = HOW

ΔA= A0 –A 10, Δt= 10 min

2-6-3 Measuring the capacity and stability of foaming

To measure the foaming capacity, 20 ml of protein solutions with pH 5-8 were prepared and poured into a 50 ml graduated cylinder. Then it was stirred for one minute with a homogenizer at a speed of 10,000 rpm. The volume of the final mixture was read. The percentage of volume increase at zero time compared to the initial volume was considered as foaming capacity (Equation 7) [18].

Equation 7:

Sample volume before foam formation / sample volume at different times after foam formation - sample volume before foam formation = foaming capacity (%)

To calculate the stability of the foam, the volume of the foam was read at 0.5, 5, 10, 40 and 60 minutes after the formation of the foam. Then, using the above relationship, the percentage of floor volume remaining in each of the mentioned times was reported as an index of floor stability [18]. (equation 8)

Equation 8: $Floor\ stability = \frac{V0 \times 100}{\Delta V}$

100 x (the volume of the floor immediately after being hit / the volume of the floor after 60 minutes) = stability of the floor

rpm for 20 min and the supernatant was collected. Then, the hydrolyzed proteins were kept in the freezer and turned into powder using a freeze dryer [9, 12].

2-3 degrees of hydrolysis

The amount of hydrolysis was measured with the help of trichloroacetic acid (TCA). The basis of this method is to measure the ratio of proteins soluble in 10% trichloroacetic acid to all the proteins in the sample. For this purpose, 5 ml of the sample was mixed with 5 ml of 20% trichloroacetic acid and then centrifuged at 6700 rpm for 10 minutes. Then, the amount of protein in the solution phase was measured and the amount of hydrolysis was calculated by formula 1 [13]:

Equation 1:

(the amount of protein dissolved in 10% trichloroacetic acid solution / the amount of protein in the sample) = degree of hydrolysis

2-4 Determining the amount of amino acids

The hydrolyzed protein powder was completely hydrolyzed for 24 hours at 110 degrees Celsius using 6 normal hydrochloric acid. Then, using phenyl isothiocyanate (PITC), amino acids were derivatized. Total amino acids were measured using a Smart line (Germany) HPLC device using a C18 column with a fluorescent detector (RF-530). [4].

2-5 antioxidant activity

For DPPH free radical inhibitory activity, 1 ml of different hydrolyzed protein treatments were added separately with 1 ml of 0.1 mM DPPH solution and the resulting mixture was shaken well and placed in a dark room for 15 minutes, and then the optical absorbance of the samples was read at a wavelength of 517 nm against the control [14].

Equation 3:

100x (witness absorption / witness absorption - sample absorption) = DPPH free radical inhibition percentage

To measure the ferric reducing power (FRAP), first, 0.5 ml of its solution sample was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferric cyanide. Then the mixture was placed in an incubator at 50°C for 20 minutes. After this period, it was mixed with 2.5 ml of 10% trichloroacetic acid and centrifuged at 3000 rpm for 10 minutes. Then 2.5 ml of the upper layer of this solution was combined with 2.5 ml of distilled water and 2.5 ml of ferric chloride (0.1% FeCl3) and placed at room temperature for 10 minutes. Then the absorbance was read at a wavelength of 700 nm [15].

6-2 Measurement of the functional characteristics of hydrolysate's

2-6-1 Solubility

Solubility of hydrolyzed protein was performed using the method of Bera and Mukherjee (1989). One gram of the sample was mixed in 100 ml of water solution and the pH was adjusted to 10-2 using soda and 0.1 normal acid and then mixed for 60 minutes at room temperature and then centrifuged for 20 minutes at a

[DOI: 10.22034/FSCT.22.161.69]

due to the higher ability of alcalase enzyme to break down protein into amino acids and dissolve it more.

2-3 Checking the values of the degree of hydrolysis

The degree of hydrolysis means the rate of breaking the protein structure and producing peptides and amino acids. Enzymatic hydrolysis causes the destruction of the natural structure of proteins and as a result, the structure of proteins is opened and the active groups of amino acids that can react with free radicals are exposed. The results of statistical analysis have shown (Table 1) that with the increase of hydrolysis time, the degree of hydrolysis increases significantly. Also, at all times of hydrolysis, the degree of hydrolysis obtained from alkalase was significantly higher than that of flowerzyme. The highest (36.45%) degree of hydrolysis was related to alkalase after 30 minutes and the lowest (14.35%) was related to flowerzyme after 10 minutes. With the passage of time of hydrolysis, enzymes continuously interact with proteins and destroy the protein structure. This hydrolysis process cuts the protein chain and produces peptides and amino acids. As the hydrolysis time increases, the number of protein chain cuts increases and therefore smaller peptides with lower molecular weight are produced. Also, the length of the peptide chain becomes shorter. In the case of alcalase enzyme, the reason for the higher degree of hydrolysis by this enzyme may be due to its higher ability to break peptides into smaller peptides and even produce free amino acids. Alcalase is a protease enzyme that is able to efficiently break protein chains and break down peptides into smaller peptides and even into amino acids. This higher ability of alcalase can lead to an increase in the degree of hydrolysis by this enzyme [4, 23, 24].

2-8 Statistical evaluation

The experiments were conducted in three repetitions and in the form of a completely randomized design. Data analysis was done using SPSS 18 software. Comparison of means was done with Duncan's test with 5% error level. Graphs were drawn using Microsoft Excel 2013 software.

3- Results and discussion

3-1 protein amounts in different treatments

The amount of primary protein in spirulina algae with an average of 1.59±57.38% has been determined, while protein values for this algae have been widely reported between 50 and 70% in different studies. For example, the study by Tańska et al. in 2017 reported the protein content as 60.7% [19], while the study by Koli et al. in 2022 reported the protein content as 65.71% [20]. Also, the study of El-Hamed et al. in 2018 reported the amount of protein as 53.92% [21]. The amount of protein, hydrolyzed protein of spirulina algae in different treatments was in the range of 96.03-70.74%. Many studies have shown that in hydrolyzed aquatic protein, the protein content is about 70 to 90 percent. This increase in protein content occurs due to protein breakdown into amino acids, protein dissolution and removal of insoluble solid matter using centrifugation [2, 4, 6, 9, 22]. In the samples, the protein content increased with the increase in hydrolysis time and the use of alcalase enzyme. A research conducted by Taghdiri et al. (2023) also reported similar results about the hydrolyzed protein of Chlorella algae by Alcalase and Flowerzyme. They declared the amount of protein in the range of 93.46-15.65% and showed that higher amounts of protein are obtained by using alcalase enzyme and longer hydrolysis time [22]. This may be

Table 1. Effect of enzyme hydrolysis type and time of protein hydrolyzed on protein content and degree of hydrolysis

Enzyme		Protein content (%)	Degree of hydrolysis (%)
Type	hour (min)		
Alcalase	10	72.96±0.78^{and}	20.99±1.04^{c}
	20	85.75±0.82^{c}	27.26±1.06^{b}
	30	96.03 ±0.55^{a}	36.45±1.11^{a}
Flavourzyme	10	70.74±0.49^{f}	14.35±1.39^{d}
	20	81.46±1.05^{d}	19.87±1.55^{c}
	30	89.73±0.65^{b}	26.25±1.19^{b}

Averages with the different letters (in same columns) indicate that there is significant difference at the P< 0.05.

3-3 amino acid composition

[DOI: 10.22034/FSCT.22.161.69]

activity. This antioxidant activity prevents oxidative damage and maintains cellular oxidant balance by reducing the direct contact of free radicals with cells. In addition, some of these amino acids act as precursors for the synthesis of effective anti-inflammatory and anti-cancer molecules such as tyrosine and phenylalanine [26, 27, 28].

In the present study, it was found that the percentage of the highest amounts of essential amino acids for alcalase and flavozyme enzymes was arginine 7.75% and 8.15%, respectively. Also, the percentage of the highest amounts of non-essential amino acids for alcalase and flowerzyme enzymes were observed, respectively glutamic acid 11.99%, 12.25%. In the study conducted by Morsy et al. in 2014, the percentage of arginine in the amino acid profile of spirulina algae was reported as 7.65% and glutamic acid as 13.79%. These results are almost consistent with the results of the present study [29].

On the other hand, according to the criteria provided by the World Food and Agriculture Organization (FAO/WHO) in 1990, the ratio of essential amino acids to total amino acids should be at least 40% and the ratio of essential to non-essential amino acids should not be less than 0.6 [25]. According to the results of the study, hydrolyzed proteins have a suitable combination of amino acids. The ratio of essential to non-essential amino acid for Alcalase and Flowerzyme enzyme is 1.07 and 0.99, respectively, and the amount of essential amino acid to the total available amino acid is 51.82 and 49.81, respectively.

Proteolytic enzymes are able to hydrolyze proteins into smaller peptides consisting of 2 to 20 amino acids. By cutting peptide bonds in the structure of proteins, these enzymes break them into smaller pieces. Amino acids resulting from this decomposition and short peptides can have various biological properties and nutritional value. For example, some of these peptides can exhibit antimicrobial, antioxidant, anti-inflammatory and other beneficial biological properties. These biological properties and nutritional value of peptides can be exploited in various applications including food and pharmaceutical industries [22].

Based on the results, the proteins obtained from hydrolysis by both proteolytic enzymes contain various amino acids. These proteins can be used as a useful source of nutrition. However, the concentration of amino acid phenylalanine in these proteins is lower than that reported by the FAO model [25].

The amounts of hydrophobic and branched amino acids for protein hydrolyzed by alcalase enzyme were 29.82% and 28.34%, respectively, and 19.10% and 17.89% for flowerzyme, respectively. Hydrophobic amino acids such as phenylalanine, proline, methionine, alanine, leucine, isoleucine, tyrosine, and valine, as well as branched amino acids such as valine, isoleucine, and leucine, play an important role in inhibiting free radicals due to their ability to absorb and transfer electrons as well as form free radicals. These amino acids, with their hydrophobic properties, reduce the access of free radicals to the target cells, and as a result, they can have potential antioxidant

Table 2: Effect of enzyme hydrolysis type (at 30 min) of protein hydrolyzed on amino acid composition

Amino acid (g 100 g^{-1})	Alcalase	Flavourzyme	FAO/ WHO, 1990
Threonine[a]	4.28	4.7	3.4
Valine[a]	4.54	4.15	3.5
Methionine[a]	2.78	2.67	
Isoleucine [a]	7.45	6.49	2.8
Leucine[a]	7.11	6.75	6.6
Phenyl alanine[a]	3.59	3.21	6.3
Histidine [a]	5.59	5.55	1.9
Lysine[a]	4.25	4.11	5.8
Arginine[a]	7.75	8.11	
Glycine	5.11	6.35	
Aspartic acid	8.95	9.11	
Glutamic acid	11.99	12.25	

[DOI: 10.22034/FSCT.22.161.69]

Serine	4.11	4.75	
Alanine	6.99	6.68	
Tyrosine	3.40	3.59	1.1
Cysteine	0.95	1.01	
Proline	2.50	2.48	
Total amino acid	91.34	92.04	
LET[b]	29.82	28.34	
BCAA[c]	19.10	17.98	

[a] Essential amino acids

[b] Combined total of hydrophobic amino acids (HAA)= alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, methionine and cysteine

c Branched-chain amino acids (valine, isoleucine, leucine)

and cause their transformation into stable compounds. These results show that by increasing the time of protein hydrolysis, the antioxidant activity of all samples increases and the chain reactions of free radicals are inhibited [33, 34]. Also, the antioxidant activity of proteins hydrolyzed by alcalase enzyme was higher than flowerzyme. This antioxidant activity directly depends on the amino acid composition, structure and molecular weight of peptides. Hydrophobic amino acids (HAA) have anti-oxidation properties. These amino acids, including tyrosine, tryptophan, and phenylalanine, have an aromatic ring structure that can directly react with free radicals and reactive oxygen species, thus exhibiting antioxidant power. The amount of HAA amino acids was higher in proteins hydrolyzed by alcalase enzyme. This shows that protein hydrolysis by alcalase enzyme can lead to the production of peptides with antioxidant activity and more free radical scavenging power [4, 35]. In the study conducted by Taghdiri et al. in 2023, with the hydrolysis of Chlorella algae proteins with florezyme and alcalase enzymes, with increasing hydrolysis time and increasing the degree of protein hydrolysis, the antioxidant power of all samples has increased significantly, and the highest values were observed in protein hydrolyzed by alcalase enzyme [23]. These results are consistent with the results of the present study and show that the hydrolysis of more proteins leads to a significant increase in the antioxidant power of the samples.

3-4 antioxidant properties

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical activity test is a fast, cheap and no negative effect method to evaluate the antioxidant activity of foods. In this method, DPPH, which is a free radical, interacts with electron donors and thus reacts. Peptides from hydrolyzed proteins can also play the role of electron donors and react with free radicals to stop oxidation chain reactions. DPPH in the stable form of free radical has the maximum absorption at the wavelength of 517 nm in ethanol. But when placed close to a proton donor, DPPH free radicals are inhibited and their absorption is reduced [30, 31, 32]. In this study, by increasing the duration of protein hydrolysis to 30 minutes and increasing the degree of hydrolysis, the DPPH free radical inhibition rate of the samples increased significantly. The highest values of DPPH free radicals were related to alkalase after 30 minutes (36.45%) and the lowest values were related to flowerzyme after 10 minutes (14.35%). Based on the results of this research, hydrolyzed proteins from spirulina algae are able to donate protons in the reaction with free radicals and thus lead to the end of the chain reactions of free radicals. These peptides with more electron-donating property, which is directly related to increasing the duration of protein hydrolysis, are able to deliver electrons to free radicals

[DOI: 10.22034/FSCT.22.161.69]

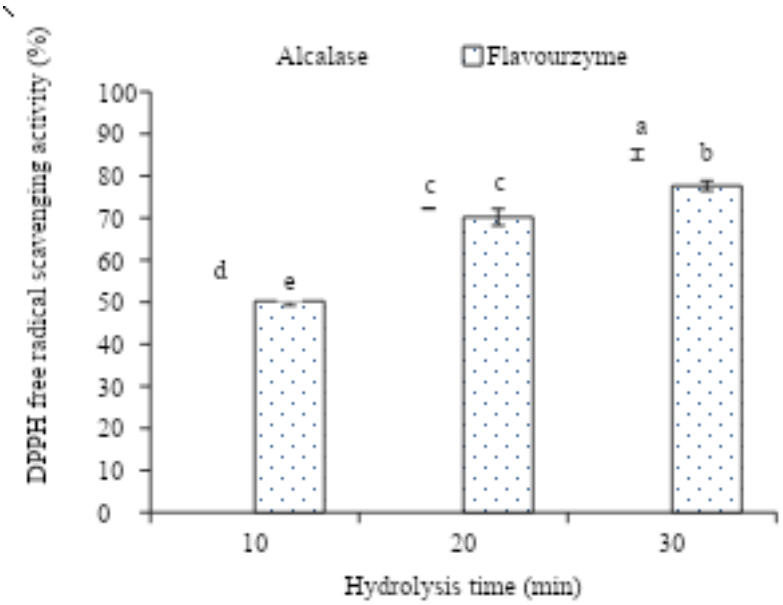

Figure 1: The DPPH free radical savaging activity of spurlina protein hydrolysates
Different letters in the same times showed a significant difference at p<0.05.

reducing power of trivalent iron (ferric) increases. The statistical results also show that this increase is significant and with the increase in hydrolysis time, the reducing power increases significantly. Therefore, using higher hydrolysis time is recommended. It should be noted that alkalase enzyme has a greater ability to increase the reductive effect. Therefore, the use of alcalase enzyme instead of florzyme can lead to an increase in the reduction power of trivalent iron [4].

One of the methods used to evaluate the antioxidant ability of a compound is the method of measuring the power of reducing iron by donating electrons or hydrogen. Numerous studies have shown that there is a direct relationship between the amount of antioxidant activity and the reducing power of a bioactive compound. In this method, the ability of hydrolyzed proteins to reduce Fe3+ ion to Fe2+ ion is evaluated [4, 10]. According to the results of using alcalase enzyme compared to flowerzyme, the

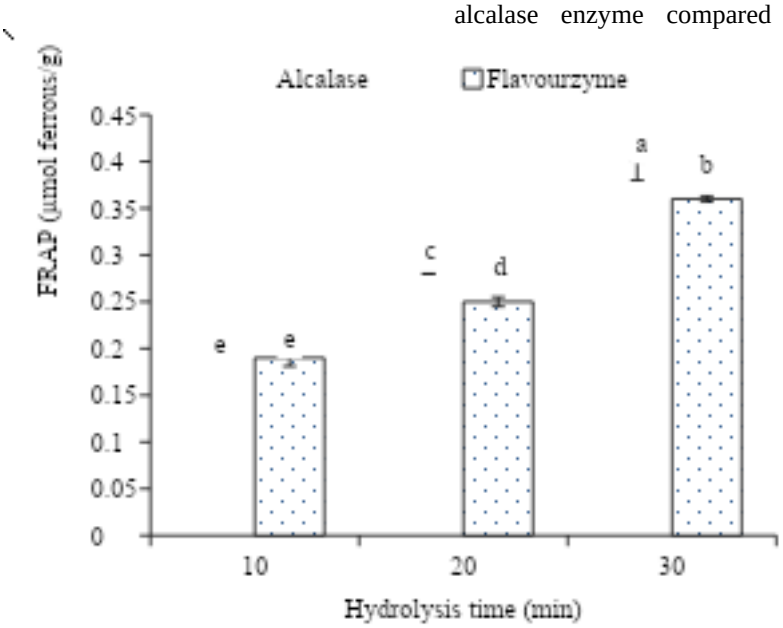

Figure 2: The FRAP of spurlina protein hydrolysates
Different letters in the same times showed a significant difference at p<0.05.

respective medium. Therefore, insoluble proteins seem to have a much lower application potential in food [36].

Based on the results of the current research, alcalase enzyme has shown a greater ability to increase the solubility of hydrolyzed proteins than flavozyme. Also, it was found that the solubility increases

3-5 functional properties

Among all the functional properties of proteins, solubility has the greatest impact on the usefulness of hydrolyzed proteins in food systems. Other functional properties such as foam and emulsion and gelling properties usually require protein solubility in the

[DOI: 10.22034/FSCT.22.161.69]

it has been shown that the solubility of hydrolyzed protein from ribbon fish (*Lepturacanthus Savala*) increases with increasing time and degree of hydrolysis [27]. These results show that the time and intensity of hydrolysis have an important effect on the solubility of proteins, and with their increase, the solubility of proteins also increases. The research of Ma et al. (2018) has shown that as the hydrolysis time increases, the solubility of the protein obtained from enzymatic hydrolysis of soy protein also increases. This increase may be due to the breakdown of proteins into smaller and soluble peptides in different environments that have higher solubility [37].

significantly with the increase of hydrolysis time. The results have shown that the degree of hydrolysis and the solubility of proteins have a direct relationship, which means that with the increase in the degree of hydrolysis, the solubility also increases. This increase in solubility is due to the release of small peptides and the formation of new carboxylic and amine groups from amino acids. These findings show that hydrolyzed proteins with high solubility have potential in food formulation [32]. These results show that hydrolyzed proteins with high solubility can be used as stability and foaming agents in food formulations. In the research of Yathisha et al. (2022),

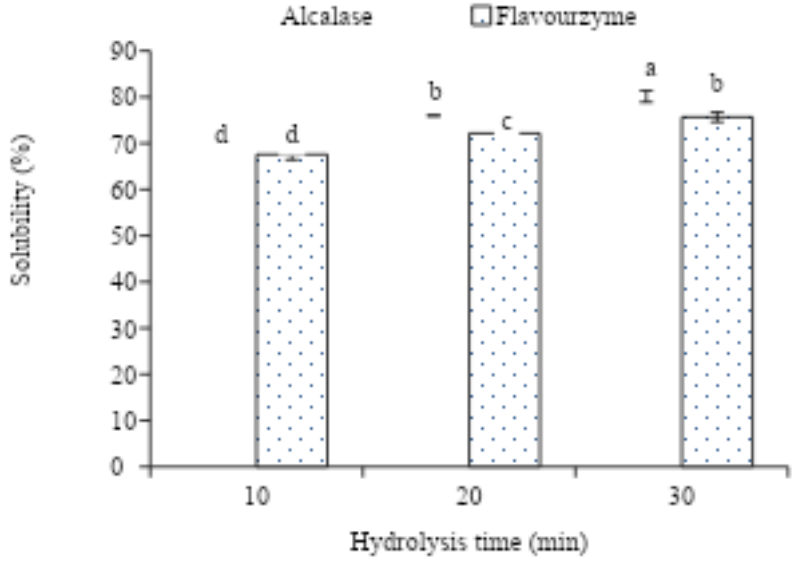

Figure 3: The solubility of spurlina protein hydrolysates
Different letters in the same times showed a significant difference at $p<0.05$.

weight, and increasing the degree of hydrolysis leads to the production of peptides with lower molecular weight. Peptides with lower molecular weight are able to create favorable emulsifying properties due to their high solubility. Therefore, in the present study, proteins hydrolyzed by alcalase enzyme have the highest emulsifying properties and emulsion stability during the hydrolysis time of 30 minutes. Ghelich et al. (2022) also announced that the emulsion properties of hydrolyzed wheat germ protein improved with the increase in hydrolysis time, and also the use of alcalase enzyme compared to flowerzyme increased the capacity of the emulsion to stain [24].

The emulsifying property of proteins can be defined as the ability of proteins to form and stabilize emulsions. The ability to form an emulsion is the basic characteristic required for any protein part that can be used as a food item with suitable functional properties in the food industry [38]. The present study has shown the results that there is a match between emulsifying properties and emulsion stability. All the hydrolyzed proteins in this study proved to have high emulsifying properties, and proteins hydrolyzed by alcalase enzyme and higher hydrolysis time had higher amounts of emulsifying properties. Molecular weight and solubility of peptides obtained from hydrolysis of proteins are two important factors in determining the index of emulsifying activity and emulsion stability. Hydrolysis of proteins decreases their molecular

[DOI: 10.22034/FSCT.22.161.69]

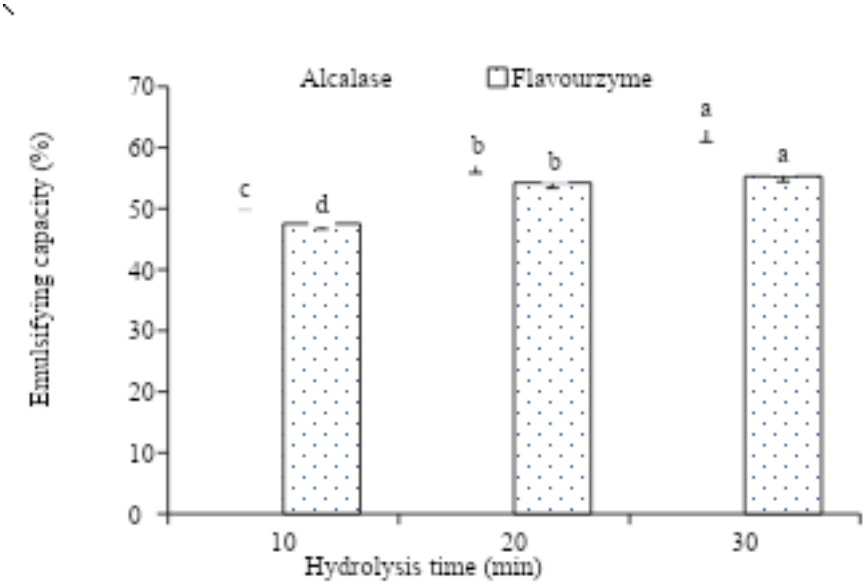

Figure 4: The emulsifying capacity of spurlina protein hydrolysates
Different letters in the same times showed a significant difference at $p<0.05$

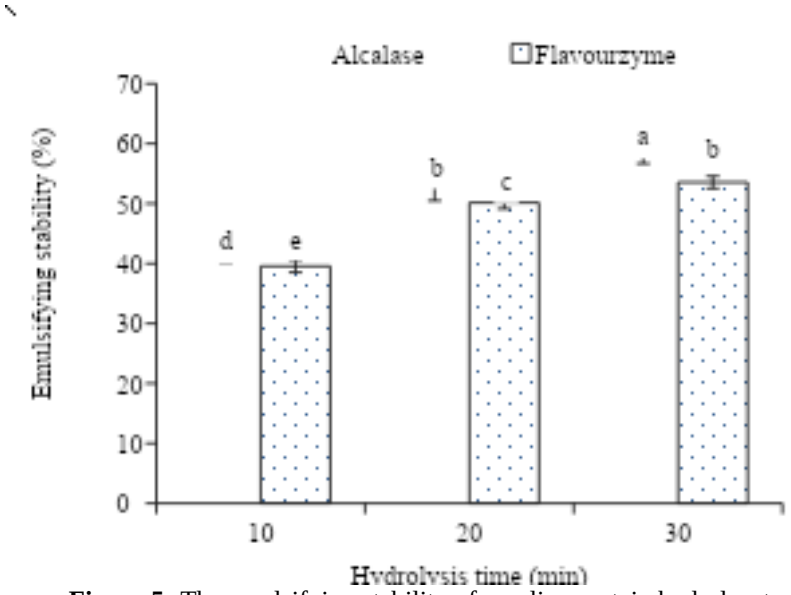

Figure 5: The emulsifying stability of spurlina protein hydrolysates
Different letters in the same times showed a significant difference at $p<0.05$.

enzyme can significantly improve the foaming and foam stability of colloidal mixture. Three important characteristics to demonstrate good foaming capacity are rapid migration at the water-air interface, release and regeneration at this interface. Hydrolyzed samples usually contain peptides with hydrophobic amino acids and more hydrophobic character, which are rapidly adsorbed at this interface. Proteins lead to the formation of foam due to having surface active compounds, and with the increase in the solubility of proteins, the surface tension in the interfacial space between bubbles and liquid shows a further decrease and the foam formation capacity increases. By carrying out the hydrolysis process, the molecular weight of proteins is reduced and as a result the Colloidal mixed foam with continuous liquid phase and dispersed gas phase is thermodynamically considered as an unstable system [39]. The results of the present study have shown that foaming and stability of the floor are consistent with each other. All hydrolyzed proteins have been shown to have high foaming capacity, and proteins hydrolyzed by alcalase enzyme have higher amounts of foaming capacity at all times of hydrolysis. As the hydrolysis time increases, the foaming capacity values also increase. Specifically, proteins hydrolyzed by alcalase enzyme with a hydrolysis time of 30 minutes have the highest foaming capacity and foam stability. These results show that the hydrolysis of proteins by alcalase

[DOI: 10.22034/FSCT.22.161.69]

These characteristics make proteins hydrolyzed by enzymes to act as suitable foaming agents [40, 41, 42]. solubility is increased and the property of foam formation by proteins becomes smaller and more.

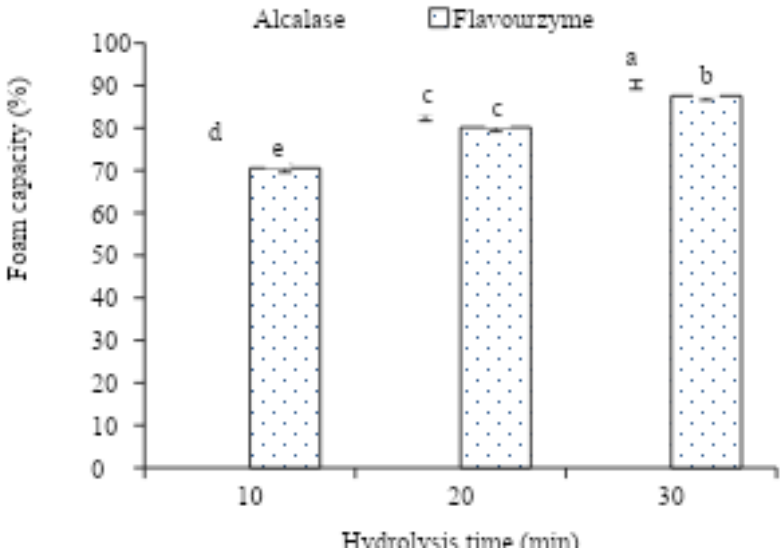

Figure 6: The foaming capacity of spurlina protein hydrolysates
Different letters in the same times showed a significant difference at $p<0.05$.

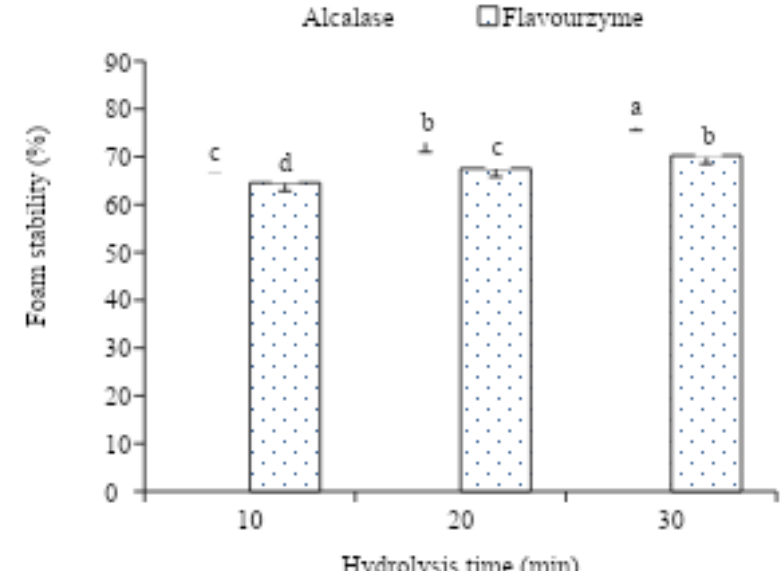

Figure 7: The foaming stability of spurlina protein hydrolysates
Different letters in the same times showed a significant difference at $p<0.05$.

4- Final conclusion:

The results of this study showed that the peptides obtained from the enzymatic hydrolysis of spirulina algae have the ability to inhibit free radicals and at the same time have high antioxidant properties. Also, these peptides can be used as useful compounds in various industries, including the food industry, due to their good functional characteristics, which include easy digestion and absorption. Also, enzymatic hydrolysis of spirulina algae using alcalase enzyme leads to an increase in protein value and the production of biological peptides with acceptable antioxidant and functional properties. Therefore, the peptides obtained from the enzymatic hydrolysis of spirulina algae are considered as useful compounds in food products and other industries.

5-Resources

[1] Jia, J., Maa, H., Zhao, W., Wang, Z., Tian, W., & Luo, L. (2010). The use of ultrasound for enzymatic preparation of ACE-inhibitory peptides from wheat germ protein. Food Chemistry , 119, 336–342.

[DOI: 10.22034/FSCT.22.161.69]

[2] Nemati, M., Shahoseini, S. R., & Ariaii, P. (2024). Review of fish protein hydrolysates: Production Methods, Antioxidant and antimicrobial activity and nanoencapsulation. Journal of Food Science and Biotechnology .

[3] Chi, C. F., Hu, F. Y., Wang, B., Li, T., & Ding, G. F. (2015). Antioxidant and anticancer peptides from the protein hydrolysate of blood clam (*Tegillarca granosa*) muscle. Journal of Functional Foods , 15, 301-313.

[4] Shahosseini, S. R., Javadian, S. R., & Safari, R. (2022). Effects of Molecular Weights -Assisted Enzymatic Hydrolysis on Antioxidant and Anticancer Activities of Liza abu Muscle Protein Hydrolysates. International Journal for Peptide Research & Therapeutics , 28, 72.

[5] Feyzi, S., Varidi, M., Zareb, F., & Varidi, M. J. (2015). Extraction Optimization of Fenugreek Seed Protein. Science of Food and Agriculture , 15, 3165–3176.

[6] Shahosseini, S. R., Javadian, S. R., & Safari, R. (2023). Evaluation of antibacterial and antioxidant activities of *Liza abu* viscera protein hydrolysate. Journal of Innovation in Food Science and Technology , 15(1), 143-155.

[7] Bumandalai, O., Bayliss, K. L., & Moheimani, N. R. (2024). Innovative processes for combating contaminants in fresh Spirulina. Algal Research , 78, 103397.

[8] Lafarga, T., Fernández-Sevilla, J. M., González-López, C., & Acién-Fernández, F. G. (2020). Spirulina for the food and functional food industries. Food Research International, 137, Article 109356.

[9] Nemati, M., Javadian, S. R., Ovissipour, M., & Keshavarz, M. (2012). A study on the properties of alosa (*Caspian shad*) by-products protein hydrolysates using commercial enzymes. World Applied Sciences Journal , 18(7), 950-956.

[10] Yaghoubzadeh, Z., Peyravii Ghadikolaii, F., Kaboosi, H., Safari, R., & Fattahi, E. (2020). Antioxidant Activity and Anticancer Effect of Bioactive Peptides from Rainbow Trout (*Oncorhynchus mykiss*) Skin Hydrolysate. International Journal of Peptide Research and Therapeutics , 26, 625–632.

[11] AOAC. (2005). Official Method of Analysis (17th ed.). Washington, DC: Association of Official Analytical Chemists.

[12] Dorvaj, Z., Javadian, S. R., Oveissipour, M., & Nemati, M. (2013). Use of Protein Hydrolysates From Caspian Sea Sprat (Clupeonella Cultiventris) As A Nitrogen Source For Bacteria Growth Media (Vibrio Anguillarum, Bacillus Licheniformis, Bacillus Subtilis). Journal of Aquatic Animals & Fisheries, 4(15), 11-18.

[13] Kristinsson, H. G., & Rasco, B. A. (2000). Fish protein hydrolysates: Production, biochemical and functional properties. Critical Reviews in Food Science and Nutrition, 40(1), 43-81.

[14] Bougatef, A., Hajji, M., Balti, R., Lassoued, I., Triki-Ellouz, Y., & Nasri, M. (2009). Antioxidant and free radical-scavenging activities of smooth hound (Mustelus mustelus) muscle protein hydrolysates obtained by gastrointestinal proteases. Food Chemistry, 114, 1198-1205.

[15] Sheih, I. C., Wu, T. K., & Fang, T. J. (2009). Antioxidant properties of a new antioxidative peptide from algae protein waste hydrolysate in different oxidation systems. Bioresource Technology, 100(13), 3419-3425.

[16] Bera, M. B., & Mukherjee, R. K. (1989). Solubility, emulsifying, and foaming properties of rice bran protein concentrates. Journal of Food Science, 54(1), 142-145.

[17] Slizyte, R., Mozuraitytė, R., Martínez-Alvarez, O., Falch, E., Fouchereau-Peron, M., & Rustad, T. (2009). Functional, bioactive, and antioxidative properties of hydrolysates obtained from cod (Gadus morhua) backbones. Process Biochemistry, 44, 668-677.

[18] Shahidi, F., & Onodenalore, A. (1995). Water dispersions of myofibrillar proteins from capelin (Mallotus villosus). Food Chemistry, 53, 51-54.

[19] Tańska, M., Konopka, I., & Ruszkowska, M. (2017). Sensory, physico-chemical, and water sorption properties of corn extrudates enriched with Spirulina. Plant Foods for Human Nutrition, 72, 250-257.

[20] Koli, D. K., Rudra, S. G., Bhowmik, A., & Pabbi, S. (2022). Nutritional, functional, textural, and sensory evaluation of Spirulina enriched green pasta: A potential dietary and health supplement. Foods, 11(7), 979. https://doi.org/10.3390/foods11070979

[21] El-Hameed , A. , Mahmoud , K. , El-Maatti , A. , El-Saidy , S. M. , & Ahmed , S. M. (2018). Effect of adding Spirulina platensis in pasta products (spaghetti). Journal of Agricultural Research, 45, 293-300.

[DOI: 10.22034/FSCT.22.161.69]

[22] Taghdiri, S., Emtyazjoo, M., Azizi, M. H., Ariaii, P., & Sedaghati, M. (2023). The effect of hydrolyzed protein obtained from Chlorella vulgaris on the shelf life and quality of oil cake during the storage period. Food Measure. Advance online publication. https://doi.org/10.1007/s11694-023-02186-y

[23] Mirsadeghi Darabi, D., Ariaii, P., Safari, R., & Ahmadi, M. (2022). Effect of clover sprouts protein hydrolysates as an egg substitute on physicochemical and sensory properties of mayonnaise. Food Science & Nutrition, 10, 253–263.

[24] Ghelich, S., Ariaii, P., & Ahmadi, M. (2022). Evaluation of Functional Properties of Wheat Germ Protein Hydrolysates and Its Effect on Physicochemical Properties of Frozen Yogurt. International Journal of Peptide Research and Therapeutics, 28(1), 69.

[25] FAO/WHO. (1990). Energy and protein requirements. Report of joint FAO/WHO/UNU Expert Consultation Technical Report. FAO/WHO and United Nations University, Geneva, Series No. 724.

[26] Firmansyah, M., & Abduh, M. Y. (2019). Production of protein hydrolysate containing antioxidant activity from Hermetia illucens. Heliyon, 5(6), e02005.

[27] Yathisha, U. G., Vaidya, S., & Bangera Sheshappa, M. (2022). Functional Properties of Protein Hydrolyzate from Ribbon Fish (Lepturacanthus Savala) as Prepared by Enzymatic hydrolysis. International Journal of Food Properties, 25(1), 187-203.

[28] Chew, R. M., Mohd Zin, Z., Ahmad, A., Mohtar, N. F., Rusli, N. D., & Zainol, M. K. (2020). Physicochemical and sensory properties of deep-fried battered squid containing Brownstripe red snapper (Lutjanus vitta) protein hydrolysate. Food Research, 4(4), 1245-1253.

[29] Morsy, O. M., Sharoba, A. M., El-Desouky, A. I., Bahlol, H. E. M., & Abd El Mawla, E. M. (2014). Production and evaluation of some extruded food products using spirulina algae. Annals of Agricultural Science, Moshtohor, 52(4), 495-510.

[30] Varedesara, M. S., Ariaii, P., & Hesari, J. (2021). The effect of grape seed protein hydrolysate on the properties of stirred yogurt and the viability of Lactobacillus casei in it. Food Science and Nutrition, 9, 2180–2190.

[31] Aderinola, T., Fagbemi, T., Enujiugha, V., Monisola Alashi, A., & Emmanuel Aluko, R. (2018). Amino acid composition and antioxidant properties of Moringa oleifera seed protein isolate and enzymatic hydrolysates. Heliyon, 4(10), e00877.

[32] Dong, S., Zeng, M., Wang, D., Liu, Z., Zhao, Y., & Yang, H. (2008). Antioxidant and biochemical properties of protein hydrolysates prepared from Silver carp (Hypophthalmichthys molitrix). Food Chemistry, 107, 1485-1493.

[33] Bahram, S., Khezri, M., & Javadian, S. R. (2020). Evaluation of antioxidant and antimicrobial properties of hydrolyzed protein of Saurida tumbil. Experimental Animal Biology, 9(2), 23-35.

[34] Sheih, I. C., Wu, T. K., & Fang, T. J. (2009). Antioxidant properties of a new antioxidative peptide from algae protein waste hydrolysate in different oxidation systems. Bioresource Technology, 100(13), 3419-3425.

[35] 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, 30: 125165.

[36] Wouters, A. G. B., Rombouts, I., Fierens, E., Brijs, K., & Delcour, J. A. (2016). Relevance of the functional properties of enzymatic plant protein hydrolysates in food systems. Comprehensive Reviews in Food Science and Food Safety, 15(4), 786-800.

[37] Ma, W., Qi, B., Sami, R., Jiang, L., Li, Y., & Wang, H. (2018). Conformational and functional properties of soybean proteins produced by extrusion-hydrolysis approach. International Journal of Analytical Chemistry, 2018, 9182508.

[38] Zayas, J. F. (1997). Emulsifying properties of proteins. In Functionality of Proteins in Food (pp. 134–227). Springer.

[39] Boye, J. I., Aksay, S., Roufik, S., Ribéreau, S., Mondor, M., Farnworth, E., & Rajamohamed, S. H. (2010). Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. Journal of Food Research International, 43, 538–546.

[DOI: 10.22034/FSCT.22.161.69]

[40] García-Moreno, P. J., Guadix, A., Guadix, E. M., & Jacobsen, C. (2016). Physical and oxidative stability of fish oil-in-water emulsions stabilized with fish protein hydrolysates. Food Chemistry, 253, 124-135.

[41] Mazloomi-Kiyapey, S. N., Sadeghi-Mahoonak, A., Ranjbar-Nedamani, E., & Nourmohammadi, E. (2019). Production of antioxidant peptides through hydrolysis of medicinal pumpkin seed protein using pepsin enzyme and the evaluation of their functional and nutritional properties. ARYA Atherosclerosis, 15(5), 218-227.

[42] Sakanaka, S., Tachibana, Y., & Okada, Y. (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food Chemistry, 89, 569-575.

[DOI: 10.22034/FSCT.22.161.69]

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی_پژوهشی

ارزیابی خواص عملکردی و آنتی اکسیدانی پپتیدهای حاصل از هیدرولیز آنزیمی جلبک اسپیرولینا

اندیشه توکلی[۱]، رضا فرهمندفر[۲]، علی معتمدزادگان[۳]، پیمان آریایی[۴]، مریم اثنی عشری[۵*]

۱- دانشجوی دکتری، گروه علوم و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران

۲- دانشیار، گروه علوم و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران

۳- استاد، گروه علوم و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران

۴- دانشیار، گروه علوم و صنایع غذایی، واحد آیت الله آملی، دانشگاه آزاد اسلامی، آمل، ایران

۵- استادیار، بخش تحقیقات فرآوری تولیدات دامی، موسسه تحقیقات علوم دامی کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۳/۰۳/۲۲

تاریخ پذیرش: ۱۴۰۳/۱۰/۲

کلمات کلیدی:

پروتئین هیدرولیز شده،
جلبک اسپیرولینا،
خاصیت آنتی اکسیدانی،
خواص عملکردی.

DOI: **10.22034/FSCT.22.161.69.**

* مسئول مکاتبات:

m.asnaashari@yahoo.com

چکیده

هیدرولیز آنزیمی پروتئین جلبک اسپیرولینا، منجر به افزایش ارزش پروتئینی آن و تولید پپتیدهای بیولوژیکی و عملکردی می‌شود که قابلیت هضم و جذب بالا و خواص آنتی‌اکسیدانی مناسبی دارند. در این پژوهش، خواص آنتی‌اکسیدانی و عملکردی پپتیدهای زیست فعال حاصل از هیدرولیز آنزیمی جلبک اسپیرولینا بررسی شود. در روند ارزیابی، ابتدا پروتئین‌های جلبک اسپیرولینا با استفاده از آنزیم‌های آلکالاز و فلاورزایم طی زمانهای مختلف ۱۰، ۲۰ و ۳۰ دقیقه هیدرولیز شدند. سپس، درجه هیدرولیز، بازیافت پروتئینی، خواص آنتی‌اکسیدانی پپتیدها به وسیله آزمون‌های مهار کنندگی رادیکال‌های آزاد (1,1-diphenyl-2-picrylhydrazyl) DPPH و (Ferric Reducing Antioxidant Power) FRAP و ویژگی‌های عملکردی پپتیدها نیز شامل حلالیت، ظرفیت و پایداری کف کنندگی و امولسیون مورد.مورد ارزیابی قرار گرفتند. نتایج این ارزیابی‌ها نشان داد که پپتیدهای حاصل از هیدرولیز آنزیمی جلبک اسپیرولینا دارای خواص آنتی‌اکسیدانی بالا بوده و می‌توانند به عنوان مهارکننده‌های رادیکال‌های آزاد عمل کنند. همچنین، ویژگی‌های عملکردی مناسبی نیز در پپتیدها مشاهده شد که نشان از قابلیت آنها در کاربردهای مختلفی مانند صنایع غذایی دارند. همچنین بر اساس نتایج آنزیم آلکالاز توانایی بالاتری در تولید پروتئین هیدرولیزی با درجه هیدرولیزاسیون، محتوای پروتئینی و بازیافت پروتئینی بالاتر، و همچنین خاصیت آنتی‌اکسیدانی و عملکردی بهتری نسبت به آنزیم فلاورزایم دارا بود و زمان هیدرولیز نیز تاثیر مثبتی بر پارامترهای فوق داشت. بنابراین، پژوهش حاضر نشان می‌دهد که پپتیدهای حاصل از هیدرولیز آنزیمی جلبک اسپیرولینا دارای خواص آنتی‌اکسیدانی و عملکردی هستند و می‌توانند به عنوان ترکیبات مفید در محصولات غذایی و سایر صنایع مورد استفاده قرار گیرند.

[DOI: 10.22034/FSCT.22.161.69]