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Investigation of Functional Properties and Antioxidant of Hydrolyzed Protein from Chia Seed Under the Influence of Hydrolysis Time and Enzyme Type

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ABSTRACT

Chia seeds (*Salvia hispanica*) are recognized as a rich source of protein and bioactive compounds, offering positive effects on human health. Enzymatic hydrolysis of these proteins can enhance their functional properties and antioxidant activity. This study aims to investigate the effect of different enzymes on the production of hydrolyzed protein from chia seed with desirable functional characteristics and high antioxidant. Hydrolyzed protein from chia seeds was produced using the enzymes Protamex and Bromelain over periods of 30, 60, and 90 minutes at optimal temperature and pH (50 °C and pH 7). Subsequently, parameters such as solubility, foaming, and emulsifying properties, along with DPPH radical scavenging activity and iron-reducing power of the hydrolyzed proteins, were evaluated. Results indicated that increasing hydrolysis time and the use of Protamex significantly affected the degree of hydrolysis and the quantities of chia seed proteins. At 90 min, the highest degree of hydrolysis (40.23%) and protein content (90.70%) were observed ($P< 0.05$), demonstrating the high efficiency of this enzyme in the hydrolysis process. These conditions also led to improvements in functional properties, including solubility, emulsification, and foaming capacity of the proteins. Furthermore, this hydrolysis resulted in a significant increase in antioxidant activity, including DPPH radical scavenging and iron chelation. The results revealed that different enzymes exert varying effects on a substrate, and enzymatic modification of chia seed proteins creates a natural source of antioxidants with considerable potential for applications in the food and pharmaceutical industries.

1- Introduction

In recent years, food has been recognized not only as a source of energy and essential compounds for the growth and health of the body, but also as a source of bioactive compounds with positive effects. Bioactive peptides, which are functional compounds in food, have received attention in recent years. These compounds, especially small protein fragments, have significant biological effects and are produced during the processes of protein digestion or hydrolysis and can help improve health and body function [1, 2]. These peptides are produced by various methods, including chemical synthesis, microbial fermentation and enzymatic hydrolysis [3]. The enzymatic hydrolysis process is a complex process and requires numerous preliminary studies to better understand and develop an effective enzymatic process. The parameters of this process, including temperature, pH, enzyme to substrate ratio and processing time, must be carefully selected, because each of these factors affects the protease activity and substrate behavior [4]. Important enzymes used in protein hydrolysis include Protamax and bromelain. Protamax is a protease derived from bacteria.*Bacillus subtilis*It is extracted and widely used due to its ability to produce protein with a high degree of hydrolysis in a short time and less bitterness than other enzymes. Bromelain is also obtained mainly from pineapple stems and some fruits of the Bromeliaceae family and contains a mixture of proteolytic enzymes that have the ability to digest metabolized proteins [3, 5, 6].

mountain (*Spanish sage*L) is an annual plant of the genus Salvia and the family Mint, which is known as a valuable food source. Chia seeds are rich in protein, antioxidants, dietary fiber, vitamins, and minerals [7, 8]. The protein content of chia seeds varies between 15 and 24% and includes all essential amino acids, including glutamic acid, arginine, aspartic acid, alanine, phenylalanine, leucine, and serine. The amino acid compounds present in chia seeds, especially arginine, aspartic acid, and glutamic acid, play an important role in improving protein function and enable these seeds to produce bioactive peptides that have positive effects on human health [7, 8, 9]. In addition, hydrolyzed chia proteins are known as a functional ingredient in a variety of food products and have been considered as a natural antioxidant [7].

Given the richness of chia seeds in terms of amino acid composition, this study investigated this plant as a suitable protein source for enzymatic hydrolysis. The main objective of the study is to analyze the antioxidant and functional properties of hydrolyzed proteins from chia seeds and their health-promoting aspects, while comprehensive information regarding the functional and antioxidant properties of hydrolyzed proteins from chia seeds is not available. This study also investigates the effect of different enzymes and their combinations in producing hydrolyzed proteins with appropriate functional and antioxidant properties.

2- Materials and methods

1-2-Raw materials

Chia seeds with scientific name(*Salvia Hispanic* L)It was obtained from Kian Food Company. Protamax enzymes (derived from bacteria)*Bacills subtilis*) and bromelain (derived from pineapple fruit) The representative of Novazyme (Denmark) in Iran, the standard of amino acids, folinic acid, and free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldridge, USA. Other chemicals used were of laboratory and production purity from Merck, Germany, and Sigma-Aldridge, USA.

2-2- Defatting the seeds

200 g of chia seeds were dried, ground, and then mixed with hexane solvent in a ratio of 1:10 (volume-weight) and stirred for 4 hours with a shaker at a speed of 440 rpm. Subsequently, the remaining solvent was removed by vacuum oven at 30°C for 2 hours. Then, the resulting powder was passed through a 40-mesh sieve [10].

2-3-Chia seed protein hydrolysis

The protein content of chia seed samples was investigated using the Koldahl method. In this method, the samples were first digested and then the total nitrogen content in the samples was determined by titration. Finally, the total protein content in the aqueous phase of the samples was calculated using a conversion factor of 6.25 [11]. For chia seed protein hydrolysis, the protein was prepared at a concentration of 4% (w/v) in phosphate buffer 7 (optimal pH) for bromelain and protamax enzymes. Hydrolysis was performed at an optimal temperature of 50°C, with an enzyme concentration of 1% and a time range of 10 minutes to 1 hour in a shaking incubator (V-8480, South Korea) at 200 rpm. To inactivate the enzyme activity, the protein solution was placed in a hot water bath at 80°C for 15 minutes. Then, the solution was centrifuged at 10,000 rpm for 20 minutes. To produce hydrolyzed protein powder, the resulting supernatant was dried in a freeze-dryer (5509 FDB Operon, South Korea) at -20°C, 40 mbar, and 48 hours. The hydrolyzed protein powder was stored at -20°C until use. [6].

2-4- Degree of hydrolysis

Degree of hydrolysis using trichloroacetic acid[1]20% (vol/vol) was measured. The basis of this measurement method is the percentage ratio of proteins soluble in trichloroacetic acid to the total proteins in the sample obtained by centrifugation after hydrolysis. For this purpose, an equal volume of the separated protein solution was mixed with the trichloroacetic acid solution and, after stirring, centrifuged at 20°C for 5 minutes (6700×g). In this method, the formation of a colored complex and the intensity of the color formed depend on the protein concentration in the sample. The degree of hydrolysis was calculated using the following equation [12].

Equation 1:

100× (Total nitrogen in hydrolyzed sample) / (Amount of dissolved nitrogen in 10% trichloroacetic acid solution) = Degree of hydrolysis (percentage)

2.5- Amino acid composition

In order to obtain the amino acid composition of hydrolyzed protein powder, the protein sample was first completely hydrolyzed for 24 hours at 110°C using 6 M hydrochloric acid. Then, the amino acids present were derivatized by adding phenyl isothiocyanate (PITC). The total amino acids were measured using a Smart line HPLC (made in Germany) equipped with a C18 column and a fluorescent detector (RF-530) [2].

6.2-Antioxidant activity

For DPPH free radical scavenging activity, 1 ml of different protein hydrolysate treatments were separately added 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. Then the optical absorbance of the samples was read at a wavelength of 517 nm against the control. [13].

Equation 1:

100× (Blank absorbance / Blank absorbance-Sample absorbance)= Percentage of DPPH free radical inhibition

In this study, the ferric reducing power (FRAP) was determined using the Gulcin (2020) was measured. First, 0.5 mL of the 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 solution. This mixture was incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of the upper layer of the solution was mixed with 2.5 mL of distilled water and 2.5 mL of 0.1% ferric chloride solution and incubated at room temperature for 10 min. The absorbance of the solution was measured at 700 nm to determine the ferric reducing power of the sample [13].

7-2-Measuring the functional properties of hydrolyzed chia seed protein

1-7-2-Solubility

The solubility of hydrolyzed protein was determined using the method of Bera and Mukherjee (1989). One gram of sample was mixed in 100 ml of water solution and adjusted to pH 2-10 using 0.1 N sodium hydroxide and acid, then mixed for 60 minutes at room temperature and then centrifuged for 20 minutes at 500 rpm and the nitrogen content in the supernatant of the sample was determined using the Koldahl method and the percentage of soluble protein was calculated using equation (5) [14].

Equation 3:

100× (gram weight of initial sample / gram weight of water soluble solids in supernate)= Water solubility index

2-7-2- Emulsifying capacity and stability

To 3 grams of sample, 50 ml of distilled water and 50 ml of rapeseed oil were added and homogenized for 30 seconds with a homogenizer (APU500b, Dilkofnavar, Iran). Then, it was divided equally into 4 test tubes and centrifuged at 2000 g for 5 minutes (Behdad, Iran). EC was reported according to equation (6) below [15].

Equation 4:

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

10 ml of rapeseed oil was mixed with 30 ml of 1% protein solution and their pH was adjusted to five different pH values 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 of the liquid portion at the bottom of the tube was taken with a microsampler, which was done at times t = 0' and t = 10'. Then, the samples obtained at times 0 and 10 minutes were mixed with 5 ml of 1% sodium dodecyl sulfate and the absorbance of the diluted solution was read with a spectrophotometer at a wavelength of 500 nm [15].

Equation 5:

AD/tDA0 ×= OUT

ΔA= A0 –A 10, Δt= 10 min

3-7-2-Measurement of foaming capacity and stability

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 increase in volume at time zero compared to the initial volume was considered as the foaming capacity (Equation 6) [16].

Equation 6:

1-TCA

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

To calculate the foam stability, the foam volume was read at 0.5, 5, 10, 40, and 60 minutes after foam formation. Then, using the above equation, the percentage of foam volume remaining at each of the aforementioned times was reported as the foam stability index [16]. (Equation 7)

Equation 7: $Floor\ stability = \frac{V0 \times 100}{\Delta V}$

100 × (volume of foam immediately after application / volume of foam after 60 minutes) = foam stability

8-2- Statistical evaluation

The experiments were conducted in three replications in a completely randomized design. Data analysis was performed using SPSS 18 software. Comparison of means was performed using Duncan's test (One way Anova) with a probability level of 5%. Graphs were drawn using Microsoft Excel 2013 software.

3- Results and discussion

1-3-Protein Amount

The initial protein content of chia seeds is 0.35.±18.29% and the protein content of chia seeds after defatting is 1.60±35.09%, which is similar to the protein content of soybean meal, which is widely used in animal and human nutrition [17]. The results obtained are almost consistent with the findings of Villanueva-Lazo et al. (2021) regarding the protein content of defatted chia seeds using hexane; in their study, the protein content was reported to be 34.95% [7]. The protein content (Table 1) in different treatments varied between 96.70-96.58%, indicating a high protein content in chia seed hydrolysate. The high protein content in chia seed hydrolysate makes this seed a useful nutritional source in food products, and enzymatic hydrolysis is known as an effective method for improving its quality and increasing its protein content, with results showing that using this process significantly increases the potential of chia seeds [4].

2-3-degree hydrolysis

The results of the study show that the type of enzyme and the duration of hydrolysis have a significant effect on the increase in the degree of hydrolysis (Table 1) ($P<0.05$). This degree increases continuously with the use of both enzymes, and the process time also has a significant effect on this trend. The increase in the degree of hydrolysis leads to a decrease in the length of peptide chains and molecular weight distribution, which in turn leads to the breaking of peptide bonds and an increase in the amount of free amino acids. These results have also been confirmed in the studies of Shahosseini et al. (2022) and Rashidi et al. (2024) [1, 6]. Based on previous findings, commercial microbial enzymes, such as Protamax, can significantly increase the efficiency of hydrolysis. On the contrary, plant proteolytic enzymes, such as bromelain, usually show lower efficiency in hydrolysis [5]. The Protamax enzyme, which is derived from bacteria*Bacillus subtilis*It is known as a widely used proteolytic enzyme in the food industry. This enzyme is very popular due to its ability to rapidly degrade proteins and produce small peptides with a high degree of hydrolysis, especially in short times and with minimal bitterness. Protamax is used in protein hydrolysis processes and can help improve the sensory and nutritional properties of food products [4, 6]. Rashidi et al. (2024) reported similar results on the degree of hydrolysis of clover hydrolyzed protein by Protamax and bromelain enzymes. They found that the degree of hydrolysis increased with increasing storage time and a higher degree of hydrolysis was observed with Protamax enzyme [6].

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

Enzyme		Protein content (%)	Degree of hydrolysis (%)
Type	Time (min)		
Protamax	30	80.21±1.52^{c}	20.55±0.78^{and}
	60	90.17±0.32^{b}	30.63±0.81^{c}
	90	96.58 ±0.16^{a}	40.23±0.28^{a}
Bromelain	30	70.96±1.35^{d}	18.14±0.56^{f}
	60	82.88±2.08^{c}	25.59±0.50^{d}
	90	90.70±0.79^{b}	35.25±0.79^{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

Amino acids are essential for protein synthesis, and the proteins produced play vital physiological roles, including structural, enzymatic, and oxygen transport, and directly or indirectly affect health maintenance. In addition to the importance of amino acid composition in the nutritional value of a protein source, this composition also determines the functional properties of proteins. In general, hydrolyzed plant proteins contain high amounts of essential amino acids, and changes in their amino acid profile are influenced by the starting material, enzyme type, and hydrolysis conditions [2, 6]. In the present study (Table 2), high amounts of essential and non-essential amino acids were identified, leucine, the highest essential amino acid related to the enzymes protamax and bromelain, 99.8 and 45.8%, respectively. Also, the highest amounts of non-essential amino acids for the same enzymes, glutamic acid, were 55.13 and 48.13%, respectively. These findings are consistent with the results of a study by Villanueva-Lazo et al. (2022) on the amino acid profile of hydrolyzed chia seed protein by alcalase enzyme [8]. In that study, leucine and glutamic acid were also identified as the dominant essential and non-essential amino acids. According to FAO/WHO (1990) standards, the ratio of essential amino acids to total amino acids should not be less than 40% and the ratio of essential to non-essential amino acids should not be less than 0.6. The results indicate that hydrolyzed proteins have a suitable composition of amino acids [18]. The ratio of essential to non-essential amino acids for Protamex and bromelain was reported to be 0.76 and 0.75, respectively, and the ratio of essential to total amino acids was 43.34 and 42.91, respectively. The studies showed that the concentration of all essential amino acids was higher than the recommended values of FAO/WHO (1990) for adult needs. This pattern of essential amino acids indicates the high nutritional value of chia seeds, which can effectively contribute to meeting the basic amino acid requirements in the adult diet. The total hydrophobic amino acids in proteins hydrolyzed by Protamax and bromelain were 36.65% and 34.65%, respectively. The presence of these amino acids in appropriate amounts indicates the high nutritional value of these proteins. In addition, these amino acids have a significant impact on the functional and biological properties of proteins and can play a role in antioxidant, antimicrobial, and cancer prevention activities, as well as helping to regulate blood sugar and blood pressure [19]. In general, hydrophobic amino acids help stabilize the structure of proteins and improve their physical properties, which is of particular importance in industrial and food processes.

Table 2: Effect of enzyme hydrolysis type (at 90 min) of protein hydrolyzed on amino acid composition

Amino acid (g 100 g^{-1})	Protamax	Bromelain	FAO/ WHO, 1990
Threonine[a]	4.20	4.40	3.4
Valine[a]	4.70	4.90	3.5
Methionine[a]	3.70	3.40	-
Isoleucine [a]	3.70	3.40	2.8
Leucine[a]	7.50	6.90	6.6
Phenyl alanine[a]	9.95	9.10	6.3
Histidine [a]	2.70	2.80	1.9
Lysine[a]	6.10	6.60	5.8
Arginine[a]	10.22	10.11	-
Tryptophan[a]	0.95	1.05	-
Glycine	4.11	4.21	-
Aspartic acid	9.22	9.88	-
Glutamic acid	17.55	18.22	-

Serine	5.10	4.99	-
Alanine	4.80	4.90	-
Tyrosine	1.90	1.95	1.1
Cysteine	1.50	1.60	-
Total amino acid	98.30	98.11	-
Essential amino acids/ Total amino acid	55.05	53.39	-
Essential amino acids/ none ersential amino acids	1.22	1.14	-
LET[b]	36.65	34.65	-

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

3-4- Antioxidant properties

The antioxidant activity of chia seed protein hydrolysates was measured using the DPPH assay, which quantifies the ability of an antioxidant (i.e., hydrogen or electron donor) to scavenge the DPPH radical cation based on the one-electron reduction of the relatively stable DPPH radical cation previously formed by an oxidation reaction. The above results show (Figure 1) that chia seed protein hydrolysates undergo single-electron transfer reactions in the DPPH reduction assay, which effectively measures the total antioxidant activity of food antioxidants and nutrients. Under the analyzed conditions, chia seed protein hydrolysates may act as electron donors and free radical scavengers, thereby providing antioxidant protection [20]. With increasing hydrolysis time, the DPPH radical scavenging activity also increased ($P<0.05$). Protein hydrolyzed by Protamax enzyme showed the highest antioxidant activity (94.55%) after 90 min of hydrolysis, and its antioxidant activity was higher than that of bromelain ($P<0.05$). This suggests that the antioxidant activity of peptides may depend on the specific proteases used for their production, the degree of hydrolysis achieved, the nature of the released peptides (e.g. molecular weight, amino acid composition and sequence), as well as the combined effects of their properties (e.g. capacity to scavenge free radicals, chelate metal ions and/or donate electrons). The size of the peptides may also play a role, as antihypertensive peptides are short and consist of only two to nine amino acids, while antioxidant peptides consist of three to sixteen amino acid residues [1, 20].

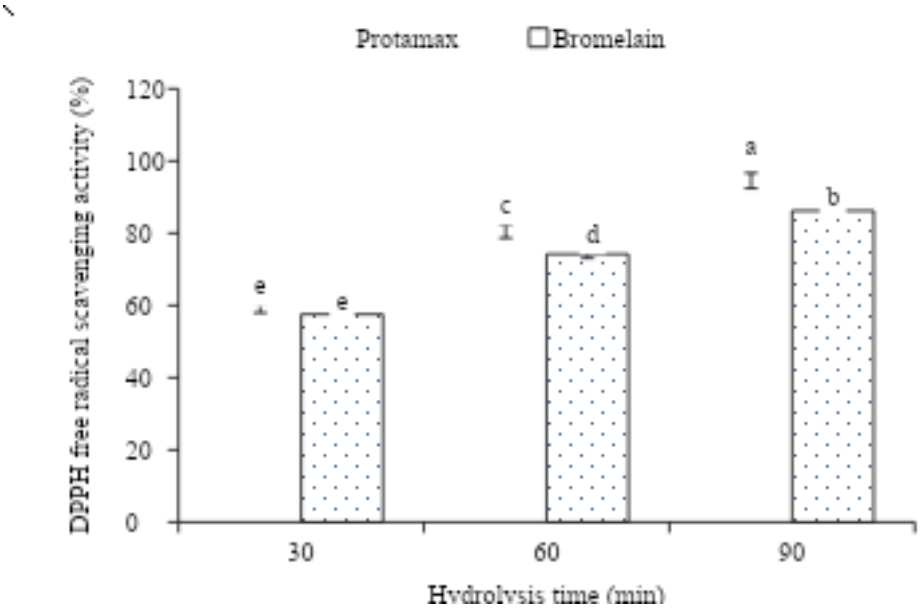

Figure 1: The DPPH free radical savaging activity of chia seed protein hydrolysates
Different letters in the same times showed a significant difference at $p<0.05$.

When the hydrolyzed protein has the property of reducing iron ions, a green color appears in the reaction medium and the stronger this color and the higher the optical absorption at a wavelength of 700 nm, the higher the reducing activity. The results show that the iron reduction power increases with increasing enzymatic hydrolysis time (Figure 2). The highest values were obtained for the protein hydrolyzed with Protamax at 90 min (0.55 µmol ferrous/g) ($P<0.05$). The increase in iron reduction power is related to the release of antioxidant amino acids such as tyrosine, tryptophan, histidine, methionine and lysine after enzymatic hydrolysis, and the concentration of these amino acids increases [21]. Compounds with higher reducing power have better potential for donating electrons or hydrogen and are known as valuable natural antioxidants [22]. In particular, protein hydrolyzed with Protamax enzyme, with a longer hydrolysis time, is likely to have the highest levels of free peptides and amino acids that are capable of donating electrons to free radicals and thus can stop the oxidation chain reaction [23]. Similar results have been reported by Silveira Coehlo et al. (2019) and Villanueva-Lazo et al. (2021), indicating that concentrations of chia seed protein hydrolyzed with protease enzymes have potential antioxidant capacity [7, 24].

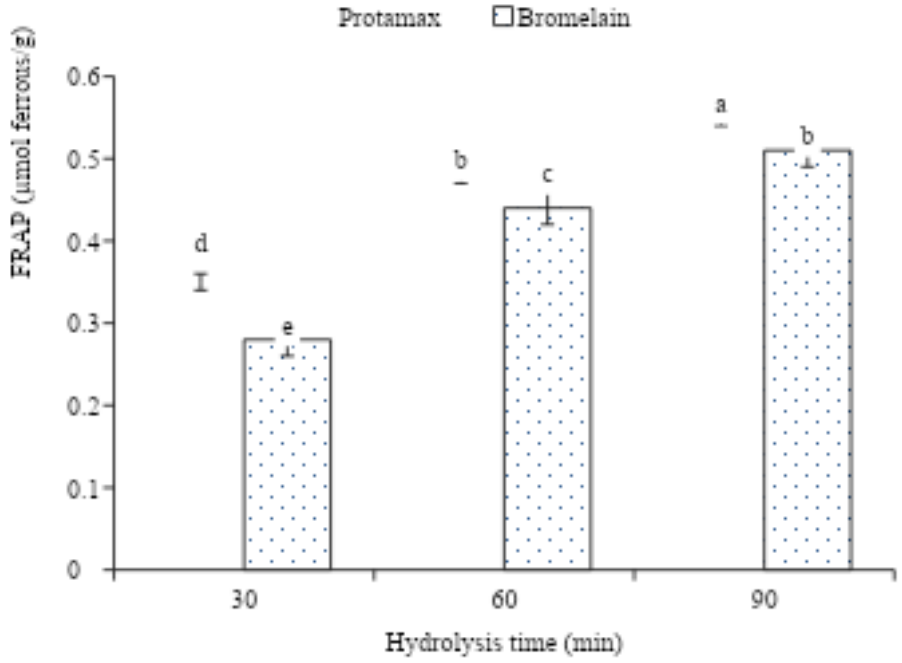

Figure 2: The FRAP of chia seed protein hydrolysates
Different letters in the same times showed a significant difference at $p<0.05$.

3.5- Functional properties

Protein solubility is a complex and critical property that can affect other functional properties such as emulsifying activity, foaming capacity, and gelling properties [25]. Chia protein solubility is not only affected by pH and temperature, but can also be changed by salt addition, different drying methods, and hydrolysis method and time. According to the results, all hydrolyzed proteins showed high solubility (Figure 3), and there was no significant difference in the solubility of proteins hydrolyzed by both enzymes at most hydrolysis times ($P<0.05$). However, the solubility values were significantly different among different hydrolysis times. Enzymatic hydrolysis can increase the number of smaller hydrophilic peptides [26]. With increasing hydrolysis time, solubility values increased significantly, such that the protein hydrolyzed at 90 min had the highest solubility ($P<0.05$). This increase in solubility is due to the breakdown of proteins into smaller peptides, which are usually more soluble. This phenomenon occurs due to an increase in surface area to volume ratio and better access to water molecules [27]. These findings are consistent with the results of a study by Villanueva-Lazo et al. (2021) on the solubility of chia seed protein hydrolyzed by the enzyme alcalase, who also reported a positive effect on protein solubility with increasing hydrolysis time [7].

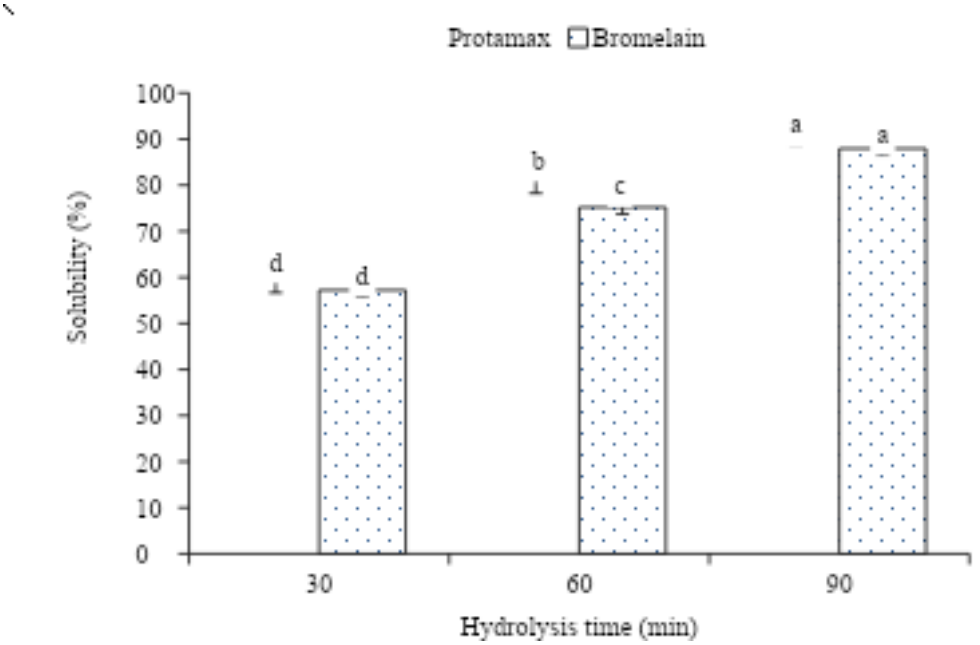

Figure 3: The solubility of chia seed protein hydrolysates
Different letters in the same times showed a significant difference at p<0.05.

Emulsifying properties are a critical property of proteins in the production of ice cream, margarine, sauces, and other foods that require the protein to act as an emulsifier to fully combine two immiscible liquids. In general, emulsifying properties can be measured through physical properties, using emulsifying activity, emulsifying capacity, and emulsion stability [28]. Chia oil [29], corn oil [7], refined sunflower oil [25], and canola oil [26] have been used to measure the emulsifying properties of chia seed protein or protein hydrolysate. The emulsifying capacity and emulsion stability (Figures 4 and 5) of chia seed protein can be strongly influenced by various methods, such as pH adjustment, separation, and enzymatic hydrolysis [27]. The results of emulsion stability and emulsion capacity were consistent with each other, with the increase in hydrolysis time, the emulsion capacity values increased, and higher emulsion properties were observed with the Protamax enzyme (P<0.05). As a key indicator in the hydrolysis process, the emulsifying properties of proteins are affected by the peptide chain length, the emulsifying capacity increases. According to the study of Villanueva-Lazo et al. (2021) and Urbizo-Reyes et al., (2019), chia seed protein hydrolyzed with alcalase had 50-fold higher emulsifying activity and 100-fold higher emulsion stability than the intact protein [7, 26].

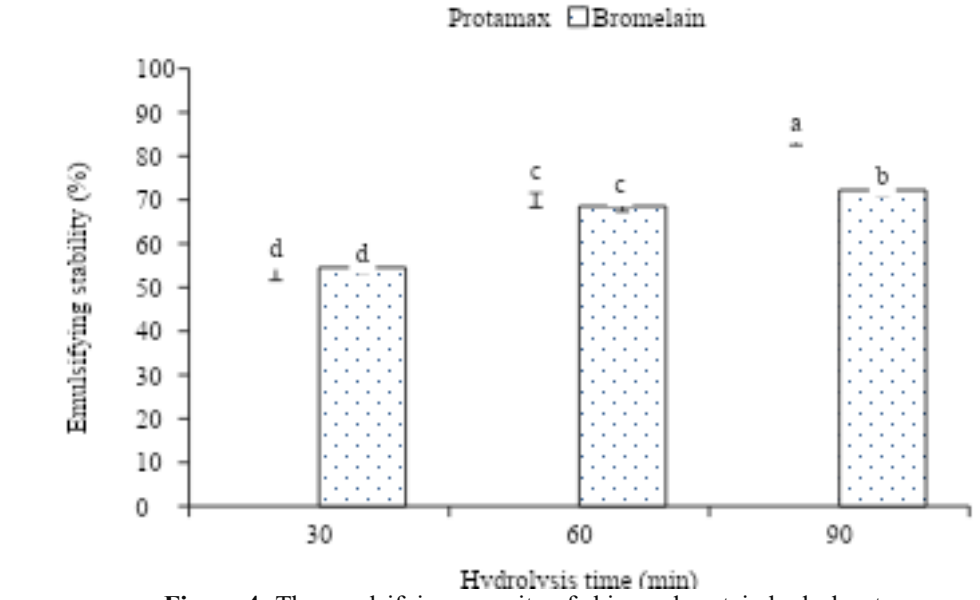

Figure 4: The emulsifying capacity of chia seed protein hydrolysates
Different letters in the same times showed a significant difference at p<0.05

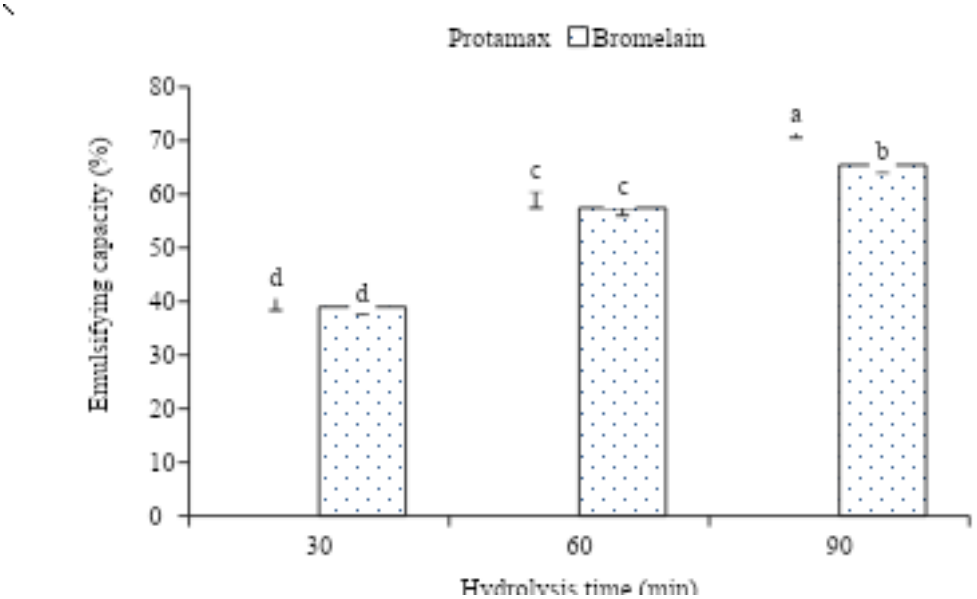

Figure 5: The emulsifying stability of chia seed protein hydrolysates
Different letters in the same times showed a significant difference at $p<0.05$.

Foaming properties are crucial in the food industry, such as beer, whipped cream, etc. Foam formation refers to the process of dispersion of air bubbles in a continuous liquid or solid phase, resulting in the formation of a flexible interfacial film [30]. Foaming capacity indicates the ability of a protein to produce foam, which can be reflected by foam volume increase or percentage foam volume increase, and foam stability indicates the durability of the formed foam over a given period of time [27]. Several factors affect the foaming capacity and stability of proteins, including pH, hydrolysis method, protein concentration, and extraction methods. The values of foam capacity and foam stability (Figures 6 and 7) were consistent with each other, and the foaming of the protein hydrolyzed by the Protamax enzyme was higher at all hydrolysis times. With increasing hydrolysis time, foaming capacity increased, such that protein hydrolyzed with Protamax enzyme showed the highest foaming capacity and foam stability at a hydrolysis time of 90 minutes ($P<0.05$). Several studies have investigated the effect of enzymatic hydrolysis process and hydrolysis time on the foaming capacity and stability of protein hydrolysates. Factors such as the solubility of proteins at different pH conditions, degree of hydrolysis, and type of peptides affect the ability to form films and, consequently, these indicators [31, 32]. Accordingly, the effect of increasing the degree of hydrolysis under the influence of process time and enzyme type is effective in reducing the chain length of peptides and their function in creating and stabilizing foam. According to the study of Villanueva-Lazo et al. (2021) and Urbizo-Reyes et al., (2019), hydrolyzed chia seed protein had very good foaming properties.

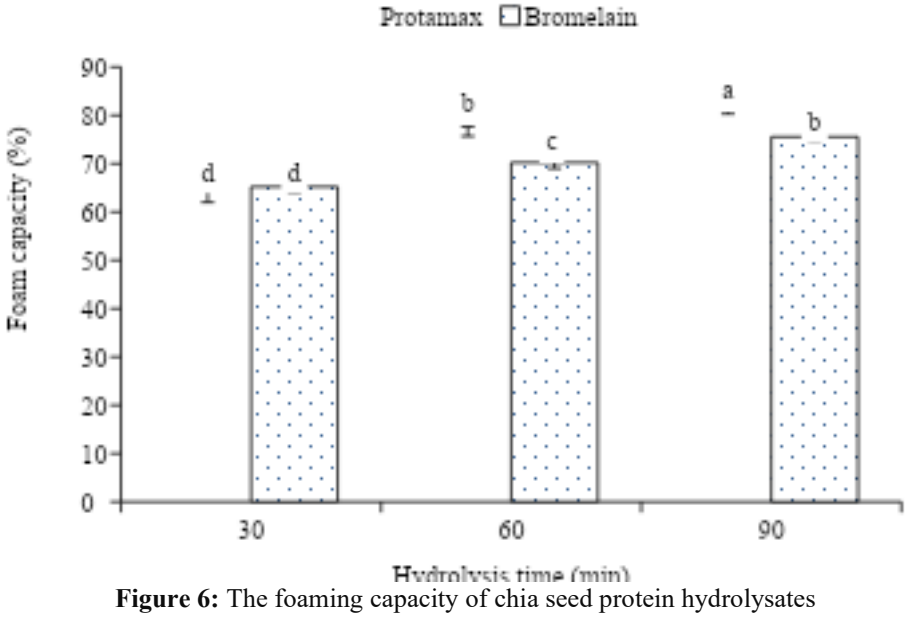

Figure 6: The foaming capacity of chia seed protein hydrolysates

Different letters in the same times showed a significant difference at p<0.05.

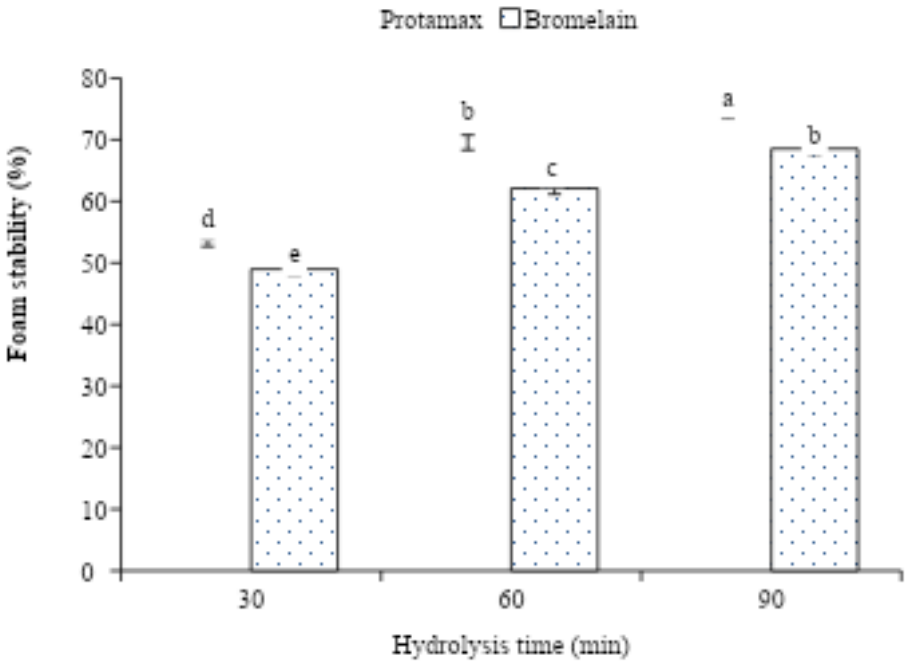

Figure 7: The foaming stability of chia seed protein hydrolysates

Different letters in the same times showed a significant difference at p<0.05.

4- Conclusion

Based on the results, enzymatic hydrolysis of chia seed proteins has acceptable functional and antioxidant properties. The type of enzyme affected the functional and antioxidant properties of hydrolyzed proteins. Hydrolyzed chia seed proteins by Protamax enzyme showed better degree of hydrolysis, protein, solubility, foaming and foam stability, DPPH inhibition and reducing power. In fact, enzymatic modification of chia seed protein creates a natural antioxidant source that can be used as a natural antioxidant source in food models.

5-Resources

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بررسی ویژگی‌های عملکردی و آنتی‌اکسیدانی پروتئین‌هیدرولیز شده از دانه چیا تحت تأثیر زمان هیدرولیز و نوع آنزیم

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چکیده

دانه‌های چیا (*Salvia hispanica*) به عنوان منبعی غنی از پروتئین و ترکیبات زیست‌فعال، تأثیرات مثبتی بر سلامت انسان دارند. هیدرولیز آنزیمی این پروتئین‌ها می‌تواند ویژگی‌های عملکردی و فعالیت آنتی‌اکسیدانی آن‌ها را بهبود بخشد. در این پژوهش، تأثیر نوع آنزیم‌ها بر تولید پروتئین‌های هیدرولیز شده از دانه چیا با خصوصیات عملکردی مطلوب و آنتی‌اکسیدانی بالا مورد بررسی قرار خواهد گرفت. پروتئین هیدرولیز شده از دانه چیا با استفاده از آنزیم‌های پروتامکس و برواملین در مدت زمان ۳۰، ۶۰ و ۹۰ دقیقه، در دما و pH بهینه (۵۰ درجه سانتی‌گراد و pH ۷) تولید شد. سپس پارامترهای حلالیت، کف‌کنندگی و امولسیون، به همراه فعالیت مهارکنندگی رادیکال DPPH و قدرت احیاکنندگی آهن پروتئین‌های هیدرولیز شده مورد ارزیابی قرار گرفت. نتایج نشان داد که افزایش زمان هیدرولیز و استفاده از آنزیم پروتامکس تأثیر قابل توجهی بر درجه هیدرولیز و مقادیر پروتئین‌های دانه چیا داشته است. در زمان ۹۰ دقیقه، بالاترین مقادیر درجه هیدرولیز (۴۰/۲۳٪) و پروتئین (۹۰/۷۰٪) مشاهده شد ($p < 0.05$)، که نشان‌دهنده کارایی بالای این آنزیم در فرآیند هیدرولیز است. این شرایط همچنین منجر به بهبود ویژگی‌های عملکردی، از جمله حلالیت، امولسیون و کف‌کنندگی پروتئین‌ها گردید، علاوه بر این، این هیدرولیز باعث افزایش قابل توجهی در فعالیت آنتی‌اکسیدانی، از جمله مهارکنندگی رادیکال DPPH و شلاته‌کنندگی یون آهن گردید. نتایج نشان داد که آنزیم‌های مختلف روی یک سوبسترا اثرات متفاوتی اعمال می‌کنند و اصلاح آنزیمی پروتئین دانه چیا منجر به ایجاد یک منبع طبیعی آنتی‌اکسیدانی می‌شود که پتانسیل بالایی برای کاربرد در صنایع غذایی و دارویی، دارد.