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Optimizing enzymatic hydrolysis of pumpkin seeds protein (*Cucurbita maxima L.*) by pancreatin with using microwave pretreatment

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ABSTRACT

The seeds of *Cucurbitaceae* plants (*pumpkin, melon, etc*.) are one of the rich sources of protein. Proteins are one of the most important nutritional sources for humans. Enzymatic hydrolysis of pumpkin seed protein by using microwave pretreatment leads to the production of hydrolyzates with bioactive properties, including antioxidant activity. The use of microwave pretreatment causes changes in the 3D structure of proteins, It opens the 3-dimensional structure of the protein and accelerates the access of the enzyme to the peptide bonds. Therefore, the use of microwave pretreatment is a suitable method to save time and enzyme concentration which are used in enzymatic hydrolysis. In this study, The solution of pumpkin seed protein concentrate was exposed to microwave energy with a power of 450-900 W for 30-90 seconds and it was used as a substrate solution in enzymatic hydrolysis experiments. Enzymatic hydrolysis by pancreatin, with the concentration of 0.5 to 2.5% compared to the protein substrate, was performed in the time from 20 to 190 minutes, at the optimum temperature and pH of pancreatin, in order to produce hydrolysates with antioxidant potential. Antioxidant power was measured by using DPPH radical scavenging activity methods, total antioxidant activity (Absorbance at 695 nm) and iron chelating activity. The highest amount of antioxidant activity by using microwave pretreatment was in 105 minutes and the ratio of 1.5% E/S and the optimal conditions that provided by the software to achieve the maximum iron chelating activity (95.5%), DPPH radical scavenging activity (51.5%) and total antioxidant (Absorbance at 695 nm) (0.976), was in 102 minutes and the ratio of 1.5% E/S of which 89.5% corresponded with the obtained results.

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1- Introduction

Proteins are important nutritional sources for humans. These compounds provide nitrogen, amino acids and energy needed for the normal functioning of body systems. The most common way to produce peptides is the hydrolysis of proteins with the help of enzymes]1[. Today, bioactive peptides derived from the hydrolysis of various dietary proteins have various biological roles, such as lowering blood pressure [2], relaxing3[,anti-cholesterol]4[, Antioxidants]5[And against cancer]6[Are. Pumpkin seeds can be used as a raw material to produce high quality protein products for food formulation as well as functional properties.7[. More importantly, pumpkin seeds are a valuable raw material for the preparation of antioxidant peptides due to their high protein content, easy access and low cost. Zakari et al. (2019) reported that hydrolyzed pumpkin seed protein has high antioxidant and nitric oxide inhibitory properties and can be used as a suitable ingredient in food formulations [8]. Hydrolyzed pumpkin seed meal with suitable functional properties can be used in different food formulations to improve physical and chemical properties, increase shelf life and as anti-hypertensive agents and antioxidants in the prevention of cardiovascular diseases [9]. Some other studies conducted on pumpkin seeds are as follows: The effect of enzymatic hydrolysis of peptides derived from pumpkin seeds (*Cucurbita pepo L.*(on zinc binding capacity and stability of digestive system in laboratory conditions [10], functional properties of isolated and hydrolyzed pumpkin seed protein)*Pumpkin pie*) [11], production of antioxidant peptides from pumpkin seed meal [12], antioxidant properties of pumpkin seed protein [13] and production of antioxidant peptides from hydrolyzed pumpkin seed protein [7]. In recent years, many physical methods, such as ultrasound]14 ،15[, very high pressure]16[, microwave]17، 18[etc. have been used to aid hydrolysis to obtain higher amounts of bioactive peptides from food protein sources. Some equipment, such as high hydrostatic pressure and radiation, are very expensive and not readily available for home-scale use.19[. Microwave to electromagnetic waves with frequency300 MHz to300 GHz and wavelengths between1 mm to1 Meter is used. Microwaves usually exhibit three characteristics: penetration, reflection, and absorption. Food protein can absorb microwaves and change the protein structure. Research shows that enzymatic hydrolysis with the help of microwaves can shorten the time of enzymatic hydrolysis, improve the reaction speed, and increase the efficiency of peptide production.20 ،21[. The purpose of this research is to optimize the conditions of enzymatic hydrolysis of pumpkin seed protein using microwave pretreatment by pancreatin enzyme and to investigate its antioxidant properties. hydrolyzed using free radical scavenging methodsDPPH, total antioxidant (absorbance at 695 nm) and iron chelating activity.

2- materials and methods

1-2- Preparation of raw materials

First, pumpkin fruit (*Cucurbita maxima L.*) was obtained from the local market of Astana Ashrafieh in Gilan province and after separating the seeds manually, it was dried in an oven at a temperature of 50 degrees Celsius for 72 hours [13]. Pancreatin

enzyme and other chemicals used in the experiments were obtained from reputable companies (Majalli and Merck).

2-2- Production of protein concentrate

The dried pumpkin seeds were oiled without peeling by a cold press and the resulting flour was completely powdered and passed through a 40 mesh sieve. Hexane solvent was added to the powder at a ratio of 10:1 (volume-weight) and mixed by a shaker at a speed of 440 rpm for 4 hours. The process of oil removal continued until it was reduced to about 5%. The residual solvent in the flour was removed by vacuum oven at 40°C for 24 hours. The oil-free flour was spread in distilled water at a ratio of 1 to 10 (weight-volume), and the pH of the solution was adjusted to 11 by 1 normal sodium bicarbonate and then mixed by a mechanical stirrer for 1 hour at laboratory temperature. After that, centrifugation was performed for 20 minutes at a speed of 5000 rpm at a temperature of 4 degrees Celsius by a refrigerated centrifuge, and by collecting the supernatant solution, the residue was re-dissolved in distilled water and the extraction of the supernatant was repeated. The supernatants were collected and mixed together and the pH was adjusted to 4 using 1 normal hydrochloric acid, the centrifugation was repeated for 20 minutes at 4°C at 5000 rpm. Finally, the resulting sediment was dried by a freeze dryer and stored in closed containers in a dry and cool environment [9].

3-2- Chemical tests

1-3-2- Protein content of the sample

2 grams of the sample along with 20 milliliters of concentrated sulfuric acid and a number of Keldal tablets (catalyst) were added to the digestion tube and a digestion tube without sample and containing only acid and catalyst was used as a control. The digestion operation was performed with the power of 80 units for 120 minutes until the contents of the unit became clear. After cooling and removing the acid vapors, distillation was done using 40% soda ash and 4% boric acid, and then the titration of the sample was continued with the help of 0.1 normal hydrochloric acid until the sample turned purple in the presence of methylated reagent. The nitrogen percentage of the sample was calculated using equation 1 [22].

Equation 1

$$1.4\times W\times(H_1\text{-}IN_2)/m\times100 = \text{percentage of sample nitrogen}$$

N normality of hydrochloric acid,IN_1 The volume of acid used for the sample,IN_2 The volume of acid consumed for the witness andm The weight of the sample is in grams. To calculate the protein percentage of the sample, we multiply the total nitrogen by the protein factor (6.25).

2-3-2- Moisture content of the sample

5 grams of the sample was weighed in an aluminum container for moisture measurement and placed in the oven at a temperature of 105 degrees Celsius for 60 minutes and after cooling for 15 minutes in the desiccator, weighing was done. This process was repeated several times until the moisture content of the sample reached a constant value. Then the amount of moisture was calculated according to equation 2 [22].

Equation 2:

$$(IN_1\text{-}IN_2)/m\times100 = \text{moisture percentage}$$

IN_1 Weight of container and sample before drying,IN_2 Weight of container and sample after drying andm It is the weight of the sample.

3-3-2- Ash content of the sample

2 grams of sample was weighed in a plant picker with a specified weight. First, it was burned on a heater, and then the crucible containing the sample was transferred to an electric furnace with a temperature of 550-

600 degrees Celsius for 6 hours, and the heating continued until the color of the ash turned white. Finally, after 15 minutes of cooling the plant in the desiccator, weighing was done. The amount of ash was calculated through equation 3 [22].

Equation 3:

$(IN_1\text{-}IN_2)/m\times100$ = percentage of ash

IN_1 Bush and ash weight, W_2 The weight of the plant and m is the weight of the sample.

4-3-2- Fat content of the sample

3 grams of the sample was weighed on filter paper and placed inside the cartridge. The cartridge was transferred to the extraction part of the Soxhlet machine. A balloon with a certain weight was half filled with hexane solvent and then connected to the device. The extraction operation was carried out for 4 hours. Finally, to remove the remaining solvent, the flask containing the sample was transferred to an oven with a temperature of 40 degrees Celsius, then the flask was weighed again. Fat percentage was calculated with equation 4 [22].

Equation 4:

$(IN_1\text{-}IN_2)/m\times100$ = fat percentage

IN_1Weight of balloon and sample, W_2The weight of the balloon and m is the weight of the sample.

4-2- Microwave pretreatment

Pumpkin seed protein concentrate solution with a ratio of 5% in phosphate buffer 0.1 M (100 gr/L) Na_2HPO_4-Now_2AFTER_4) pH 7.4,) was prepared. The solution was stirred for 30 minutes and exposed to microwave energy with a specific power of 900-450 W for 90-30 seconds for each treatment. Protein solution pretreated with microwave was used as substrate solution in enzymatic hydrolysis experiments [23]. It should be noted that after measuring the total antioxidant activity (absorbance at 695 nm) in different powers and times of microwaves, the power of 600 watts was selected for 30 seconds and applied before enzymatic hydrolysis.

5-2- Enzymatic hydrolysis

In order to optimize the enzymatic hydrolysis conditions, the response surface method was used. Pancreatin enzyme was added in concentrations of 0.5 to 2.5% compared to the protein substrate, and the hydrolysis time was performed as an independent factor for each, within 20 to 190 minutes in a shaker incubator at a speed of 200 rpm. The temperature and pH of hydrolysis were considered according to optimum temperature (40 °C) and optimum pH (pH=7.4). In order to inactivate the enzyme, the protein solution was placed in a hot water bath with a temperature of 85 degrees Celsius for 15 minutes. Then the hydrolysates (supernatant) were centrifuged at 4000 rpm at 4°C for 15 minutes. The resulting supernatant was dried with a freeze dryer to obtain hydrolyzed protein powder and stored at -20°C until use [13, 24]. Enzymatic hydrolysis was performed according to Table 1.

2-6- Measurement of antioxidant power

1-6-2- DPPH free radical inhibition

The hydrolysates were dissolved in distilled water with an optimal concentration (40 mg/ml). Then 200 microliters of the sample was mixed with 600 microliters of methanol and 200 microliters of DPPH (0.15 mmol in methanol). Vigorously for 2 minutes

It was shaken and kept for 30 minutes in the dark at room temperature. Absorbance was measured at a wavelength of 517 nm using a UV-vis spectrophotometer. Control contains 800 µL of methanol and 200 µL of DPPH (0.15 mM). DPPH radical inhibition ability was calculated using equation 5 [25].

Equation 5 100x absorbance of the control / (absorbance of the sample - absorbance of the control) = percentage of free radical inhibition DPPH DPPH

2-6-2- Iron chelating activity

200 µL of optimized sample, with 10 µL of $FeCl_2$ (2 mmol) and 600 microliters of distilled water were mixed. Then 20 microliters of ferrosin solution[1] (5 mM) was added to the mixture and mixed vigorously for 2 minutes. Then the mixture was kept at room temperature for 10 minutes. Color reduction, due to iron chelation, was recorded by measuring absorbance at 562 nm. Control sample containing 800 µL of distilled water, 10 µL of $FeCl_2$ and 20 microliters of ferrozine solution (5 mM). The percentage of chelation was calculated using equation 6 [25].

Equation 6 100x absorption of the control / (absorption of the sample - absorption of the control) = percentage of chelation

3-6-2- Total antioxidant capacity (absorbance at 695 nm)

In this method, 0.1 ml of the optimal sample with 1 ml of the reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was poured into 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. More absorbance indicates higher total antioxidant capacity (absorbance at 695 nm) [26].

2-7- Optimizing the process

Hydrolysis conditions were optimized using Design Expert software version 11 and response surface method in the form of central compound design. The independent variables are the ratio of enzyme to substrate concentration (X_1) and hydrolysis time (X_2) were tested at five levels (α+, +1, 0, -1, α-) and three central points. The investigated response was total antioxidant activity, iron ion chelation and DPPH free radical inhibition. The effect of independent variables on antioxidant properties was evaluated using SPSS version 26 software. Means were compared using Duncan's test at the 95% confidence level. The different levels of independent variables are presented in Table 2.

Table 2: Levels of independent variables used to optimize the antioxidant activities of pumpkin seed protein

a-	-1	0	+1	a+	independent variables
0.5	0.8	1.5	2.2	2.5	E/S
20	45	105	165	190	time

3. Results and Discussion

1-3- Chemical compounds

The amount of moisture, ash, protein and fat is shown in Table 3.

[1] -ferrozine

Table 3: Chemical compounds present in whole grain, defatted flour and pumpkin seed protein concentrate

moisture	ash	fat	protein	sample
8.46%	6.9%	31.57%	33.92%	whole grain
7.65%	4.65%	5.12%	59.5%	defatted flour
5.45%	1.45%	2.29%	65.62%	pumpkin seed protein concentrate

Results are averages of 3 replicates.

As can be seen in Table 3, defatting has reduced the fat content of protein concentrate compared to whole grains. First of all, this reduction is due to cold pressing of whole grain, which has reduced the amount of fat in the resulting flour (from 31.57% to 12.5%), and the final reduction of fat in the concentrate is due to the use of hexane solvent, protein extraction in the solution. Alkalinity and protein precipitation is at its isoelectric point [24]. The lowest amount of moisture is related to the protein concentrate, the reason for which is the use of an oven with a temperature of 30 degrees Celsius to remove the remaining hexane after the degreasing process and the use of a freeze dryer to dry the protein sediment after the extraction process [27]. The amount of protein and fat in whole grain is slightly different from the findings of Ardabili et al. According to the table, the lowest amount of ash is related to protein concentrate, which is due to the protein extraction process and the removal of large amounts of non-protein compounds.

Table 1: random treatments and antioxidant activity of hydrolyzed pumpkin seed protein with using microwave pretreatment

Total Oxidant	DPPH%	Chilating Activity%	E/S %	time	treatment
0.835	45.2	83.3	1.5	20	1
0.87	49.5	89	0.8	45	2
0.825	44.1	83.5	2.2	45	3
0.825	47.3	86.9	0.5	105	4
0.955	51.5	90.3	1.5	105	5
0.975	50.4	91.1	1.5	105	6
1	52.6	90. 2	1.5	105	7
0.835	48.6	87.4	2.5	105	8
0.81	45	83.5	0.8	165	9
0.895	48	85.5	2.2	165	10
0.825	43.6	83.2	1.5	190	11

2-3- The effect of hydrolysis time and ratioE/S on chelation activity using pretreatmentMW

According to the values of the numberP in the table4 The values are deducedA^2، B^2 AndAB have a significant effect on iron chelating activity ($p<0.05$). VariableA^2, is more effective than other variables. The non-significance of the lack of fit factor ($p>0.05$), indicates the appropriateness of the model for predicting the range of the studied variables. The relationship of chelation of iron ion with independent variables, in quadratic form and with correlation coefficientR^2=0.9566 The good ability of the model in predicting the effect of independent variables on dependent variables shows that from Eq7 follow.

$90.53+1.87AB-3.60A^2-1.65B^2$

Table 4: Effect of time and E/S ratio on iron ion chelating activity

df	P_{Value}	Regression coefficient	
5	0.0020	90.53	model
1	0.2177	-0.4552	A
1	0.3290	-0.3491	B
1	0.0093	1.87	AB
1	0.0002	-3.60	A^2
1	0.0078	-1.65	B^2
		0.7161	Pre-R^2
		0.9132	adj-R^2
3	0.1698		Lack of fit

A: Time,B: E/S

In the chelation reaction, ferrozin creates a violet complex with iron ion. When the composition of chelating agents is present in the environment, the concentration of iron ions will decrease and the intensity of the color of this complex will decrease. Therefore, by increasing the chelating ability of iron ion, the color intensity of the complex is created and as a result, the absorption intensity in562 nanometer will decrease]12[. According to Figure 1, the highest level of chelating activity with the use of pretreatment, related to time105 Minutes and ratios5/1 PercentE/S and its lowest amount belongs to time190 It was minutes.It has been reported that some amino acids and their derivatives such as cysteine, histidine, tryptophan, lysine, arginine, leucine, tyrosine and valine have antioxidant activity [29]. In addition, tripeptides containing tryptophan or tyrosine amino acids at their C-terminus show significant antioxidant activity [30]. Noormohammadi et al. (2017) reported that the highest chelating activity of hydrolyzed pumpkin seeds was within 2 hours and the enzyme concentration was 2% [12]. The difference in different reports is probably due to the difference in hydrolysis conditions, the species used, the type of enzyme, etc. Pretreatment of proteins before enzymatic hydrolysis works to improve the release of bioactive peptides from different proteins. MW causes the 3D structure of the protein to open and helps to increase the access of enzymes to peptide bonds [31]. Oloko et al. (2015) pointed out that the use of microwave energy increases the degree of hydrolysis and the stability of hydrolyzed whey proteins [31]. Gazikalovic et al. (2021) stated that compared to heat treatment, it seems that microwave treatment affects gluten structure and allergenicity and in combination with enzymatic hydrolysis, finally produces hydrolyzed protein with antioxidant properties and proper function [32].

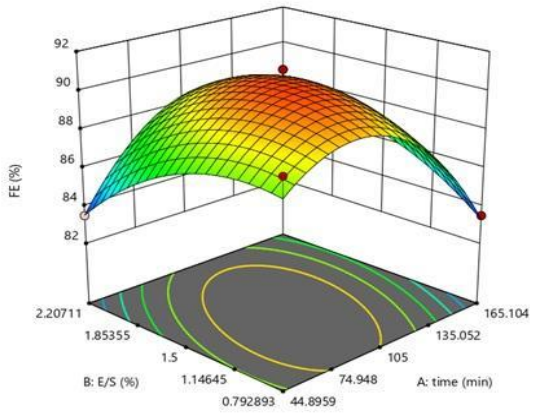

Figure 1: Investigating the effect of hydrolysis time and E/S ratio on iron chelating activity by using microwave pretreatment

3-3- Effect of hydrolysis time and ratioE/S on free radical inhibitionDPPH Using pretreatmentMW

According to the values of P number in Table 5, the values of A^2 ،B^2 and AB affect DPPH free radical inhibition ($p<0.05$). A^2 It is more effective than other variables. The non-significance of the misfit factor

($p>0.05$) indicates that the model fits well with the data related to DPPH free radical inhibition. The relationship of DPPH free radical inhibition with independent variables, quadratic and with correlation coefficient R^2= 0.9414, which shows that the model was able to predict 94.14% of the total changes in the range of the studied values. Equation 8 shows the relationships between reaction variables and DPPH free radical inhibition.

$$51.50+2.10AB-3.43A^2-1.66B^2$$

Table 5: Effect of time and E/S ratio on DPPH radical scavenging activity

df	P_{Value}	Regression coefficient	
5	0.0042	51.50	model
1	0.3773	-0.3578	A
1	0.8568	-0.0702	B
1	0.0101	2.10	AB
1	0.0006	-3.43	A^2
1	0.0131	-1.66	B^2
		0.7096	Pre-R^2
		0.8828	adj-R^2
3	0.5843		Lack of fit

A: Time,B: E/S

according to the picture2, the highest rate of free radical inhibitionDPPH In the age of105 Minutes and ratios5/1 PercentIt is E/S And its lowest amount belongs to time190 It is minutes. As can be seen from the results, with the increase in hydrolysis time and ratioE/S,From the amount of free radical inhibitionDPPH has been reduced From the free radical inhibitory propertyDPPH It is used to check the hydrogenation ability of hydrolyzed proteins. Elimination of free radicals is a mechanism by which antioxidant compounds are able to prevent oxidative reactions. free radicalDPPH It is one of the few stable radicals at room temperature. When this compound is placed in the vicinity of a hydrogenating compound such as an antioxidant, a hydrogen is accepted, it becomes a stable compound, and as a result, radical inhibition, noticeable color change from purple to yellow, and a decrease in the amount of absorption in 517 nanometer is observed. The type of raw material, the specificity of the enzyme, the conditions of hydrolysis, and the size and structure of the amino acids and peptides produced are among the factors affecting the antioxidant activity [33]. The reduction of radical inhibitory ability with increasing enzyme concentration can be due to the enzyme's digestive effect on the produced peptides. With the increase in the concentration of the enzyme, the possibility of effect on the protein substance increases and this leads to the breakdown of a number of antioxidant peptides produced in the initial stages of hydrolysis.34[. YoungEt al (2022) Effect of microwave pretreatment on free radical inhibitory activityDPPH studied milk protein hydrolysates and stated that all microwave pretreatments of milk protein hydrolysates with different power, inhibitory activityDPPH compared to the control showed a higher and free radical inhibition powerDPPH, with the help of a powerful microwave300 Watt was the highest value [35].StunnedEt al (2015) They reported microwave pretreatment with power400 Watt causes the highest amount of antioxidant activity in hydrolyzed sunflower meal [36]. The difference in the powers used in different studies may be related to the solubility of protein substances. Milk protein hydrolysates are affected by less microwave power compared to insoluble proteins.35[.

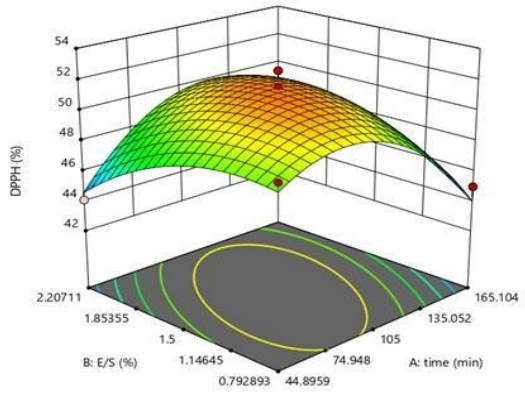

Figure 2: The effect of hydrolysis time and E/S on DPPH radical scavenging activity by using microwave pretreatment

4-3- Effect of hydrolysis time and ratioE/S on total antioxidant activity (absorbance at 695 nm) under pretreatmentMW

According to the values of P number in Table 6, the values of A^2 ،B^2 and AB affect the total antioxidant activity (absorbance at 695 nm) ($p<0.05$). The non-significance of the misfit factor ($p>0.05$) indicates that the model fits well with the data related to the total antioxidant activity (absorbance at 695 nm). The relationship of total antioxidant activity (absorbance at 695 nm) with independent variables, quadratic and with correlation coefficient R^2=0.9583. which shows that the model was able to predict 95.83% of the total changes in the studied range of values. Equation 9 shows the relationship between reaction variables and total antioxidant activity.

$$0.9767+0.0325AB-0.0683A^2-0.0683B^2$$

Table 6: Effect of hydrolysis time and E/S ratio on total antioxidant activity (absorbance at 695 nm)

df	P_{Value}	Regression coefficient	
5	0.0018	0.9767	model
1	0.9441	-0.0005	A
1	0.3795	0.0068	B
1	0.0221	0.0325	AB
1	0.0004	-0.0683	A^2
1	0.0004	-0.0683	B^2
		0.8079	Pre-R^2
		0.9166	adj-R^2
3	0.6625		Lack of fit

A: Time,B: E/S

Figure 3 showed the highest total antioxidant activity (absorbance at 695 nm) at 105 minutes and the E/S ratio was 1.5%. According to the data, the total antioxidant activity (absorbance at 695 nm) decreased with increasing hydrolysis time and decreasing enzyme concentration. The evaluation of the total antioxidant capacity (absorbance at 695 nm) is based on the reduction of 6-valent molybdenum to 5-valent molybdenum and is a quantitative method to check the antioxidant power of water-soluble and fat-soluble (total antioxidant activity) which is formed by the formation of a green phosphomolybdenum complex in an acidic environment. Accompanied. Yang et al. (2022) investigated the effect of microwave pretreatment on the antioxidant activity and stability of milk protein enzyme products and stated that the power of 300 watts causes the highest total antioxidant activity (absorbance at 695 nm) [35]. Colleagues (2019) investigated the antioxidant activity of olive leaves with microwave and ultrasound pretreatment and reported that the highest amount of antioxidant activity is obtained with microwave pretreatment at a temperature of 86 degrees Celsius and a time of 3 minutes [37]. The difference in

power and time used in Various studies may be related to the solubility of protein materials [35]. Nguyen et al. (2017) reported that enzymatic hydrolysis with microwave pretreatment is a suitable method for producing hydrolyzed fish protein with appropriate antioxidant properties [38].

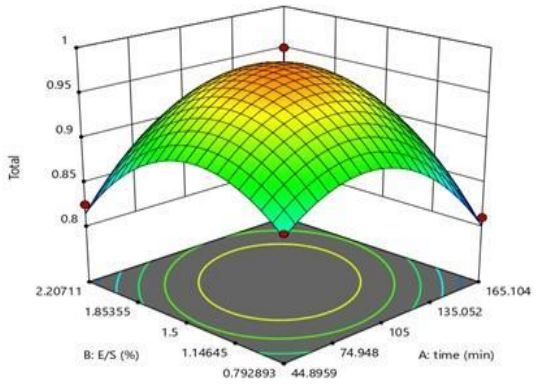

Figure 3: Effect of hydrolysis time and E/S ratio on total antioxidant activity (absorbance at 695 nm) by using microwave pretreatment.

5-3- Selection of optimal treatment and validation of the model

Optimal conditions suggested by the software, time102 Minutes and ratios5/1 PercentIt was E/Sthat with the degree of acceptability5/89% In order to achieve maximum iron chelating activity (5/90%), free radical inhibitionDPPH (5/%51) and total antioxidant (976/0) matched. To validate the model in time and ratioE/S Suggested, antioxidant tests were performed again and the results showed 86.4% iron chelating activity, 46.5% free radical inhibition.DPPH and total antioxidant activity (absorbance at 695 nm) was 0.915. These results indicate the good ability of the model to investigate the effect of independent variables on the antioxidant activity of hydrolyzed pumpkin seed protein.

4 - Conclusion

The results show that the optimal conditions for achieving the highest amount of iron chelating activity (5/95%), free radical inhibitionDPPH (5/%51) and total antioxidant (976/0), Time105 Accuracy and ratio5/1 PercentE/S is. The research results indicate that the hydrolyzed protein of pumpkin seeds using microwave pretreatment has significant antioxidant activity and has a high ability to enrich and formulate food in order to improve the health level of the society.

5- Resources

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مقاله علمی_پژوهشی

بهینه‌سازی هیدرولیز آنزیمی پروتئین دانه کدو (*Cucurbita maxima L.*) توسط پانکراتین با استفاده از پیش‌تیمار مایکروویو

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چکیده

دانه‌های گیاهان *Cucurbitaceae* (کدو، خربزه و غیره) یکی از منابع غنی پروتئین، بشمار می‌آیند. پروتئین‌ها از جمله منابع تغذیه‌ای مهم انسان هستند. هیدرولیز آنزیمی پروتئین دانه کدو با استفاده از پیش‌تیمار مایکروویو منجر به تولید هیدرولیزشده‌هایی با خواص زیست فعالی، از جمله فعالیت آنتی‌اکسیدانی می‌شود. استفاده از پیش‌تیمار مایکروویو باعث ایجاد تغییراتی در ساختمان ۳ بعدی پروتئین‌ها می‌شود، ساختار ۳ بعدی پروتئین را باز کرده و دسترسی آنزیم به پیوندهای پپتیدی را تسریع می‌کند. بنابراین، استفاده از پیش‌تیمار مایکروویو، روشی مناسب برای صرفه‌جویی در زمان و غلظت آنزیم مورد استفاده در هیدرولیز آنزیمی می‌باشد. در این پژوهش محلول محتوی کنسانتره پروتئین دانه کدو در معرض انرژی مایکروویو با توان ۴۵۰-۹۰۰ وات طی ۳۰-۹۰ ثانیه قرار گرفت و به‌عنوان محلول سوبسترا در آزمایشات هیدرولیز آنزیمی استفاده شد. هیدرولیز آنزیمی توسط آنزیم پانکراتین، با غلظت ۰/۵ تا ۲/۵ درصد نسبت به سوبسترای پروتئینی در بازه زمانی ۲۰ تا ۱۹۰ دقیقه، دما و pH اپتیمم پانکراتین، به‌منظور تولید هیدرولیز شده‌هایی با پتانسیل آنتی‌اکسیدانی انجام شد. قدرت آنتی‌اکسیدانی با استفاده از روش‌های مهار رادیکال آزاد DPPH، آنتی‌اکسیدانی کل (جذب در ۶۹۵ نانومتر) و فعالیت شلاته‌کنندگی آهن، اندازه‌گیری شد. بیشترین میزان فعالیت آنتی‌اکسیدانی با استفاده از پیش-تیمار مایکروویو در زمان ۱۰۵ دقیقه و نسبت E/S ۱/۵ درصد بود و شرایط بهینه ارائه شده توسط نرم‌افزار برای دست‌یابی بـه بیشینه فعالیت شلاته‌کنندگی آهن (۹۵/۵٪)، مهار رادیکال آزاد DPPH (۵۱٪/۵) و آنتی‌اکسیدانی کل (جذب در ۶۹۵ نانومتر) (۰/۹۷۶)، زمان ۱۰۲ دقیقـه و نسبت E/S ۱/۵ درصد بود که ۸۹/۵٪ با نتایج بدست آمده مطابقت داشت.