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Evaluation the effect of microwave pretreatment on the antioxidant properties of flaxseed protein hydrolysate

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ABSTRACT

In this study, the effect of microwave pretreatment at the power of 500 and 900 (W) on the antioxidant properties (DPPH radical scavenging activity and total antioxidant capacity) of flaxseed protein hydrolysate was investigated in the period of 30-210 minutes. In the next step, the effect of different concentrations (20-100 mg/ml) of the optimum treatment on the antioxidant properties (total antioxidant capacity, Fe reducing power, DPPH radical scavenging activity and Fe chelating activity) was investigated and was compared with the antioxidant capacity of vitamin C as a synthetic antioxidant and unhydrolyzed flaxseed protein. The results showed that microwave pretreatment at a power of 500 W significantly increased the antioxidant properties (DPPH radical scavenging activity and total antioxidant capacity) of flaxseed protein hydrolysate, but higher microwave power (900 W) led to reduction of antioxidant activity in comparison to the sample without pretreatment or the sample with microwave pretreatment with a power of 500 W. The sample with microwave pretreatment with a power of 500 W and hydrolysis time of 180 minutes was selected as the optimum treatment with the highest total antioxidant capacity and DPPH radical scavenging activity. Investigating the effect of concentration on the antioxidant properties of hydrolyzed protein showed that the highest DPPH radical scavenging activity (52.7 %), total antioxidant capacity (1.35 absorbance at 695 nm), Fe reducing power (0.859 absorbance at 700 nm) were achieved at the concentration of 60 (mg/ml) and the highest Fe chelating activity (50.83%) was obtained at the concentration of 80 (mg/ml). As a result, the flaxseed protein hydrolysate with considerable antioxidant capacity, can be used in the production of functional food products, nutritional supplements for athletes and the elderly.

1.Introduction

Free radicals play an important role in the occurrence of dangerous diseases such as cancer, gastric ulcers, and Alzheimer's disease. It is impossible to form free radicals in aerobic organs during respiration. These radicals are unstable and quickly react with other compounds in the body and cause cell damage (1). Oxidation of lipids is one of the most important challenges of the food industry because, in addition to the dangerous effects on human health, it causes the formation of dangerous compounds as well as smelly and tasteless substances that cause irreparable economic losses to producers. Slow (2). Synthetic antioxidants such as BHA, BHT, PG, and TBHQ have very acceptable antioxidant properties and have a reasonable price, but their adverse effects on human health have caused concern among researchers; as a result, in many countries, their use in the formulation of substances Food is limited or stopped (3). Therefore, in recent years, the identification and extraction of natural antioxidant compounds has become one of the topics of great interest to researchers. Among natural compounds with antioxidant capacity, bioactive peptides contain 2-20 amino acids and have a molecular weight of less than 6000 daltons, and are produced by enzymatic hydrolysis, chemical synthesis, or microbial fermentation (4). In recent years, peptides and hydrolyzed proteins with antioxidant properties have been extracted from various plant sources, including peanut (5), grape seed (6), wheat germ (7), pumpkin (8), and fenugreek (9). Among the suitable plant sources for extracting bioactive peptides, flax seed is a suitable option. Flax seeds have been used as food for centuries. Most of its fatty acid profile is alpha-linolenic acid, and it has a lot of omega-3, which reduces cardiovascular diseases, blood pressure, depression, osteoporosis, rheumatism, weight loss, diabetes, and digestive system diseases. It has 25% fiber, 19-29% protein, 8% mucilage compounds, and 3.67% ash and is used as a fortifier in the United States and European countries (10). The effectiveness of the enzymatic hydrolysis process in improving the functional properties and bioactivity of food proteins has been proven in many studies. Nevertheless, the use of pre-treatments that lead to better enzyme performance and the production of hydrolyzed protein with more antioxidant properties has a special place. Among suitable pretreatments such as microwave, ultrasound, ohmic, and heat, microwave is a suitable technology that has been used in recent years to improve the health properties of hydrolyzed proteins (11). Microwave is a fast, convenient, and cost-effective method of heating that creates its thermal effect by polarizing macromolecules; this leads to the alignment of the poles of the electromagnetic field, which may cause breakage. hydrogen bonds and better exposure of proteins to the enzyme (12). Therefore, according to the above, the aim of this research is to investigate the effect of microwave pretreatment at different powers (900 and 500 watts) on antioxidant properties (DPPH radical inhibition and total antioxidant) of hydrolyzed flax seed protein using Pancreatin enzyme was 20-210 minutes. In the next step, the effect of different concentrations (20-100 mg/ml) on the antioxidant properties (DPPH radical inhibition, total antioxidant, iron ion reduction and iron ion chelation) of optimally hydrolyzed protein was investigated.

2.Materials and methods

2-1- Materials

Ammonium molybdate, pancreatin, iron dichloride, ferric chloride, ferrozine, ascorbic acid, and DPPH from Merck and

ethanol, sodium triphosphate, sulfuric acid, soda, hydrochloric acid, potassium dihydrogen phosphate, dipotassium hydrogen phosphate from Sigma and flax seed was obtained from a shop in the center of Tehran.

2-2- protein extraction

At first, the flaxseed meal was ground with an electric powder mill, and in order to decrease, the resulting flour was mixed with hexane at a ratio of 1:4 (weight/volume) and stirred for 3 hours at room temperature. Then, using a Buchner funnel, hexane was separated, and the defatted powder was dried at 35°C and then passed through a 40-mesh sieve. The process of protein extraction from the defatted powder was that the resulting powder was mixed with distilled water at a ratio of 1:10 with pH=10 and stirred for 2 hours, then the resulting solution was centrifuged at 4500×g for 30 minutes. In the next step, the pH of the supernatant was set at 4pH (isoelectric pH). Then, to precipitate the proteins, the resulting solution was centrifuged at 4500 x g for 20 minutes, and the protein precipitate was washed twice with distilled water and centrifuged again at 4500 x g for 5 minutes. Then, the resulting protein isolate was dried with a freeze dryer and kept at 4°C until the next tests (6).

2-3- Microwave pretreatment

First, a 5% flax protein solution was prepared in phosphate buffer (0.2 M, pH=7). The resulting solution was dissolved at 4°C for 24 hours. Microwave pretreatment was applied at 700 and 500 watts for 1 minute (13).

2-4-The production of hydrolyzed flax seed protein

Flax seed protein isolate (sample without microwave application, samples treated with microwave) was mixed in a concentration of 5% in phosphate buffer and stirred for 30 minutes for complete hydration at ambient temperature. Then, the samples were placed in the incubator (temperature 40°C), and pancreatin was added at a ratio of 1.5%. The reaction was carried out in 30-210 minutes. In each step, after the necessary time has passed, in order to inactivate the enzyme, the samples were transferred into a water bath with a temperature of 90 °C, and after 10 minutes, they were placed in an ice container to reach the ambient temperature. In the next step, the samples were centrifuged (8000 x g for 20 minutes), and the resulting supernatant was stored in a dark container away from light and at a temperature of -20 °C until the tests were performed (14).

2-4- antioxidant properties

2-4-1- DPPH radical scavenging activity

In order to evaluate DPPH radical scavenging activity, hydrolyzed flax seed protein samples were prepared at a concentration of 40 mg/ml in distilled water. Then, at a ratio of 1:1, 1.5 ml of DPPH ethanol solution at a concentration of 0.15 mM was added to 1.5 ml of each sample and vortexed for 20 seconds for complete mixing. Then, the resulting mixture was kept in the dark for 30 minutes and finally centrifuged at 2500 rpm for 15 minutes. The absorbance of the separated supernatant was read at 517 nm (15). DPPH radical scavenging activity was calculated using formula number 1:

$$I\ (\%) = [\frac{Ablank - Asample}{Ablank}] \times 100 \quad (1)$$

2-4-2- Total antioxidant capacity

In order to measure the antioxidant capacity of all the samples, 0.1 ml of the sample dissolved in distilled water (concentration 40 mg/ml) was mixed with 1 ml of the reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). and placed in a water bath with a temperature of 90°C for 90 minutes. Finally, after cooling the samples and reaching the ambient temperature, the absorbance of the samples was read at 695 nm. Higher absorption indicates higher total antioxidant capacity (16).

2-5-Investigating the effect of concentration on the antioxidant properties of optimal treatment of hydrolyzed flax seed protein

To evaluate the effect of the concentration (20-100 mg/ml) on DPPH radical scavenging activity and the total antioxidant capacity of the optimal treatment, the methods of Kaveh et al. (2024) and Prieto et al. (1999) mentioned in the previous section were used respectively (15) and (16).

2-5-1-Fe^{2+}chelating activity

In order to measure the ability of the samples to chelate iron ions, first, 1 ml of hydrolyzed protein dissolved in distilled water at a concentration of (20-100 mg/ml) with 0.05 ml of iron dichloride solution (mM2) and 1.85 ml of distilled water was mixed. Then 0.1 ml of ferrozine solution (5 mM) was added and the mixture was stirred. In the last step, it was kept at room temperature for 10 minutes and then its absorbance was read at 562 nm (5). The chelating activity of the samples was calculated using equation (2):

(2)

Chelating effect (%) = [(A control – A sample/A control)] × 100

A blank (control sample absorption without active compound) and A sample (hydrolyzed sample absorption).

2-5-2- Fe reducing power

To evaluate the Fe reducing power of the resulting hydrolyzed protein, 0.5 ml of the sample dissolved in distilled water at a concentration of 20-100 (mg/ml) with 0.5 ml of 0.2 M phosphate buffer (pH = 6.6) and 0.5 ml of potassium Ferricyanide (W/V) 1% was mixed and kept at 50°C for 20 minutes. Then, in the next step, 0.5 ml of 10% trichloroacetic acid solution was added to the mixture and centrifuged at 2500 rpm for 10 minutes. Finally, 1 ml of the resulting supernatant was mixed with 1 ml of distilled water and 0.2 ml of 0.1% ferric chloride (W/V) and kept at room temperature for 10 minutes. The absorbance of the samples was read at 700 nm. The increase in absorption of the mixture indicates an increase in the reductive power (17).

2-6- Statistical analysis

The statistical analysis was performed using the SPSS 16.0 software. All experiments were determined in triplicates and the data were expressed as means ± SD. The Significance differences between means were investigated using Duncan's

test at 5% significance level using analysis of variance (ANOVA).

3.Results and discussion

3-1- DPPH radical scavenging activity

DPPH is a fat-soluble free radical that has the highest absorption at the wavelength of 517 nm, and by receiving hydrogen from an antioxidant combination, it becomes stable and its absorption decreases (18). The results of the hydrolysis time investigation showed (Figure 1) that in the samples without microwave pretreatment and the sample with microwave pretreatment with a power of 500 watts, increasing the hydrolysis time up to 180 minutes increased the DPPH radical inhibition activity, respectively. The amount was 43.75% and 51.43%, and with a further increase in hydrolysis time, there was no significant effect ($p<0.05$). On the other hand, in the treatment by applying microwave pretreatment with 900 W power, increasing the hydrolysis time up to 90 minutes caused a significant increase in DPPH radical inhibition activity; the hydrolysis for 120 minutes was not significantly different from the sample hydrolyzed in 90 minutes, and the increase was more Hydrolysis time caused a significant decrease in the antioxidant activity of the hydrolyzed protein. The progress of the hydrolysis process over time leads to the production of proton-donating peptides that can react with the DPPH free radical and produce stable compounds and end the radical chain reactions. The adverse effect of increasing the enzymatic hydrolysis time may be due to the greater effect of the enzyme, which leads to the breaking and decomposition of a number of antioxidant peptides that are produced in the initial stages of hydrolysis, which this process causes the reduction of DPPH free radical inhibition power (19). In accordance with these results, Batista et al. (2010) reported that increasing the degree of hydrolysis and time increases the DPPH radical scavenging activity of hydrolyzed crab[1] protein. They attributed these results to the increase in the amount of hydrogen-donating peptides that have the ability to react with free radicals (20). Similar to these results, Kaveh et al. (2023), Jamdar et al. (2010) and You et al. (2009) reported the positive effect of hydrolysis time on DPPH radical inhibition ability with the hydrolysis of proteins from the intestines and viscera of Hoover Muscat and peanuts, respectively. did (3 and 5). Also, Kaveh et al. (2018) with fenugreek seed protein hydrolysis stated that increasing the hydrolysis time up to 160 minutes increased the activity of the hydrolyzed protein in inhibiting DPPH radical by about 48% and further increase

1-black scabbardfish

in time had no significant effect (18). On the other hand, applying microwave pretreatment with 500W power increased the DPPH radical scavenging activity compared to the sample without microwave pretreatment, but only the hydrolyzed treatment in 30 minutes had more antioxidant activity than the sample with 500W pretreatment. Hydrolysis times up to 120 minutes, no significant difference was observed between the samples with 500 and 900 W pretreatment, and at longer hydrolysis times up to 210 minutes, samples with 900 W pretreatment had lower DPPH radical scavenging activity than They had 500 watts to pretreated samples. Improving the ability of hydrolyzed protein to inhibit DPPH radical by using microwave pretreatment with 500 watts power can be because microwaves cause water evaporation in cells and increase the pressure in the internal environment, which leads to Decomposition of intracellular compounds, disintegration of the membrane and as a result its decomposition, which facilitates the process of protein enzymolysis (21), it should be noted that microwaves can have different biological and chemical effects depending on the strength and time of microwave pretreatment. The negative effect of microwave pretreatment at a power of 900 watts on the antioxidant activity of the hydrolyzed products can be due to the adverse changes in the second structure of the protein and the reduction of the alpha helix[2] and beta sheet[3] structure (22). In general, it can be said that changes in the size, amount, and composition of free amino acids and peptides, especially peptides with low molecular weight, have a significant effect on the antioxidant property of hydrolyzed protein produced (23). In accordance with these findings, Uluko et al. (2015) investigated the effect of microwave pretreatment on DPPH radical scavenging activity of hydrolyzed protein of milk concentrate and reported an increase in the antioxidant activity of the resulting hydrolysates (24). While Noman et al. (2020) reported the negative effect of microwave pretreatment on the antioxidant activity of hydrolyzed protein of Chinese caviar[4] (25).

2-α -helix
3-β -sheet
4- Chinese sturgeon (*Acipenser sinensis*)

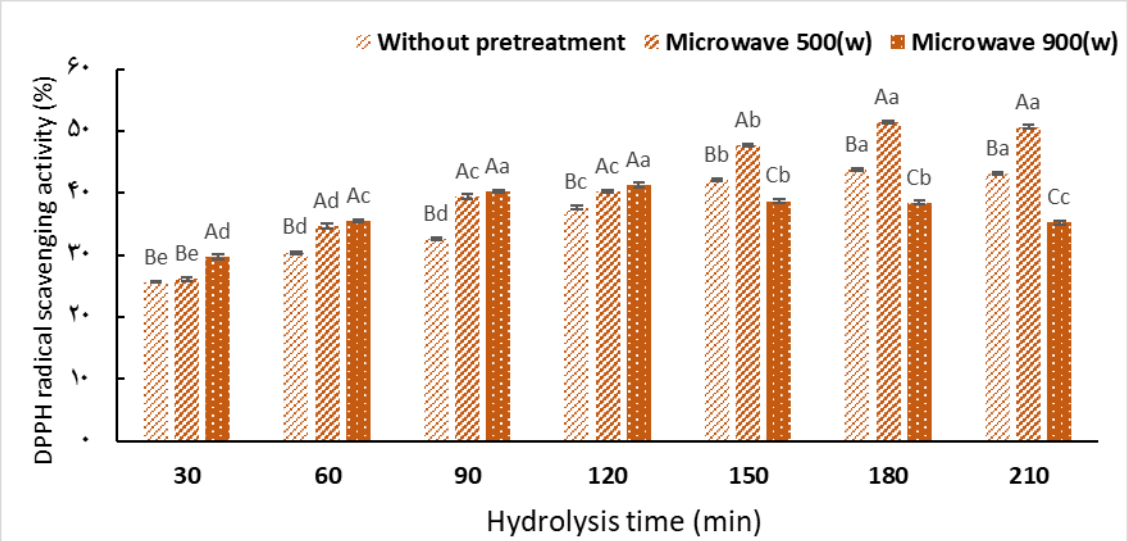

Figure 1- DPPH radical scavenging activity of flax seed protein hydrolysate

2-2- Total antioxidant capacity

Total antioxidant capacity evaluation test is a quantitative method to check the water-soluble and fat-soluble antioxidant power (total antioxidant power) of antioxidant compounds. This method is based on the reduction of 6-valent molybdenum ion to 5-valent molybdenum ion, which is accompanied by the formation of a green phosphomolybdenum complex in an acidic medium (18). As shown in Figure 2, in the samples without microwave pretreatment, increasing the hydrolysis time up to 180 minutes increased the total antioxidant capacity to 0.963 (absorbance at 695 nm) and further increasing the hydrolysis time in the control sample. It had no significant effect on the antioxidant capacity of all samples ($p<0.05$). On the other hand, in the sample with 500 W microwave pretreatment, increasing the hydrolysis time up to 150 minutes increased the total antioxidant capacity by 1.245 (absorbance at 695 nm), increasing the time up to 180 minutes did not have a significant effect, and increasing the time to 210 minutes caused a significant decrease in the total antioxidant capacity to 1.041 ($p<0.05$). In the samples with microwave pretreatment with 900 W power, increasing the hydrolysis time to 150 minutes, the total antioxidant capacity increased, but a further increase in the hydrolysis time had a negative effect. In general, during the hydrolysis time of more than 180 minutes, the antioxidant capacity of the whole hydrolyzed protein was, respectively, the sample with 500W microwave pretreatment > the sample without pretreatment > the sample with 900W microwave pretreatment. In hydrolysis times of 120 and 60, 30 minutes, the antioxidant capacity of the sample with 500 W microwave pre-treatment was the highest, and there was no significant difference between the sample with 900 W microwave pre-treatment and the sample without pre-treatment ($p<0.05$). The positive effect of microwave pretreatment on the antioxidant activity of the hydrolyzed protein can be due to its ability to break down molecular aggregates of flaxseed protein, which has increased the sensitivity of protein-peptide bands to hydrolysis by protease enzyme (pancreatin). It is worth noting that the result of this process has a lot to do with

the power and length of exposure to microwaves (22), as it was observed that the power of 900 watts of microwaves compared to the power of 500 watts had a negative effect on antioxidant activity. The negative effect of microwave pretreatment at 900 W power can be due to the adverse changes in the protein structure that prevented the release of antioxidant peptides (24). On the other hand, the positive effect of the increase in hydrolysis time may be due to the release of peptides with electron-donating ability, which leads to the transformation of free radicals into more stable compounds, which leads to an increase in the antioxidant capacity of the entire hydrolyzed protein (26). Similar to these results, Mazloomi et al. (2018) with the hydrolysis of orange core protein with alcalase stated that increasing the hydrolysis time increased the total antioxidant capacity of the hydrolyzed proteins and the highest total antioxidant capacity after hydrolysis for 4.8 The hour was obtained (27). In this regard, Kaveh et al. (2018) reported similar results in the fenugreek protein hydrolysis process. They stated that increasing the hydrolysis time up to 160 minutes by pancreatin enzyme led to an increase in the antioxidant activity of the total hydrolyzed protein produced, but further increase in hydrolysis did not have a significant effect on the antioxidant capacity of the total hydrolyzates produced (17). Also, similar to these findings, Yang et al. (2013) reported the positive effect of microwave pretreatment on the antioxidant activity of egg white hydrolyzed protein (28). Zhang et al. (2019) also reported the positive effect of microwave pretreatment on the antioxidant activity of sweet potato hydrolyzed protein (29).

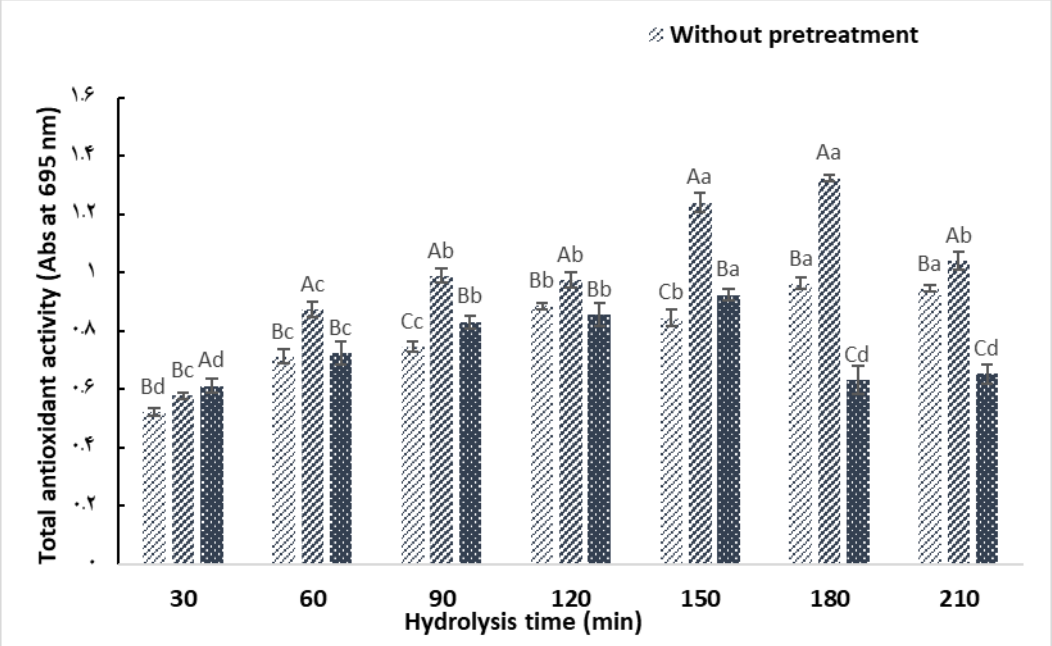

Figure 2- Total antioxidant activity of flax seed protein hydrolysate

3-3- Optimization

After evaluating the antioxidant activity (DPPH radical scavenging activity and total antioxidant capacity) of the produced hydrolyzed protein samples, the treatment with microwave pretreatment of 500 watts and hydrolysis time of 180 minutes had the

highest antioxidant activity. Therefore, it was selected in the next step to investigate the effect of concentration on its antioxidant activity (DPPH radical scavenging activity, total antioxidant capacity, Fe-reducing power, and Fe^{2+} chelating activity).

3-4- Evaluation of the effect of concentration on the antioxidant activity of the optimal treatment

3-4-1- DPPH radical scavenging activity

Figure 3 shows the effect of concentration on DPPH free radical scavenging activity of optimal treatment of hydrolyzed flaxseed protein and its comparison with unhydrolyzed protein and vitamin C at a concentration of 100 (mg/mL). The results showed that the antioxidant activity of the resulting hydrolyzed protein was dependent on the concentration and increasing the concentration from 20 (mg/mL) to 60 (mg/mL) significantly increased the DPPH radical scavenging activity from 35.68% to 52.70 %. Increasing the concentration up to 80 (mg/mL) did not have a significant effect, but the concentration of 100 (mg/mL) decreased the ability of the resulting hydrolyzate to inhibit the DPPH radical ($p<0.05$). Hydrolyzed treatment in all concentrations had more antioxidant activity than unhydrolyzed flax seed protein. which shows the success of the hydrolysis process in releasing antioxidant peptides ($p<0.05$). Despite the appropriate antioxidant activity of hydrolyzed flaxseed protein, its ability to inhibit DPPH free radical was significantly lower than the antioxidant activity of vitamin C in all concentrations ($p<0.05$). The remarkable activity of hydrolyzed flax seed protein in inhibiting DPPH free radical can be due to the presence of aromatic amino acids that have hydroxyl groups with proton-donating properties, on the other hand, the negative effect of excessive concentration increases on antioxidant activity. Oxidation may be related to saturation of active sites (30). Similar to these results, Zhao et al. (2012) with enzymatic hydrolysis of rice protein with protease enzymes trypsin, alcalase, protamax, flavorzyme and nutrease stated that the DPPH radical scavenging activity of the resulting hydrolysates was dependent on the concentration and the hydrolysates Production with Protamax enzyme had the highest antioxidant properties (31). In accordance with the results of Umayaparvathi et al. (2014), Chi et al. (2015), and Batista et al. (2010), respectively, the DPPH free radical scavenging activity of the hydrolyzed proteins of shellfish, a type of fish, and black crab depend on the concentration. reported (32, 33 and 20).

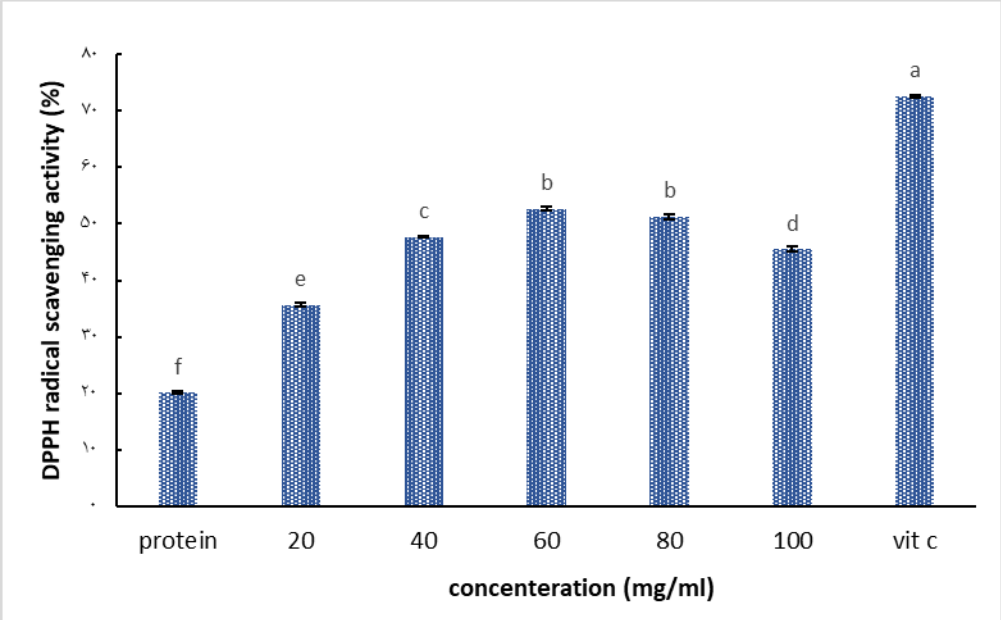

Figure 3- The effect of concentration on DPPH radical scavenging activity of flax seed protein hydrolysate

2-4-3-Total antioxidant capacity

According to Figure 4, the antioxidant capacity of the total hydrolyzed flax seed protein was dependent on the concentration, and increasing the hydrolyzed concentration from 20 (mg/ml) to 60 (mg/ml) significantly increased the antioxidant capacity. from 0.752 to 1.35 (absorbance at 695 nm) ($p<0.05$), but increasing the concentration up to 100 (mg/ml) significantly decreased the antioxidant capacity of the produced hydrolyzed protein. The lowest and highest total antioxidant capacity of hydrolyzed protein with concentration 20 (mg/ml) and 60 (mg/ml) was 0.752 and 1.35 (absorbance at 695 nm). On the other hand, in all investigated concentrations, the antioxidant capacity of the total hydrolyzed protein was significantly higher than the non-hydrolyzed flaxseed protein, which indicates the appropriate ability of pancreatin enzyme to release antioxidant peptides. On the other hand, in all the investigated concentrations, the total antioxidant capacity was lower than that of vitamin C in the concentration of 100 (mg/ml) ($p<0.05$). In general, the hydrolyzed flaxseed protein had a significant total antioxidant capacity, which indicates the success of the hydrolysis process with pancreatin enzyme in releasing peptides with electron-donating ability, which are able to act as a natural antioxidant compound. inhibit free radicals (26). In accordance with these results, Bougatef et al. (2009) expressed the positive effect of concentration on the antioxidant capacity of the total hydrolyzed protein obtained from a type of shark[5] and reported that despite the significant antioxidant capacity of the total hydrolyzed protein, Its antioxidant property was lower than the synthetic antioxidant BHA (34). Also, Kaveh et al. (2018) with the hydrolysis of fenugreek seed protein stated that the antioxidant capacity of the total hydrolyzed fenugreek protein was dependent on the concentration and increasing its

5 -smooth hound

concentration from (mg/ml) 10 to (mg/ml) 40 increased the mean It was found to have total antioxidant capacity, but increasing the concentration did not have a significant effect; They also reported that in all concentrations, the total antioxidant capacity of fenugreek hydrolyzed protein was lower than the total antioxidant capacity of vitamin C with a concentration (mg/ml) of 50 (19).

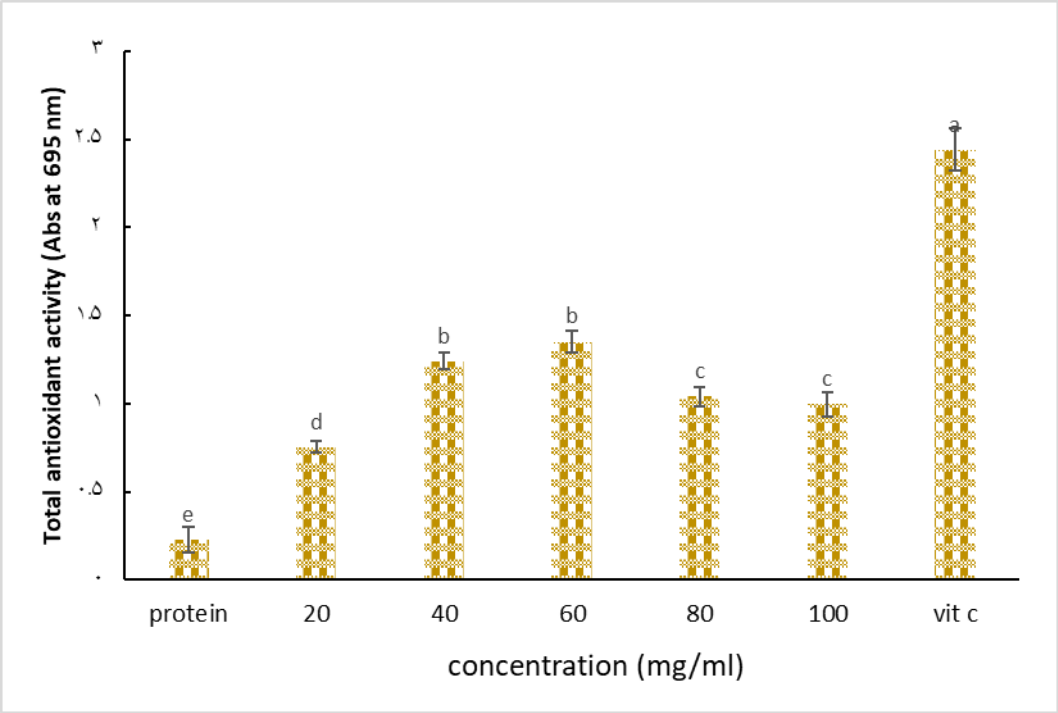

Figure 4- The effect of concentration on total antioxidant activity of flax seed protein hydrolysate

3-4-3- Fe chelating activity

In oxidation reactions, metal ions such as Fe^{2+} play an important role as a catalyst, which leads to the production of dangerous hydroxyl radicals from hydrogen superoxide. These radical compounds quickly react with nearby biological molecules and cause damage to body cells and tissues (35). Therefore, the inhibition and stabilization of metal ions plays a very important role in preventing oxidation. As shown in Figure 5, increasing the concentration increased the iron ion chelating activity of hydrolyzed flax seed protein; Increasing the concentration from 20 (mg/ml) to 80 (mg/ml) significantly increased the chelating activity of hydrolyzed protein from 34.68% to 54.83% ($p<0.05$) and increasing the concentration further did not have a significant effect. Comparing the antioxidant activity of hydrolyzed products with non-hydrolyzed protein showed that hydrolysis significantly increased the iron ion chelating activity of flaxseed protein, but in all concentrations, the chelating ability was lower than that of vitamin He had In general, studies have shown that various factors depend on the iron ion chelating ability of hydrolyzed proteins, such as: the type of primary protein, the amino acid composition, the type of protease used, and the degree of hydrolysis (36). In agreement with these findings, Xie et al. (2008) and Klompong (2007) reported the concentration-dependent activity of Fe^{2+} chelating protein hydrolyzed alfalfa and yellow line fish[6], respectively (37 and 38).

6 -yellow stripetrevally

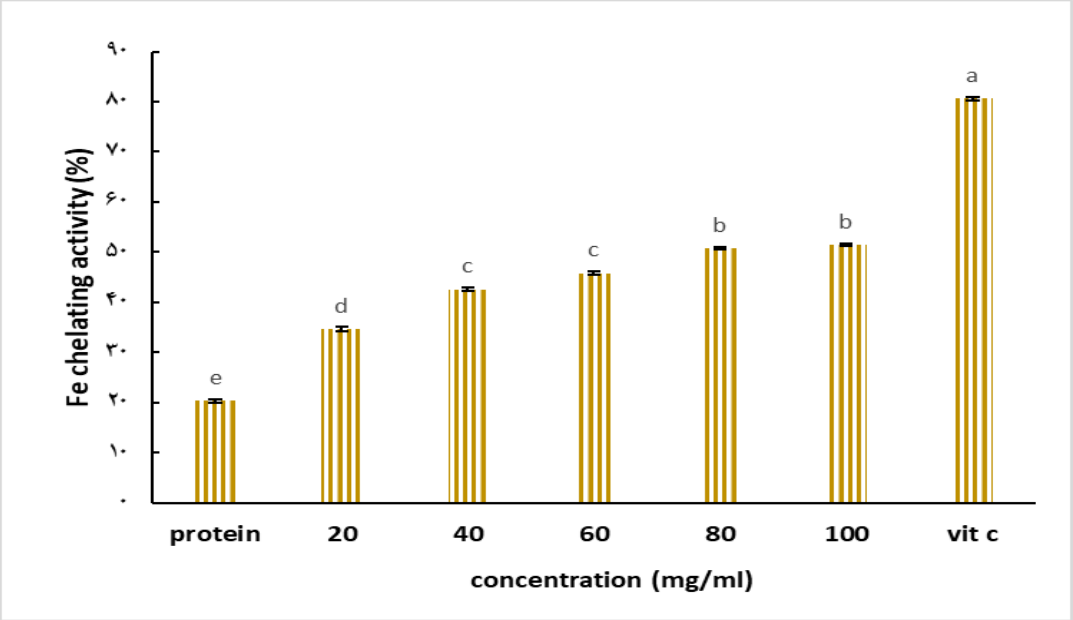

Figure 5- The effect of concentration on Fe chelating activity of flax seed protein hydrolysate.

3-4-4- Fe Reducing power

The reducing power test examines the antioxidant ability of a compound based on its potential to convert Fe^{3+} to Fe^{2+} by donating electrons. According to Figure 6, the regenerative power of hydrolyzed flaxseed proteins is significantly higher than the power of non-hydrolyzed protein, which shows the success and positive effect of the hydrolysis process with pancreatin enzyme in releasing regenerative peptides and amino acids with antioxidant capabilities such as tryptophan. and Lysine Dunst (5). On the other hand, increasing the concentration from 20 (mg/ml) to 60 (mg/ml), caused an increase in the reducing power of the produced hydrolyzates from 0.457 to 0.859 (absorbance at 700 nm); Increasing the concentration to 80 (mg/ml) did not have a significant effect, and further increasing the concentration to 100 (mg/ml), caused a significant decrease in the regenerative power ($p<0.05$). Comparison of the reducing power of the samples with the antioxidant power of vitamin C showed that the hydrolyzed protein of flax seed had less reducing power in all concentrations. In general, the reason for the reduction of hydrolyzed flaxseed proteins can be seen as the

release of amino acids with reductive capabilities such as tryptophan, lysine and methionine (5). These results were in agreement with the findings of Xie et al. (2008), Cumby et al. (2008), Zhao et al. (2012) and Umayaparvathi et al. Canola, rice and oysters have been investigated (37, 39, 31 and 32).

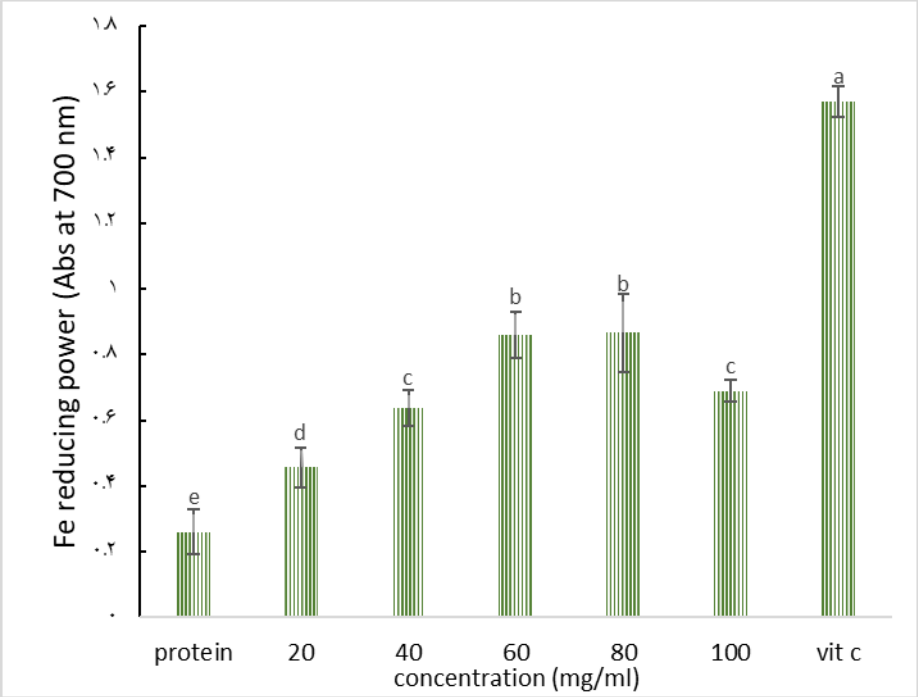

Figure 5- The effect of concentration on Fe reducing power of flax seed protein hydrolysate

3-5-Conclusion

The results of this research showed that microwave pretreatment with a power of 500 watts had a positive and significant effect on DPPH radical scavenging activity and antioxidant capacity of the total hydrolyzed protein of flaxseed. In general, the antioxidant property of the hydrolyzed protein resulting from enzymatic hydrolysis depends on various factors such as: the type and intensity of pretreatment applied, the conditions of the hydrolysis process (enzyme type, temperature, hydrolysis time and enzyme percentage), the type of primary protein and it depends on the amino acid composition of the produced peptides. In this research, antioxidant activity (DPPH radical scavenging activity, Fe^{2+} chelating activity, Fe reducing power and total antioxidant capacity) of hydrolyzed flax seed protein was concentration dependent. So that with increasing concentration from 20 (mg/mL) to60 (mg/mL) DPPH radical scavenging activity, total antioxidant capacity and Fe reducing power and with increasing concentration up to 80 (mg/mL) Fe^{2+} chelating activity increased significantly. In general, according to the results of this research, it can be stated that microwave pretreatment with 500 watts power is a very suitable solution for increasing the antioxidant property of hydrolyzed flaxseed protein. Due to the appropriate antioxidant capacity, the resulting hydrolyzed proteins have the potential to be used in food formulations to

produce useful products and compete with synthetic antioxidants.

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بررسی تاثیر پیش تیمار مایکروویو بر ویژگی‌های آنتی اکسیدانی پروتئین هیدرولیز شده بذر کتان

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چکیده

در این پژوهش تاثیر پیش‌تیمار مایکروویو در توان‌های ۵۰۰ و ۹۰۰ وات بر ویژگی‌های آنتی‌اکسیدانی (مهار رادیکال DPPH و آنتی اکسیدانی کل) پروتئین هیدرولیز شده بذر کتان در بازه زمانی ۲۱۰۰-۳۰ دقیقه بررسی گردید. در مرحله بعد تاثیر غلظت‌های متفاوت (۲۰-۱۰۰ mg/ml) تیمار بهینه بر ویژگی‌های آنتی‌اکسیدانی (ظرفیت آنتی اکسیدانی کل، فعالیت احیاء کنندگی یون آهن، مهار رادیکال DPPH و شلاته کنندگی یون آهن) بررسی و با خاصیت آنتی اکسیدانی ویتامین ث به‌عنوان یک آنتی اکسیدان سنتزی و پروتئین هیدرولیز نشده بذر کتان مقایسه شد. نتایج نشان داد که پیش‌تیمار مایکروویو در توان ۵۰۰ وات به‌طور معنی‌داری باعث افزایش خواص آنتی اکسیدانی (مهار رادیکال DPPH و آنتی اکسیدانی کل) پروتئین هیدرولیز شده بذر کتان شد اما توان بیشتر مایکروویو (۹۰۰ وات) باعث کاهش خاصیت آنتی اکسیدانی پروتئین هیدرولیز شده تولیدی نسبت به نمونه بدون اعمال پیش تیمار و یا نمونه با اعمال پیش تیمار مایکروویو با توان ۵۰۰ وات شد. نمونه با اعمال پیش تیمار مایکروویو با توان ۵۰۰ وات و زمان هیدرولیز ۱۸۰ دقیقه به‌نوان تیمار بهینه با بیشترین ظرفیت آنتی اکسیدانی کل و فعالیت مهار رادیکال آزاد DPPH به‌عنوان تیمار بهینه انتخاب شد. بررسی تاثیر غلظت بر خواص آنتی اکسیدانی پروتئین هیدرولیز شده نشان داد که بیشترین مهار رادیکال آزاد DPPH (۵۲/۷ درصد)، ظرفیت آنتی اکسیدانی کل (۱/۳۵ جذب در ۶۹۵ نانومتر)، فعالیت احیاء کنندگی یون آهن (۰/۸۵۹ جذب در ۷۰۰ نانومتر) در غلظت (۶۰ mg/ml) و بیشترین فعالیت شلاته‌کنندگی یون آهن (۵۰/۸۳ درصد) در غلظت (۸۰ mg/ml) حاصل شد. در نتیجه پروتئین هیدرولیز شده بذر کتان با دارا بودن قابلیت آنتی اکسیدانی مناسب قابلیت کاربرد در تولید محصولات غذایی فراسودمند، مکمل‌های غذایی ورزشکاران و سالمندان را دارد.