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Production of bioactive peptides from flaxseed meal: the effect of protease type and concentration, hydrolysis time, and microwave pretreatment

Faezeh Farzanfar[1], Alireza Sadeghi Mahoonak[2*], Mohammad Ghorbani[3], Seyed Hossein Hosseini Qaboos[4], Shima Kaveh[5]

1- Master's student in food chemistry, Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources

2-Professor, Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources

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

4-Assistant Professor, Department of Food Science and Technology, Islamic Azad University, Azadshahr branch

5-Ph.D. of Food Chemistry, Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources

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*Corresponding Author E-Sadeghiaz@yahoo.com

ABSTRACT

Every year, during the processing of agricultural products and food production, many waste materials and by-products are produced. Most of these by-products have bioactive properties, such as antioxidants, which can be extracted to produce products with bioactive properties. In the present study, flaxseed meal, which is obtained as a byproduct of the oil extraction process from flaxseed, was hydrolyzed using two enzymes, trypsin, and pancreatin, with time (15-210 minutes) and enzyme-to-substrate ratio (1-3%) variables. The effect of microwave pretreatment on the antioxidant properties of hydrolyzed protein was investigated by the response surface methodology. Treatment of hydrolyzed protein with trypsin and microwave pretreatment in optimal hydrolysis conditions of 84.02 minutes and enzyme to substrate ratio1.77%, as the optimal treatment with the most antioxidant properties (total antioxidant activity 0.745 (absorbance at 695 nm), DPPH radical scavenging activity of 71.35% and 76.12% Fe chelating activity) were selected. As a result, flaxseed protein hydrolysate, a bioactive product with antioxidant properties, can be used as a natural antioxidant in producing health products and nutritional supplements for athletes.

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1- Introduction

In recent years, the potential toxicity and adverse effects of synthetic antioxidants used in the production of food products have attracted the attention of researchers. On the other hand, due to the increase in awareness and as a result the demand of consumers for food without synthetic preservatives, scientists have increased their attention to the potential of plant products to be used as antioxidants to protect against ROS and various diseases caused by free radicals [1].

Every year, many waste materials and by-products are produced during the processing of agricultural products and food production. Most of these secondary wastes are consumed as animal feed or thrown away. Most of these by-products have bioactive compounds, such as natural antioxidants, phenolic compounds, etc., which can be extracted and produced into products with health-giving properties [2-3].

Flaxseed is an important source of protein due to its high amounts of sulfur amino acids such as methionine and cysteine. Also, this seed has branched amino acids such as isoleucine and leucine and essential amino acids such as lysine, threonine, tyrosine, asparagine, glutamine, and arginine, which increase the amide content of flaxseed. It has been reported that flax seeds contain cyclic proteins and peptides that have anti-hypertensive, anti-diabetic, antioxidant, and anti-inflammatory properties [4]. Antioxidant compounds found in flax seeds (especially antioxidant peptides) are a group of compounds that disable or delay oxidation processes caused by reactive oxygen species or atmospheric oxygen [5-6]. These compounds perform various activities, including the production of endogenous antioxidants by stimulating gene expression, blocking the production of metal radicals, repairing damaged molecules, and preventing various diseases [7].

Bioactive peptides are specific protein parts whose molecular mass is less than 6000 daltons and have 2-20 amino acids. These peptides are inactive in the main protein structure and after being released according to their amino acid type and sequence, they have a positive effect on the function and condition of the body and as a result the health of the person. Bioactive peptides obtained from plant proteins have recently become popular due to their properties and numerous applications [7-8]. The peptides in the side compounds of flax seeds are released as bioactive compounds with optimal properties with the help of chemical and enzymatic reactions [9]. Among the suitable methods for the production of bioactive peptides, enzymatic hydrolysis, a process that is usually performed under controlled conditions (pH, temperature, substrate concentration and enzyme activity), is the most common method for the production of bioactive peptides [10]. The process of proteolysis breaks sensitive peptide bonds and releases bioactive peptides [11].

Pretreatment with microwaves is a new technique used to accelerate protein hydrolysis and improve their functional properties due to the effect on proteins in a short time. The biological changes that can occur on proteins after microwave pretreatment are a function of field strength. Microwave radiation can be responsible for these structural changes of proteins, which lead to changes in the second and third structures of proteins after absorption of microwave energy [12]. In this regard, Ketnawa and Liceaga (2017) in a study they conducted on salmon protein, reported that the use of microwave pretreatment for 5 minutes at 90°C followed by enzymatic hydrolysis with alkalase improved the antioxidant activity of the protein. It was hydrolyzed [13]. Also, in Wang et al.'s (2022) research, the modulating effects of microwave pretreatment at different powers and times on the structure of tartaric buckwheat protein (TBP) were investigated. Compared to the germinated TBP without pretreatment, after microwave pretreatment, the content of free sulfhydryl groups in the germinated TBP increased, and secondary structure changes showed a significant decrease

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in α helix and an increase in random helix content with increasing UV intensity [14].

Therefore, this research aims to investigate the effect of type (pancreatin and trypsin) and concentration (1-3%) of protease enzyme, hydrolysis time (15-210 minutes) and microwave pretreatment on antioxidant properties (total antioxidant activity, DPPH free radical inhibition activity and Iron ion chelation) was the hydrolyzed protein of flaxseed meal.

2- Materials and methods

Chemicals: trypsin, pancreatin, trichloroacetic acid, brilliant blue (G250), DPPH, potassium ferricyanide, iron chloride, iron sulfate, hydrogen peroxide, iron dichloride, sodium chloride, iron, hydrochloric acid, potassium dihydrogen phosphate, and phosphoric acid from Merck and Ketan Seed Company. A reputable store was purchased in Gorgan.

2-1-preparation of flaxseed meal protein concentrate

First, the flour obtained from flax seeds was powdered with the help of an electric mill; Then, to further degrease the semolina powder, the resulting powder was mixed with hexane at a ratio of 1:4 (weight/volume) for 4 hours using a shaker at 200 rpm. After separating the hexane, the flour powder was placed in a 40°C oven for 3 hours. To extract the protein of flax seed meal, the resulting powder was mixed with 0.3 M sodium chloride solution at a ratio of 1:10, and by adding 1 normal soda, we brought the pH to 9.2 and put it on a magnetic stirrer for 1 hour. , to mix; The produced solution was centrifuged at 4°C and at 8000 rpm for 10 minutes. In the next step, the pH of the supernatant was adjusted to 4.5 (isoelectric pH) and centrifuged at 8000 rpm for 10 minutes. After washing the protein precipitate with distilled water, the produced flaxseed meal protein concentrate was dried using a freeze dryer [15].

2-2- applying microwave pretreatment

First, a 5% flax seed protein solution was prepared in phosphate buffer (0.2 M, pH = 7). To apply microwave pretreatment, 300, 600, 750 and 900 watts were used for 30, 60 and 90 seconds. After performing the total antioxidant test on the treatments, the treatment with a power of 300 watts and a duration of 90 seconds was selected as the optimal treatment containing the highest amount of total antioxidants [12].

2-3-preparation of hydrolyzed protein of flaxseed meal

Flax seed meal protein was hydrolyzed at the points determined by Design Expert 11 software (Table 1) with two variables of time and enzyme to substrate ratio, by pancreatin and trypsin enzymes in two cases without pretreatment and with microwave pretreatment. To perform enzymatic hydrolysis, protein concentrate was dissolved in 0.2 M phosphate buffer at a concentration of 5% (weight/volume). After applying the pretreatment, and by adding the enzyme in the determined amounts (Table 1) to the protein solution, the samples were placed in the incubator. After passing the desired time intervals (15-210 minutes), the Erlenmeyer flasks were placed in a 90°C water bath for 10 minutes to inactivate the enzyme. Then the samples were centrifuged at 8000 rpm for 10 minutes. After separating the supernatant, the hydrolyzed protein was dried using a freeze dryer [16].

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Table 1- Different treatment conditions used for protein hydrolysis of flaxseed meal (pretreated and non- pretreated using trypsin and Pancreatin)

Hydrolysis points	time (minutes)	Enzyme concentration (%)
1	15	2
2	43.56	2.7
3	43.56	1.29
4	112.5	2
5	112.5	3
6	112.5	2
7	112.5	2
8	112.5	1
9	112.5	2
10	112.5	2
11	181.45	1.29
12	181.45	2.70
13	210	2

After examining the antioxidant responses of treatments in Table 1, for hydrolysis with pancreatin without pretreatment (treatment 1), hydrolysis with pancreatin with microwave pretreatment (treatment 2), hydrolysis with trypsin without pretreatment (treatment 3) and hydrolysis with trypsin with microwave pretreatment (treatment 4)), an optimal point was selected by Design Expert 11 software. Antioxidant tests of DPPH free radical inhibition, iron ion chelating activity and total antioxidant activity were investigated in these four treatments.

2-3-1-DPPH free radical inhibition activity

First, the hydrolyzed protein was dissolved in distilled water with a concentration of 20 mg/ml. Then, 0.5 ml of the prepared sample was mixed with 0.5 ml of DPPH ethanol solution (0.15 mM) and vortexed for 20 seconds. The obtained solution was kept in a dark place for 30 minutes and then it was centrifuged for 10 minutes at 5000 rpm and the absorbance of the supernatant solution was read at 517 nm wavelength. DPPH free radical inhibition percentage was calculated using equation 1 [18]:

DPPH free radical scavenging activity (%) = (Acontrol-Asample)/Acontrol×100
(equation 1)

Asample is sample absorption and Acontrol is control absorption

2-3-2-chelating activity of iron ion

1 ml of hydrolyzed flax meal protein dissolved in distilled water (concentration 20 mg/ml) was mixed with 1.85 ml of distilled water and 0.05 ml of iron dichloride (Mm2) solution. Next, 0.1 ml of ferrozine solution (5 Mm) was added to the resulting solution. The absorbance of the solution was read after keeping at room

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temperature (24°C) for 10 minutes at a wavelength of 562 nm. The chelating activity of the samples was calculated using equation 2 [19].

Fe chelating activity (%) = (Acontrol-Asample)/Acontrol×100 (equation 2)

Acontrol is the absorption of the control and Asample is the absorption of the sample.

2-3-3-Total antioxidant activity

To obtain the total antioxidant capacity, first, 0.1 ml of hydrolyzed protein dissolved in distilled water (concentration 20 mg/ml) was mixed with 1 ml of reagent (0.6 M sulfuric acid, 4 M ammonium molybdate, and 28 M sodium phosphate) and The water bath was placed at 90°C for 90 minutes. Then, the absorbance of the samples was read at 695 nm [20].

2-4-Choosing the optimal treatment of hydrolyzed protein to have higher antioxidant activity

The response of antioxidant tests (iron ion chelation, DPPH radical inhibition and total antioxidant activity) for all 13 hydrolysis points determined by Design Expert 11 software were investigated in both hydrolysis modes with and without microwave pretreatment; Optimum points were obtained by Design Expert 11 software for all four treatments and evaluated and optimized by SPSS software. To compare the optimal treatments, Duncan's multi-range test was used at the 95% confidence level. All tests were performed in three repetitions.

3. Results and Discussion

3-1-DPPH free radical inhibition activity

According to Figure 1, in treatment 1 with increasing time, the highest percentage of DPPH free radical inhibition was observed at low ratios of enzyme to substrate. Probably, increasing the ratio of enzyme to substrate causes excessive hydrolysis of flaxseed meal protein by the enzyme, which causes the release of hydrophilic amino acids and makes the interaction of the released active amino acids with the DPPH radical difficult [21]. The obtained results were similar to the findings of Maqsoodlou et al. (2016) in pollen protein hydrolysis; They reported a decrease in the DPPH radical scavenging activity of hydrolyzed protein by increasing the ratio of enzyme to substrate [22]. In treatment 2, by increasing the time and increasing the ratio of enzyme to substrate to 2%, an increase in the percentage of DPPH free radical inhibition was observed, and then by increasing the time and increasing the ratio of enzyme to substrate, the percentage of DPPH free radical inhibition decreased. Xue et al. (2008) also reported that an increase in DPPH free radical scavenging activity was observed with an increase in the enzyme-to-substrate ratio up to 2% and then decreased. They stated that the cause of this could be excessive protein hydrolysis and the complete release of hydrophilic amino acids and disruption of the connection of active amino acids with the DPPH free radical [23]. The use of microwave pretreatment in hydrolysis with pancreatin enzyme (treatments 1 and 2) had an improving effect on DPPH free radical inhibition activity. Gui et al. (2022) in their study on milk protein hydrolysis, reported the positive effect of microwave pretreatment with 300 W power on DPPH free radical inhibitory activity [24]. Ketnawa and Liceaga (2017) reported the use of microwave pretreatment for 5 minutes at 90°C for 2 to 10 minutes as the best conditions for the production of fish peptides with high antioxidant activity [13]. Esmaeili and Hoseyni (2023) stated that 10 minutes of microwave pretreatment at 90°C has a favorable effect on DPPH radical inhibition properties of beluga visceral protein hydrolysis, which can indicate the application of this technology in the production process of fish protein hydrolysis [25].

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According to Figure 1, in treatment 3, the DPPH free radical inhibition activity increased with increasing time and varying enzyme-to-substrate ratio. This trend in treatment 4 was such that with increasing time, the highest DPPH free radical inhibition activity was observed at low ratios of the enzyme to substrate. Research have shown that peptides with high antioxidant properties are probably lost with the progress of the hydrolysis process [26]. In hydrolysis with trypsin enzyme, the use of pretreatment did not have an improving effect on DPPH free radical inhibition activity. The negative effect of pretreatment in hydrolysis with trypsin enzyme can be due to the change in the structure of the hydrolyzed protein and the placement of hydrophobic groups towards the inside of the protein molecule, which reduces the interaction between the enzyme used and the hydrophobic groups and reduces the antioxidant activity. [27].

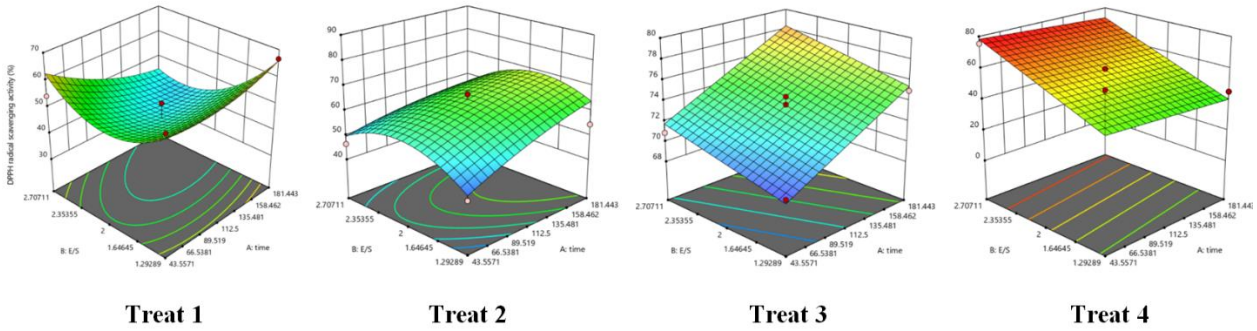

Figure 1- The effect of hydrolysis time, enzyme to substrate ratio and proteases type on DPPH radical scavenging activity of protein hydrolysate

3-2- Iron ion chelation activity

According to Figure 2, the iron ion chelating activity of treatment 1 increased up to 2.1% with increasing time and increasing enzyme-to-substrate ratio and then decreased with increasing enzyme-to-substrate ratio. In treatment 2, the highest percentage of iron ion chelation was observed in the early stages of the process and high enzyme-to-substrate ratios. Similar to these results, Xie et al. (2008), Klompong et al. (2007) and Maqsoodlou et al. (2016) showed the positive and improving effect of the enzyme-to-substrate ratio on the iron ion chelating activity of alfalfa hydrolyzed proteins, respectively. They reported yellow line fish and flower pollen [22-28-29]. The effect of microwave pretreatment on protein hydrolysis of flaxseed meal with pancreatin enzyme was improved. Gazikalović et al. (2023) stated that microwave pretreatment along with enzymatic hydrolysis of gluten increases its antioxidant properties [30]. Zheng et al. (2015) reported that microwave can improve the effect of enzymatic hydrolysis on beef bone powder protein, and improve the antioxidant function and taste of hydrolyzed protein [31].

According to Figure 2, iron ion chelating activity was observed in treatment 3 with increasing time and decreasing the ratio of enzyme to substrate. In treatment 4, by increasing the time to 117 minutes and increasing the ratio of enzyme to substrate to 2.2%, the chelating activity of iron ion increased, and then by increasing the time and ratio of enzyme to substrate, the activity of chelating iron ion decreased. The decrease in iron ion chelating activity due to the excessive increase in the protein hydrolysis time can be due to the decrease in the efficiency and effectiveness of the enzyme in producing peptides with suitable antioxidant capacity due to the release of enzyme inhibitory compounds during the long hydrolysis time [32]. Kaveh et al. (2022) observed in fenugreek seed protein hydrolysis that increasing the hydrolysis time

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up to the first 150 minutes increased the iron ion chelating activity of the hydrolyzed protein, But its further increase did not have a significant effect on the iron ion chelation activity [33]. The use of microwave pretreatment in hydrolysis with trypsin enzyme had an improving effect on the percentage of iron ion chelating activity of hydrolyzed protein. The use of microwave pretreatment before enzymatic hydrolysis of proteins improves the release of bioactive peptides from proteins and opens the three-dimensional structure of proteins and helps to increase the access of enzymes to peptide bonds [34].

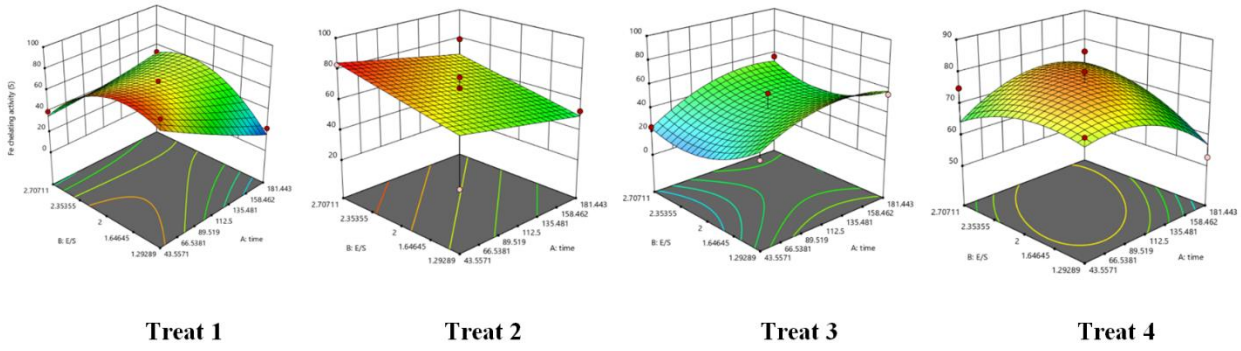

Figure 2- The effect of hydrolysis time, enzyme to substrate ratio and proteases type on Fe^{2+} chelating activity activity of protein hydrolysate

3-3-Total antioxidant activity

According to Figure 3, the total antioxidant activity of treatment 1 increased with the increase of two variables, time and enzyme-to-substrate ratio. Similar to these findings, Mazloumi et al. (2018) reported that in the hydrolysis of orange seed protein, increasing the hydrolysis time had a positive effect on the total antioxidant capacity of the hydrolyzed proteins, and the highest total antioxidant capacity after 4.8 Hour was obtained from hydrolysis [35]. In treatment 2, by increasing the time to 90 minutes and increasing the ratio of enzyme to substrate to 2.2%, the total antioxidant activity increased. After that, with the increase of both variables, a decrease in the total antioxidant activity was observed. Microwave pretreatment had an improving effect on increasing the antioxidant activity of total protein hydrolyzed with pancreatin enzyme. da Rosa et al. 2019 reported the highest antioxidant activity with microwave pretreatment at 86°C and 3 minutes in olive leaf protein hydrolysis [36]. Lin et al. (2010) conducted a study on the method of preparing antioxidant peptides by enzymatic hydrolysis of bone collagen after microwave pretreatment and reported an increase in antioxidant properties [37].

In treatment 3, the highest total antioxidant activity was observed with increasing time in low ratios of the enzyme to substrate. The increase in the antioxidant capacity of the total hydrolyzed protein following the increase in the hydrolysis time can be due to the production of peptides containing electron-donating properties, which cause the conversion of free radicals into more stable compounds, which, as a result, increase the total antioxidant capacity [38]. The total antioxidant activity of treatment 4 increased with increasing time up to 103 minutes and increasing the ratio of enzyme to substrate to 2.5% and then decreased with increasing time. Kaveh et al. (2018) in a study they conducted on the hydrolysis of fenugreek protein, reported that increasing the hydrolysis time up to 160 minutes increased the antioxidant activity of the total hydrolyzed protein, and further hydrolysis of the fenugreek protein had a significant effect on the total antioxidant activity of these samples. did not have [20]. Microwave pretreatment in hydrolyzing flaxseed meal protein with trypsin enzyme had an improving effect on total

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antioxidant activity. Nguyen et al. (2017) reported that the use of microwave pretreatment in enzymatic hydrolysis of fish protein results in the production of hydrolyzed protein with appropriate antioxidant properties [39].

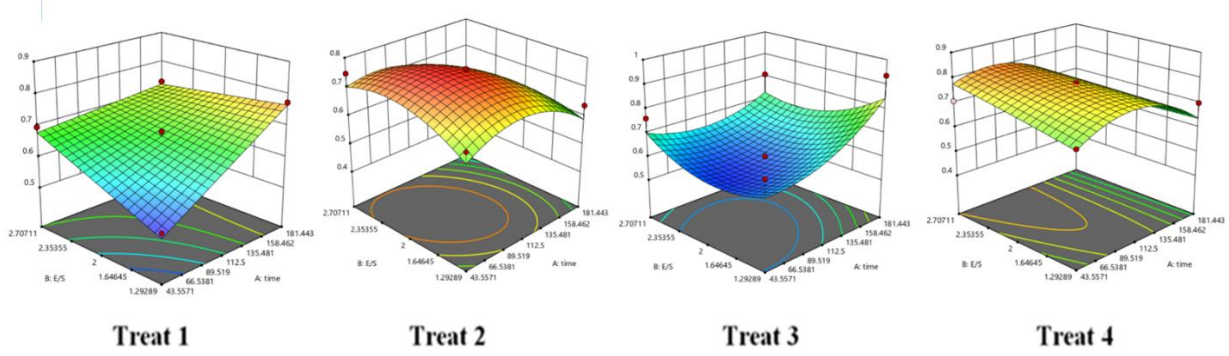

Figure 3- The effect of hydrolysis time, enzyme to substrate ratio and protease type on the total antioxidant capacity of protein hydrolysate

3-4-Optimization of the hydrolysis process

Four optimal points of flaxseed meal protein hydrolysis (hydrolysis with pancreatin without pretreatment (treatment 1), with pancreatin and pretreatment (treatment 2), with trypsin without pretreatment (treatment 3) and with trypsin with pretreatment (treatment 4)) by Design Expert software 11 was obtained.

Table 2- Examining the variables of time and ratio of enzyme to substrate of optimal treatments of hydrolyzed protein of flax seed meal. Treatment 1 (protein hydrolyzed with pancreatin without pretreatment), treatment 2 (protein hydrolyzed with pancreatin and microwave pretreatment), treatment 3 (protein hydrolyzed with trypsin without pretreatment) and treatment 4 (protein hydrolyzed with trypsin and microwave pretreatment)

Treatment	Hydrolysis time (min)	Enzyme to substrate ratio (%)
1	112.01	2.46
2	44.09	2.19
3	59.26	1.92
4	84.02	1.77

3-4-1- DPPH free radical inhibition activity

According to Figure 4, among treatments hydrolyzed with pancreatin and trypsin (with and without pretreatment), treatment 3 (protein hydrolyzed with trypsin) had the highest DPPH free radical inhibition percentage (72.22%). Microwave pretreatment had a significant effect on increasing DPPH free radical inhibition percentage of hydrolyzed pancreatin treatment ($p<0.05$). However the effect of pretreatment on DPPH free radical inhibition in hydrolysis with trypsin enzyme was not improved. In this regard, studies have shown that microwaves can produce different biological and chemical effects on biological molecules, including proteins, depending on the strength and time of their application. The negative effect of microwave pretreatment on the DPPH radical scavenging activity of the hydrolyzed protein of flaxseed meal can be due to the unfavorable changes in the second structure of the protein and the decrease in the amount of alpha helix and beta sheet structure [40].

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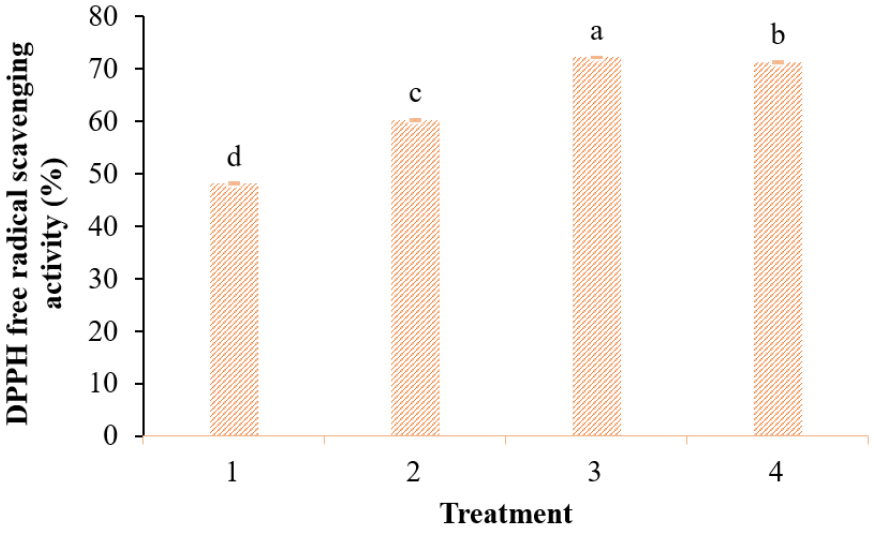

Figure 4- DPPH radical scavenging activity of optimized treatments; Means with the same letters in each column show no significant difference at the 0.05 level. Treatment 1 (protein hydrolyzed with pancreatin without pretreatment), treatment 2 (protein hydrolyzed with pancreatin and microwave pretreatment), treatment 3 (protein hydrolyzed with trypsin without pretreatment) and treatment 4 (protein hydrolyzed with trypsin and microwave pretreatment)

3-4-2- Iron ion chelation activity

According to the graph obtained from the results (Figure 5), among the treatments of this research, the highest percentage of iron ion chelation (76.12%) was related to treatment 4 (protein hydrolyzed with trypsin and microwave pretreatment). The effect of microwave pretreatment on increasing the iron ion chelation percentage in hydrolysis with both enzymes was significant ($p<0.05$). The increase in iron ion chelating potential of the hydrolyzed protein of flaxseed meal after microwave pretreatment can be due to the evaporation of water in the cells and the increase of pressure in the internal environment by the application of microwave waves, which causes the decomposition of intracellular compounds, disintegration membrane and as a result its decomposition, which ultimately leads to the ease of the process of enzymolysis of proteins [41].

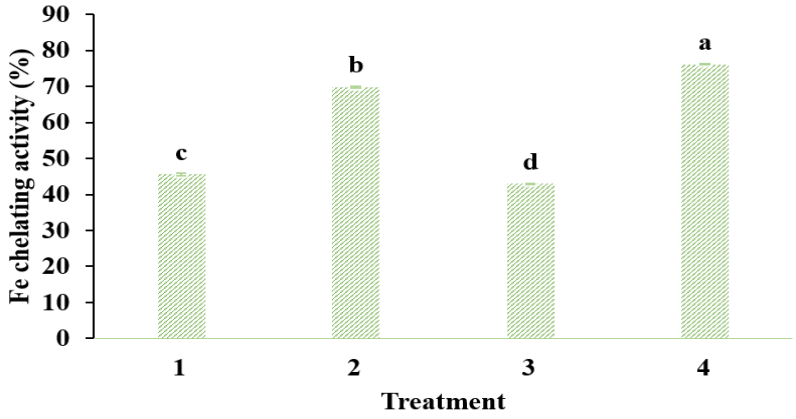

Figure 5. Fe chelating activity of optimized treatments.; Means with the same letters in each column show no significant difference at the 0.05 level. Treatment 1 (protein hydrolyzed with pancreatin without

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pretreatment), treatment 2 (protein hydrolyzed with pancreatin and microwave pretreatment), treatment 3 (protein hydrolyzed with trypsin without pretreatment) and treatment 4 (protein hydrolyzed with trypsin and microwave pretreatment)

3-4-3-Total antioxidant activity

According to Figure 6, the highest total antioxidant absorption (0.745 (absorbance at 695 nm)) obtained among the treatments of this research was related to treatment 4 (hydrolyzed protein with pancreatin enzyme and microwave pretreatment). Microwave pretreatment had a significant effect on increasing absorption in the measurement of antioxidant activity of all treatments hydrolyzed with pancreatin and trypsin ($p<0.05$). The increase in the antioxidant activity of the total hydrolyzed protein under the influence of microwave pretreatment is probably due to the decomposition of protein molecular aggregates, which has led to an increase in the sensitivity of the peptide bonds of flaxseed protein to degradation under the influence of proteases. In general, studies have shown that the positive or negative effect of microwave pre-timer depends on many factors, including its time and power [40].

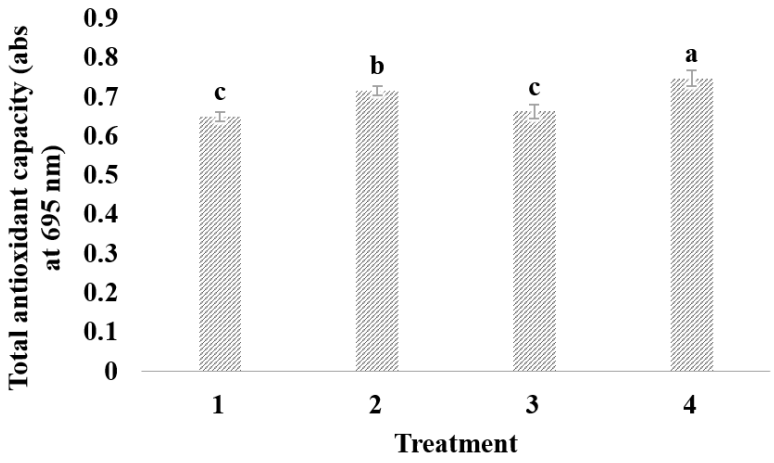

Figure 6. Total antioxidant capacity of optimized treatments: Means with the same letters in each column show no significant difference at the 0.05 level. Treatment 1 (protein hydrolyzed with pancreatin without pretreatment), treatment 2 (protein hydrolyzed with pancreatin and microwave pretreatment), treatment 3 (protein hydrolyzed with trypsin without pretreatment) and treatment 4 (protein hydrolyzed with trypsin and microwave pretreatment)

4 - Conclusion

In this research, flax seed meal protein using two protease enzymes trypsin and pancreatin and each enzyme with two treatments "with pretreatment" and "without pretreatment", with two time variables (15-210 minutes) and enzyme to substrate ratio (1 -3%) was hydrolyzed. The effect of microwave pretreatment on antioxidant properties (iron ion chelation, total antioxidant activity and DPPH free radical inhibition) of all four treatments of this research was investigated. According to the results obtained from the antioxidant tests of the treatments and its investigation by the response surface method with the help of Design Expert 11 software, the hydrolyzed protein treatment with trypsin enzyme and microwave pretreatment, with the highest total antioxidant activity value of 0.745 (absorbance at 695 nm), the activity DPPH free radical inhibition 71.35% and iron ion chelating activity 76.12% were selected as the optimal treatment. The optimum conditions for the production of the treatment with the highest antioxidant activity were determined by the hydrolysis time of 84.02 minutes and the ratio of enzyme to substrate was 1.77%. Therefore, due to the significant antioxidant capacity of the hydrolyzed protein of flaxseed meal as a value-

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added product, it can be used as a health-giving compound to enrich food formulations and supplements for athletes.

5-Resources

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تولید پپتیدهای آنتی‌اکسیدانی از کنجاله بذر کتان: تاثیر نوع و غلظت پروتئاز، زمان هیدرولیز و پیش‌تیمار مایکروویو

فائزه فرزان‌فر[۱]، علیرضا صادقی ماهونک[۲*]، محمد قربانی[۳]، سید حسین حسینی قابوس[٤]، شیما کاوه[٥]

۱-دانشجوی کارشناسی ارشد شیمی مواد غذایی، گروه علوم و صنایع غذایی، دانشکده صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان

۲- استاد گروه علوم و صنایع غذایی، دانشکده صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان

۳- استاد گروه علوم و صنایع غذایی، دانشکده صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان

٤-استادیار گروه علوم و صنایع غذایی، دانشگاه آزاد اسلامی واحد آزادشهر

٥-دانش‌آموخته دکتری شیمی مواد غذایی، گروه علوم و صنایع غذایی، دانشکده صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان.

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تریپسین،

کتان،

هیدرولیز آنزیمی

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* مسئول مکاتبات:

Sadeghiaz@yahoo.com

چکیده

هر ساله در طی فرآوری محصولات کشاورزی و تولید مواد غذایی، مواد زائد و فرآورده‌های جانبی زیادی تولید می‌شوند. اکثر این محصولات فرعی دارای خواص زیست‌فعالی مانند آنتی‌اکسیدان هستند که می‌توان آن‌ها را استخراج و در تولید محصولات سلامتی بخش به کار برد. در پژوهش حاضر، کنجاله بذر کتان که به عنوان محصول فرعی فرایند روغن‌گیری از بذر کتان حاصل می‌شود با استفاده از دو آنزیم تریپسین و پانکراتین با دو متغیر زمان (۲۱۰-۱۵ دقیقه) و نسبت آنزیم به سوبسترا (۱-۳ ٪) هیدرولیز شد. تاثیر پیش‌تیمار مایکروویو بر خواص آنتی‌اکسیدانی پروتئین هیدرولیزشده توسط روش سطح‌پاسخ بررسی شد. تیمار پروتئین هیدرولیزشده تولیدی با تریپسین و پیش‌تیمار مایکروویو در شرایط زمان هیدرولیز ۸٤/۰۲ دقیقه و نسبت آنزیم به سوبسترا ۱/۷۷ ٪ به عنوان تیمار بهینه با بیشترین خواص آنتی‌اکسیدانی (فعالیت آنتی‌اکسیدانی کل ۰/۷٤٥ (جذب در ٦۹٥ نانومتر)، فعالیت مهار رادیکال آزاد DPPH ۷۱/۳٥ ٪ و فعالیت شلاته‌کنندگی یون آهن ۷٦/۱۲ ٪) انتخاب شد. پروتئین هیدرولیزشده بذر کتان به عنوان یک محصول زیست‌فعال با خواص آنتی‌اکسیدانی، می‌تواند به‌عنوان یک آنتی اکسیدان طبیعی در تولید محصولات سلامتی‌بخش و مکمل غذایی ورزشکاران مورد استفاده قرار گیرد.

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