Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Optimizing the production of hydrolyzed protein with antioxidant properties from Tarem rice bran by alcalase enzyme

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ARTICLE INFO

Article History:
Received:2023/10/3
Accepted: 2023/12/28

Keywords:

optimization,
hydrolyzed protein,
antioxidant property,
rice bran protein,
enzymatic hydrolysis.

DOI: **10.22034/FSCT.21.148.112.**

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ABSTRACT

Peptides obtained from hydrolysis have many bioactive properties and have strong biological activities against free radicals and prevent antioxidant processes that cause damage to biological macromolecules and degradation and quality reduction. Due to the large amount of rice production in the world, a large number of rice bran is produced and available. As a suitable and cheap production source, rice bran can be used for the production of plant-derived peptides. Optimizing the conditions of enzymatic hydrolysis of rice by alcalase enzyme was done with the aim of achieving anti-allergenic properties. In order to investigate the antioxidant activities of peptides, iron 3 reduction power tests and DPPH free radical activities and total antioxidant activities were used. In order to optimize the time of the Expert design software and the response surface method with three different changes: this enzyme was applied to the substrate 1-3%, temperature 40-55 degrees Celsius and hydrolysis 30-210 minutes. In the set conditions of the treatment including temperature of 51.5 degrees Celsius, 131.5 minutes and enzyme substrate 3%, it was obtained that DPPH free radical is 37.172%, total antioxidant activity is 1.109% and reducing agent is 2.084%. The obtained results showed that the process of rice bran production by alcalase enzyme leads to the production of peptides with high anti-anxiety properties and it is noteworthy that they can be used in the production of super-beneficial foods and pharmaceutical industries and also replace them.

1. Introduction

Bioactive peptides as products of protein hydrolysis are special protein components that have significant biological effects such as antioxidant, antimicrobial, anti-diabetic, anti-cancer activities and have a positive effect on the function or condition of the body and in terms of Economically, due to its application in the production of super-beneficial foods and medicines, they have been given much attention [1]. Bioactive peptides are generally short (containing 3-20 amino acids). These peptides are often functionally inactive in the main proteins and must be released in some way to achieve their specific "bioactive" roles [2]. The most common method of producing bioactive peptides is the hydrolysis of proteins with the help of enzymes, which is a gentle and controllable process [3]. Rice is one of the most important crops (cereals) in the world. Rice production is geographically concentrated in South and East Asia [4]. Rice belongs to the genus Oryza, family Poaceae (Gramineae), family Oryzeae [5]. The rice grain consists of two main parts, the shell and the caryopsis (a total of bran, endosperm and embryo). Rice bran is separated from the starchy endosperm during milling and is rich in B vitamins and minerals. More than half of bran is made up of cellulose and pentosan, polymers based on xylose and arabinose that are tightly bound to proteins. The main protein of rice bran is albumin and globulins, while prolamin and glutelin are secondary components. Proteins and carbohydrates each account for approximately 16% of total bran dry matter. The composition of rice grain for most varieties is approximately 20% husk, 11% bran and 69% starchy endosperm [6 and 7]. Considering the huge amount of rice production worldwide and the rich protein content of rice bran, there is both a need and potential for added value to this major by-product of rice milling. Oxidative stress occurs when the balance between the production of reactive oxygen/nitrogen species (ROS/NOS) and the antioxidant defense system is upset and potentially leads to biological damage to important cellular macromolecules [8]. Spontaneous oxidation is delayed by preventing the formation of free radicals through metal ion chelating mechanisms, switching off singlet oxygen, stopping the propagation step of the chain reaction in auto-oxidation processes and reducing oxygen concentration. drop or inhibit it [9.] In the study of Hayta et al. (2020), protein extraction with antihypertensive and antioxidant properties using enzymatic hydrolysis in order to increase the added value of rice bran, optimization and the results showed that biological activity and antioxidant capacity increase with alkalase treatment [10]. Vatanasiritham et al. (2016) evaluated bran protein fractions and those hydrolyzed with papain and trypsin in terms of antioxidant activity and showed that rice bran albumin hydrolyzed with trypsin can be a natural food antioxidant. be used [11]. Oraipong and Zhao (2016), reported that hydrolyzed rice bran protein by four protease enzymes showed the properties of inhibiting the activity of α-amylase and β-glucosidase and angiotensin converting enzyme [12]. The aim of this research was to optimize the enzymatic hydrolysis of tarem rice bran protein by alkalase enzyme using the response surface method by examining three factors: time, temperature and enzyme-to-substrate ratio in order to achieve the highest iron-3 reducing power and DPPH free radical inhibition and power Total antioxidant.

2.Materials and methods

2-1- Materials

Alkalase enzyme, trichloroacetic acid, ammonium molybdate, dipotassium hydrogen phosphate, sodium tripolyphosphate, potassium ferricyanide, hydrochloric acid, ethanol, hexane, DPPH, iron chloride (lll). All these materials were obtained from Merck and Sigma companies and with high purity of laboratory grade. This research was conducted in the laboratories of the Department of Food Science and Industry of Gorgan University of Agricultural Sciences and Natural Resources. Tarem rice bran was obtained from Bandapi Eastern Rice Factory of Babol city.

2-2- Preparation and degreasing of raw materials

Rice bran was first passed through a sieve with 80micron mesh and bran defatting was done using hexane according to the method of Wang et al. (1999) [13]. In the next step, to remove the solvent, the bran was placed in an oven with a temperature of 45 degrees Celsius, and the defatted bran powder was packed in a polyethylene bag and stored in the refrigerator for testing.

2-3- protein extraction

Protein extraction from rice bran was performed according to Yum et al.'s (2010) method [14]. Defatted rice bran was mixed with distilled water at a ratio of 5:1 and then stirred for 5 minutes on a mixer at a speed of 250 rpm. Then, the pH of the mixture was adjusted to 9 with 1.0 M sodium hydroxide and stirred for 2 hours, and then centrifuged (10,000 g for 15 minutes at 4 degrees Celsius) until the insoluble materials were removed. Then, the protein in the supernatant was precipitated by adjusting the pH to 4 with the help of 1.0 M hydrochloric acid and centrifuged again (10,000 g for 15 minutes at 4 degrees Celsius). The resulting precipitate was washed twice with water to remove the dissolved substances. The sediment was then redispersed in distilled water (1:1), neutralized by adjusting the pH to 7.0 using a pH meter, and then freeze-dried. The resulting protein was stored at -18 degrees Celsius until use.

2-4- Production of hydrolyzed protein

Alkalase enzyme was used for hydrolysis. 50 grams of rice bran protein was weighed in a volumetric flask and mixed in 1000 ml of phosphate buffer (pH=8). Then, in order to optimize the production of hydrolyzed proteins with maximum antioxidant activity, the response surface method was used. The temperatures used were in the range of 40-55 degrees Celsius, the time was between 30 and 210 minutes, and the concentration of enzyme to substrate was 1 to 3%, which was optimized by the response surface method. Greenhouse placement of the treatments was done. In order to inactivate the enzyme, the protein solution inoculated with the enzyme was placed in a water bath at a temperature of 85 degrees Celsius for 15 minutes. The treatments were placed in a centrifuge with 5000 revolutions and a time of 20 minutes at a temperature of 4 degrees Celsius, and the resulting supernatant was dried with a dryer and the resulting powder was kept at -18 degrees Celsius until use [15].

2-5- Measurement of protein amount

The amount of protein in the sample was measured by the Kjeldahl method. 1 gram of the sample was added to the Kjeldahl digestion tube, and a tablet of Kjeldahl catalyst and 20 ml of concentrated sulfuric acid were added to it. A digestion tube without sample and also containing acid and catalyst was used as a control. Digestion was performed with a power of 80 units and for 120 minutes until the contents of the digestion balloon became clear. Distillation stage was done using 40% soda and 4% boric acid using a distillation apparatus after cooling down and removing acidic vapors. At the end, titration of the sample with 0.1 normal hydrochloric acid and methyl red was added until the sample turned light lemonThe nitrogen percentage of the sample was calculated using equation (1) [16].

$$(1)\ Nitrogen\ percentage = \frac{1.4 \times N \times (V1 - V2)}{m} \times 100$$

In the above relationship, N is the normality of hydrochloric acid, V1 is the volume of acid used for the sample, V2 is the volume used for the control, and m is the weight of the sample in grams. To calculate the protein percentage, the amount of nitrogen was multiplied by the protein factor (6.25) and its amount was calculated**.**

Analysis of chemical compounds

The percentage of moisture, protein, fat and ash (based on wet weight) was measured by AOAC (2005) method [16].

2-7- Measurement of DPPH radical inhibition activity

DPPH free radical inhibition percentage was determined by the method of Wu et al. (2003). The hydrolyzed powders were first dissolved in distilled water (40 mg/ml). Then, 1.5 ml of each sample was mixed with 1.5 ml of 0.15 mM ethanolic solution (DPPH 0.15) and mixed for 20 seconds. Then, the resulting mixture was centrifuged at 2500 g for 10 minutes and kept in the dark for 20 minutes. Then the absorbance of the supernatant solution was read at a wavelength of 517 nm. The DPPH radical inhibition percentage was calculated using formula (2). In the control sample, 1500 microliters of distilled water was used instead of the hydrolyzed protein sample [17].

$$(2) DPPH \text{Percentage inhibition of radical activity} = \frac{\text{absorption witness} - \text{absorption sample}}{absorption\ witness} \times 100$$

2-8- Regenerative power test

This test was performed based on investigating the reduction power of iron atom (lll) by hydrolyzed protein. The hydrolyzed powders were dissolved in distilled water (40 mg/ml) and 1 ml of the samples were mixed with 2.5 ml of 0.2 M phosphate buffer (pH=6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was kept in a greenhouse at 50 degrees Celsius for 30 minutes, and then 2.5 ml of 10% (weight-volume) trichloroacetic acid solution was added. The mixture was centrifuged for 10 minutes at 3000 rpm, and finally, 0.5 ml of 0.1% iron chloride solution, 2.5 ml of supernatant solution was mixed with 2.5 ml of distilled water. After a few minutes of reaction, the absorbance of the resulting solution was read at 700 nm. More absorption indicates an increase in its regenerative power [18].

2-9- Total antioxidant capacity measurement test

First, 0.1 ml of the sample dissolved in distilled water with 1 ml (40 mg/ml) of the reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) was poured into the Eppendorf tube and It was placed in a 90 degree Celsius water bath for 90 minutes. After cooling the samples, the absorption of the samples was read at 659 nm and the absorption result was reported as total antioxidant power. Double distilled water was used as a control sample. More absorption indicates more total antioxidant capacity [19].

3.Experiment design in order to optimize hydrolysis conditions by response surface method

In order to optimize the process using the Expert Design software and the response surface method with the central composite design for three independent variables: enzyme concentration to the substrate (X1), temperature (X2) and hydrolysis time (X3) in three levels (1, +0, 1) and the dependent variable i.e. DPPH free radical inhibition power, Fe3+ ion reduction power and total antioxidant property were used. For this purpose, 20 random treatments were selected by applying the central compound design and with the number of 6 central points without blocks.

Table 1 - Levels of Independent variables used to optimize the antioxidant activity of the Rice bran protein hydrolysate

Independent variables	Levels variables		
	1-	0	1+

Enzyme to substrateratio (%)	1	2	3
Temperature (°C)	40	47.5	55
Time(h)	30	120	210

4.Results and discussion

4-1- Chemical compounds

At first, the amount of moisture, ash and protein in defatted, non-defatted bran powder and protein concentrate and hydrolyzed protein were measured (Table 2).

Table 2 - Chemical compounds of rice bran

Sample	Ash)%(	Oil) %(	Moisture)%(	Protein) %(
Bran powder with fat	7.78	13.66	8.9	12.54
Non defatted bran powder	9.0	4.8	6.5	16.4
Rice bran protein concentrate	3.96	1.76	9.1	52.5
Hydrolyzed rice bran protein (optimal sample)	8.85	1.8	10.2	69.13

According to the results, the increase in protein percentage in hydrolyzed protein is due to the increase in protein solubility during hydrolysis and the removal of other additional compounds by centrifugation. Altor et al. (2002) reported the amount of bran concentrate protein produced between 52-58% depending on different varieties [20]. Amisa et al. (2003), respectively, expressed the protein content of rice bran protein concentrate between 17 and 14% [21]. The reason for the reduction of hydrolyzed protein fat compared to primary bran and concentrate is the defatting process of primary bran. In the research of Naib Gholami et al. (2018), the amount of fat in the non-defatted bran sample was 14.97%, the amount of ash in the bran sample was 11.43%, and the hydrolyzed protein ash was 4.99% [22]. In the research of Amisa et al. (2003), the amount of ash was reported in the range of 11.55-6.68% [21]. The amount of ash in the hydrolyzed protein sample in this research is more than the original bran sample, which can be related to the presence of solutes in the buffer or salt formed in the pH adjustment of the sample. The reason for the difference in the chemical composition of the sample in this research with the previous research can be attributed to the difference in the type of variety and the type of processes.

4-2- Optimization of rice bran protein hydrolysis conditions

The response surface method was used to achieve the highest DPPH free radical inhibition, iron (III) reduction and total antioxidant activities. After performing the hydrolysis process at the specified points by the Design expert software, each of the treatments was evaluated in terms of the desired parameters and the values are shown in Table [3].

Table 3 - Antioxidant tests of hydrolyzed rice bran protein

Treatment	Enzymeto substrate ratio **(%)**	Time (Hr)	Temperature °)C(	DPPH radical scavenging activity**(%)**	Iron reduction	total antioxidant activity
1	2	30	47.5	13.817	0.591	0.591
2	2	120	47.5	47.36	1.76	1.76
3	2	210	47.5	51.24	1.72	1.72
4	2	120	47.5	38.56	1.752	1.752
5	1	30	55	9.083	0.69	0.69
6	3	30	40	10.976	0.638	0.638
7	2	120	55	17.459	1.54	1.54
8	1	120	47.5	27.99	1.706	1.706
9	2	120	47.5	37.46	1.562	1.562
10	3	120	47.5	45.25	1.831	1.831
11	3	30	55	19.475	1.67	1.67
12	1	210	55	24.786	1.793	1.793
13	3	210	40	39.628	1.75	1.75
14	1	30	40	14.616	0.697	0.697
15	2	120	40	29.566	1.774	1.774
16	3	210	55	25.1	1.899	1.899
17	1	210	40	44.874	1.675	1.675
18	2	120	47.5	37.1	1.65	1.641
19	2	120	47.5	37.1	1.662	1.692
20	2	120	47.5	37.1	1.462	1.462

4-3- DPPH free radical inhibitory activity

The use of 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a quick, easy and cost-effective method for measuring antioxidant properties. In order to determine the optimal conditions of each variable in enzymatic hydrolysis to obtain the highest DPPH free radical inhibitory power, three-dimensional diagrams were drawn for the variables. Each figure shows the effects of two variables on the response when the third variable is located at the center point. Figure (1) shows the simultaneous effect of enzyme concentration to substrate and hydrolysis time on DPPH free radical scavenging activity of hydrolyzed rice bran protein. By increasing the enzyme concentration from 1 to 3%, an increase in antioxidant activity was observed, also according to Figure 1, the antioxidant properties increased with increasing temperature. With increasing enzyme activity and increasing temperature, the destruction of the natural structure of proteins by the enzyme leads to the availability of the active form of amino acid with antioxidant capacity [23]. According to figure (1), it can be seen that the effect of hydrolysis time on DPPH free radical inhibition activity was more than the effect of enzyme. Molecular weight is one of the parameters that influence antioxidant properties, so that peptides containing 3-20 amino acids and with a molecular weight of less than 6000 daltons have strong antioxidant properties. At the concentration of 3% of the enzyme used in this research, it can be said that the production of this type of peptides was higher. As shown in the graph, the antioxidant property was low in 30 minutes, which can be attributed to the low time of the enzyme's access to the substrate to achieve protein hydrolysis and the low production of antioxidant peptides. In Figure (2), the antioxidant power increases to a certain extent with increasing temperature, then decreases, and with increasing hydrolysis time, the antioxidant property has increased. According to the results, the highest amount of antioxidant was related to the bioactive peptides produced under the temperature conditions of 51 degrees Celsius and time of 131 minutes. According to the diagram (2), it can be seen that the antioxidant properties have decreased with increasing temperature. Gurard et al. (2002) stated that with increasing enzyme concentration, the production of hydrolyzed proteins with antioxidant properties increases [24]. Depending on the molecular weight, charge and spatial structure of the end chain of the produced peptides, the free radical trapping power in them also increases [25]. Hydrolyzed protein with a molecular weight of 1-3 kDa has the highest free radical scavenging power and is more accessible to radicals and can trap radicals more easily [26]. Peptides that contain amino acid histidine cause antioxidant activity by giving hydrogen or trapping radicals, and also the presence of amino acids containing SH group causes a very suitable antioxidant activity through direct reaction with free radicals [27]. Hay et al. (2013) showed that negatively charged amino acids have strong antioxidant effects due to their extra electrons and can act as hydrogen donors [28]. In the research of Wang et al. (2020), the antioxidant activity of Hairtail fish surimi was related to the richness of Asp, Glu, and Leu amino acids [29]. In graph (3), it can be seen that the effect of temperature on free radical scavenging activity was greater than the effect of enzyme concentration. The effect of temperature on antioxidant properties showed that this characteristic is increasing from 40 degrees and reaches its maximum value at 51 degrees Celsius, while further increase in temperature had a negative effect on antioxidant capacity and this characteristic decreases. to be The reduction of antioxidant activity at lower temperature due to incomplete hydrolysis and also at high temperature can be caused by thermal denaturation of proteins and loss of optimal enzyme activity [30].

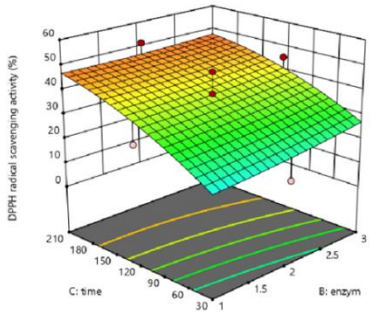

Figure 1. Diagram of the effect of enzyme concentration and hydrolysis time on DPPH free radical inhibition activity of hydrolyzed rice bran protein

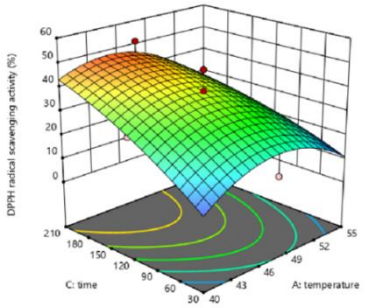

Figure 2. Diagram of the effect of hydrolysis time and temperature on DPPH free radical scavenging activity of hydrolyzed rice bran protein

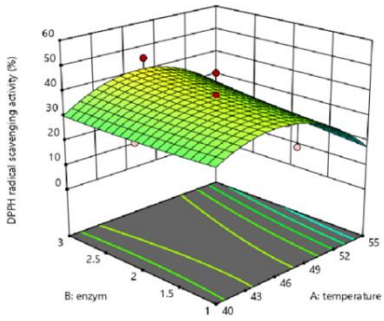

Figure 3. Effect diagram of alcalase enzyme concentration and hydrolysis temperature on DPPH free radical inhibition activity of hydrolyzed rice bran protein

4-4- Investigating the reductive power of Fe3+

In this method, the ability of treatments to regenerate trivalent iron and convert it into divalent iron is measured. The presence of reducing agents (antioxidants) leads to the reduction of ferricyanide complexes and their conversion to ferrous form [31]. The evaluation of reducing power shows the electron donating ability of a compound. According to figure (4), the temperature increase, and the hydrolysis time, the reductive activity of the produced hydrolyzates has increased. The increasing trend of the reducing power of hydrolyzed proteins can be attributed to the type of enzyme

and the conditions of the reaction, the amino acids present in the active site of the enzyme, which are very effective in reducing the iron ion [25]. Figure (5) shows the effect of time and concentration of enzyme to the substrate on the reductive ability of Fe3+, that increasing the concentration of the enzyme to the substrate from 1 to 3% and also increasing the hydrolysis time from 30 to 131 minutes increases the reductive activity. Researches have shown that the breaking of the peptide bond and release from the main structure of proteins, the molecular weight decreases, and then with the increase of time and the progress of the hydrolysis process and the breaking of more chains, the regenerative peptides increase [32]. Ji et al. (2009) in the study of the reductive activity of hydrolyzed protein obtained from tuna waste with alcalase enzyme stated that the reductive activity increases with the increase of the enzyme amount [32].

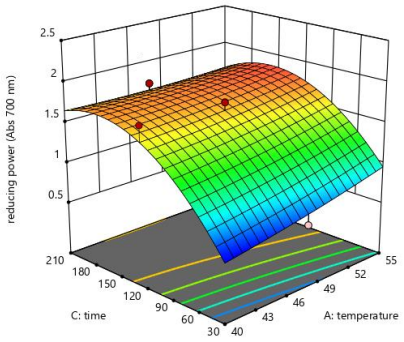

Figure 4. The graph of the effect of temperature and hydrolysis time on the Fe $^{3+}$ reductive activity of hydrolyzed rice bran protein

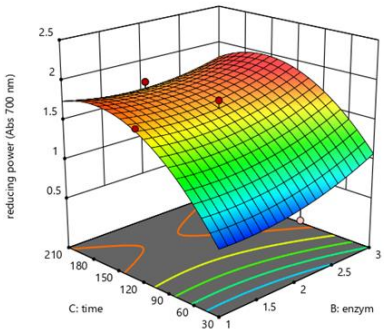

Figure 5. Effect diagram of substrate enzyme concentration and hydrolysis time on Fe $^{3+}$ reductive activity of rice bran hydrolyzed protein

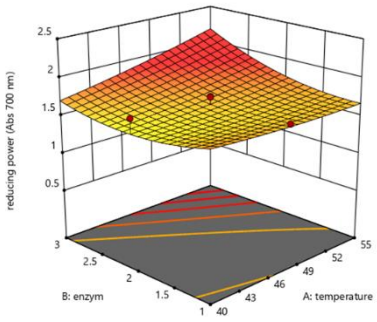

Figure 6. Effect diagram of substrate enzyme concentration and hydrolysis temperature on Fe 3+ reductive activity of hydrolyzed rice bran protein

4-5- Total antioxidant capacity

The basis of work in this method is the reduction of 6-valent molybdenum to 5-valent molybdenum in acidic environment and high temperature. This reaction is associated with the formation of green phospho-molybdenum complexes, which have the maximum absorption at the wavelength of 695 nm [33]. In figure (7), total antioxidant N has increased and it can be seen that this property has also increased with increasing enzyme concentration. Research results have shown that the presence of hydrophobic amino acids such as valine, phenylalanine, isoleucine, and leucine in proteins hydrolyzed with alcalase enzyme play a very important role in total antioxidant activity. According to figure (8), with the increase in the hydrolysis time, the antioxidant properties first increase and then decrease. The decrease in the antioxidant properties of peptides after more than 150 minutes of hydrolysis may be related to the higher hydrolysis due to the greater effect of the enzyme on the substrate, which causes the peptides to break down too much and break some of the initial peptide chains with high antioxidant activity and from loss of these peptides [34]. Taheri et al. (2011), in the study of the antioxidant activity of rainbow sardine fish, stated that the antioxidant activity increased up to its optimum point, after that it decreased with the increase of time and concentration of the enzyme to the substrate, which is due to the effect of time and temperature. It is on enzyme activities. According to Figure (9), increasing the concentration of the enzyme led to an increase in the total antioxidant activity, and also increasing the time first causes an increase and then a decrease in this property [35].

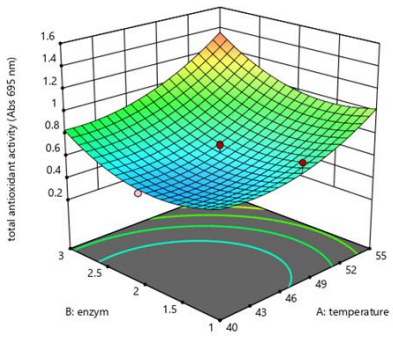

Figure 7. Diagram of the effect of enzyme concentration on the substrate and hydrolysis temperature on the antioxidant activity of the total hydrolyzed rice bran protein.

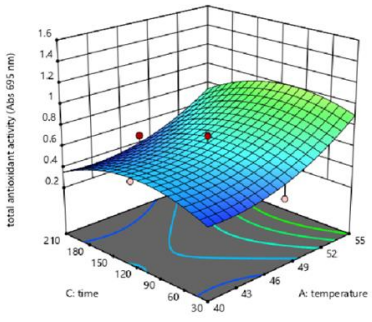

Figure 8. Diagram of the effect of hydrolysis time and temperature on the antioxidant activity of the total hydrolyzed protein of rice bran

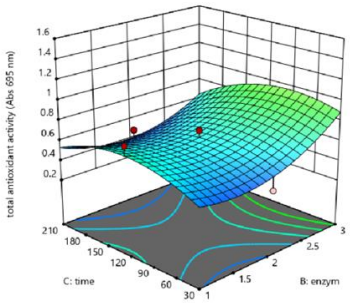

Figure 9. Effect diagram of enzyme concentration to substrate and hydrolysis time on antioxidant activity of hydrolyzed rice bran protein

5. Conclusion

Optimum conditions have been obtained using design expert software. Hydrolysis condition for hydrolyzed rice bran protein with optimal activity (DPPH free radical inhibitory power, Fe3+ iron inhibitory power and total antioxidant capacity) by Alcalase enzyme was that the optimum value was at 51.5 degrees Celsius, time 131.5 minutes and enzyme concentration to 3% substrate has been obtained. The maximum free radical inhibitory activity was 37.172%, the total antioxidant activity was 1.109% and the reductive activity was 2.084%. According to the results and antioxidant properties obtained from rice bran protein and the high production volume of this by-product, it can be used for the production of bioactive peptides and a useful product with antioxidant capacity can be produced as natural antioxidants. And also the combination of food and medicine should be proposed as a suitable solution for the prevention and treatment of related diseases.

6. Resources

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مقاله علمی_پژوهشی

بهینه سازی تولید پروتئین هیدرولیز شده با ویژگی آنتی اکسیدانی از سبوس برنج طارم توسط آنزیم آلکالاز

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۲/۷/۱۱

تاریخ پذیرش: ۱۴۰۲/۱۰/۷

کلمات کلیدی:

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DOI: 10.22034/FSCT.21.148.112.

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چکیده

پپتیدهای حاصل از هیدرولیز پروتئین دارای خواص زیست فعالی متعددی بوده و دارای فعالیت آنتی اکسیدانی قوی در برابر رادیکال های آزاد هستند و از فرآیندهای اکسیداسیون که سبب آسیب به ماکرومولکول‌های بیولوژیکی و تخریب و افت کیفیت غذا میگردد، جلوگیری می‌کنند. با توجه به حجم زیاد تولید برنج در دنیا، مقدار زیادی سبوس برنج تولید و در دسترس می باشد. با توجه به عنوان منبع پروتئینی مناسب و ارزان، سبوس برنج میتواند برای تولید پپتید با منشاء گیاهی مورد استفاده قرار گیرد. بهینه سازی شرایط هیدرولیز آنزیمی پروتئین سبوس برنج توسط آنزیم آلکالاز با هدف دستیابی به حداکثر خاصیت آنتی اکسیدانی صورت گرفت. به منظور بررسی فعالیت آنتی اکسیدانی پپتیدهای حاصله از آزمون های قدرت احیاکنندگی آهن۳ و سنجش فعالیت مهار کنندگی رادیکال های آزاد DPPH و فعالیت آنتی اکسیدانی کل استفاده شد. جهت بهینه سازی فرآیند از نرم افزار Design Expert و روش سطح پاسخ با سه متغیر مستقل : غلظت آنزیم به سوبسترا ۳ – ۱ درصد، دمای ۵۵ – ٤۰ درجه سلسیوس و زمان هیدرولیز ۲۱۰ – ۳۰ دقیقه انجام شد. تیمار بهینه در شرایط تعیین شده شامل دمای ۵۱.۵ درجه سلسیوس، زمان ۱۳۱.۵ دقیقه و غلظت آنزیم به سوبسترا ۳ درصد به دست آمد که دارای حداکثر مهارکنندگی رادیکال آزاد DPPH 37.172 درصد، فعالیت آنتی اکسیدانی کل ۱.۱۰۹ درصد و احیاکنندگی ۲.۰۸٤ درصد تعیین شده است. نتایج به دست آمده نشان داد است فرآیند هیدرولیز پروتئین سبوس برنج توسط آنزیم آلکالاز، منجر به تولید پپتیدهایی با ویژگی آنتی اکسیدانی بالا و قابل توجه شده که می تواند در تولید غذاهای فراسودمند و صنایع دارویی استفاده و همچنین جایگزین آنتی اکسیدان های سنتزی شود.