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Scientific Research

Optimization Microwave treatment on improving antioxidant properties corn gluten hydrolyzed

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ABSTRACT

The purpose of this research was to investigate the effect of hydrolysis time and microwave pretreatment on the enzymatic hydrolysis of corn gluten with alcalase enzyme for producing corn hydrolyzed with the highest antioxidant capacity. First, corn gluten powder was hydrolyzed at 90 watts with a ratio of enzyme to substrate of 1% and at the temperature of 50 ºC in optimization design by applying changes in the pretreatment time (1-2 minutes) and enzymatic hydrolysis time (30-360 minutes) compared with the control sample. The results show that increasing the microwave time increased the ability of free radicals and total oxidative composition in shorter hydrolysis times. Applying microwave pretreatment increased the degree of hydrolysis linearly compared to the sample. The highest level of free radical ability was observed in the samples treated and treated by microwave with 2 minutes in 195 minutes of hydrolysis, 55.40 and 59.63, respectively. The amount of total oxidative value equal to 1.01% was obtained for 2 minutes of microwave treatment and 360 minutes of enzymatic hydrolysis. The best pretreatment conditions to achieve the highest antioxidant properties were 2 minutes and 205 minutes of enzymatic hydrolysis. Therefore, microwave pretreatment with high power shortens the hydrolysis time to obtain peptides with higher oxidative resistance and increases the efficiency of enzymatic hydrolysis.

1-Introduction

In recent years, bioactive peptides derived from animal and plant sources have attracted the attention of researchers. These compounds are produced through microbial, enzymatic, and chemical methods. Among these, enzymatic hydrolysis is more common due to its milder conditions and the fact that it does not destroy amino acids [1]. Bioactive peptides can be obtained from various protein sources, including animal sources (milk, meat, eggs), marine sources (fish, shrimp, and shellfish), and plant sources (cereals, legumes, and oilseeds). Nowadays, plant sources have received more attention due to their lower cost [2]. The antioxidant properties of bioactive peptides derived from various protein sources, such as egg white and peanut, have been investigated [3, 4]. Corn is the third most consumed cereal grain in the world after wheat and rice. Corn gluten meal, with a protein content of 62–71% (w/w), is the main product of the corn wet-milling process [5]. Under the pH and acidity conditions prevalent in most food products, corn gluten meal has low solubility in aqueous systems due to its hydrophobic amino acids and disulfide bonds within its two protein components, namely zein and glutelin. Consequently, it is rarely used in the food industry [6]. Microwave is a fast, convenient, and cost-effective method for heating. Microwaves increase the degree of hydrolysis and solubility of hydrolyzed protein [7]. Enzymatic hydrolysis of proteins following microwave irradiation is a novel method to accelerate protein hydrolysis and improve their functional properties due to its effect on the spatial structure of proteins within a short period of time [8]. The biological changes that can occur in proteins after microwave treatment are a function of microwave intensity, and as a result of microwave energy absorption, changes occur in the chemical bonds of the secondary and tertiary structure of the protein [9]. Alain Gohi et al. (2019) investigated the effect of microwave pretreatment and optimization of lotus seed protein hydrolysis. In this study, lotus seed protein was first subjected to microwave treatment at 500–900 W for 15–210 seconds, and then hydrolyzed using papain enzyme at concentrations of 0.2–0.8 g/L [10]. The results showed that microwave treatment weakened the protein bond structure and led to an increase in protein hydrolysis. Microwave treatment at 800 W for 120 seconds had the greatest effect on lotus seed protein. Furthermore, an enzyme concentration of 0.5 g/L was the optimal papain concentration for hydrolyzing lotus seed protein. In the present study, the effect of microwave pretreatment on the antioxidant properties of hydrolyzed corn gluten protein was evaluated in order to achieve the highest antioxidant capacity.

2-Materials and Methods

Corn gluten meal was obtained from Nab Fructose Factory and ground using a mechanical mill, then sieved through a 50-mesh sieve. Subsequently, the corn gluten meal powder was stored in airtight packages at refrigerator temperature until the experiments were performed. All chemical materials and the enzyme Alcalase were purchased from reputable companies.

Determination of Chemical Composition

Chemical composition analysis was performed according to the AOAC method. Total protein content in the raw materials was determined using a Kjeldahl apparatus, fat content using a Soxhlet apparatus, ash content using an electric furnace, and moisture content by placing the sample in an oven at 105°C for 24 hours [11].

Preparation of Hydrolyzed Protein

For enzymatic hydrolysis using Alcalase, the method of Zhou et al. (2015) was employed [12]. An 1.8% (w/v) solution of corn gluten meal powder was prepared in potassium phosphate buffer (pH 8.0). Subsequently, to optimize the production of hydrolyzed proteins with maximum antioxidant activity, the prepared solution was subjected to microwave pretreatment at 90 W based on predefined treatments before enzyme addition (microwave power and time were selected based on preliminary tests and achieving a constant temperature of 70°C). The enzyme was then added at an enzyme-to-substrate ratio of 1% (based on preliminary tests), and hydrolysis was carried out at 50°C according to Table 1. After the completion of hydrolysis, to inactivate the enzyme, the solution was placed in a water bath at 85°C for 15 minutes. Each treatment was separately centrifuged in a refrigerated centrifuge at 5000 rpm and 4°C for 20 minutes. The resulting supernatant was dried using a freeze-dryer to obtain hydrolyzed protein powder. The obtained powder was then stored at -18°C until use. Subsequently, the degree of hydrolysis and antioxidant capacity of the hydrolyzed proteins were measured using DPPH radical scavenging activity, ferric ion reducing power, ferrous ion chelating activity, and total antioxidant capacity methods.

Determination of Degree of Hydrolysis

To measure the degree of hydrolysis, 5 mL of the sample was mixed with 5 mL of 20% trichloroacetic

acid (TCA). After vortexing, the mixture was centrifuged at 3750 rpm for 20 minutes. The protein content in the soluble phase was then measured using the Bradford method, and the degree of hydrolysis (DH) was calculated using the following equation [13].

$$DH = \frac{N2\ liquid\ in\ TCA\ 10\%}{Total\ N2\ in\ Sample} \times 100$$

Antioxidant Properties of Hydrolyzed Corn Gluten Protein

Total Antioxidant Capacity

First, 0.1 mL of the sample dissolved in distilled water (40 mg/mL) was mixed with 1 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a microtube (Eppendorf) and placed in a 90°C water bath for 90 minutes. After cooling, the absorbance of the samples was measured at 695 nm. Double-distilled water was used as a blank. Higher absorbance indicates greater total antioxidant capacity [14].

DPPH Radical Scavenging Activity

First, the hydrolyzed powders were dissolved in distilled water (40 mg/mL). Then, 1.5 mL of each sample was mixed with 1.5 mL of DPPH ethanolic solution (0.15 µM) and vortexed for 20 seconds. The resulting mixture was then centrifuged at 2500 rpm for 10 minutes and kept in the dark for 30 minutes. The absorbance of the supernatant solution was measured at 517 nm [15]. The DPPH radical scavenging percentage was calculated using the following equation:

$$\%) = [\frac{Ablank - Asample}{Ablank}]$$)Scavenging percentage × 100

Where A blank is the absorbance of the control sample (the same volume of distilled water instead of the sample mixed with DPPH solution) and A sample is the absorbance of the sample.

Ferric Reducing Power

First, the hydrolyzed powders were dissolved in distilled water (40 mg/mL). Then, 1 mL of each sample was 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 incubated at 50°C for 20 minutes. After that, 0.5 mL of 10% trichloroacetic acid (TCA) solution was added to the mixture, followed by centrifugation at 2500 rpm for 10 minutes. Then, 1 mL of the supernatant was mixed with 1 mL of distilled water and 0.2 mL of 0.1% (w/v) ferric chloride. Finally, the absorbance of the samples was measured at 700 nm after 10 minutes of incubation at room temperature. The same volume of distilled water was used instead of the sample to prepare the control sample. Increased absorbance of the reaction mixture indicates increased reducing power [16].

Ferrous Ion Chelating Activity

First, 1 mL of the sample dissolved in distilled water at a concentration of 40 mg/mL was mixed with 0.05 mL of ferric chloride solution (2 mM) and 1.85 mL of double-distilled water. Then, 0.1 mL of ferrozine solution (5 mM) was added and the mixture was vigorously shaken. The absorbance was measured at 562 nm after 10 minutes of incubation at room temperature. Double-distilled water was used as a blank [4]. The chelating activity of the samples was calculated using the following equation:

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

Chelating activity

Where A blank is the absorbance of the control sample (the same volume of distilled water instead of the sample mixed with the solution) and A sample is the absorbance of the sample.

Statistical Analysis

In this study, the effect of different hydrolysis times (30 to 360 minutes) on the antioxidant properties of hydrolyzed corn gluten protein, as well as the effect of pretreatment on antioxidant properties, was investigated using an optimization method based on a central composite design (CCD). The analysis was performed using Design Expert software version 9 according to Table 1.

3-Result

Table 1: Designing treatment hydrolysis corn gluten by microwave

Treatment	Microwave time (min)	Hydrolysis time (min)
1	0	30
2	2	30
3	0	360
4	2	360
5	0	195
6	2	195
7	1	30
8	1	360
9	1	195
10	1	195
11	1	195

Evaluation of Chemical Characteristics of Corn Gluten Meal Powder

Table 1- Chemical composition of Corn gluten powder

Chemical Composition	Amount
Protein (N× 6.25)	81.23 ± 0.17
Fat	2.2 ± 0.10
Moisture	7.14 ± 0.30
Ash	2.20 ± 0.10
Carbohydrate (by Difference)	7.23 ± 0.11

Data reported as dry basis
three replications ± Mena

Effect of Microwave Pretreatment on the Degree of Hydrolysis of Hydrolyzed Corn Gluten Protein

The degree of hydrolysis determines the size and amino acid composition of peptides, as well as the biological activity and flavor of hydrolyzed proteins. The effect of microwave pretreatment on the degree of hydrolysis of hydrolyzed corn gluten protein has been investigated in Figure 1.

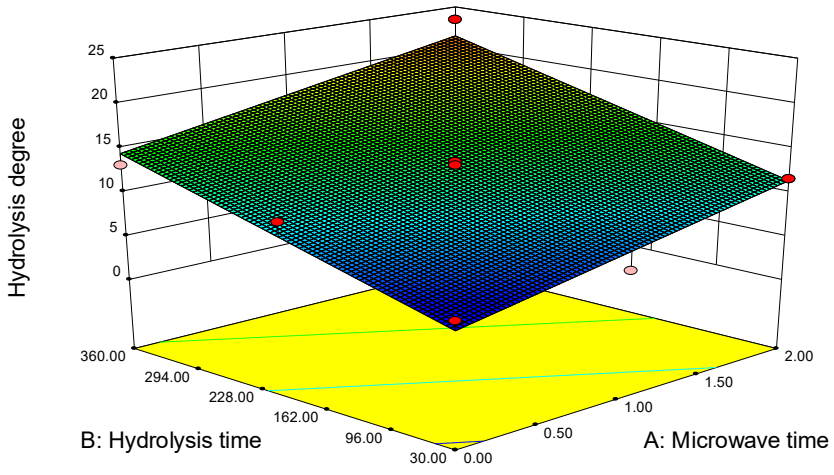

Figure 1: The effect of microwave time and hydrolysis time on the degree of hydrolysis of hydrolyzed corn gluten protein

According to Figure 1, with increasing hydrolysis time, the degree of hydrolysis of microwave-pretreated hydrolyzed corn gluten protein showed a significant increasing trend. Based on the results, the highest degree of hydrolysis (23.45%) was observed in the sample pretreated

with microwave for 2 minutes and hydrolyzed for 360 minutes, while the lowest degree of hydrolysis (5.45%) was observed in the control sample hydrolyzed for 30 minutes. It is likely that microwave pretreatment caused weakening of chemical and spatial bonds in the corn gluten protein structure, which increased enzyme accessibility to peptide bonds and consequently increased the degree of hydrolysis. Increased accessibility and enzymatic reaction time lead to a higher degree of hydrolysis, further cleavage of peptide bonds, shortening of peptide chain length, reduction in molecular weight distribution, and an increase in free amino acid content [17]. In a similar study, increasing the time and concentration of Alcalase increased the degree of hydrolysis of almond protein from 10% to 40% [4].

Effect of Microwave Pretreatment on the Total Antioxidant Capacity of Hydrolyzed Corn Gluten Protein

Total antioxidant capacity is a method for evaluating the antioxidant capacity of water-soluble and fat-soluble compounds. Compounds with electron-donating activity can terminate the radical chain and convert active free radicals into more stable products [18]. The effect of microwave pretreatment on the total antioxidant capacity of hydrolyzed corn gluten protein has been investigated in Figure 2.

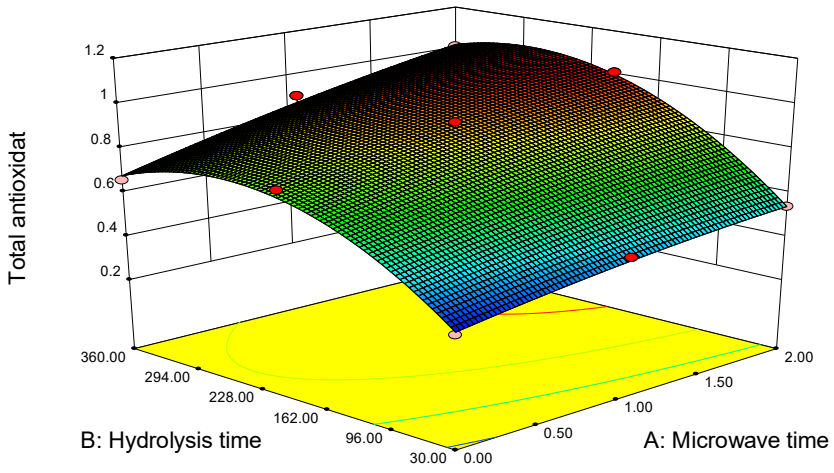

Figure 2: Effect of microwave time and hydrolysis time on total antioxidant activity of hydrolyzed corn gluten protein

According to Figure 2, with increasing microwave pretreatment time, the total antioxidant capacity of microwave-pretreated hydrolyzed corn gluten protein samples showed a significant increasing trend compared to the control sample. The highest and lowest total antioxidant capacity values were observed in the sample pretreated with microwave for 2 minutes and hydrolyzed for 360 minutes, and the control sample, with values of 1.01 and 0.36 (absorbance at 695 nm), respectively. Based on the results, it can be stated that with increasing microwave pretreatment time from 1 to 2 minutes at a constant thermal power of 90 W and increasing the degree of hydrolysis, the amount of low molecular weight peptides showed an increasing trend, and these hydrolyzed corn gluten proteins exhibited higher antioxidant capacity. Furthermore, the results in Figure 2 indicate that with increasing hydrolysis time from 200 to 360 minutes, the increase in antioxidant capacity of hydrolyzed corn gluten protein samples showed a very slow trend. The microwave heating process weakens chemical bonds (add reference). Increasing the enzymatic hydrolysis time increases the release of peptides with electron-donating properties, and these compounds are able to stabilize free radicals, which in turn increases the total antioxidant activity. Therefore, it can be concluded that there is a significant correlation between the degree of hydrolysis and total antioxidant activity to a large extent. These peptides can convert free radicals into stable compounds; consequently, with increasing hydrolysis duration, the total antioxidant activity of the samples increased [18]. The antioxidant activity of hydrolyzed proteins depends on the protein source, the type of hydrolysis method, the type of pretreatment, and some other factors [19].

Compared to their parent proteins, peptides exhibit better antioxidant activities due to the electron-rich peptide bonds generated by enzymatic hydrolysis and increased accessibility of the side chain functional groups to reactive species [20].

Effect of Microwave Pretreatment on DPPH Radical Scavenging Activity of Hydrolyzed Corn Gluten Protein

Measurement of DPPH radical scavenging activity is a reliable, accurate, simple, and cost-effective method with high reproducibility, which is used to evaluate the antioxidant activity of plant compounds under in vitro conditions. The effect of microwave pretreatment on the DPPH radical scavenging activity of hydrolyzed corn gluten protein has been investigated in Figure 3.

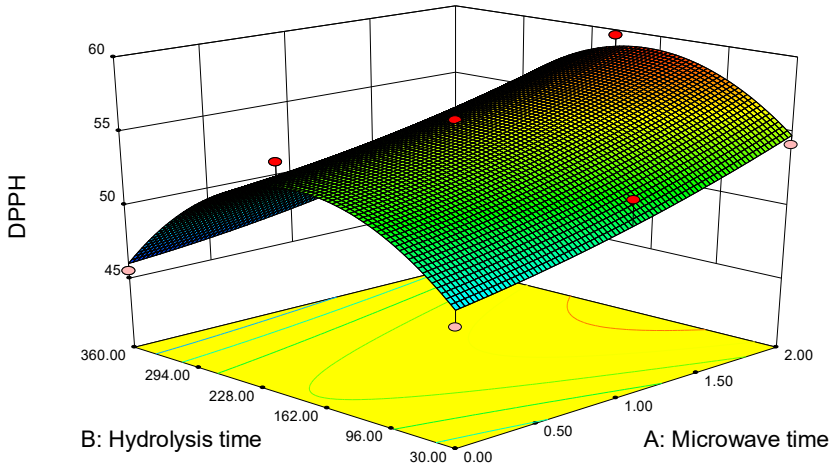

Figure 3: Effect of microwave time and hydrolysis time on DPPH radical scavenging (%) activity of hydrolyzed corn gluten protein

According to Figure 3, with increasing microwave pretreatment time from 1 to 2 minutes at a constant power of 90 W, the DPPH radical scavenging percentage of microwave-pretreated hydrolyzed corn gluten protein samples showed a significant increasing trend compared to the control sample. Based on the results, the highest and lowest DPPH radical scavenging percentages were observed in the sample pretreated with microwave for 2 minutes and hydrolyzed for 195 minutes (56.63%) and the control sample (45.5%), respectively. The results in Figure 3 indicate that with increasing hydrolysis time from 195 to 360 minutes, the increase in DPPH radical scavenging activity of hydrolyzed corn gluten protein samples showed a very slow trend. The microwave heating process, by weakening chemical bonds, improved the DPPH radical scavenging activity. One of the most important factors in radical scavenging activity is the position and number of hydroxyl groups that directly participate in oxidation-reduction reactions. Compounds with lower molecular weight have a greater ability to participate in oxidation-reduction reactions; therefore, these compounds have a greater capacity to transfer hydroxyl groups and exhibit higher DPPH radical scavenging activity [21]. Sarabandi et al. (2018) investigated the effect of enzymatic hydrolysis conditions of casein with pancreatin on the antioxidant and functional properties of hydrolyzed casein and reported that hydrolysis of casein with 2.5% pancreatin led to an increase in DPPH radical scavenging activity (from 11.13% to 42.17%) [22]. The hydrolysis process releases antioxidant peptides from the protein; however, increasing hydrolysis time and further enzyme action leads to the breakdown of some antioxidant peptides produced in the early stages of hydrolysis, consequently reducing DPPH radical scavenging activity [23]. After microwave treatment, the DPPH radical scavenging capacity of chia seeds increased by 35.13% compared to the control [24]. It is likely that the progression of hydrolysis and the action of the enzyme on peptides produced in the early stages leads to the degradation of peptides with high antioxidant activity, consequently reducing the antioxidant capacity produced in later stages.

Effect of Microwave Pretreatment on the Ferrous Ion Chelating Activity of Hydrolyzed Corn Gluten Protein

The effect of microwave pretreatment on the ferrous ion chelating activity of hydrolyzed corn gluten protein has been investigated in Figure 4.

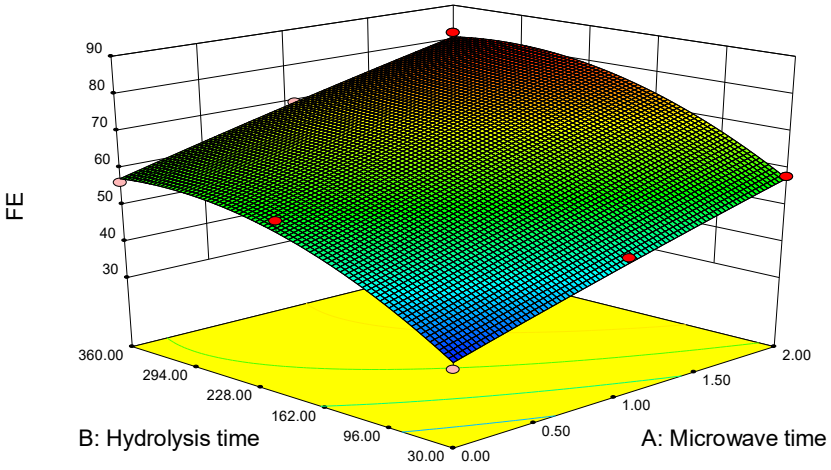

Figure 4: Effect of microwave time and hydrolysis time Iron chelating activity of hydrolyzed corn gluten protein

According to Figure 4, with increasing microwave pretreatment time from 1 to 2 minutes at a constant power of 90 W, the ferrous ion chelating activity of microwave-pretreated hydrolyzed corn gluten protein samples showed a significant increasing trend compared to the control sample. Based on the results, the highest and lowest ferrous ion chelating activity values were observed in the sample pretreated with microwave for 2 minutes and hydrolyzed for 360 minutes (81.96%) and the control sample (30.35%), respectively. Based on the results, it can be stated that with increasing microwave pretreatment time from 1 to 2 minutes at a constant thermal power of 90 W and increasing the degree of hydrolysis, there is a significant correlation between the degree of hydrolysis and ferrous ion chelating activity. The results in Figure 4 indicate that with increasing hydrolysis time from 195 to 360 minutes, the increase in ferrous ion chelating activity of hydrolyzed corn gluten protein samples showed a very slow trend. The use of pretreatment improved the effect of the enzyme on the substrate, and in this state, high molecular weight fractions of the protein are broken down into relatively small molecular weight fractions with electron-donating capacity, which improves antioxidant properties. However, with excessive progression of the degree of hydrolysis and the formation of very low molecular weight peptide fragments, the antioxidant activity of these protein compounds decreases [25]. Protease enzymes lead to the digestion of peptide bonds by breaking the bonds between hydrophobic amino acids and other amino acids [26]. The characteristics of the protease influence the size, quantity, free amino acid composition, peptides, and their amino acid sequence, which in turn affects the antioxidant activity of the hydrolysates. The chelating ability of hydrolyzed proteins is influenced by the type of enzyme, the degree of hydrolysis, and the amino acid structure of the parent protein [27]. Therefore, Alcalase likely produces peptides with an appropriate amino acid composition and the ability to chelate iron ions.

Effect of Microwave Pretreatment on the Ferric Reducing Power of Hydrolyzed Corn Gluten Protein

One of the most important characteristics of antioxidant compounds in food samples is their ferric reducing power. The effect of microwave pretreatment on the ferric reducing power of hydrolyzed corn gluten protein has been investigated in Figure 5.

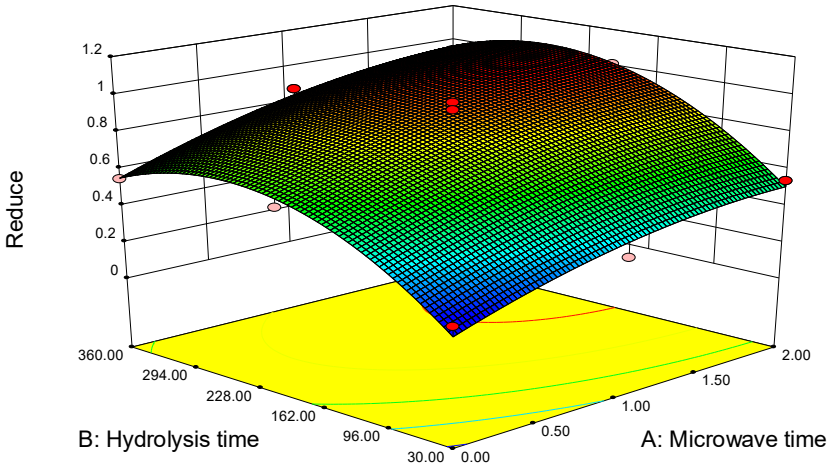

Figure 5: Effect of microwave time and hydrolysis time on Fe^{3+} reducing power of hydrolyzed corn gluten protein

According to Figure 5, with increasing microwave pretreatment time from 1 to 2 minutes at a constant power of 90 W, the reducing power of microwave-pretreated hydrolyzed corn gluten protein samples showed a significant increasing trend compared to the control sample. Based on the results, the highest and lowest ferric reducing power values of hydrolyzed corn gluten protein were observed in the sample pretreated with microwave for 2 minutes and hydrolyzed for 195 minutes (1.01) and the control sample (0.22), respectively. Increasing the enzymatic hydrolysis time of corn gluten protein led to an increasing trend in the amount of low molecular weight peptides, which in turn increased the reducing power. The increase in reducing power of hydrolyzed proteins can be attributed to the breakdown of the peptide chain, increased release of amino acids with antioxidant activity (such as tryptophan, methionine, lysine, histidine, and tyrosine), and free radical scavenging ability [22]. Enzymatic hydrolysis breaks the bonds adjacent to the amino acids phenylalanine, tyrosine, and tryptophan. As these peptide bonds are broken and these amino acids are released from the native protein structure, the molecular weight of the hydrolyzed proteins and peptides decreases [16]. Furthermore, increasing the hydrolysis time and progression of the hydrolysis process leads to further cleavage of peptide chains, resulting in an increase in peptides with electron-donating ability for the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) [28]. Increasing the duration of enzyme activity leads to increased release of peptides with electron-donating properties, which can convert free radicals into stable compounds. Consequently, with increasing hydrolysis duration, the total antioxidant activity of the samples increased [18].

Optimization of Hydrolyzed Corn Gluten Protein

Based on the analysis of the results and in order to achieve the highest level of antioxidant properties in hydrolyzed corn gluten protein samples, the highest antioxidant properties were observed under the following conditions: microwave pretreatment for 2 minutes at 90 W power and hydrolysis of corn gluten protein with Alcalase for 205 minutes (Figure 6). Replication of experiments to validate the output data of the software against reality confirmed these results.

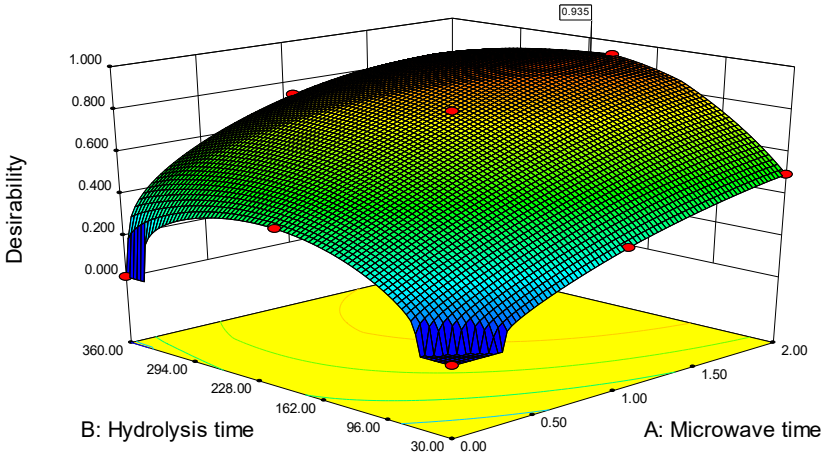

Figure 6: The selection of the proposed optimal treatment with the highest antioxidant activity for hydrolyzed corn gluten protein derived from the enzymatic hydrolysis using Alcalase enzyme

4-Conclusion

Due to the increasing prevalence of diseases, the use of natural compounds such as plant proteins, including corn gluten protein, has become more common. The use of enzymatic methods improves the antioxidant properties of hydrolyzed proteins; however, due to the high cost of enzymes, researchers are seeking to use pretreatments to reduce enzyme consumption and activity time in order to achieve the highest functional properties. The results of this study indicate that the use of microwave heating improved the antioxidant properties of corn gluten protein compared to the control sample. Increasing microwave pretreatment time caused a linear increase in the degree of hydrolysis. The highest antioxidant properties were observed under the following conditions: microwave pretreatment for 2 minutes at 90 W power and hydrolysis of corn gluten protein with Alcalase for 205 minutes. Therefore, the use of microwave as a pretreatment is an appropriate method to enhance antioxidant properties.

Conflict of Interest

There are none to declare.

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Author Contributions

All authors have contributed to the design, analysis, writing, and approval of the final manuscript in this manner

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مقاله علمی_پژوهشی

بهینه‌سازی شرایط پیش تیمار حرارتی ماکروویو بر بهبود ویژگی‌های آنتی‌اکسیدانی پروتئین هیدرولیز شده گلوتن ذرت

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چکیده

هدف از این پژوهش بررسی اثر زمان هیدرولیز و پیش تیمار ماکروویو بر هیدرولیز آنزیمی پروتئین گلوتن ذرت به‌وسیله آنزیم آلکالاز جهت تولید پروتئین هیدرولیز شده با قابلیت آنتی‌اکسیدانی‌بالا بود. ابتدا گلوتن ذرت به پودر تبدیل و سپس عمل هیدرولیز در توان ۹۰ وات با نسبت آنزیم به سوبسترا ۱ درصد و در دمای ۵۰ درجه‌سانتی‌گراد در قالب طرح بهینه‌سازی با اعمال متغیرهای زمان پیش تیمار (۲ – ۱ دقیقه) و زمان هیدرولیز آنزیمی (۳۶۰ – ۳۰ دقیقه) در مقایسه با نمونه شاهد صورت گرفت. نتایج نشان داد که افزایش زمان تیمار ماکروویو باعث افزایش قابلیت مهار رادیکال‌آزاد و ظرفیت آنتی‌اکسیدانی‌کل در زمان‌های هیدرولیز کوتاه‌تر گردید. اعمال پیش تیمار ماکروویو سبب افزایش خطی درجه هیدرولیز نسبت به نمونه شاهد شد. بالاترین میزان مهار رادیکال آزاد در نمونه‌های تیمارشده توسط ماکروویو با ۲ دقیقه در مدت زمان ۱۹۵ دقیقه هیدرولیز به‌ترتیب ۵۹/۶۳ مشاهده شد. حداکثر مقدار ظرفیت آنتی‌اکسیدانی‌کل برابر ۱/۰۱ درصد برای تیمار ماکروویو ۲ دقیقه و ۳۶۰ دقیقه هیدرولیز آنزیمی بدست آمد. با تحلیل نتایج بهترین شرایط پیش تیمار برای رسیدن به بالاترین ویژگی‌های آنتی‌اکسیدانی زمان ۲ دقیقه و مدت زمان ۲۰۵ دقیقه هیدولیز آنزیمی بود. بنابراین استفاده از پیش‌تیمار ماکروویو با توان بالا موجب کوتاه نمودن زمان هیدرولیز جهت دستیابی به پپتیدهای با قابلیت آنتی‌اکسیدانی بالاتر و افزایش کارآیی هیدرولیز آنزیمی می‌گردد.