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Preparation of isolated and hydrolyzed proteins from rice bran and study of their chemical composition and antioxidant capacity

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ABSTRACT
The present study aimed to prepare isolated and hydrolyzed proteins from Iraqi rice bran variety Amber 33 and study the antioxidant capacity. The results of chemical analysis of moisture, protein, fat, ash, and carbohydrates showed that they reached 8.41, 12.85, 18.39, 6.28, and 54.07% respectively for whole rice bran and 9.54, 18.20, 2.16, 9.14, 60.96% respectively for defatted bran and 3.21, 84.69, 1.3, 7.43, 3.54% respectively for isolated protein. The degree of degradation was observed to increase with the progression of degradation time and the HRBPI7 degradant achieved the highest degree of degradation reaching 34.73% after 120 minutes without the appearance of a bitter taste, the free radical inhibition capacity reached 51.72% outperforming all other degradation treatments while the RBPI protein isolate achieved a free radical inhibition capacity of 49.63% compared to what the synthetic antioxidant BHT achieved, which recorded a free radical inhibition capacity of 72.89%. The HRBPI7 degradant achieved a reducing power capacity of 49.86% outperforming all other degradation treatments and close to what the RBPI protein isolate achieved in terms of reducing power capacity, which reached 48.86% compared to what the synthetic antioxidant BHT achieved, which recorded a reducing power capacity of 63.89%. The HRBPI7 hydrolysate achieved a ferrous ion binding capacity of 61.06%, outperforming all other hydrolysis treatments and approaching the ferrous ion binding capacity achieved by the RBPI protein isolate, which reached 52.22%, compared to the synthetic antioxidant BHT, which recorded a ferrous ion binding capacity of 71.83%.

Keywords: biological properties, protein, Rice manufacturing, by-products, fat.

INTRODUCTION

Rice (Oryza sativa L.) is one of the world's major cereal crops and the second most important food crop in the world after wheat among more than 30 food crops (Yaoet al.,2020;MohammadAliet al., 2024). Rice is an annual plant, native to the continent of Asia, especially India, Vietnam, Indonesia, and China. The production of these countries is estimated at 50% of the total global production of rice (Hammoud and Saddam, 2024; Whaib and Mousa, 2022), in addition to its cultivation in many countries of the world, including countries in the Mediterranean Sea, which produces about 26% of grain production and about 20% of the total grain trade in the world (Jasim and Nasser 2020; Kareem and Shakir 2016). As for Iraq, rice cultivation has spread in many central and southern governorates, the local production of this crop in the years 2009, 2010, 2011, and 2012 amounted to about 3,720, 3,506, 4,270, and 3,513 million tons respectively (Abood andMohammed, 2017;Shamuradet al., 2019).

The composition of the whole grain of rice consists of 63-73% carbohydrates, 1-2% fat, 6-8% protein, 7-10% fiber and 3%. -5% ash and rice grains contain 3 main parts including the endosperm (~70%), the hull (~20%), and the bran (~10%) (Zhaoet al.,2018;AbadiandNaser,2019;Najamuddinet al.,2021).

Bran is one of the by-products that result from the rice manufacturing process, as it produces three main parts: bleached rice, bran, and hull (Xiaoet al.,2020; Xuet al.,2021). Bran is produced in large quantities during the rice milling process and constitutes about 8-10% of rice grains and, is not sufficiently exploited for many activities. It can be used as animal feed. Thus, more than 80 million tons of bran are the byproduct of rice milling worldwide (Zulfafamyet al., 2018;Yuwanget al., 2018;Shuet al.,2021). Rice bran contains many components such as phenolics, γ-oryzanol, tocopherols, and tocotrienols, along with other nutritional components that are beneficial for health (AL-Abdulla and AL-Nasery, 2021;Puniaet al.,2021). It also contains a high percentage oftotal protein (11-15%), about 20% of fat, and an excellent source of fiber (7-11%). In addition, rice bran contains much more nutrients and vitamins than other parts of rice. (Rahman, 2018; Andrianiet al.,2022;Jasim and Nasser 2023).

Recently, the potential biological properties of rice bran have been studied, including anti-inflammatory, anti-cancer, antibacterial, and antioxidant properties (Udenigwe, 2016;Sapna and Jayadeep, 2021;Sapwarobolet al.,2021). These functional properties of rice bran indicate that it is ideal for commercial uses in the food and beverage industry, as well as in the food and pharmaceutical industries (Amarowiczand Pegg, 2019; Wasanet al.,2022).
Yamwangmorn and Prum (2021) pointed out that the antioxidant capacity of rice bran is ideal for commercial uses in the food and beverage industry, as well as the food and pharmaceutical industries. Based on this, the present study aims to investigate the chemical composition of defatted rice bran and protein isolate from Iraqi rice bran variety Anbar 33 and the antioxidant capacity of the isolate and protein hydrolysates, which may help to determine their potential use in food products.

MATERIALS AND METHODS

Rice bran samples

Rice bran variety Amber 33 was obtained from the Agricultural Research Department/Rice Research Station in Al-Mishkhab.

Preparing of defatted rice bran (DRB)

Fat was extracted from rice bran according to Mao and Hua (2012) by treating the rice bran with hexane at a ratio of 1/10 (weight/volume) with continuous stirring for three hours. The mixture was filteredusing a Buechner funnel and filter paper No. 1, The hexane was eliminated using a rotary evaporator. The extraction process was repeated until the hexane wasclear. The extraction product was collected are dried, then ground and sieved using a No. 60 mesh sieve, and stored at -20°C until use.

Preparation of protein isolate from defatted rice bran (DRBPI)

The method mentioned by Cho (2020) was used with some modifications. Mix the sample with distilled water at a ratio of 1:10 and place it on a magnetic stirrer for an hour. Adinst the pH of the suspension to 9, and mix for 1 h using a magnetic mixer centrifugated the suspension at 5000 rpm for 20 min at 4 °C. The pH of the filtrate was adjusted to 4.5, the residuewas washed three times with distilled water. The protein was dried and stored until.

Enzymatic hydrolysis of rice bran protein isolate

Preparation of isolated rice bran protein hydrolysate using pepsin enzyme

The isolated rice bran hydrolysate was prepared using pepsin enzyme according to Chatterjee et al., (2015) with some modification. The isolated rice bran protein was mixed with 0.1 M hydrochloric acid at a ratio of 20:1 (w/v) and incubated at 50 °C for 1 h. The pH was then adjusted to 2 using NaOH (1) M, and incubated in a Water bath at 37 °C for 15 min, followed by the addition of enzyme at 1000, 2000, and 3000 U/g of the isolated rice bran protein. The digested material was taken at different times (15, 30, 60, 90, 120, 180, 240, 300, 360) min and the enzyme action was stopped by raising the pH to 7 and the Centrifugation was performed at 5000xg for 15 min to control the lift and it was stored at -18°C.

Preparation of isolated rice bran protein hydrolysate using trypsin enzyme

Rice bran protein isolate hydrolysate was prepared using trypsin enzyme according to Liu and Chiang, (2008) with some modification. Rice bran protein isolate was mixed with distilled water at a ratio of 20:1 (w/v) and the pH was adjusted to 8, and then incubated at 50 °C for 1 h. The mixture was then placed in a water bath at 37 °C for 15 min, followed by enzyme addition at 1000, 2000, and 3000 units/g of rice bran protein isolate. Then, the digested samples were taken at time intervals (15, 30, 60, 90, 120, 180, 240, 300, 360) minutes. The enzyme action was stopped using a boiling water bath for 5 minutes and centrifugation at 5000 xg for 15 minutes and the filtrate was collected and stored at -18 °C until use.

Preparation of isolated rice bran hydrolysate using synergistic enzymes pepsin and trypsin

Rice bran protein isolate was prepared synergistically with pepsin and trypsin enzymes. The protein solution was prepared according to paragraph (3-8-1). Pepsin enzyme was used at 2000 enzyme units per 1 g of rice bran protein isolate. After the end of the decomposition period, the pH was adjusted to 8 and trypsin enzyme was added at a concentration of 2000 enzyme units per 1 g of rice bran protein isolate. Then, the digested samples were taken at time intervals (15, 30, 60, 90, 120, 180, 240, 300, 360) minutes and the enzyme action was stopped using a boiling water bath for 5 minutes and centrifugation at 5000 xg for 15 minutes. The filtrate was collected and stored at -18 °C until use.

Determination of Degree of Hydrolysis (DH)

The degree of decomposition was measured according to Liu and Chiang, (2008) with some modifications as follows:

Preparation of the standard curve for the amino acid leucine

The stock solution (55 mM) was prepared by taking 0.361 g of the amino acid leucine transferring it to a 50 ml volumetric flask and filling the volume to the mark. Then the required concentrations were made as shown in the table below:

Table 1: Concentrations of the amino acid L-leucine used in preparing the standard curve to estimate the degree of degradation:

Concentration (mM)	Stock solution (µl)	DW(µl)	Final volume(µl)
0	0	1000	1000
5	91	909	1000
15	273	727	1000
25	455	545	1000
35	636	364	1000
45	818	182	1000
55	1000	0	1000

Mix 0.250 ml of each of the above dilutions with 2 ml of 1% SDS solution, 2 ml of 0.2125 M sodium phosphate buffer, pH 8.2, and 2 ml of 1% TNBS solution. The mixture was incubated at 50 °C for 1 h in isolation from light, after which the reaction was stopped by adding 4 ml of 0.1 M hydrochloric acid solution. The tubes were allowed to cool at room temperature (2±30 °C) for 30 min, the absorbance was measured at 340 nm, and a standard curve was drawn between the amino acid concentration and the reading obtained at the above wavelength.

As for the samples under study, 0.250 ml were transferred to a glass tube and subjected to the above work steps. The terminal NH3 groups were calculated using the standard curve for the amino acid leucine (Figure 1) and the degree of decomposition was calculated according to the equation below, described by (Jamdar et al., 2010):

$$\mathbf{DH} = [(\mathbf{L_t} - \mathbf{L_0})/(\mathbf{L_{MAX}} - \mathbf{L_0})] \times \mathbf{100}$$

L_t= amount of terminal amino acids at time.

L_0= the amount of amino acids present in the original protein sample.

L_{MAX} = the amount of total amino acids in the undigested sample that can be obtained after acid hydrolysis with 6 N hydrochloric acid at 120 °C for 24 h.

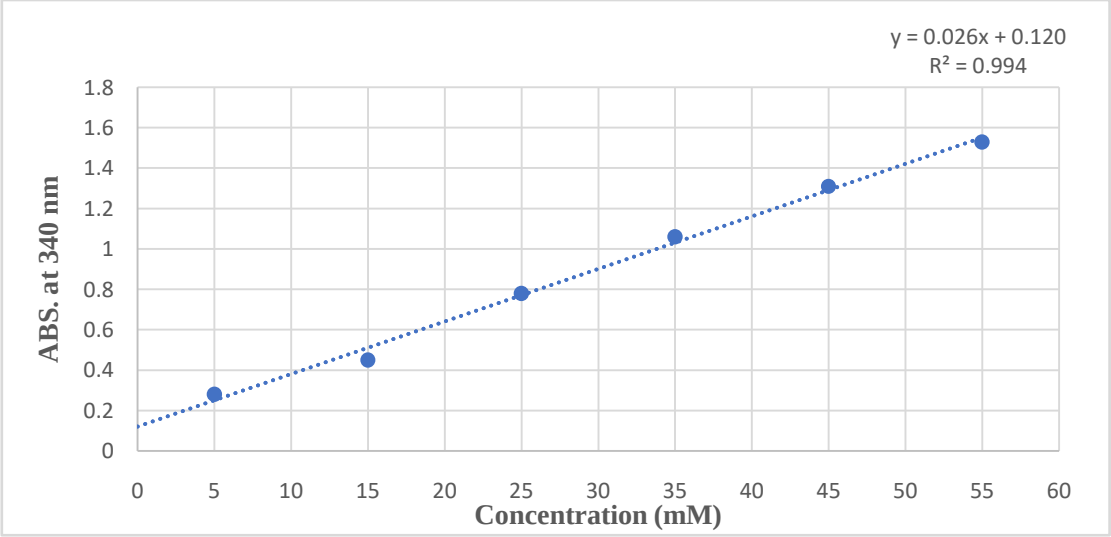


Figure 1: Standard curve for the amino acid L-Leucine

Measurement of antioxidant activity

Radical-Scavenging Activity (DPPH)

The free radical scavenging ability was measured according to Laohakunjitet al., (2017) with some modification. 0.1 ml of the studied sample (concentration 3 mg/ml) was mixed with 0.9 ml distilled water and 1

ml DPPH solution prepared at 0.1 mM (ethanol 99%). The mixture was left in the dark at room temperature (2±30 °C) for 30 min and centrifuged at 10000 xg for 5 min. After that, the absorbance was measured at 517 nm. The synthetic antioxidant Butylated hydroxyl toluene (BHT) prepared at 0.1 mg/ml was used as a comparison sample. The percentage of free radical scavenging ability of the samples was calculated using the following equation:

Free Radicals scavenging activity=$[\mathbf{C} - (\mathbf{B} - \mathbf{A})/\mathbf{C}] \times \mathbf{100}$

A = Absorbance reading of the sample under study at a wavelength of 517 nm
B = Absorbance reading of control sample at 517 nm (prepared by mixing 1 ml of sample with 1 ml of ethanol)
C = Light absorbance reading of the control sample at a wavelength of 517 nm (prepared by mixing 1 ml of distilled water with 1 ml of DPPH solution)

Reductive power estimation

The reducing power of the protein hydrolysates of the rice bran protein isolate was estimated according to Li et al., (2015). 1 ml of the sample was taken and mixed with 2.5 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%) solution. The mixture was kept at 50°C for 20 min. 1 ml of trichloroacetic acid (10%) solution was added and centrifugation was carried out at 10000 xg for 10 min. 2.5 ml of the clear mixture was taken and 2.5 ml of distilled water and 300 µl of ferric chloride FeCL3 (1%) solution were added. The optical absorbance was measured at a wavelength of 700 nm. The control sample was prepared using distilled water instead of the sample. The comparison sample was prepared (BHT) at a concentration of 3 mg/ml and the same steps were carried out, as the higher the absorbance, the higher the reducing power.

Metal bonding ability

The metal binding capacity of the protein hydrolysates of isolated and hydrolyzed rice bran proteins was estimated according to Boyer and Mccleary, (1987). 4.7 ml of the sample was taken and mixed with 0.1 ml of 2 mM ferrous chloride FeCL2 solution, and 0.2 ml of 5 mM ferrozine solution, the mixture was left for 20 min at room temperature, and the absorbance was measured at a wavelength of 562 nm, distilled water was used instead of the sample when preparing the reaction mixture of the control sample. The metal binding capacity was calculated according to the following equation:

Chelating activity (%) $=[(\mathrm{B} - \mathrm{A})/\mathrm{B}] \times 100$

A = Absorbance reading of the sample at a wavelength of 562 nm
B = Absorbance reading of the control sample at a wavelength of 562 nm

Statistical analysis

The ready-made statistical program GenStat v.12.1 (GenStat, 2009) was used in data analysis to study the effect of different factors on the studied traits according to a completely randomized design (CRD), and the significant differences between the means were compared with the least significant difference (Least Significant Difference-LSD).

RESULTS AND DISCUSSION

Chemical analysis

The chemical composition of whole, defatted, and protein isolate rice bran was analyzed, and is shown in Table 2.

Table 2: Chemical composition of whole rice bran, defatted rice bran, and protein isolate.

Components%	WRB	DRB	PIRB
Moistuer	8.41b	9.54c	3.21a
Protein	12.85c	18.20b	84.69a
Fat	18.39c	2.16b	1.13a
Ash	6.28b	9.14a	3.54c
Carbohydrate	54.07b	60.96a	7.43c

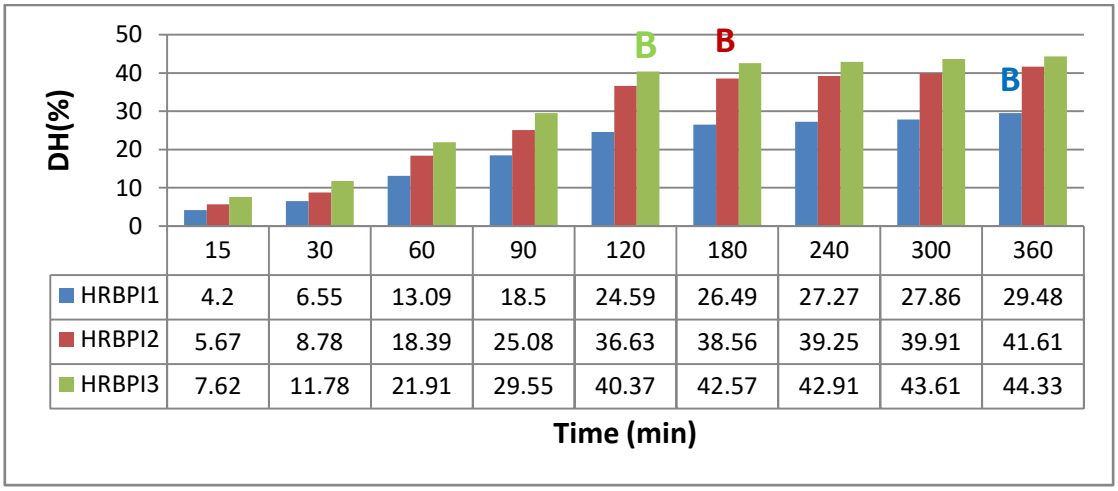
WRB:Wholerice bran, RBD: defatted Ricebran, RBPI:Ricebran Proteinisolate.

The percentage of moisture, protein, fat, ash, and carbohydrates were 8.41, 12.85, 18.39, 6.28, and 54.07%, respectively, in whole rice bran and 9.54, 18.20, 2.16, 9.14, and 60.96% respectively in the defatted rice bran and 3.21, 84.69, 1.3, 3.54, and 7.43%,respectively in the protein isolate.

These results are close or consistent with some components of previous related studies and may differ. Wisetkomolmatet al., (2022) findings indicated that the percentage of moisture, protein, fat, and ash reached 9.54%, 12.82%, 16.48%, and 9.35%, respectively, for whole rice bran. Kalschneet al., (2020) reported that the

percentage of moisture, protein, fat, ash, and carbohydrates reached 11.28%, 14.14%, 10.40%, 5.40%, and 54%, respectively, for whole rice bran.
While Abadi and Naser (2019) found that the percentage of protein, fat, ash, and carbohydrates ranged between 18.16-19.05%, 0.54-0.57%, 9.51-10.15%, 52.51-60.01% respectively in the defatted bran. Wang et al., (2015)found, that the percentage of moisture, protein, fat, ash, and carbohydrates reached 8.58%, 12.45%, 26.59%, 8.57%, and 43.73%, respectively, for whole rice bran, and 7.31%, 15.59%, 5.96%, 7.43%, 62.94%, respectively for defatted rice bran.
Jasim and Nasser (2020) stated that the percentage of moisture, protein, fat, ash, and carbohydrates reached 10.31%, 19.3%, 0.89%, 12.3%, and 41.07%, respectively, in defatted rice bran, and 3.82%, 77.62%, 0.75%, 4.98%, and 10.27%, respectively in protein isolate, Casas et al., (2019) reported that the protein and ash was 18.63, 13.97%, respectively, in defatted rice bran.

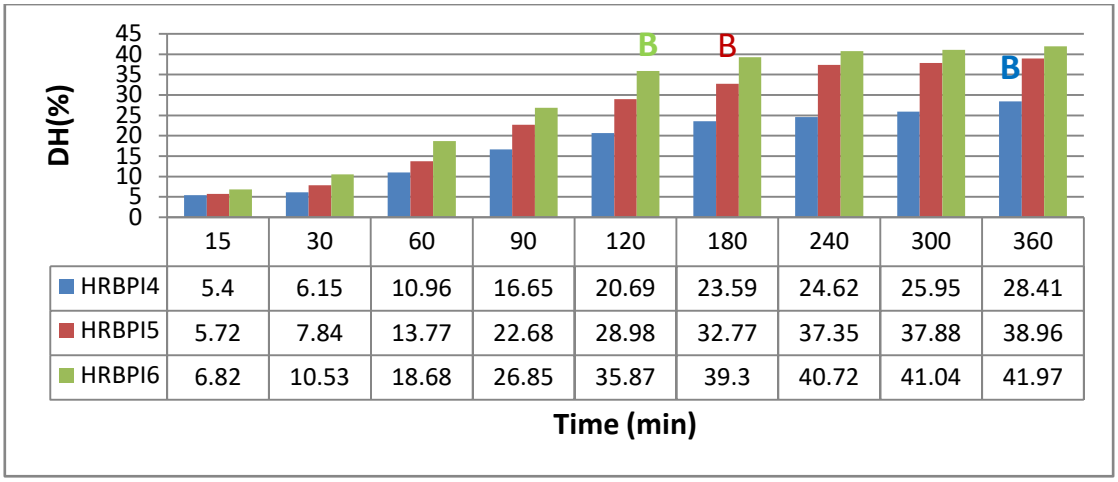
Enzymatic hydrolysis of proteins
Figure 2 shows the degrees of degradation of rice bran protein isolate treated with pepsin enzyme and enzyme concentrations (1000, 2000, 3000) enzyme units/g isolate, symbolized by (HRBPI1, HRBPI2, HRBPI3), respectively. Different periods were used to estimate the degree of degradation (15, 30, 60, 90, 120, 180, 240, 300, 360) minutes, as it was observed that the degree of degradation increased with the increase in the enzyme concentrations used and with the advancement of the degradation time. The degree of degradation of HRBPI1 treatment was (4.2, 6.55, 13.09, 18.5, 24.59, 26.49, 27.27, 27.86, and 29.48)% respectively, while HRBPI2 treatment was (5.67, 8.78, 18.39, 25.08, 36.63, 38.56, 39.25, 39.91, 41.61)% respectively, while HRBPI3 treatment was (7.62, 11.78, 21.91, 29.55, 40.37, 42.57, 42.91, 43.61, 44.33)% respectively.



	15	30	60	90	120	180	240	300	360
HRBPI1	4.2	6.55	13.09	18.5	24.59	26.49	27.27	27.86	29.48
HRBPI2	5.67	8.78	18.39	25.08	36.63	38.56	39.25	39.91	41.61
HRBPI3	7.62	11.78	21.91	29.55	40.37	42.57	42.91	43.61	44.33

Figure 2: The degrees of degradation of the rice bran protein isolate treated with pepsin enzyme and enzyme concentrations (1000, 2000, 3000) units/g isolated at a temperature of 37°C and a pH of 2. The results represent the average of two replicates. The letter B indicates the time of the appearance of the bitter taste in the protein hydrolysate.

The appearance of bitter taste was observed in the hydrolyzed rice bran protein treated with pepsin enzyme after (360, 180, 120) minutes from the start of the enzyme treatment and at the indicated concentrations. The treatment represented by adding pepsin enzyme at a rate of 2000 units/g isolated was chosen based on the amount of enzyme added, the degree of degradation and antioxidant capacity reached, and the time of appearance of bitter taste.
Figure 3 shows the degrees of degradation of rice bran protein isolate treated with Trypsin enzyme and enzyme concentrations (1000, 2000, 3000) enzyme units/g isolate, symbolized by (HRBPI4, HRBPI5, HRBPI6), respectively. Different periods were used to estimate the degree of degradation (15, 30, 60, 90, 120, 180, 240, 300, 360) minutes, as it was observed that the degree of degradation increased with the increase in the enzyme concentrations used and with the advancement of the degradation time. The degree of degradation of HRBPI4 treatment reached (5.4, 6.15, 10.96, 16.65, 20.69, 23.59, 24.62, 25.95, 28.41)% respectively, while HRBPI5 treatment reached (5.72, 7.84, 13.77, 22.68, 28.98, 32.77, 37.35, 37.88, 38.96)% respectively, while HRBPI6 treatment reached (6.82, 10.53, 18.68, 26.85, 35.87, 39.30, 40.72, 41.04, 41.97)% respectively.

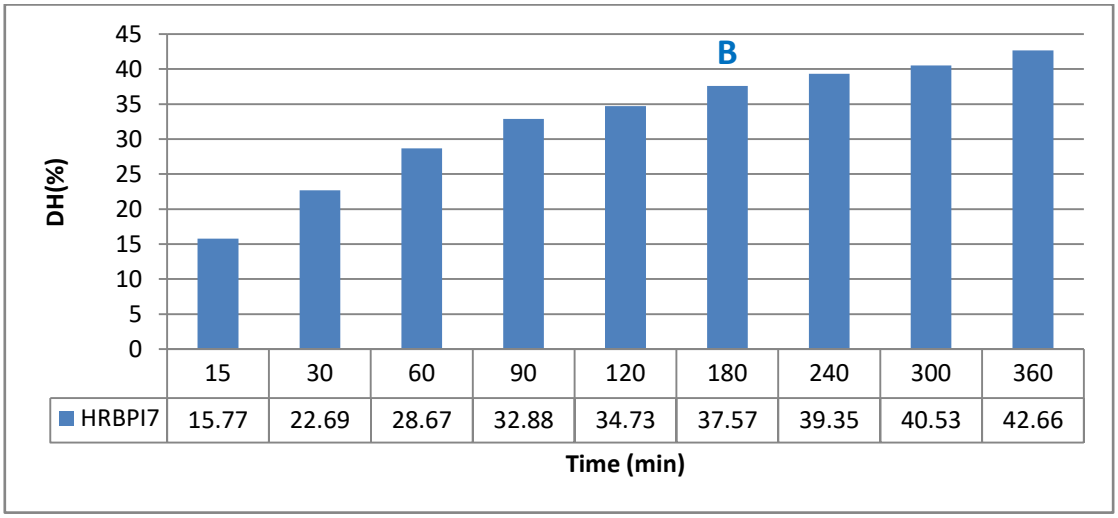


	15	30	60	90	120	180	240	300	360
HRBPI4	5.4	6.15	10.96	16.65	20.69	23.59	24.62	25.95	28.41
HRBPI5	5.72	7.84	13.77	22.68	28.98	32.77	37.35	37.88	38.96
HRBPI6	6.82	10.53	18.68	26.85	35.87	39.3	40.72	41.04	41.97

Figure 3: The degrees of degradation of the rice bran protein isolate treated with trypsin enzyme and enzyme concentrations (1000, 2000, 3000) units/g isolated at 37°C and a pH of 8. The results represent the average of two replicates. The letter B indicates the time of the appearance of the bitter taste in the protein hydrolysate.

The appearance of bitter taste was observed in the hydrolyzed rice bran protein treated with the Trypsin enzyme after (360, 180, 120) minutes from the start of the enzyme treatment and at the indicated concentrations. The treatment represented by adding the Trypsin enzyme at a rate of 2000 units/g isolate was chosen based on the amount of enzyme added, the degree of degradation and antioxidant capacity reached, and the time of appearance of bitter taste.

Figure 4 shows the degrees of degradation of rice bran protein isolate treated with Pepsin and Trypsin enzymessynergistically and with enzyme concentrations (2000) enzyme units/g isolate for each enzyme, symbolized by (HSPI7), respectively. Different periods were used to estimate the degree of degradation (15, 30, 60, 90, 120, 180, 240, 300, 360) minutes. The degree of degradation for HRBPI7 treatment reached (15.77, 22.69, 28.67, 32.88, 34.73, 37.57, 39.35, 40.53, and 42.66) %, respectively, as it was observed that the degree of degradation increased with the advancement of the degradation time. It was observed that the bitter taste appeared in the treated rice bran protein hydrolysate after (120) minutes from the start of the enzymatic treatment.



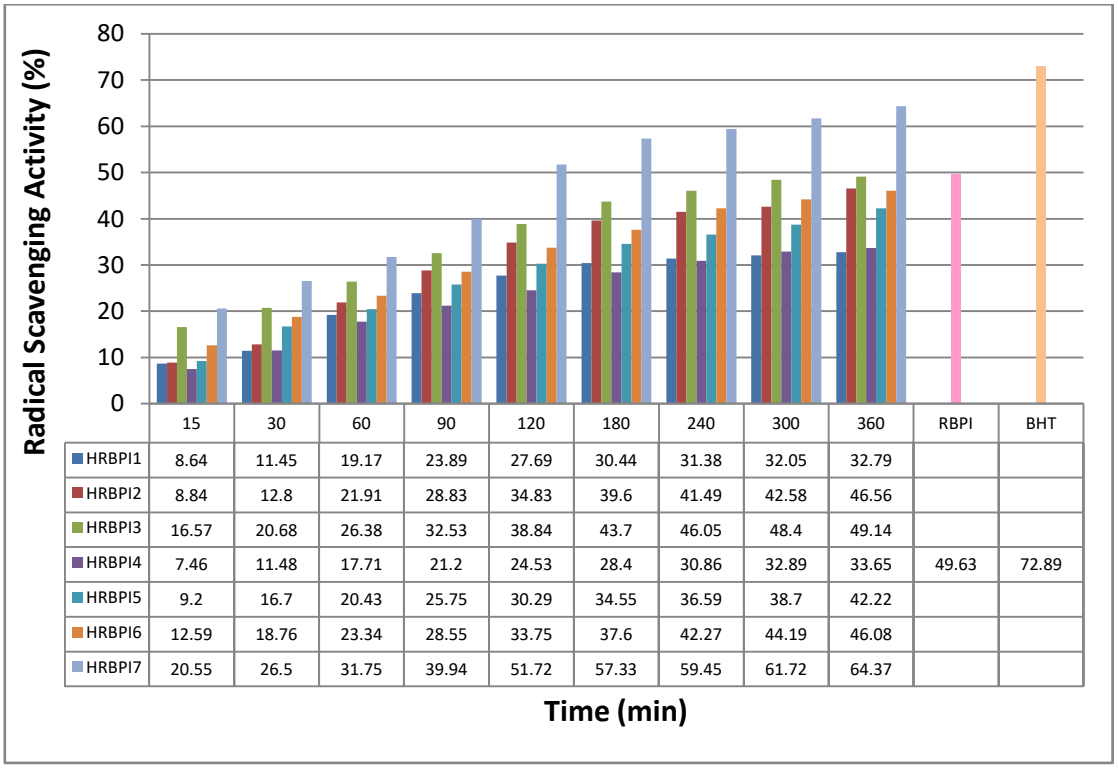
	15	30	60	90	120	180	240	300	360
HRBPI7	15.77	22.69	28.67	32.88	34.73	37.57	39.35	40.53	42.66

Figure 4: The degrees of degradation of the rice bran protein isolate treated with pepsin and trypsin enzymes synergistically. The results represent the average of two replicates. The letter B indicates the time of the appearance of the bitter taste in the protein hydrolysate.

Zaky et al., (2020) studied the digestion of rice bran protein concentrate (RBPC) using four commercial enzymes, Alcalase, Trypsin, Protamex, and Flavourzyme at different digestion times (2, 4, 6) hours. The degree of hydrolysis was estimated and the results showed that DH varies depending on the type of enzyme used, its concentration, and the duration of hydrolysis. All enzymes recorded a high degree of enzymatic hydrolysis throughout the first two hours and then the rates of increase in values decreased after that.

Singh et al, (2019) indicated that the degree of degradation of protein concentrate from rice bran using papain enzyme at different concentrations (0.020, 0.025, 0.030, 0.035, 0.040) w/w and at times (60, 90, 120, 150, 180) minutes ranged between (14.54-34.13)%.
Phongthaiet al, (2016) found that the degree of hydrolysis (DH) of protein concentrate from alcalase-treated rice bran increased rapidly during the initial 60 min, then decreased slightly before maintaining a constant DH of 15.04% until the end of hydrolysis. To produce protein hydrolysates with DH of 5.04, 10.37, and 15.04%, for periods of 5, 50, and 270 min.

Antioxidant activity
Radical-Scavenging Activity (DPPH)
Figure 5 shows the ability of free radical scavenging using DPPH for the protein isolate and the hydrolysates under study (HRBPI1, HRBPI2, HRBPI3, HRBPI4, HRBPI5, HRBPI6, HRBPI7) compared with the synthetic antioxidant BHT. It was observed that the ability of free radical scavenging for all protein hydrolysates increases with increasing degradation time.



	15	30	60	90	120	180	240	300	360	RBPI	BHT
HRBPI1	8.64	11.45	19.17	23.89	27.69	30.44	31.38	32.05	32.79		
HRBPI2	8.84	12.8	21.91	28.83	34.83	39.6	41.49	42.58	46.56		
HRBPI3	16.57	20.68	26.38	32.53	38.84	43.7	46.05	48.4	49.14		
HRBPI4	7.46	11.48	17.71	21.2	24.53	28.4	30.86	32.89	33.65	49.63	72.89
HRBPI5	9.2	16.7	20.43	25.75	30.29	34.55	36.59	38.7	42.22		
HRBPI6	12.59	18.76	23.34	28.55	33.75	37.6	42.27	44.19	46.08		
HRBPI7	20.55	26.5	31.75	39.94	51.72	57.33	59.45	61.72	64.37		

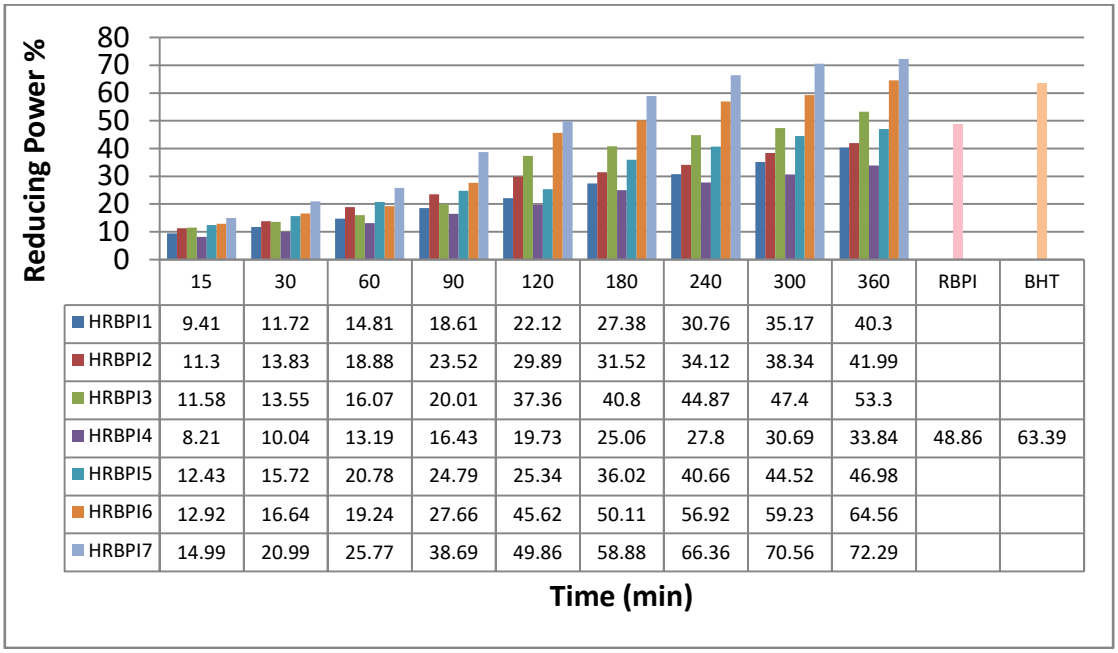
Figure 5: Free radical scavenging capacity of isolated rice bran protein hydrolysates estimated using DPPH assay, symbols represent isolated rice bran protein hydrolysates treated with enzymes: (HRBPI1) pepsin (u/gm1000), (HRBPI2) pepsin (u/gm2000), (HRBPI3) pepsin (u/gm3000), (HRBPI4) trypsin (u/gm1000), (HRBPI5) trypsin (u/gm2000), (HRBPI6) trypsin (u/gm3000), (HRBPI7) pepsin and trypsin synergistically (u/gm2000).

The results of the elected protein solutions show depending on the degree and time of the decomposition, which showed the highest effectiveness of curbing free radicals without the appearance of bitter taste, the HRBPI7 has achieved a degree of decomposition of 43.73 % and with a decomposition time of 120 minutes, recording a curriculum for free radicals amounting to 51.72 %, outperforming all transactions of decomposition The other, while the RBPI isphalin is the ability to curb free radicals of 49.63 % compared to what was achieved by the BHT antioxidant, which recorded the ability to curb free radicals of 72.89 %, through what was mentioned in the number of HRBPI7, as an elected treatment.
Liu et al., (2019) reported that the difference in the free radical scavenging ability of proteolytic could be attributed to the different degrees of enzymatic degradation, the type of resulting peptides, their molecular weight, size, and conformation, as well as the type and sequence of amino acids.
The results of this study were consistent with Yan et al., (2015) when studying the antioxidant property of peptides with molecular weight (3, 10) kDa isolated from rice residue hydrolysate treated with Alcalase, Flavourzyme, Protamex, Pepsin, Papain, Trypsin enzymes individually and synergistically. The hydrolysate treated with Papain, Flavourzyme, and Protamex enzymes synergistically gave an inverse relationship between

the ability to inhibit free radicals and molecular weight, as peptides with a molecular weight of 3 kDa outperformed peptides with a molecular weight of 10000 kDa.

Reductive power

Figure 6 shows the reductive power of each of the degraders (HRBPI1, HRBPI2, HRBPI3, HRBPI4, HRBPI5, HRBPI6, HRBPI7). It was observed that the reductive power of all protein degraders increases with the progression of the decomposition time and the increase in the degree of decomposition for each treatment separately. This is due to the role of the enzymatic treatment in increasing the amount of amino acids exposed to the medium as the decomposition process progresses, which in turn has the ability to donate hydrogen ions or electrons, which is positively reflected in the reductive power of the protein degrader (Zhang et al., 2019).



	15	30	60	90	120	180	240	300	360	RBPI	BHT
HRBPI1	9.41	11.72	14.81	18.61	22.12	27.38	30.76	35.17	40.3		
HRBPI2	11.3	13.83	18.88	23.52	29.89	31.52	34.12	38.34	41.99		
HRBPI3	11.58	13.55	16.07	20.01	37.36	40.8	44.87	47.4	53.3		
HRBPI4	8.21	10.04	13.19	16.43	19.73	25.06	27.8	30.69	33.84	48.86	63.39
HRBPI5	12.43	15.72	20.78	24.79	25.34	36.02	40.66	44.52	46.98		
HRBPI6	12.92	16.64	19.24	27.66	45.62	50.11	56.92	59.23	64.56		
HRBPI7	14.99	20.99	25.77	38.69	49.86	58.88	66.36	70.56	72.29		

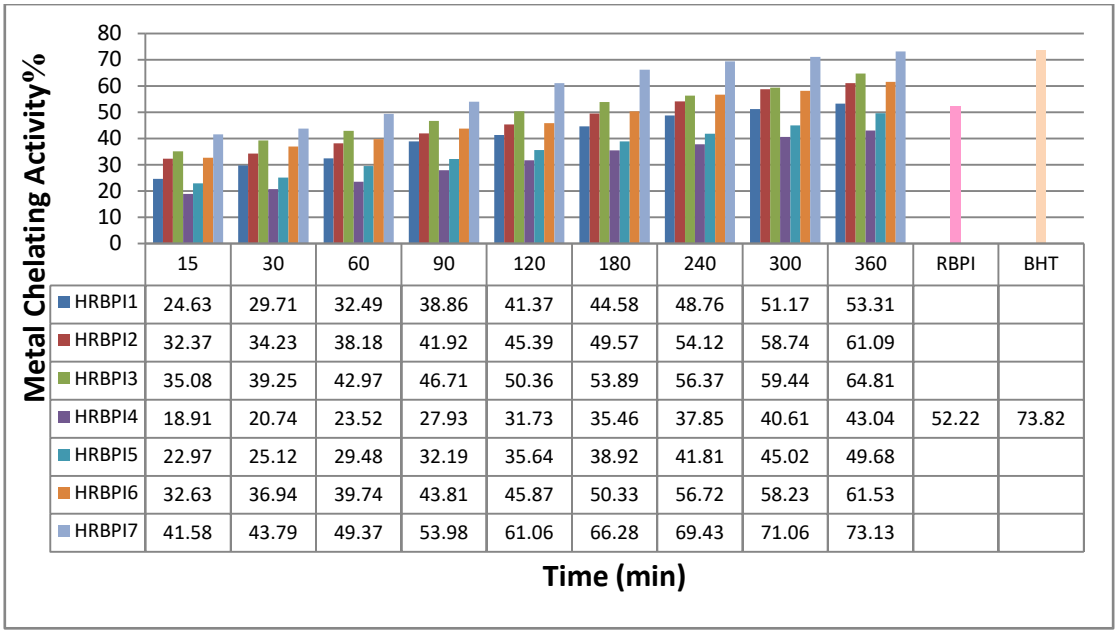
Figure 6: Reducing power of isolated rice bran protein hydrolysates. The symbols represent isolated rice bran protein hydrolysates treated with enzymes: (HRBPI1) pepsin (u/gm1000), (HRBPI2) pepsin (u/gm2000), (HRBPI3) pepsin (u/gm3000), (HRBPI4) trypsin (u/gm1000), (HRBPI5) trypsin (u/gm2000), (HRBPI6) trypsin (u/gm3000), (HRBPI7) pepsin and trypsin synergistically (u/gm2000).

The results of the selected protein hydrolysates based on the degree and time of hydrolysis showed the highest reductive power without the appearance of bitter taste, as the hydrolysate HRBPI7 achieved a degree of hydrolysis of 43.73% with a hydrolysis time of 120 minutes, recording a reductive power of 49.86%, outperforming all other hydrolysis treatments and approaching what the protein isolate RBPI achieved in terms of reductive power of 48.86% compared to what the synthetic antioxidant BHT achieved, which recorded a reductive power of 63.89%. Based on what was mentioned above, the treatment of the hydrolysate HRBPI7 was considered a selected treatment.

Salboukh, (2016) when studying the oxidative activity of protein isolates from rice bran and local oat bran showed that the reductive power capacity reached (84, 69)% respectively. Ma et al., (2014) indicated that the activity of peptides resulting from the protein hydrolysate of oat bran using Alcalas enzyme at a concentration of 2.5 mg/ml gave a reductive power capacity of 70%.

Metal bonding ability

Figure 7 shows the ferrous ion binding capacity percentage for each degrader (HRBPI1, HRBPI2, HRBPI3, HRBPI4, HRBPI5, HRBPI6, HRBPI7). It was observed that the ferrous ion binding capacity for all protein degraders increases with the progression of decomposition time and the increase in the degree of decomposition for each treatment separately. This is due to the role of the enzyme treatment in increasing the amount of amino acids exposed to the medium as the decomposition process progresses, which in turn has the ability to donate hydrogen ions or electrons, which is positively reflected in the ferrous ion binding capacity for the protein degrader (Zhang et al., 2019).



	15	30	60	90	120	180	240	300	360	RBPI	BHT
HRBPI1	24.63	29.71	32.49	38.86	41.37	44.58	48.76	51.17	53.31		
HRBPI2	32.37	34.23	38.18	41.92	45.39	49.57	54.12	58.74	61.09		
HRBPI3	35.08	39.25	42.97	46.71	50.36	53.89	56.37	59.44	64.81		
HRBPI4	18.91	20.74	23.52	27.93	31.73	35.46	37.85	40.61	43.04	52.22	73.82
HRBPI5	22.97	25.12	29.48	32.19	35.64	38.92	41.81	45.02	49.68		
HRBPI6	32.63	36.94	39.74	43.81	45.87	50.33	56.72	58.23	61.53		
HRBPI7	41.58	43.79	49.37	53.98	61.06	66.28	69.43	71.06	73.13		

Figure 7: Ion binding capacity of isolated rice bran protein hydrolysates. The symbols represent isolated rice bran protein hydrolysates treated with enzymes: (HRBPI1) pepsin (u/gm1000), (HRBPI2) pepsin (u/gm2000), (HRBPI3) pepsin (u/gm3000), (HRBPI4) trypsin (u/gm1000), (HRBPI5) trypsin (u/gm2000), (HRBPI6) trypsin (u/gm3000), (HRBPI7) pepsin and trypsin synergistically (u/gm2000).

The results of the selected protein hydrolysates based on the degree and time of hydrolysis showed the highest ability to bind ferrous ions without the appearance of a bitter taste, as the hydrolysate HRBPI7 achieved a degree of hydrolysis of 43.73% and a hydrolysis time of 120 minutes, recording a ferrous ion binding ability of 61.06%, outperforming all other hydrolysis treatments and approaching what the protein isolate RBPI achieved in terms of ferrous ion binding ability, which reached 52.22%, compared to what the industrial antioxidant BHT achieved, which recorded a ferrous ion binding ability of 71.83%. Based on what was mentioned above, the treatment of the hydrolysate HRBPI7 was considered as a selected treatment.

The results indicated significant differences ($p \leq 0.05$) between the treatments to bind ferrous ions between different concentrations of enzymatic hydrolysates. This difference may be due to the specificity of pepsin and trypsin enzymes in producing effective peptides that can chelate to bind metal ions (Biswas et al., 2017). The increased ability of the peptides prepared by pepsin and trypsin enzymes in the ability to bind ferrous ions may be attributed to the presence of a high concentration of metal-binding amino acids such as (aspartic, glutamic, arginine, lysine, and methionine) which were observed in pepsin and trypsin enzyme hydrolysates. The sequence of amino acids in the peptide and the molecular weight also affect its ability to bind ferrous ions (Zhu et al., 2017).

CONCLUSION

Rice bran protein isolates and hydrolysate have good antioxidant properties, making them ideal for commercial use in the food and beverage and pharmaceutical industries.

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