Measurement: Food 13 (2024) 100141

Contents lists available at ScienceDirect

Measurement: Food

journal homepage: www.elsevier.com/locate/meafoo

Optimizing protein hydrolysates of rice bran: Physicochemical, antioxidant, antibacterial properties, and chemometric analysis for functional food potential

Kah Yaw Ee [a,b,*], Wen Jie Ng [c,d], Peir Shinn Hew [e], Kai Sheng Mah [a], Li Xin Lim [a], Shen Yun Kee [a], Mei Yi Law [a]

[a] Department of Agricultural and Food Science, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia
[b] Centre for Agriculture and Food Research, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia
[c] Department of Allied Health Sciences, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia
[d] Centre for Biomedical and Nutrition Research, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia
[e] Laboratory of Food Safety and Food Integrity, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ARTICLE INFO

Keywords:
Rice bran (*Oryza sativa L.*)
Protein hydrolysate
Physicochemical property
Antioxidant activity
Antibacterial activity
Chemometric analysis

ABSTRACT

Rice bran is one of the major by-products of the rice milling process. This study examined the physicochemical alterations and potential functions of protein hydrolysates of rice bran. After removal of fats, rice bran protein was hydrolyzed by using bromelain (Br), trypsin (Tr), papain (Pa), and pepsin (Pe) for different durations (1–5 h). The dry yield of the hydrolysates (3.43–6.30%), the peptide (34.87–85.68 mg/g), and the total phenolic contents (TPC) (21.94–74.59 mg GAE/g) displayed a weak correlation with the duration of the hydrolysis. Hydrolysates Br5h, Tr1h, Pa1h, and Pe4h exhibited the most potent ferric reducing antioxidant power (FRAP) and strong 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging abilities among all samples. Following exposure to elevated temperatures (65–100°C), the antioxidant properties of Br5h (ABTS 136.67 mg TE/g; FRAP 35.83 mg Fe(II)/g) and Pe4h (ABTS 44.78 mg TE/g; FRAP 49.77 mg Fe(II)/g) remained potent. Furthermore, it is worth noting that Br5h and Pe4h exhibited the most robust antibacterial effects, particularly against *Staphylococcus aureus* and *Escherichia coli*. Additionally, principal component analysis revealed comprehensive physicochemical and antioxidant properties within the hydrolysates. Notably, strong associations were identified between FRAP and peptide content, as well as TPC in Br4h, Pe3h, Pe4h, and Pe5h. Overall, the hydrolysates Br5h and Pe4h, which demonstrate exceptional thermal stability, show significant potential as components for the future development of functional food products with antioxidant and antibacterial properties.

1. Introduction

Rice (*Oryza sativa* L.) is a primary staple grain and a key ingredient in a wide variety of culinary products. The process of producing brown rice begins with the removal of husk from rough paddy rice. Following this, brown rice is further polished, resulting in the separation of rice bran and white rice. It has been documented that the production of 500.8 million tonnes of white rice leads to the generation of approximately 100 million tonnes of bran and husk [1]. Despite constituting approximately 10% of the by-product's dry weight, rice bran is frequently discarded as agricultural waste or utilized sparingly as animal feed [2]. In recent years, there has been a growing focus on upcycling initiatives, which aim to promote a circular economy with a more effective management on food and agricultural waste.

In addition to the production of rice bran oil for human consumption, defatted rice bran offers various recycling opportunities. Rice bran protein is notable for its high nutritional value, functionality, and digestibility [1,3]. This protein can undergo further processing to develop products with enhanced value and multifunctional properties, as observed in previous studies [4–6]. Enzymatic hydrolysis is a common choice for this purpose due to its low toxicity, minimal side effects, and gentle operating conditions [7]. This technique efficiently liberates bioactive compounds from rice bran protein while preserving its nutritional content and augmenting its functional properties [3,8].

* Corresponding author.
E-mail address: eeky@utar.edu.my (K.Y. Ee).

https://doi.org/10.1016/j.meafoo.2024.100141
Received 19 November 2023; Received in revised form 4 January 2024; Accepted 29 January 2024
Available online 3 February 2024

Various enzymes can be utilized in this process, including plant-derived papain and bromelain, animal-derived pepsin and pancreatin, and microorganism-derived alcalase [9,10]. To optimize protein hydrolysis, enzymes require specific conditions encompassing temperature, pH, duration, and enzyme concentration [11]. The extent of hydrolysis depends on the enzymatic cleavage activity, which is influenced by multiple factors, such as protein molecular weight, amino acid composition, and peptide sequence [8,11–13].

Enzymatic hydrolysis has been observed to enhance the release of health-promoting bioactive peptides and phenolic compounds, such as those with antioxidant and antibacterial properties [3,10,14]. For example, the antioxidant activities of protein hydrolysates of rice bran were enhanced through enzymolysis using alcalase and flavourzyme, applied at various durations [11]. It is believed that these documented effects are not attributed to a single compound within the hydrolysates but rather to the synergetic actions of functional peptides and phenolic compounds [5,10,15]. Additionally, the ability to withstand high temperatures is crucial when utilizing protein hydrolysates as functional ingredients in food processing to ensure their effectiveness [16].

Hence, this study was carried out to investigate how the duration of hydrolysis affects several aspects, including the yield of protein hydrolysates, peptide content, total phenolic content (TPC), and the antioxidant and antibacterial properties of these hydrolysates. The enzymes employed in this study were plant-derived bromelain and papain, and animal-derived trypsin and pepsin. The hydrolysates demonstrating the most robust antioxidant activity underwent a thermal stability assessment. Furthermore, chemometric analyses were employed to elucidate the interrelationship and dispersion of antioxidant and physicochemical properties.

2. Materials and methods

2.1. Materials

Rice bran was obtained from Hock Ju Edar Sdn. Bhd., Malaysia. All chemical and reagents were of analytical grade and purchased from Merck KGaA, Germany, *e.g.* gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), etc.; SIME Scientific, Germany, *e.g.* 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), potassium persulfate, etc.; R&M Chemicals, UK, *e.g.* Folin-Ciocalteu reagent; Nacalai Tesque, Japan, *e.g.* 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) diammonium salt; unless stated otherwise.

2.2. Extraction of crude protein from defatted rice bran

Rice bran was defatted in hexane (1:5 ratio) by stirring for 2 h. The resulting pallet was collected after centrifugation (2000 × *g*) for 10 min. Then, the pallet was mixed with distilled water (1:4 ratio), adjusted to pH 9 using 2 M sodium hydroxide solution, and stirred for 2 h [3]. Next, the supernatant was collected after centrifugation (8000 × *g*) for 10 min. It was adjusted to pH 4.5 using 2 M hydrochloric acid and stirred for another 20 min. After that, the resulting protein pallet was collected after centrifugation (12,000 × *g*) for 10 min. The protein pallet was then adjusted to pH 7 and dried at 70°C. The yield of the crude rice bran protein was calculated using Eq. (1).

$$\text{Yield } (\%) = \frac{\text{Dried protein pallet (g)}}{\text{Defatted rice bran (g)}} \times 100\% \quad (1)$$

2.3. Enzymatic hydrolysis of rice bran protein

Rice bran protein underwent hydrolysis with bromelain, papain, pepsin, and trypsin at an enzyme-to-substrate (E/S) ratio of 1:100 (w/w) [1]. In the case of bromelain, papain, and trypsin hydrolysis, 1 mg of the enzyme was added to 40 mL of a 0.1 M potassium phosphate buffer (pH 7) along with 0.1 g of the substrate for each enzyme individually. For pepsin hydrolysis, 0.1 M potassium chloride buffer (pH 1.5) was used. Bromelain and papain hydrolyses were conducted at 50°C, while pepsin and trypsin hydrolyses were performed at 37°C with continuous gentle shaking. Subsequently, hydrolysis was terminated by placing the samples in a boiling water bath for 10 minutes. The supernatant was collected after centrifugation (5000 × *g*) for 15 min, oven-dried at 70°C, and stored at 4°C prior to analysis. The yield of each hydrolysate was calculated using Eq. (2).

$$\text{Yield } (\%) = \frac{\text{Dried protein hydrolysate (g)}}{\text{Dried protein pallet (g)}} \times 100\% \quad (2)$$

2.4. Determination of peptide content

O-phthaldialdehyde (OPA) reagent was prepared freshly by dissolving 40 mg of OPA powder in 1 mL of 80% (v/v) methanol, 25 mL of 100 mM sodium tetraborate solution, 2.5 mL of 20% (w/w) sodium dodecyl sulfate solution, and 100 μL of 2-mercaptoethanol [17]. The volume was adjusted to 50 mL with distilled water. For assay, 100 μL of sample (200–2000 μg/mL) was mixed with 1 mL of OPA reagent. The mixture was then kept in dark at room temperature for 2 min before the absorbance was read at 340 nm using a UV–Vis spectrophotometer. Soy peptone solution (200–1000 μg/mL) was assayed as standard. Results were calculated as milligrams per gram (mg/g) of the dry sample.

2.5. Determination of total phenolic content (TPC)

Briefly, 100 μL sample (200–2000 μg/mL) was mixed with 200 μL of Folin-Ciocalteu's phenol reagent and incubated in dark at room temperature for 10 min [15]. Subsequently, 1 mL of 7% (w/v) sodium carbonate solution was added and further incubated for 30 min. The absorbance was read at 765 nm. Gallic acid solution (20–100 μg/mL in 80% ethanol) was assayed as standard. Results were expressed as milligrams of gallic acid equivalent (GAE) per gram of the dry sample.

2.6. Determination of antioxidant activity

For the 2,2′-azinobis-(3-ethylbenozothiazoline-6-sulfonate) (ABTS) assay, 20 μL of sample (200–2000 μg/mL) was mixed with the ABTS working solution (0.7 ± 0.02 at 734 nm) which was diluted from a 7 mM ABTS stock solution consisting of 2.45 mM potassium persulfate [15]. Then, the absorbance was read at 734 nm after incubating the mixture in the dark at room temperature for 6 min. Trolox solution (20–100 μg/mL in 80% ethanol) was assayed as standard. Results were expressed as milligrams of Trolox equivalent (TE) per gram of the dry sample.

For the ferric reducing antioxidant power (FRAP) assay, 30 μL of sample (200–2000 μg/mL) was mixed with 90 μL of distilled water and 1.5 mL of FRAP reagent which consisting of 50 mL of 300 mM acetate buffer (pH 3.6), 5 mL of 20 mM ferric chloride hexahydrate solution, and 5 mL of 10 mM TPTZ solution [15,18]. It was followed by incubation at 37°C for 30 min, and the absorbance was recorded at 593 nm. Ferrous sulfate heptahydrate solution (200–1000 μM) was assayed as standard. Results were calculated as milligrams of Fe(II) per gram of the dry sample.

2.7. Thermal stability test

Briefly, 5 mL of crude protein and hydrolysates (2 mg/mL) were incubated in water bath at various temperatures (65°C, 75°C, 85°C, and 100°C) for 30 min [16]. Meanwhile, one sample was kept at room temperature to serve as a control. After incubation, all samples were examined for antioxidant activity.

2.8. Determination of antibacterial activity

The minimum inhibitory concentration (MIC) and minimum

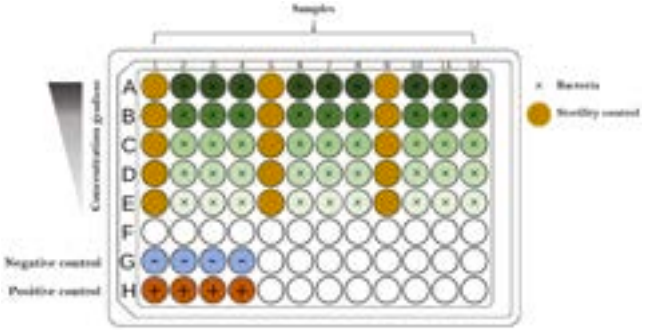

Fig. 1. Layout of minimum inhibitory concentration (MIC) assay.

bactericidal concentration (MBC) were determined on the samples with the highest antioxidant activity [17]. *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC BAA-1026) suspensions were prepared with optical density (OD) of 0.08–0.1 at 625 nm. For the MIC test, 200 μL samples (100 mg/mL) were filtered through a 0.45 μm syringe filter and pipetted into four wells in a 96-well flat bottom microplate individually. Each sample was then serially diluted, starting with 100 μL of sample (100 mg/mL) mixed with 100 μL of distilled water, resulting in concentrations of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL, individually. One well of each sample concentration served as a sterility control, while the remaining three wells had 5 μL of bacterial suspension added. Distilled water (100 μL) was assayed as negative control in row G, and the bacterial suspension was added into the distilled water in row H as a positive control (Fig. 1). The microplate was incubated at 37°C for 24 h, and then absorbance was measured at 590 nm. To indicate bacterial growth, 25 μL of 0.2 mg/mL iodonitrotetrazolium chloride dye was added to each well, and the development of pinkish-red color after 30-min incubation signify bacterial growth. For the MBC test, a loopful of the content from each well without bacterial growth was spread onto a Mueller Hinton agar plate, followed by incubation at 37°C for 24 h. The MBC of the sample was determined by the absence of bacterial colonies.

2.9. Statistical and chemometric analyses

All the experiments were carried out in triplicate, and the results were presented as mean values along with their respective standard deviations. The differences between these mean values were further assessed through one-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test at a significance level of 0.05, using IBM SPSS version 28.0.1.0. In addition, JMP® Pro version 16.0.0 was employed for various chemometric analyses, including Pearson correlation analysis, Bartlett's sphericity test, Kaiser-Meyer-Olkin (KMO) test, Principal Component Analysis (PCA), and Hierarchical Cluster Analysis (HCA).

3. Results and discussion

3.1. Yield of rice bran protein and hydrolysates

The protein yield from rice bran is 11.34 ± 0.25% (Table 1). Subsequent enzymatic hydrolysis resulted in approximately 50% and more reduction in dry yield of hydrolysates relative to the yield of the extracted crude protein. It is worth noting that the protein content of rice bran (10–16%) can vary due to factors, such as its origin, cultivation method, and extraction condition [3,8]. A similar study [19] reported a protein yield of 11.85%, while another study [20] achieved a higher protein content of 20.78% using a pH shift extraction method. Additionally, different combinations of solvent-to-solid ratio, pH, temperature, and centrifugation speed can lead to variations in protein yield [19]. To enhance protein yield, researchers have explored techniques such as mild heating, microwave treatment, and ultrasonication during pH shift extraction [3,10,21,22]. While rice bran protein underwent hydrolysis for various durations, there were only minimal significant differences in yield observed within the same enzyme group. Extrinsic factors, such as pH and electrolytes, can potentially lead to enzyme deactivation and affect the rate of hydrolysis [11]. A similar study reported an initial increase in the hydrolysis rate up to 3 h, followed by a decrease [23]. This trend was different with the results observed in the hydrolysis in this study.

3.2. Peptide content in the hydrolysates

The yield of hydrolysates is closely related to the degree of hydrolysis, which indicates how efficiently an enzyme can cleave peptide bonds. In simpler terms, the increasing trend in peptide content with longer duration of enzymatic hydrolysis suggests that the degree of hydrolysis rises as the hydrolysis process continues (Table 1). Trypsin hydrolysis primarily cleaves peptide bonds at the carboxyl side of arginine and lysine, whereas pepsin hydrolysis mainly targets tyrosine, tryptophan, and phenylalanine [7,21]. In the current study, both enzymatic hydrolyses released a significant number of peptides from rice bran protein.

In contrast, the enzyme bromelain exhibits a broader range of preferential cleavage sites when compared to trypsin. Bromelain primarily cleaves peptide bonds at lysine, glutamic acid, glycine, ornithine, and alanine residues [24]. Similarly, a study on corn protein hydrolysis using bromelain found that the peptide content increased gradually with extended hydrolysis duration, ultimately reaching the highest degree of hydrolysis (12.1%) after 5 h [25]. These results are consistent with our findings, suggesting that an extended hydrolysis duration is necessary for bromelain to release a greater quantity of peptides. Additionally, our results indicated that pepsin hydrolysates contain significantly higher levels of peptides compared to papain hydrolysates, despite papain having a broader specificity for cleaving peptide bonds at lysine, phenylalanine, and arginine [9]. The distinctions in their structure-function relationships could be a promising area for further exploration in future studies.

Table 1
Yield, peptide and total phenolic contents of crude protein and protein hydrolysates of rice bran.

Sample	Yield (%)	Peptide (mg/g)	Total phenolic content (mg GAE/g)
Crude protein	11.34 ± 0.25^{A}	37.46 ± 0.64^{F}	21.23 ± 0.53^{M}
Br1h	6.00 ± 0.00^{BCDE}	54.89 ± 0.64^{DE}	28.35 ± 1.01^{HIJK}
Br2h	6.30 ± 0.20^{B}	55.64 ± 0.56^{DE}	27.00 ± 0.58^{IJKL}
Br3h	6.17 ± 0.06^{BCD}	67.50 ± 0.32^{B}	31.73 ± 1.17^{GHI}
Br4h	6.17 ± 0.12^{BCD}	67.50 ± 0.64^{B}	40.50 ± 0.10^{E}
Br5h	6.23 ± 0.12^{BC}	70.47 ± 0.32^{B}	45.90 ± 0.58^{D}
Tr1h	5.67 ± 0.21^{CDE}	48.96 ± 0.96^{E}	38.14 ± 3.09^{EF}
Tr2h	5.80 ± 0.17^{BCDE}	57.12 ± 1.40^{D}	29.03 ± 0.58^{HIJK}
Tr3h	6.00 ± 0.17^{BCDE}	57.12 ± 0.64^{D}	28.01 ± 0.58^{HIJK}
Tr4h	5.90 ± 0.10^{BCDE}	54.89 ± 0.32^{DE}	29.70 ± 1.17^{HIJ}
Tr5h	5.63 ± 0.15^{DE}	66.76 ± 1.11^{BC}	32.04 ± 0.10^{GH}
Pa1h	5.83 ± 0.15^{BCDE}	34.87 ± 0.32^{F}	23.96 ± 4.22^{KLM}
Pa2h	5.93 ± 0.15^{BCDE}	54.15 ± 0.32^{DE}	24.98 ± 1.55^{JKLM}
Pa3h	6.17 ± 0.25^{BCD}	56.38 ± 0.32^{D}	21.94 ± 1.55^{LM}
Pa4h	5.43 ± 0.31^{E}	60.09 ± 0.56^{CD}	46.58 ± 2.03^{D}
Pa5h	5.57 ± 0.12^{E}	55.64 ± 0.10^{DE}	35.10 ± 1.17^{FG}
Pe1h	3.67 ± 0.06^{F}	56.56 ± 0.32^{D}	54.34 ± 1.55^{C}
Pe2h	3.70 ± 0.44^{F}	57.49 ± 0.64^{D}	54.68 ± 1.01^{C}
Pe3h	3.43 ± 0.06^{F}	68.06 ± 1.28^{B}	60.41 ± 0.58^{B}
Pe4h	3.47 ± 0.21^{F}	79.56 ± 0.56^{A}	72.23 ± 2.92^{A}
Pe5h	3.47 ± 0.06^{F}	85.68 ± 0.56^{A}	74.59 ± 2.11^{A}

Br = bromelain; Tr = trypsin; Pa = papain; Pe = pepsin; 1–5 h = hydrolysis duration (hour).
$^{A–M}$Data in the same column with different superscripts are significantly ($p < 0.05$) different ($n = 3$).

3.3. TPC in the hydrolysates

The results clearly demonstrated the effectiveness of enzymatic hydrolysis in releasing phenolic compounds (21.23 mg GAE/g) from rice bran crude protein. There was a general upward trend in TPC with increasing duration of hydrolysis observed in both bromelain and pepsin hydrolyses (Table 1). Notably, pepsin hydrolysis was found to release the most phenolic compounds from rice bran hydrolysates compared to the other enzymes used at various durations. These findings align with similar studies that have indicated a connection between prolonged hydrolysis duration and the liberation of more phenolic compounds [25,26]. Moreover, previous research has shown that phytochemical compounds bound to protein complexes can also be released through enzymatic hydrolysis [14,15,27]. Approximately 74% of the phenolic compounds in rice exist in the form of insoluble bound compounds. Therefore, employing enzymes such as protease, cellulase, and glucoamylase can be more effective in converting these compounds into free or soluble conjugates [28]. Additionally, higher levels of phenolic compounds, such as ferulic, protocatechuic, *p*-hydroxybenzoic, chlorogenic, caffeic, coumaric, gallic acids, quercetin, and (-)-epicatechin, were observed after the rice bran was enzymatic hydrolyzed [28]. This enhancement in TPC in the protein hydrolysates aligns with the results of a similar study that used alcalase, neutrase, and flavourzyme to hydrolyze rice bran protein [29].

It is worth noting that various hydrolysis processes released phenolic compounds differently. For instance, maximum TPC (38.14 mg GAE/g) was released at the initial 1 h during trypsin hydrolysis and then decreased with prolonged hydrolysis. In the case of papain hydrolysis, TPC (46.58 mg GAE/g) significantly increased at the 4 h duration in the current study. The difference in TPC release aligns with findings from similar studies that have reported varrying amounts of TPC in protein hydrolysates of rice bran using trypsin [1,30]. Several factors have been proposed to account for these differences, including the interference of enzymatic activity by enzyme inhibitors, variations in the origin and cultivar of rice, and the termination of enzymes due to high temperature. In comparison to the present study, research conducted with rice bran from China over various hydrolysis durations (2–6 h) resulted in a lower range of TPC (1.03–1.12 mg GAE/g) [30].

3.4. Antioxidant activities of hydrolysates

ABTS and FRAP assays were employed to determine the optimal duration of enzymatic hydrolysis, as illustrated in Fig. 2. These assays operate on different mechanisms, the ABTS assay primarily involving hydrogen atom transfer, while the FRAP assay is based on single electron transfer reactions. In the ABTS assay, a general upward trend in

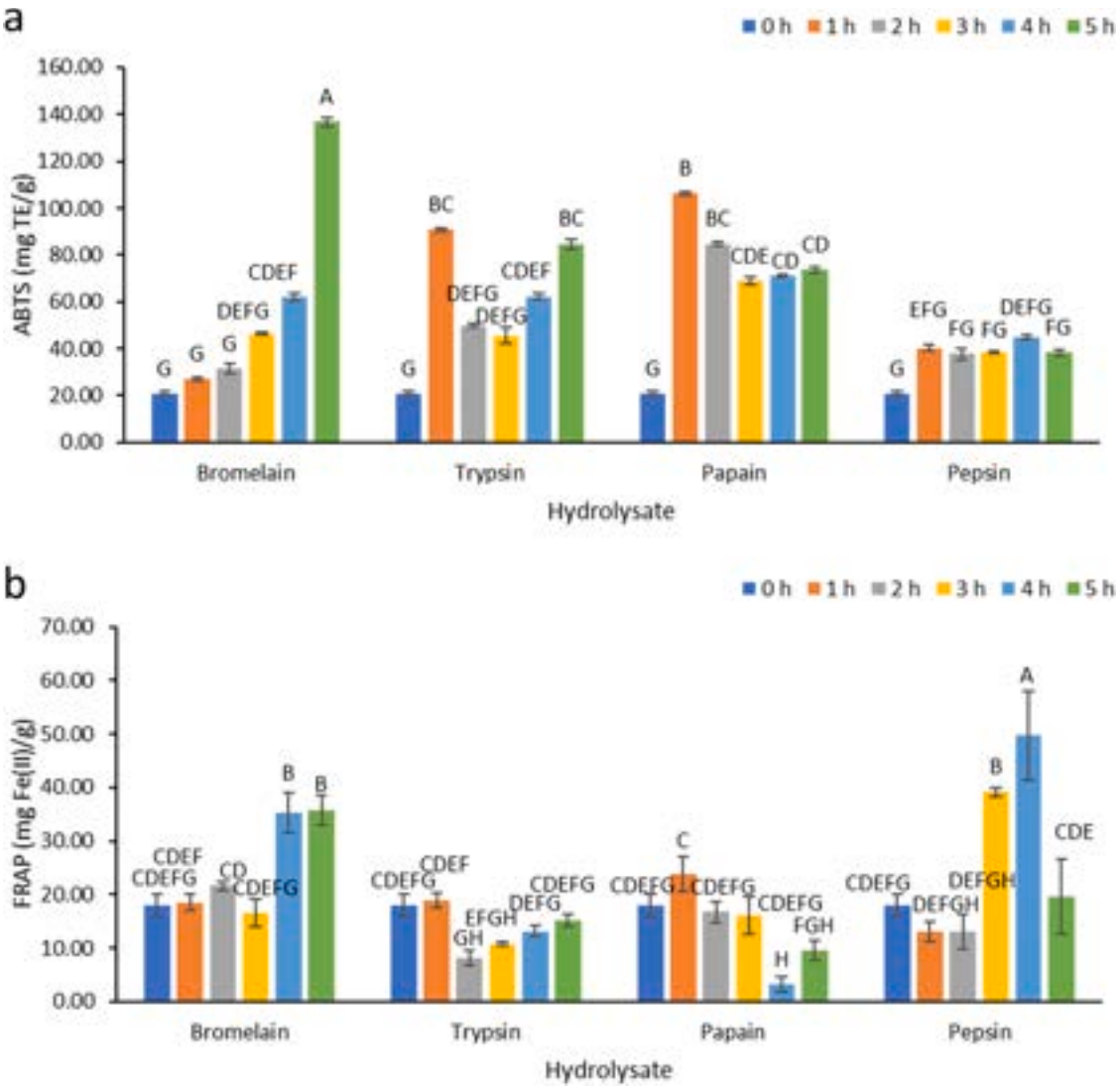

Fig. 2. (a) ABTS radical scavenging activity and (b) FRAP values of crude protein and protein hydrolysates of rice bran at various durations. $^{A–H}$Different superscripts indicate significant ($p < 0.05$) difference ($n = 3$).

antioxidant activity was observed during the initial 1-h hydrolysis. However, in the case of the FRAP assay, a decreasing trend in values was noticed during the initial 1–2 h for trypsin, papain, and pepsin hydrolyses, followed by an increase later for trypsin and pepsin hydrolyses.

Within the same enzyme group, the bromelain hydrolysate exhibited the highest antioxidant activity (ABTS 136.67 mg TE/g; FRAP 35.83 mg Fe(II)/g) after 5 h of hydrolysis, labeled as Br5h. In contrast, trypsin hydrolysate showed the highest antioxidant activity (ABTS 90.73 mg TE/g; FRAP 19.00 mg Fe(II)/g) during the initial 1-h hydrolysis, labeled as Tr1h, followed by a reduction at 2–3 h and a subsequent increase. Similarly, during the initial 1-h hydrolysis, papain hydrolysate exhibited the highest antioxidant activity (ABTS 106.25 mg TE/g; FRAP 23.85 mg Fe(II)/g), labeled as Pa1h, followed by a reduction during prolonged hydrolysis. In the case of pepsin hydrolysis, there was an insignificant difference in the ABTS assay (44.78 mg TE/g), but a significant increase in the FRAP value (49.77 mg Fe(II)/g) was observed at 4-h hydrolysis, labeled as Pe4h.

The results highlighted that a longer hydrolysis duration (e.g. 5 hours) does not consistently improve the antioxidant activity of hydrolysates, despite greater release of peptides and TPC. Our results shared similarities with other research findings. Previous studies involving enzymatic hydrolysis using alcalase, neutrase, and flavourzyme showed weak relationship between hydrolysis duration and the antioxidant activities of protein hydrolysates of rice bran [11,29]. For example, in a recent study, prolonged trypsin hydrolysis (6 h) of rice bran protein exhibited lower ABTS radical scavenging activity (10.26%) compared to its crude protein (27.31%) [30]. In contrast, another study reported that the protein hydrolysate of corn silk produced through trypsin hydrolysis for only 1 h exhibited the highest ABTS radical scavenging activity (66.66%) [16], aligning with the findings of Tr1h in our study. These results indicate that the connection between hydrolysis duration and antioxidant activity is complex.

In general, longer hydrolysis duration tends to produce lower molecular weight peptides and more phenolic compounds, resulting in higher antioxidant potential [31]. This phenomenon is evident in the present study, where the extended hydrolysis by bromelain and pepsin demonstrated the increase of ABTS radical scavenging activity and FRAP values over the tested hydrolysis durations (1–5 h). The antioxidant activity might be attributed to the presence of bioactive peptides with specific properties, including sulfur-containing, negatively-charged acidic, and hydrophobic amino acids, as well as the presence of phenolic compounds that are potentially capable of donating protons and electrons, depending on their functional group arrangement, configuration, and the number of free hydroxyl groups [32]. The quantity of these compounds was notably higher in Br5h and Pe4h compared to the native protein. Further study should focus on analyzing the potential functional groups.

A similar study on the protein hydrolysate of rice reported that the antioxidant effect was directly proportional to the content of polyphenols and peptides [33]. Another similar study also noted that the pepsin hydrolysates of orange by-products exhibited the highest antioxidant activity after 3.5 h of hydrolysis [31], which closely resembles the findings for Pe4h in the present study. These results highlighted the importance of the content and properties of peptides and phenolic compounds in determining the antioxidant potential of hydrolysates.

In this study, papain and trypsin hydrolyses exhibited different trend compared to bromelain and pepsin hydrolyses. Even though Pa4h released the highest concentrations of peptides and phenolic compounds, the highest antioxidant activity was found in Pa1h. This suggests that the antioxidant activity of Pa1h was not directly related to the quantity of released peptides and phenolic compounds, but rather to the inherent antioxidant properties of the compounds themselves. This finding aligns with a study [34], which also observed that papain hydrolysates displayed the highest ABTS radical scavenging activity after a 1-h hydrolysis.

On the other hand, the results indicated that the antioxidant activity of Tr1h was primarily attributed to the TPC, while Tr5h was mainly contributed by antioxidant peptides. Both samples exhibited antioxidant activity without significant difference ($p > 0.05$). This is consistent with a similar study that reported how trypsin hydrolysis enhanced the FRAP value of protein hydrolysate of Portuguese rice bran due to the liberation of more antioxidant phenolic compounds [1]. These findings underscore the complex relationship between hydrolysis duration and antioxidant activity, which is influenced by various factors, including the properties of the released compounds.

In addition to the presence of phenolic compounds, the variations in antioxidant activity among the hydrolysates are closely related to the size and quantity of antioxidant peptides generated during enzymatic hydrolysis. Small peptides with molecular weights (1–3 kDa) are known to exhibit potent biological activity [15]. Similar studies found that enzymatic hydrolysates of rice bran protein [8,35] and corn protein meal [25] with 1–3 kDa sizes displayed high antioxidant activity. Furthermore, the choice of enzyme, hydrolysis condition, and the presence of hydrophobic and aromatic amino acids at the C-terminal of the peptide chain are closely linked to the antioxidant potential of hydrolysates [6,36].

To gain deeper insights into this topic, further studies are recommended. These studies could integrate antioxidant assays with advance technology, such as gas or liquid chromatography mass spectrometry (GCMS or LCMS), Fourier-transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and amperometry. The combination of these methods can enhance the accuracy and precision of the outcomes, thereby providing a more comprehensive understanding of the structure-function relationship.

3.5. Thermal stability of the antioxidant hydrolysates

Thermal stability is a crucial characteristic of antioxidants because heat treatment is frequently employed in various stages of food processing. In this study, heat treatment at different temperatures was applied to crude protein and hydrolysates, including Br5h, Tr1h, Pa1h, and Pe4h, to investigate the thermal impact on their antioxidant activities, as shown in Fig. 3.

While it is generally known that antioxidant peptides [37] and phenolic compounds [38] are sensitive to heat treatment, this study revealed that the rice bran crude protein displayed remarkable stability, with an insignificant change in ABTS radical scavenging activity and a gradual increase in FRAP value even after heating to 100°C. The fluctuations in antioxidant activity among the hydrolysates indicate that the response to heat treatment can vary depending on the specific components and conditions of the samples. It is possible that some compounds within the samples are more resistant to heat-induced degradation, contributing to the observed differences in thermal stability.

The structural properties of proteins and peptides can indeed be altered after heating, potentially leading to the release of more peptides with antioxidant activity [33]. This could explain the observed gradual increase in antioxidant activity in certain samples in the study. Aggregation of rice bran crude protein during heating may expose their hydrophobic domains associated with antioxidant activity. Similar studies have reported increased antioxidant activity after heat treatment at various temperatures. For instance, the antioxidant activity of egg white protein [39] and its pepsin hydrolysates increased after heat treatment at 60°C, 65°C, and 100°C compared to non-heated samples [33]. Additionally, similar findings were reported in the case of protein hydrolysates of corn silk [16] and jackfruit seed peptides [40], where the antioxidant activity remained stable at high temperatures, up to 100°C. These results highlighted the potential for heat treatment to enhance the antioxidant properties of certain protein and peptide samples.

3.6. Antibacterial properties

Table 2 presents the MIC and MBC of protein hydrolysates of rice

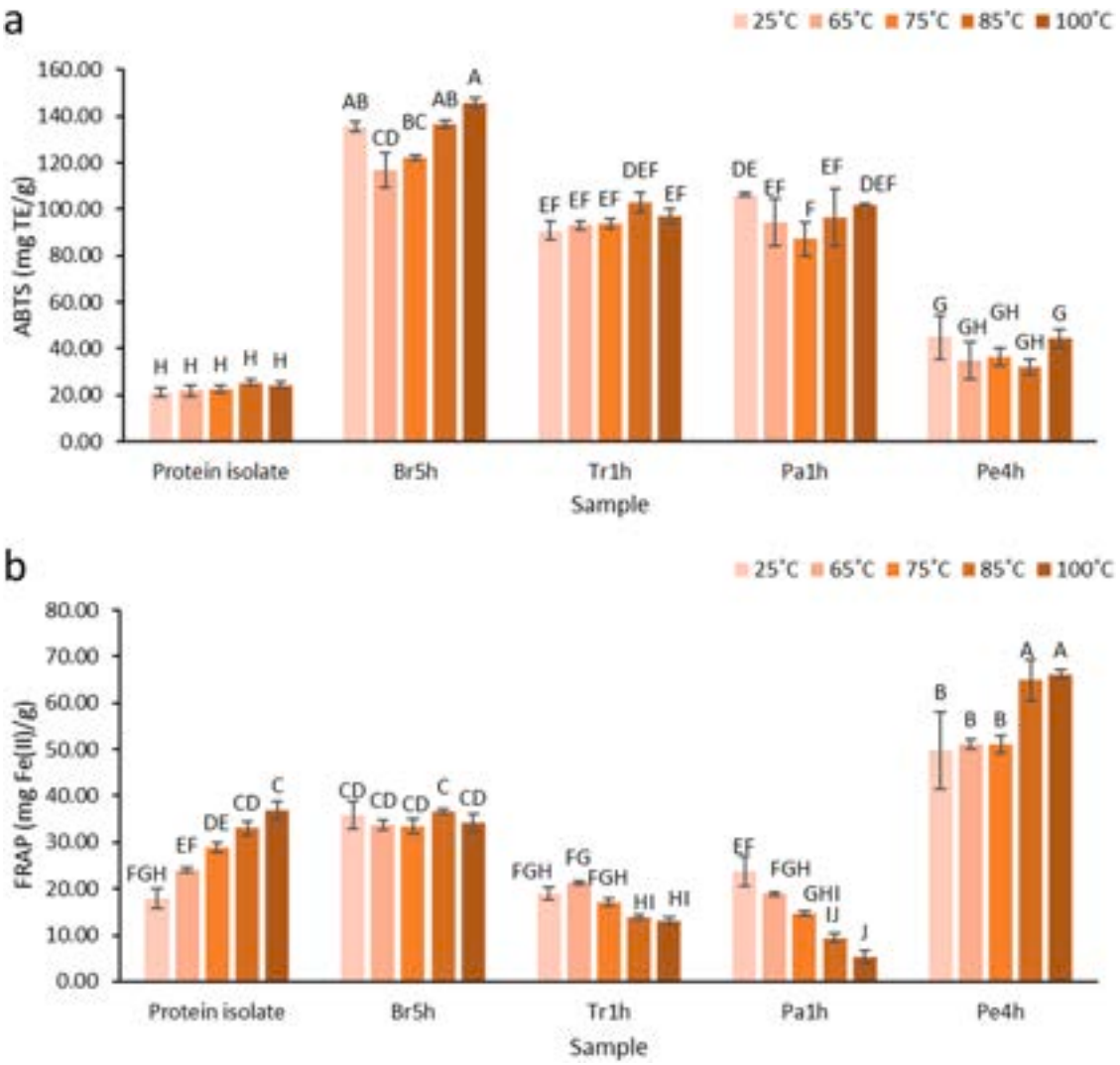

Fig. 3. Thermal stability test of crude protein and selected protein hydrolysates of rice bran with the highest antioxidant activity within the same enzyme group using (a) ABTS and (b) FRAP assays. $^{A–J}$Different superscripts indicate significant ($p < 0.05$) difference ($n = 3$).

Table 2
Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of protein hydrolysates of rice bran.

Hydrolysate	*E. coli*		*Staphylococcus aureus*	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Br5h	> 100	ND	6.25	50
Tr1h	> 100	ND	12.5	50
Pa1h	> 100	ND	6.25	100
Pe4h	12.5	100	6.25	100

Br = bromelain; Tr = trypsin; Pa = papain; Pe = pepsin; 1–5 h = hydrolysis duration (hour); ND = not determined.

bran against *E. coli* and *S. aureus*. The results showed that only Pe4h (MIC 12.5 mg/mL and MBC 100 mg/mL) displayed antibacterial activity against *E. coli*, while Br5h, Tr1h, and Pa1h did not exhibit any antibacterial effects. On the other hand, all the hydrolysates, with MIC 6.25–12.5 mg/mL, inhibited the growth of *S. aureus*. The MBC results revealed that 50 mg/mL of Br5h and Tr1h, as well as 100 mg/mL of Pa1h and Pe4h, exhibited bactericidal effects against *S. aureus*. Importantly, all the hydrolysates were more potent against *S. aureus* compared to *E. coli*. While Br5h was found to be the most effective against *S. aureus*, Pe4h exhibited a broader spectrum of antibacterial activity against both *E. coli* and *S. aureus*.

The varying susceptibility of Gram-negative bacteria, such as *E. coli*, to antibacterial compounds can be attributed to their protective structures, which include the peptidoglycan cell wall and an outer lipopolysaccharide membrane. These structures limit the penetration of hydrophilic compounds into the cells. However, certain chemical compounds, such as phenolic compounds and esters, can affect the cell membrane target sites of both Gram-positive and Gram-negative bacteria [41]. Despite the noticeable increase in peptide content and total phenolic content (TPC) in the hydrolysates in this study, these

Table 3
Pearson correlation coefficients (r) analysis between physicochemical and antioxidant properties of crude protein and protein hydrolysates of rice bran.

Parameters	Peptide	TPC	ABTS	FRAP
Peptide	1.0000	0.7249**	-0.0809	0.3960**
TPC	0.7249**	1.0000	-0.1587	0.4369**
ABTS	-0.0809	-0.1587	1.0000	0.1002
FRAP	0.3960**	0.4369**	0.1002	1.0000

(r) near to +1 or −1 indicates strong relationship and near to 0 indicates weak/no relationship.
$|0.000| < r < |0.250|$ weak correlation; $|0.250| < r < |0.750|$ moderate correlation; $|0.750| < r < |1.000|$ strong correlation
**Correlation is significant at $p < 0.01$.

Table 4
Eigenvalues, variability, cumulative variability, Bartlett's sphericity test, and Kaiser-Meyer-Olkin (KMO) test that are associated with each principal component.

Variables	PC1	PC2
Physicochemical and antioxidant properties		
Eigenvalues	2.0636	1.0657
Variability (%)	51.591	26.643
Cumulative variability (%)	51.591	78.233
Bartlett's sphericity test	$p < 0.0001$, there is at least one of the correlations between the variables that is significantly different from 0.	
Kaiser-Meyer-Olkin (KMO) test	0.607, KMO > 0.5 is deemed as adequate for performing PCA.	

compounds did not significantly contribute to the antibacterial properties. It is notable that the improvement in antibacterial activity for both protein hydrolysates in this study was relatively modest compared to the results from a similar study on protein hydrolysates of string beans [42], where the MIC and MBC values for *S. aureus* were considerably lower. Furthermore, a peptic hydrolysate of rice bran protein, containing cationic peptides, was reported to be effective against Gram-negative *Porphyromonas gingiva* [43]. However, a similar study using Protease G6 did not show such effects [17]. Additionally, other antimicrobial protein hydrolysates derived from *Vicia faba* demonstrated antibiofilm

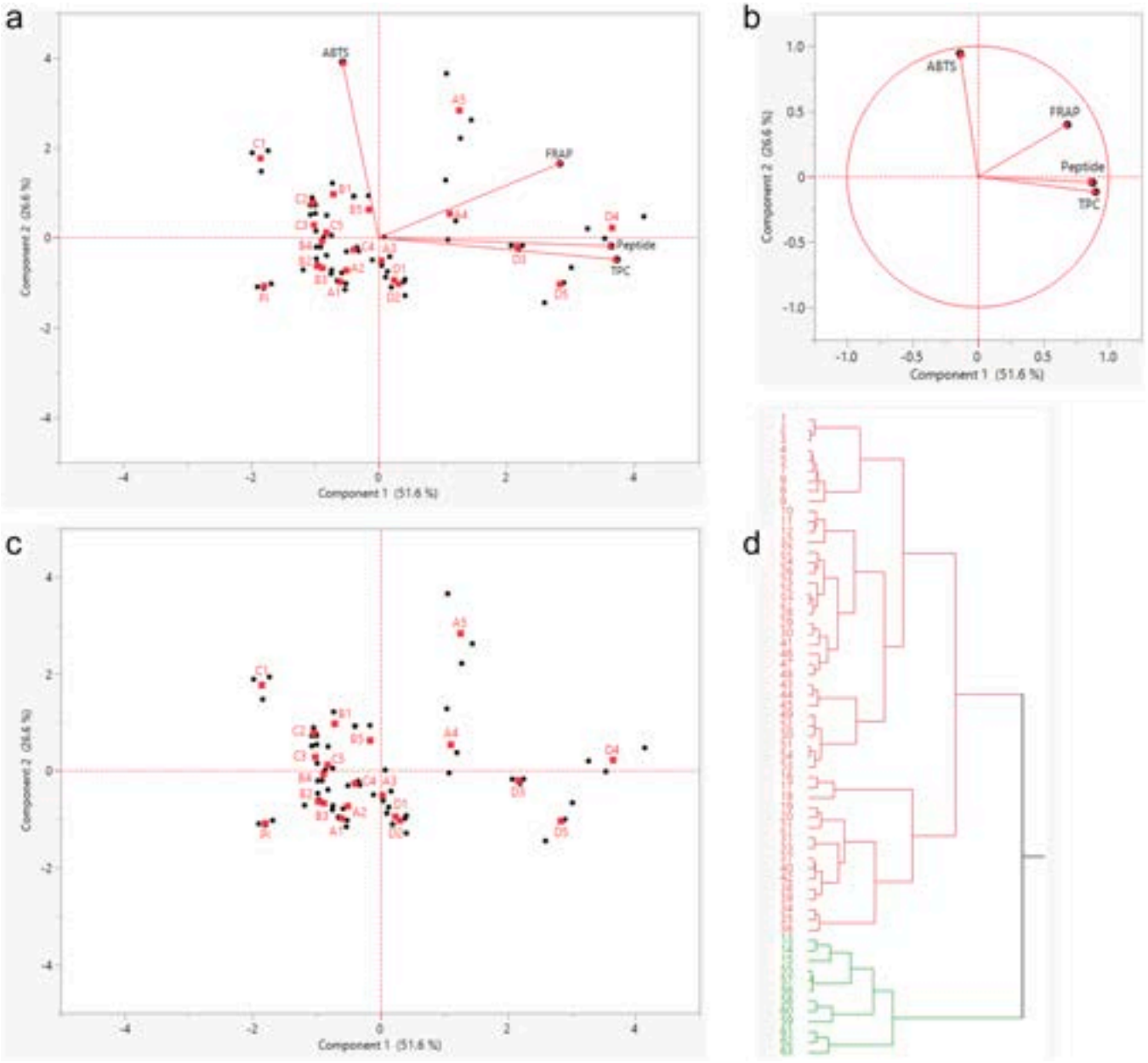

Fig. 4. Chemometric analysis of physicochemical and antioxidant properties of crude protein and protein hydrolysates of rice bran. (a) PCA biplot; (b) PCA loading plot; (c) PCA score plot; (d) Dendrogram of HCA. Note: PI = protein; A = bromelain; B = trypsin; C = papain; D = pepsin; 1–5 = hydrolysis duration (h).

activities against *Pseudomonas aeruginosa* PA14 [44]. These variations highlight the complex interactions between specific hydrolysates, bacterial strains, and their resistance mechanisms, which can vary from one study to another [45]. Further study in this area could benefit from identifying the specific antibacterial compounds within the hydrolysates and expanding the scope to encompass a variety of food spoilage microorganisms. This broader investigation would provide a more comprehensive understanding of the potential applications and effectiveness of protein hydrolysates in combating microbial food spoilage.

3.7. Chemometric analysis

Pearson correlation coefficient was determined to investigate the relationship between physicochemical and antioxidant properties of the rice bran crude protein and its hydrolysates (Table 3). PCA was conducted (Table 4, Fig. 4) to further explain the correlation and distribution of these physicochemical and antioxidant properties. Prior to the PCA, Bartlett's sphericity test and KMO test were performed to validate the dataset's suitability and sampling adequacy for a suitable PCA application. The results of Bartlett's sphericity test ($p < 0.05$) and KMO test (KMO > 0.5) indicated that the dataset was suitable and adequate, respectively. Then, the dataset was subjected to PCA. PC1 and PC2 with an eigenvalue (EV) > 1 explained a cumulative variance of 78.233%, in which PC1 representing 51.591% of the variance and PC2 representing 26.643% of the variance, respectively. HCA was also applied to further explain the differences between the rice bran crude protein and its hydrolysates.

Table 3 shows significant moderate correlations ($p < 0.01$), particularly the strong associations between TPC and FRAP, as well as peptide content (r = 0.4369 and 0.7249, respectively). Additionally, a moderate correlation (r = 0.3960) was observed between FRAP and peptide content, given their close positioning. In contrast, a weak correlation ($p > 0.01$) was found for ABTS radical scavenging activity against FRAP value, TPC, and peptide contents (r = 0.1002, -0.1587, and -0.0809, respectively). Notably, our ABTS radical scavenging activity differs from those of previous studies [8,35,46]. It is essential to consider other factors that might influence physicochemical properties, such as sample preparation, enzyme, extraction method, and environmental conditions, such as temperature and electrolytes in the medium.

According to the PCA results, Br4h, Pe3h, Pe4h, and Pe5h are clearly separated from the other samples by PC1. Samples located close to each other share similar physicochemical and antioxidant properties, while those situated farther apart are dissimilar. The samples positioned near the center of the plot generally have more comprehensive properties. The proximity of Br4h, Pe3h, Pe4h, and Pe5h to TPC, FRAP, and peptide content suggests that the phytochemicals they produced are quite similar compared to other samples.

In line with the PCA results, the dendrogram from HCA also demonstrates a clear separation into two distinct clusters, represented in red and green colors. These results indicated a positive correlation between the FRAP values of most bromelain and pepsin hydrolysates with released peptides and TPC (green clusters). Conversely, the ABTS radical scavenging activity of crude protein, trypsin, and papain hydrolysates shows a negative correlation with released peptides and TPC (red clusters). These relationships warrant further investigation to understand their structure-function connections. Consequently, the dendrogram helps illustrate both the differences between samples and the similarity of properties in Br4h, Pe3h, Pe4h, and Pe5h.

Overall, Br3h, Tr5h, Pa4h, and Pe1h are positioned near the center of the plot, indicating their generally more comprehensive physicochemical and antioxidant properties compared to other samples within the same enzyme group. Notably, Pe4h stands out as the only sample with high total phenolic and peptide contents. As an extension of this study, we recommended selecting these five hydrolysates for further fractionation and purification, coupled with omics approaches and bioinformatics. It is essential to extract and identify these bioactive compounds, followed by *in vivo* experiments and animal trials to better understand their efficacy.

4. Conclusion

In summary, rice bran protein was extracted and subjected to enzymatic hydrolysis using plant-derived bromelain and papain, and animal-derived trypsin and pepsin at various time intervals. The choice of enzyme and hydrolysis duration significantly impacted the physicochemical and antioxidant properties of the resulting hydrolysates. Protein hydrolysates of rice bran produced by using bromelain (Br5h) and pepsin (Pe4h) demonstrated superior antioxidant activity, particularly in ABTS and FRAP assays. Prolonged hydrolysis generally increased antioxidant potential, with a close connection with the release of TPC. The physicochemical and antioxidant properties were correlated, while PCA and HCA analyses revealed distinct groupings of hydrolysates based on their properties. Some hydrolysates exhibited antibacterial activity, Pe4h was the most potent against *E. coli* and *S. aureus*. These variations highlight the complex interactions between hydrolysates and bacterial strains. Furthermore, Br5h and Pe4h exhibited excellent thermal stability and hold promise as potential ingredients for antioxidant and antibacterial applications in the development of functional food products. Overall, this study provides valuable insights in optimizing the enzymatic hydrolysis and the potential application of protein hydrolysates of rice bran. Further research is recommended to identify bioactive compounds and assess their efficacy through *in vivo* experiments.

Statements and declarations

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

CRediT authorship contribution statement

Kah Yaw Ee: Conceptualization, Data curation, Formal analysis, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Wen Jie Ng:** Conceptualization, Data curation, Formal analysis, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Peir Shinn Hew:** Data curation, Software, Writing – original draft, Writing – review & editing. **Kai Sheng Mah:** Data curation, Formal analysis, Writing – original draft. **Li Xin Lim:** Data curation, Formal analysis, Writing – original draft. **Shen Yun Kee:** Data curation, Formal analysis, Writing – original draft. **Mei Yi Law:** Data curation, Formal analysis, Writing – original draft.

Declaration of competing interest

The authors declare no conflict of interest, financial, or otherwise.

Acknowledgements

The authors would like to thank the Faculty of Science, Universiti Tunku Abdul Rahman for providing financial support and research facilities. The authors thank Hock Ju Edar Sdn. Bhd., Malaysia for providing rice bran.

References

[1] Y.A. Rodríguez-Restrepo, P. Ferreira-Santos, C.E. Orrego, J.A. Teixeira, C.M. R. Rocha, Valorization of rice by-products: protein-phenolic based fractions with bioactive potential, J. Cereal Sci. 95 (2020) 103039, https://doi.org/10.1016/j.jcs.2020.103039.

[2] X. Zhuang, T. Yin, W. Han, X. Zhang, Chapter 10 – nutritional ingredients and active compositions of defatted rice bran, L. Z. Cheong, X. Xu. Rice Bran and Rice Bran Oil, Academic Press, Cambridge, 2019, pp. 247–270, https://doi.org/10.1016/B978-0-12-812828-2.00010-X.

[3] Y. Zheng, N. Gao, J. Wu, B. Yin, Chapter 11 – rice bran protein: extraction, nutraceutical properties, and potential applications, L. Z. Cheong, X. Xu. Rice Bran and Rice Bran Oil, Academic Press, Cambridge, 2019, pp. 271–293, https://doi.org/10.1016/B978-0-12-812828-2.00011-1.

[4] C. Uraipong, J. Zhao, Rice bran protein hydrolysates exhibit strong *in vitro* α-amylase, β-glucosidase and ACE-inhibition activities, J. Sci. Food Agric. 96 (2016) 1101–1110, https://doi.org/10.1002/jsfa.7182.

[5] Y.Q. Liu, P. Strappe, W.T. Shang, Z.K. Zhou, Functional peptides derived from rice bran proteins, Crit. Rev. Food Sci. Nutr. 59 (2017) 349–356, https://doi.org/10.1080/10408398.2017.1374923.

[6] Z. Karami, S.H. Peighambardoust, J. Hesari, B. Akbari-Adergani, D. Andreu, Antioxidant, anticancer and ACE-inhibitory activities of bioactive peptides from wheat germ protein hydrolysates, Food Biosci. 32 (2019) 100450, https://doi.org/10.1016/j.fbio.2019.100450.

[7] Y. Hou, Z. Wu, Z. Dai, G. Wang, G. Wu, Protein hydrolysates in animal nutrition: industrial production, bioactive peptides and functional significance, J. Anim. Sci. Biotechnol. 8 (2017) 24, https://doi.org/10.1186/s40104-017-0153-9.

[8] S. Phongthai, S. D'Amico, R. Schoenlechner, W. Homthawornchoo, S. Rawdkuen, Fractionation and antioxidant properties of rice bran protein hydrolysates stimulated by *in vitro* gastrointestinal digestion, Food Chem. 240 (2018) 156–164, https://doi.org/10.1016/j.foodchem.2017.07.080.

[9] A. Tapal, P.K. Tiku, Chapter 27 – nutritional and nutraceutical improvement by enzymatic modification of food proteins, M. Kuddus. Enzymes in Food Biotechnology Production, Applications, and Future Prospects Rice Bran, Academic Press, Cambridge, 2019, pp. 471–481, https://doi.org/10.1016/B978-0-12-813280-7.00027-X.

[10] P. Fathi, M. Moosavi-Nasab, A. Mirzapour-Kouhdasht, M. Khalesi, Generation of hydrolysates from rice bran proteins using a combined ultrasonication-alcalase hydrolysis treatment, Food Biosci. 42 (2021) 101110, https://doi.org/10.1016/j.fbio.2021.101110.

[11] K. Hunsakul, T. Laokuldilok, V. Sakdatorn, W. Klangpetch, C.S. Brennan, N. Utama-ang, Optimization of enzymatic hydrolysis by alcalase and flavourzyme to enhance the antioxidant properties of jasmine rice bran protein hydrolysate, Sci. Rep. 12 (2022) 12582, https://doi.org/10.1038/s41598-022-16821-z.

[12] Q.Q. Qiao, Q.B. Luo, S.K. Suo, Y.Q. Zhao, C.F. Chi, B. Wang, Preparation, characterization, and cytoprotective effects on HUVECs of fourteen novel angiotensin-I-converting enzyme inhibitory peptides from protein hydrolysate of tuna processing by-products, Front. Nutr. 9 (2022) 868681, https://doi.org/10.3389/fnut.2022.868681.

[13] Y.D. Hu, Q.H. Xi, J. Kong, Y.Q. Zhao, C.F. Chi, B. Wang, Angiotensin-I-converting enzyme (ACE)-inhibitory peptides from the collagens of Monkfish (*Lophius litulon*) swim bladders: isolation, characterization, molecular docking analysis and activity evaluation, Mar. Drugs 21 (2023) 526, https://doi.org/10.3390/md21100516.

[14] C. Sun, Y. Shan, X. Tang, D. Han, X. Wu, H. Wu, M. Hosseininezhad, Effects of enzymatic hydrolysis on physicochemical property and antioxidant activity of mulberry (*Morus atropurpurea* Roxb.) leaf protein, Food Sci. Nutr. 9 (2021) 5379–5390, https://doi.org/10.1002/fsn3.2474.

[15] K.Y. Ee, L.Y. Khoo, W.J. Ng, F.C. Wong, T.T. Chai, Effects of bromelain and trypsin hydrolysis on the phytochemical content, antioxidant activity, and antibacterial activity of roasted butterfly pea seeds, Processes 7 (2019) 534, https://doi.org/10.3390/pr7080534.

[16] T.T. Chai, S.Y. Ang, K. Goh, Y.H. Lee, J.M. Ngoo, L.K. Teh, F.C. Wong, Trypsin-hydrolyzed corn silk proteins: Antioxidant activities, *in vitro* gastrointestinal and thermal stability, and hematoprotective effects, eFood 1 (2020) 156–164, https://doi.org/10.2991/efood.k.200323.001.

[17] H.T.H. Trang, P. Pasuwan, Screening antimicrobial activity against pathogens from protein hydrolysate of rice bran and Nile Tilapia by-products, Int. Food Res. J. 25 (2018) 2157–2163. http://www.ifrj.upm.edu.my/25%20(05)%202018/(53).pdf.

[18] Y. Sheng, W.Y. Wang, M.F. Wu, Y.M. Wang, W.Y. Zhu, C.F. Chi, B. Wang, Eighteen novel bioactive peptides from Monkfish (*Lophius litulon*) swim bladders: Production, identification, antioxidant activity, and stability, Mar. Drugs 21 (2023) 169, https://doi.org/10.3390/md21030169.

[19] L.C. Foong, M.U. Imam, M. Ismail, Iron-binding capacity of defatted rice bran hydrolysate and bioavailability of iron in Caco-2 cells, J. Agric. Food Chem. 63 (2015) 9029–9036, https://doi.org/10.1021/acs.jafc.5b03420.

[20] D. Akter, R. Begum, M.N. Rahman, N. Talukder, M.J. Alam, Optimization of extraction process parameter for rice bran protein concentrate and its utilization in high protein biscuit formulation, Curr. Res. Nutr. Food Sci. 8 (2020) 596–608, https://doi.org/10.12944/CRNFSJ.8.2.25.

[21] S. Phongthai, S.T. Lim, S. Rawdkuen, Optimization of microwave-assisted extraction of rice bran protein and its hydrolysates properties, J. Cereal Sci. 70 (2016) 146–154, https://doi.org/10.1016/j.jcs.2016.06.001.

[22] S. Phongthai, W. Homthawornchoo, S. Rawdkuen, Preparation, properties and application of rice bran protein: a review, Int. Food Res. J. 24 (2017) 25–34. http://www.ifrj.upm.edu.my/24%20(01)%202017/(2).pdf.

[23] T.P. Singh, R.A. Siddiqi, D.S. Sogi, Statistical optimization of enzymatic hydrolysis of rice bran protein concentrate for enhanced hydrolysate production by papain, LWT Food Sci. Technol. 99 (2019) 77–83, https://doi.org/10.1016/j.lwt.2018.09.014.

[24] X. Feng, S. Hang, Y. Zhou, Q. Liu, H. Yang, Bromelain kinetics and mechanism on myofibril from golden pomfret (*Trachinotus blochii*), J. Food Sci. 83 (2018) 2148–2158, https://doi.org/10.1111/1750-3841.14212.

[25] R. Hu, G. Chen, Y. Li, Production and characterization of antioxidative hydrolysates and peptides from corn gluten meal using papain, ficin, and bromelain, Molecules 25 (2020) 4091, https://doi.org/10.3390/molecules25184091.

[26] M. Irakli, F. Kleisaris, K. Kadoglidou, D. Katsantonis, Optimizing extraction conditions of free and bound phenolic compounds from rice by-products and their antioxidant effects, Foods 7 (2018) 93, https://doi.org/10.3390/foods7060093.

[27] A. Ghasemzadeh, M.T. Karbalaii, H.Z.E. Jaafar, A. Rahmat, Phytochemical constituents, antioxidant activity, and antiproliferative properties of black, red, and brown rice bran, Chem. Cent. J. 12 (2018) 17, https://doi.org/10.1186/s13065-018-0382-9.

[28] L. Liu, W. Wen, R. Zhang, Z. Wei, Y. Deng, J. Xiao, M. Zhang, Complex enzyme hydrolysis releases antioxidative phenolics from rice bran, Food Chem. 214 (2017) 1–8, https://doi.org/10.1016/j.foodchem.2016.07.038.

[29] P. Thamnarathip, K. Jangchud, A. Jangchud, S. Nitisinprasert, S. Tadakittisarn, B. Vardhanabhuti, Extraction and characterisation of Riceberry bran protein hydrolysate using enzymatic hydrolysis, Int. J. Food Sci. Technol. 51 (2016) 194–202, https://doi.org/10.1111/ijfs.13008.

[30] A.A. Zaky, Z. Chen, Y. Liu, S. Li, Y. Jia, Preparation and assessment of bioactive extracts having antioxidant activity from rice bran protein hydrolysates, J. Food. Meas. Charact. 13 (2019) 2542–2548, https://doi.org/10.1007/s11694-019-00174-9.

[31] S.N. Mazloomi, A.S. Mahoonak, L. Mora, M. Ghorbani, G. Houshmand, F. Toldra, Pepsin hydrolysis of orange by-products for the production of bioactive peptides with gastrointestinal resistant properties, Foods 10 (2021) 679, https://doi.org/10.3390/foods10030679.

[32] H. Jin, C. Liu, S. Zhang, Z. Guo, J. Li, Q. Zhao, Y. Zhang, J. Xu, Comparison of protein hydrolysates against their native counterparts in terms of structural and antioxidant properties, and when used as emulsifiers for curcumin nanoemulsions, Food Funct. 11 (2020) 10205–10218, https://doi.org/10.1039/D0FO01830A.

[33] Y. Chen, P. Han, B. Ma, X. Wang, M. Ma, N. Qiu, X. Fu, Effect of thermal treatment on the antioxidant activity of egg white hydrolysate and the preparation of novel antioxidant peptides, Int. J. Food Sci. Technol. 57 (2021) 15036, https://doi.org/10.1111/ijfs.15036.

[34] E.S.K. Ibrahim, M.A. Ghani, The effect of enzymatic hydrolysis on the antioxidant activities and amino acid profiles of defatted chia (*Salvia hispanica* l.) flour, Food Res. 4 (2020) 38–50, https://doi.org/10.26656/fr.2017.4(S4).003.

[35] L. Wattanasiritham, C. Theerakulkait, S. Wickramasekara, C.S. Maier, J.F. Stevens, Isolation and identification of antioxidant peptides from enzymatically hydrolyzed rice bran protein, Food Chem. 192 (2016) 156–162, https://doi.org/10.1016/j.foodchem.2015.06.057.

[36] Z. Karami, B. Akbari-Adergani, Bioactive food derived peptides: a review on correlation between structure of bioactive peptides and their functional properties, J. Food Sci. Technol. 56 (2019) 535–547, https://doi.org/10.1007/s13197-018-3549-4.

[37] C.Z. Zhu, W.G. Zhang, Z.L. Kang, G.H. Zhou, X.L. Xu, Stability of an antioxidant peptide extracted from Jinhua Ham, Meat. Sci. 96 (2014) 783–789, https://doi.org/10.1016/j.meatsci.2013.09.004.

[38] I. Volf, I. Ignat, M. Neamtu, V.I. Popa, Thermal stability, antioxidant activity, and photo-oxidation of natural polyphenols, Chem. Pap. 68 (2014) 121–129, https://doi.org/10.2478/s11696-013-0417-6.

[39] P.S. Rao, E. Nolasco, A. Handa, M.J. Naldrett, S. Alvarez, K. Majumder, Effect of pH and heat treatment on the antioxidant activity of egg white protein-derived peptides after simulated *in-vitro* gastrointestinal digestion, Antioxid 9 (2020) 1114–1128, https://doi.org/10.3390/antiox9111114.

[40] T.T. Chai, J. Xiao, S. Mohana Dass, J.Y. Teoh, K.Y. Ee, W.J. Ng, F.C. Wong, Identification of antioxidant peptides derived from tropical jackfruit seed and investigation of the stability profiles, Food Chem. 340 (2021) 127876, https://doi.org/10.1016/j.foodchem.2020.127876.

[41] Z. Breijyeh, B. Jubeh, R. Karaman, Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it, Molecules 25 (2020) 1340, https://doi.org/10.3390/molecules25061340.

[42] A. Jakubczyk, M. Karaś, P. Stanikowski, B. Rutkowska, M. Dziedzic, E. Zielińska, K. A. Szychowski, U.E. Binduga, K. Rybczyńska-Tkaczyk, B. Baraniak, Characterisation of biologically active hydrolysates and peptide fractions of vacuum packaging string bean (*Phaseolus vulgaris* L.), Foods 9 (2020) 842, https://doi.org/10.3390/foods9070842.

[43] M. Taniguchi, M. Kameda, T. Namae, A. Ochiai, E. Saitoh, T. Tanaka, Identification and characterization of multifunctional cationic peptides derived from peptic hydrolysates of rice bran protein, J. Funct. Foods 34 (2017) 287–296, https://doi.org/10.1016/j.jff.2017.04.046.

[44] I. Karkouch, O. Tabbene, D. Gharbi, M.A.B. Mlouka, S. Elkahoui, C. Rihouey, L. Coquet, P. Cosette, T. Jouenne, F. Limam, Antioxidant, antityrosinase and antibiofilm activities of synthesized peptides derived from *Vicia faba* protein hydrolysate: a powerful agents in cosmetic application, Ind. Crops Prod. 109 (2017) 310–319, https://doi.org/10.1016/j.indcrop.2017.08.025.

[45] Y. Huan, Q. Kong, H. Mou, H. Yi, Antimicrobial peptides: classification, design, application and research progress in multiple fields, Front. Microbiol. 11 (2020) 582779, https://doi.org/10.3389/fmicb.2020.582779.

[46] O. Selamassakul, N. Laohakunjit, O. Kerdchoechuen, L. Yang, C.S. Maier, Isolation and characterisation of antioxidative peptides from bromelain-hydrolysed brown rice protein by proteomic technique, Process. Biochem. 70 (2018) 179–187, https://doi.org/10.1016/j.procbio.2018.03.024.