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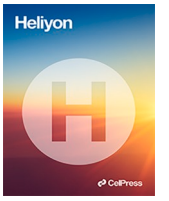

Research article

# Gamma radiation vs high pressure pretreatment on physicochemical characteristics of rice bran hydrolysate

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ABSTRACT

This study investigated the effect of using gamma radiation and high-pressure processing as pretreatment, to consider the structural and amino acid composition changes in rice bran hydrolysate (RBH). The extraction yield and degree of hydrolysis of the irradiated sample were greater than those of the pressurized and control samples, which radiation at 10 kGy gave 31 % yield. Protein content of the control was the highest at 36.1 %, with 32.4 % in pressurized sample at 500 MPa. Control had the highest concentration of total and branched-chain amino acids, with a value of 25,834 mg/100g. Before and after extraction, the microstructure changed visibly and protein agglomeration can be significantly induced by applying a high-pressure. Therefore, this study showed the potential of using both pretreatment methods prior to enzymolysis extraction, with radiation producing more extract. High-pressure produced more protein content, but neither method produced any difference in amino acid content.

## 1. Introduction

Rice constitutes one of the most important agricultural products worldwide, and is grown in over a hundred countries on every continent except Antarctica [1]. Consumers choose refined white rice, especially in Asian countries. These will yield an essential by-product, rice bran, from which rice bran oil can be extracted. While the defatted rice bran is offered as inexpensive animal feed. Current studies indicate that rice bran provides numerous nutritional benefits such as high protein content, vitamins, minerals, and antioxidants [2]. There have been reports that rice has a higher protein digestibility and biological value than other major cereals (i.e., wheat, corn, and barley) [1,3]. However, plant proteins have usage limitations since certain structures are not suitable for chemical processing. Therefore, they need to be processed or modified. The hydrolysis of plant proteins by enzymes is a common technique for modifying functional properties. According to Zhao et al. [4], protein hydrolysate demonstrated higher protein solubility under varying pH and temperature than the native protein. In addition, enzymatic hydrolysis also generates short-chain peptides with greater nutritional value than natural protein [5].

Despite extensive knowledge of food preservation by heat treatment, new concepts and reduction in thermal processing severity are being explored to minimize nutrient loss. Alternative techniques to conventional thermal treatment comprise high pressure processing (HPP), pulsed electric fields (PEF), irradiation, and ultra-filtration [6]. Gamma radiation is becoming more widely accepted as a phytosanitary treatment for food and herbal materials worldwide. It enhances the hygienic quality of various foods and herbal

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materials and reduces losses from microbial contamination [7]. It can also produce direct and indirect impacts on solid and liquid food, resulting in modifications of the molecular structure of the food itself [8]. The effect of gamma irradiation on sunflower seed protein isolates was studied by Malik et al. [9]. Gamma irradiation was found to have an effect on secondary protein. The α-helix content decreased while the β-sheet content increased as the gamma radiation dose increased. Ahmed et al. [10] studied the nutritional and functional effects of gamma irradiation on sorghum seeds. Due to protein denaturation, gamma irradiation at 0.5–5 kGy increased protein digestibility and solubility.

High pressure processing, also known as cold pasteurization. By relying on the Le Chatelier's law, that is when a liquid is subjected to high pressure (100-1000 MPa). The overall volume of the liquid is reduced and causing various reactions such as breaking of hydrogen bonds in food molecules. High pressure processing can significantly alter the structure and texture of whey proteins, as well as reduce droplet size, which may lead to an improvement in the quality of the food [11,12]. Perreault et al. [13] investigated the effect of high-pressure pre-treatment extraction of flax seed protein hydrolysate. They found that 600 MPa alters protein molecules. Zhao et al. [14] investigated the extraction of peptides from mushroom foot meals utilizing protease enzymes and high-pressure pre--treatment. Results showed that 400 MPa for 10 min was the optimal extracting condition since it had the highest protein concentration and degree of hydrolysis. After reviewing many studies related to gamma radiation and high-pressure processing, we found that there was scant research comparing these two techniques and have not been fully understood. Therefore, the researchers aimed to investigate the effect of two pre-treatment methods using gamma radiation and high-pressure processing on the physical and chemical properties of rice bran protein hydrolysate.

## 2. Materials and methods

### 2.1. Materials

Defatted rice bran (DRB) (10.59 % moisture content, 1.26 % oil content, 13.72 % fiber and 12.00 % impurities) from Kasisuri Co. Ltd. (Phra Nakhon Si Ayutthaya, Thailand) was filtered through a 50-mesh sieve, divided into 5 portions and stored in polyethylene bags at 4 °C for further examination. Protease G6 (EC 3.4.21.62; alkaline serine endo-protease) produced by *Bacillus licheniformis* (Enzyme activity of 5.8 x $10^5$ DU/g) was purchased from Siam Victory Chemical Co. Ltd. (North Klongtoey, Bangkok, Thailand). Other chemicals and reagents used were of analytical grade.

### 2.2. Pre-treatment samples preparation

The preparation method for pressurized DRB (DRB-P) was adapted from Perreault et al. [13]. Using water as a medium, the bags of DRB were placed in a high pressure vessel (HPP600 MPa, BaoTou KeFa High Pressure Technology Co., China). Two pressure levels, 200 and 500 MPa (DRB-P200 and DRB-P500), were used at 25 °C for 20 min, then the samples were kept at 4 °C for further analysis.

The preparation method for the gamma-irradiated rice bran was as follows: 5 and 10 kGy (DRB-G5 and DRB-G10) were irradiated using a gamma chamber (Gamma chamber 5000, BRIT, India) with cobalt-60 as the radiation source, at a dose rate of 2.2 kGy/h [15].

### 2.3. Rice bran hydrolysate preparation

Distilled water was added to the DRB (1:7) and the pH of the solution adjusted to 9.5 using 1 M NaOH, and stirred with an overhead stirrer (RW20, IKA, Malaysia). It was hydrolyzed with 2 % (v/w) protease G6 when the temperature reached 60 °C for 3 h. The enzyme was inactivated with a high heat for 2 min and allowed to cool at room temperature. Then centrifuged at 10000×*g* for 15 min, 4 °C, the supernatant solution was freeze-dried. Rice bran hydrolysate powders (RBH) were divided into 5 treatments; a non-pretreatment sample as control (RBH-C), irradiated with gamma rays at 5 kGy was RBH-G5 and at 10 kGy was RBH-G10. Pressurized sample at 200 MPa was RBH-P200 and 500 MPa was RBH-P500.

### 2.4. Analysis of the physicochemical properties

#### 2.4.1. Extraction yield

The freeze-dried extracts were weighed and calculated based on the initial weight of DRB using the equation as below

$$\text{Extraction yield } (\%) = \frac{weight\ of\ RBH\ (\mathbf{g})}{weight\ of\ DRB\ (\mathbf{g})} \times 100$$

#### 2.4.2. Protein content determination

Protein quantification was made using the Kjeldahl method and conversion factor for rice of 6.25 [16].

#### 2.4.3. Degree of hydrolysis

According to Adler-Nissen [17], the formol titration method was used to calculate the percentage of dissociated peptide bonds in the protein molecule to evaluate the degree of protein degradation. 2.5 ml of RBH solution was prepared and the pH adjusted to 8.5 using 0.01 N NaOH. Then, 1 ml of 35 % formaldehyde was added and incubated for 1 min at room temperature. Then titrated with 0.01 N NaOH to achieve pH 8.5. The amount of NaOH used was noted and calculated using the equation:

$$DH\ (\%) = B \times N_B \times \frac{1}{\alpha} \times \frac{1}{M_p} \times \frac{1}{h_{tot}} \times 100$$

where B = volume of alkali solution used (ml), $N_B$ = concentration of alkali solution in normality, $1/\alpha$ = the specific degree of amino acid hydrolysis is proportional to the pK value at a particular temperature and pH (at 60 °C and pH 9.5 is 1.00) [17], $M_p$ = total protein mass in the sample, and $h_{tot}$ = total number of peptide bonds in the protein (the specific value for rice bran protein is 8.4).

#### 2.4.4. Color

Each sample was measured using the CIE L*a*b* system (illuminant d65/10°) (Hunter lab CX2678, Hunter lab, USA), where L* represents the brightness value, a* represents the red value, and b* represents the yellow value.

#### 2.4.5. Amino acid composition

The amino acid composition of freeze-dried RBH samples was evaluated using high performance liquid chromatography (HPLC) with equivalent standard amino acids according to in-house method WI-TMC-06 [18].

#### 2.4.6. Microstructure

The microstructure of rice bran before and after enzymatic hydrolysis was assessed using a scanning electron microscope (JSM7800F, JEOL, Japan). With accelerating voltage of 5.0 kV and 500x and 2000× magnification. Samples were coated with gold-plate to examine workpieces.

#### 2.4.7. Fourier transform infrared spectroscopy

Freeze-dried RBH samples were evaluated for conformation and functional groups using an ATR-FTIR spectrometer (BrukerBio-SpinCorp, Billerica, MA, USA) with a wave number range from 400 to 4000 $cm^{-1}$. An average of 32 scans at 4 $cm^{-1}$ resolution and spectrums were reported in transmittance mode.

#### 2.4.8. Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics. Completely Randomized Design (CRD) with tri-replicate was used and reported as mean ± standard deviation. Analysis of variance (ANOVA) was used to assess the variance of the data, and Duncan's new multiple range test was used to compare significant difference at 95 % level of confidence.

## 3. Results and discussion

### 3.1. Extraction yield of RBH

The extraction yield, total protein content, and degree of hydrolysis were all investigated. The results are shown in Table 1. It was discovered that the extraction yield varied significantly ($p < 0.05$). The rice bran that was irradiated with gamma rays gave the maximum extraction yield at 10 kGy (RBH-G10) followed by RBH-G5, as gamma rays stimulate the disintegration of water molecules, resulting in free radicals. These radicals will compete to continuously capture electrons with other compounds, and can break down hydrogen bonds in the cellulose structure of rice bran and also denature proteins [19]. This is consistent with Khattak et al. [7], in which the extract from gamma-irradiated (2–16 kGy) *Nigella sativa* seeds was substantially higher than that of the unirradiated sample. Kim et al. [20] observed that gamma radiation increased the amount of extracts from Korean herbs by 5–30 %. Therefore, the free radicals in the irradiated sample caused by the effects of gamma rays induced denaturation of the protein structure [19,21]. The control sample and the high-pressure treated samples exhibited a significant decrease in extraction yield compared to those treated with gamma rays. There was no significant difference between the two pressure levels ($p > 0.05$) due to the fact that pressure affected the rupture of hydrogen bonds and destroyed the amorphous regions of cellulose, causing cellulose to rearrange into a tightly packed cell that decreases enzymatic permeability [22]. It can be confirmed that radiation was found to be superior for increasing enzymatic extraction yield.

**Table 1**
Extraction yield, protein content, and degree of hydrolysate of RBH.

| Treatments | Extraction yield (%) | Protein content (%) | Degree of hydrolysis (%) |
|---|---|---|---|
| RBH-C | $24.98 \pm 0.60^{c}$ | $36.08 \pm 0.43^{a}$ | $11.78 \pm 0.27^{c}$ |
| RBH-G5 | $28.94 \pm 0.35^{b}$ | $30.31 \pm 0.13^{c}$ | $13.52 \pm 0.41^{a}$ |
| RBH-G10 | $31.04 \pm 0.47^{a}$ | $28.80 \pm 1.09^{d}$ | $12.99 \pm 0.15^{b}$ |
| RBH-P200 | $24.87 \pm 1.24^{c}$ | $31.33 \pm 0.76^{bc}$ | $11.92 \pm 0.18^{c}$ |
| RBH-P500 | $24.47 \pm 1.05^{c}$ | $32.39 \pm 0.27^{b}$ | $12.06 \pm 0.17^{c}$ |

Results are mean of tri-replicate ± standard deviation. Superscripts with different letters in same column (a, b, c) are significantly different ($p < 0.05$) between treatments.
RBH-C = control rice bran hydrolysate, RBH-G5 = irradiated 5 kGy rice bran hydrolysate, RBH-G10 = irradiated 10 kGy rice bran hydrolysate, RBH-P200 = pressurized 200 MPa rice bran hydrolysate, RBH-P500 = pressurized 500 MPa rice bran hydrolysate.

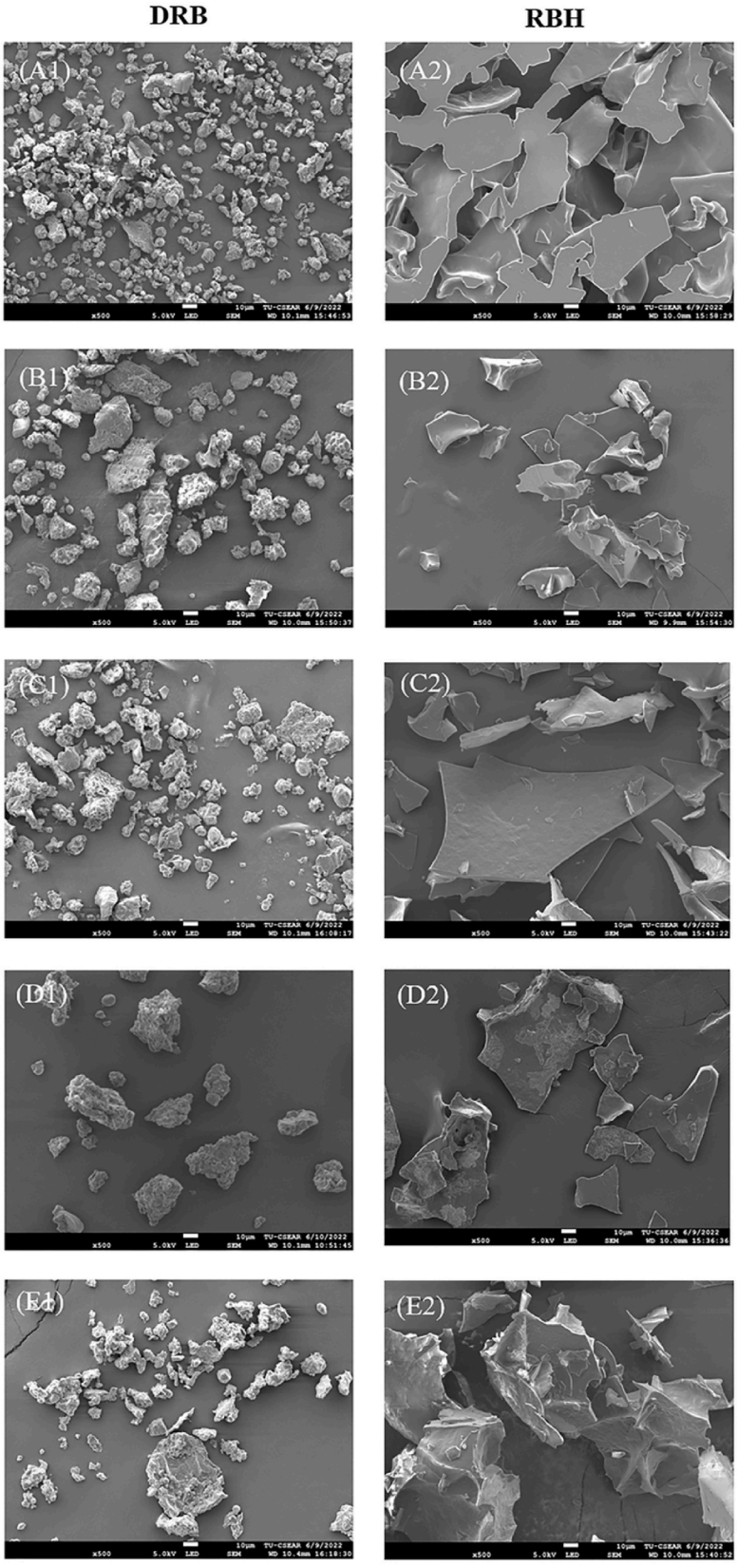


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**Fig. 1.** Microstructures of (1) defatted rice bran and (DRB) and (2) rice bran hydrolysate (RBH); A–E. samples are (A) control, (B) irradiated 5 kGy, (C) irradiated 10 kGy, (D) pressurized 200 MPa, and (E) pressurized 500 MPa.

### 3.2. Protein content and degree of hydrolysis of RBH

RBH-C samples showed the highest protein content ($p < 0.05$), followed by RBH-P500, RBH-P200, and RBH-G10, which appeared to have the lowest protein content. The reduction in the irradiated samples is due to the impacts of free radicals causing protein denaturation. These free radicals may initiate a chain reaction with other molecules to generate nitrite, nitrate, and nitro compounds, such as peroxynitrite or nitrotyrosine, which cannot be converted back to ammonium sulfate via the Kjeldahl digesting process. Consequently, the total amount of nitrogen captured from samples decreased [23,24]. The protein content of pressurized samples was slightly lower than that of RBH-C, which may be due to the densely packed cells, as shown in Fig. 1, so that the enzyme cannot effectively penetrate.

The degree of hydrolysis was calculated from the ratio of broken peptide bonds to total peptide bonds in the protein structure [25]. According to Table 1, RBH-G5 exhibited the highest degree of hydrolysis ($p < 0.05$) with 13.52 %, followed by RBH-G10 with 12.99 %. This was consistent with the results of the extraction yield and the microstructure image (Fig. 1) in that the presence of free radicals from the disintegration of other molecules in the irradiated sample increased the denaturation of the polypeptide chain, allowing NaOH to react with the carboxyl group in the polypeptide chain more. RBH-C, RBH-P200, and RBH-P500 samples did not differ significantly ($p > 0.05$), consistent with Dong et al. [26] in which peanut protein isolates were subjected to high pressure at 100–500 MPa before being extracted into protein hydrolysates, and it was found that the degree of hydrolysis of the pressure-treated samples was comparable to or less than that of the control samples. In addition, there was also no significant change in the degree of hydrolysis between the control and pressurized samples, probably due to the influence of pressure that unfolded the native proteins. When the pressure is relieved, polypeptide chains will reagglomerate into larger particles as a result of disulfide bonds, hydrophobic-hydrophilic interactions, and hydrogen bonds, making it more difficult for enzymes to infiltrate the cleavage site [13,27].

### 3.3. Color of DRB and RBH

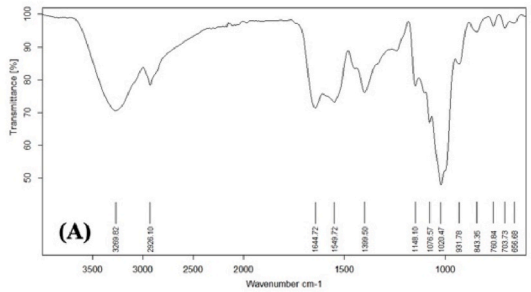
Some rice cultivars have pigments on the pericarp and seed coats that impart yellow, red, black, or purple hues. The pericarp and aleurone layers of the grains consist of hydrophilic and semi-hydrophilic compounds, such as carotenoids, anthocyanins, and polyphenols [28]. As shown in Table 2, the color of defatted rice bran before and after extraction revealed that the brightness (L*) of samples before and after extraction was significantly different. Comparing the DRB samples, it was discovered that the irradiated samples were brighter than the pressurized samples, likely due to particle size and number changes that altered the degree of light scattering [29]. RBH samples were less bright than DRB samples, and both DRB and RBH of pressurized samples were significantly darker than radiated samples, except for RBH-P200.

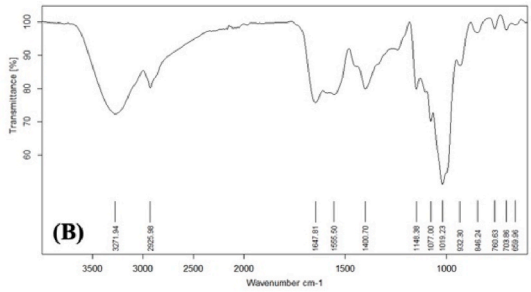
The yellowness (b*) of the RBH did not differ significantly ($p > 0.05$), but b* increased in all treatments relative to the pre-extraction sample (DRB). In all RBH samples, the red value (a*) increased in the same way as the yellowness. According to Hemmler et al. [30], the side chains of lysine and cysteine are the strongest nucleophiles and can undergo protein glycation with reducing sugars to form Maillard reaction products (MRPs) the quickest. The protein unfolding from pressure allows the reducing sugar to attach to amino acids more readily after enzymolysis. When the Maillard reaction causes an increase in browning, the yellowness and redness of all RBH samples increase. RBH-P200 was significantly darkened, which corresponded to the shifting of the saccharides region in the FTIR spectrum (Fig. 2).

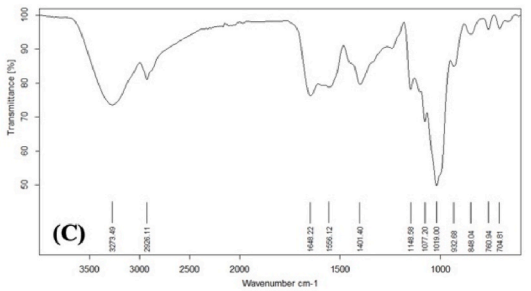
**Table 2**
Color of DRB and RBH.

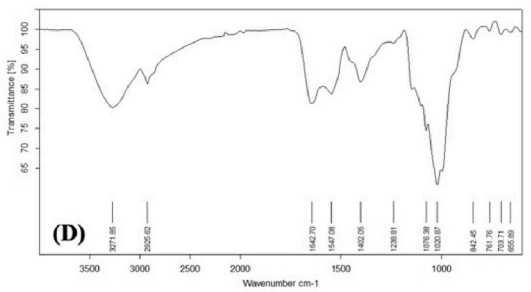
| Treatments | Lightness (L*) | Yellowness (b*) | Redness (a*) |
|---|---|---|---|
| DRB-C | $66.11 \pm 0.82^{x}$ | $15.55 \pm 0.59^{x}$ | $2.75 \pm 0.16^{x}$ |
| DRB-G5 | $66.09 \pm 0.54^{x}$ | $15.36 \pm 0.30^{x}$ | $2.63 \pm 0.04^{x}$ |
| DRB-G10 | $66.58 \pm 0.36^{x}$ | $15.29 \pm 0.44^{x}$ | $2.65 \pm 0.07^{x}$ |
| DRB-P200 | $64.98 \pm 0.35^{y}$ | $14.84 \pm 0.67^{x}$ | $2.58 \pm 0.15^{x}$ |
| DRB-P500 | $65.06 \pm 0.24^{y}$ | $14.87 \pm 0.32^{x}$ | $2.59 \pm 0.11^{x}$ |
| RBH-C | $51.51 \pm 0.70^{b}$ | $19.77 \pm 0.43^{a}$ | $5.80 \pm 0.21^{a}$ |
| RBH-G5 | $53.51 \pm 0.54^{a}$ | $19.20 \pm 0.31^{a}$ | $5.65 \pm 0.04^{ab}$ |
| RBH-G10 | $54.33 \pm 0.38^{a}$ | $18.92 \pm 0.16^{a}$ | $5.62 \pm 0.09^{ab}$ |
| RBH-P200 | $49.65 \pm 0.58^{c}$ | $17.42 \pm 0.83^{b}$ | $5.46 \pm 0.24^{b}$ |
| RBH-P500 | $51.91 \pm 0.11^{b}$ | $19.37 \pm 0.18^{a}$ | $5.73 \pm 0.01^{ab}$ |

$^{x, y, z}$ superscripts are comparing between defatted rice bran samples (DRB) where the numbers of different letters from the same column were significantly different ($p < 0.05$).
$^{a, b, c}$ superscripts are comparing between rice bran hydrolysate samples (RBH) where the numbers of different letters from the same column were significantly different ($p < 0.05$).
C = control, G5 = irradiated 5 kGy, G10 = irradiated 10 kGy, P200 = pressurized 200 MPa, P500 = pressurized 500 MPa.



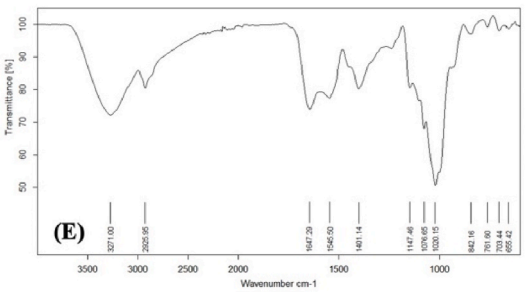




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**Fig. 2.** FTIR spectra of Rice bran hydrolysate (RBH). (A) RBH-C = control rice bran hydrolysate, (B) RBH-G5 = irradiated 5 kGy rice bran hydrolysate, (C) RBH-G10 = irradiated 10 kGy rice bran hydrolysate, (D) RBH-P200 = pressurized 200 MPa rice bran hydrolysate, (E) RBH-P500 = pressurized 500 MPa rice bran hydrolysate.

### 3.4. Amino acid composition of RBH

The content of total amino acids in RBH is shown in Table 3. RBH-C contained the highest concentration of branched-chain amino acids (BCAA) with 4215.34 mg/100g, followed by RBH-P500 with 4175.84 mg/100g, and RBH-G10 with 3895.68 mg/100g. The BCAA content of all samples yielded the same direction as the total amino acid content (TAA). Li et al. [25] investigated the influence of irradiation on hydrolyzed rice protein, with increasing radiation intensity up to 50 kGy, the amount of amino acids was observed to decrease. Davies [31] reported broken protein fragments associated with the hydrogen of α-carbon atoms of the amino acids, which are scavenged by hydroxyl radicals from the breakdown of water molecules, followed by the reaction with oxygen to create various compounds.

Elias and Cohen [32] reported that sulfur-containing amino acids are the most sensitive to radiation. This aligns with the results shown in Tables 3 and in which methionine has the highest reduction at 17.85 % in RBH-G10. Some free radicals produced by radiation, according to Hellwig [21] and Kuan et al. [33], may cause the loss of amino acids like histidine, cysteine, and methionine through oxidation, deamination, and molecular breakdown. The total amino acid content of RBH-P500 is comparable to that of the control, and showed the opposite direction to that of the irradiated sample. This may be due to the effect of protein reaggregation into larger particles, reducing enzyme permeability, resulting in the amino acids being less degraded as the degree of hydrolysis values were the lowest among all treatments. Although while radiation and high-pressure did affect the structure of proteins, nearly all of the amino acids, as measured by the total amount of amino acid composition, were still preserved, including all of the essential amino acids.

### 3.5. Microstructure of DRB and RBH

SEM images demonstrated the distinctions between pre-extraction rice bran (DRB) and hydrolyzed rice bran extract (RBH). Fig. 1 shows that DRB is more spherical while RBH exhibited polygonality, consistent with the findings of Li et al. [25] RBH has a smoother surface and more small cracks than DRB. Among DRB samples, it was found that the control hydrolysate was rounder, similar in size, with a smoother surface. Whereas the irradiated samples exhibited larger particles, undulating texture, and small spherical particles that form large clusters as the intensity of the radiation increased. Wang et al. [34] discovered a correlation between increased irradiation and an increase of protein molecular cracking. This is consistent with the findings of Malik et al. [9], who also discovered cracks on the surface of sunflower isolate after gamma irradiation. In addition, the pressurized rice bran exhibited larger agglomeration than the control. Regarding the rice bran hydrolysate (RBH) samples, the RBH-C sample had the most homogeneous particle distribution and the same size plaque as the DRB-C, whereas the pressurized sample had the most uniform and bigger particle

**Table 3**
Amino acid composition of RBH.

| Free amino acids (mg/100g) | RBH-C | RBH-G5 | RBH-G10 | RBH-P200 | RBH-P500 |
|---|---|---|---|---|---|
| Alanine | 1755.41 ± 0.44 | 1663.39 ± 0.42 | 1660.39 ± 0.42 | 1690.39 ± 0.43 | 1750.41 ± 0.44 |
| Arginine | 2112.49 ± 0.53 | 1961.46 ± 0.49 | 1934.45 ± 0.49 | 1954.46 ± 0.49 | 2030.47 ± 0.51 |
| Aspartic acid | 2369.55 ± 0.60 | 2259.53 ± 0.57 | 2205.51 ± 0.55 | 2316.54 ± 0.58 | 2386.56 ± 0.60 |
| Cystine | 947.76 ± 0.24 | 987.41 ± 0.25 | 973.44 ± 0.24 | 1044.24 ± 0.26 | 937.25 ± 0.24 |
| Glutamic acid | 4061.95 ± 1.02 | 3887.91 ± 0.98 | 3790.88 ± 0.95 | 3990.93 ± 1.00 | 4052.95 ± 1.02 |
| Glycine | 1473.34 ± 0.37 | 1408.33 ± 0.35 | 1371.32 ± 0.35 | 1467.34 ± 0.37 | 1456.34 ± 0.37 |
| Histidine[a] | 809.65 ± 0.20 | 745.11 ± 0.19 | 753.08 ± 0.19 | 777.89 ± 0.20 | 856.71 ± 0.22 |
| Isoleucine[a] | 944.58 ± 0.24 | 902.27 ± 0.23 | 871.97 ± 0.22 | 876.89 ± 0.22 | 934.09 ± 0.24 |
| Leucine[a] | 1836.43 ± 0.46 | 1770.41 ± 0.45 | 1703.40 ± 0.43 | 1731.40 ± 0.44 | 1812.42 ± 0.46 |
| Lysine[a] | 1136.27 ± 0.29 | 1084.25 ± 0.27 | 1042.24 ± 0.26 | 1063.25 ± 0.27 | 1043.24 ± 0.26 |
| Methionine[a] | 349.95 ± 0.09 | 334.37 ± 0.08 | 287.50 ± 0.07 | 385.28 ± 0.10 | 291.53 ± 0.07 |
| Phenylalanine[a] | 1154.27 ± 0.29 | 1105.26 ± 0.28 | 1064.25 ± 0.27 | 1088.25 ± 0.27 | 1150.27 ± 0.29 |
| Proline | 1864.43 ± 0.47 | 1842.43 ± 0.46 | 1567.37 ± 0.39 | 1695.40 ± 0.43 | 1501.35 ± 0.38 |
| Serine | 1278.30 ± 0.32 | 1218.28 ± 0.31 | 1195.28 ± 0.30 | 1216.28 ± 0.31 | 1180.28 ± 0.30 |
| Threonine[a] | 1105.26 ± 0.28 | 1042.24 ± 0.26 | 1013.24 ± 0.25 | 1012.24 ± 0.25 | 1060.25 ± 0.27 |
| Tryptophan[a] | 350.01 ± 0.09 | 418.19 ± 0.11 | 365.20 ± 0.09 | 134.00 ± 0.03 | 203.23 ± 0.05 |
| Tyrosine | 850.28 ± 0.21 | 780.25 ± 0.20 | 797.87 ± 0.20 | 783.80 ± 0.20 | 865.70 ± 0.22 |
| Valine[a] | 1434.33 ± 0.36 | 1386.32 ± 0.35 | 1320.31 ± 0.33 | 1349.31 ± 0.34 | 1429.33 ± 0.36 |
| BCAA | 4215.34 | 4059.01 | 3895.68 | 3957.61 | 4175.84 |
| TEAA | 9120.75 | 8788.43 | 8421.17 | 8418.52 | 8781.07 |
| TAA | 25834.27 | 24797.41 | 23917.68 | 24577.91 | 24942.37 |

BCAA represents Branched-Chain Amino Acids.
TEAA and TAA are Total Essential Amino Acids and Total Amino Acids.
RBH-C = control rice bran hydrolysate, RBH-G5 = irradiated 5 kGy rice bran hydrolysate, RBH-G10 = irradiated 10 kGy rice bran hydrolysate, RBH-P200 = pressurized 200 MPa rice bran hydrolysate, RBH-P500 = pressurized 500 MPa rice bran hydrolysate.
[a] represents essential amino acids.

distribution. RBH-P500 exhibited greater consolidation than the irradiated samples, with the largest particles. The radiation and pressure may have caused the protein to unfold, revealing a buried hydrophobic group that boosted the contact between the polypeptide chain's non-polar sides, but radicals may interfere with the binding action, and induced the highest DH. Alternatively, there were less radicals in the pressurized sample so all the hydrophobic and hydrophilic interactions effectively led to reagglomeration. Also, the density of the particles generated during pressured treatments may have prevented the enzyme from penetrating the particle membrane. Wang et al. [35] discovered larger rice protein hydrolysate particles when subjected to a pressure of 300 MPa, which may be the result of protein agglomeration. These morphological findings supported the previous finding on the degree of hydrolysis that when protein hydrolysates exhibited the highest degree of hydrolysis this will result in small and dispersed particles.

### *3.6. Fourier transform infrared spectroscopy*

FTIR is a method employed to investigate the functional groups by using infrared radiation (IR radiation) at various wavelengths through organic matter. Chemical bonds absorb energy at specific wavelengths and the energy of each wavelength is interpreted as a distinct spectrum [36]. Fig. 2 shows the FTIR spectrum, in which the peak spectra of protein and carbohydrate do not overlap in the FTIR spectrum of rice bran hydrolysate [37]. The wave number 3600-3200 $cm^{-1}$ represents –OH group or hydrogen-bonded compounds, such as phenol and 3000-2800 $cm^{-1}$ for –CH group [38]. Peaks were also found in 850-700 $cm^{-1}$, which is in the oscillating aromatic band, and the main characteristic of phenol compounds [39]. Wave number 3400-3380 $cm^{-1}$ demonstrated N–H stretching vibration and at greater than 3000 $cm^{-1}$ were related to aromatic compounds [36].

In terms of protein structure, it involves two main bands, protein amide I (1640 $cm^{-1}$), amide II (1562 $cm^{-1}$), and secondary structures which are α-helix (1660-1653 $cm^{-1}$) and β-sheet (1645-1643 $cm^{-1}$). In the amide phase, RBH-G10 peaked at 1648.22 $cm^{-1}$, followed by RBH-G5 (1647.81 $cm^{-1}$), RBH-P500 (1647.29 $cm^{-1}$), RBH-C (1644.72 $cm^{-1}$), and RBH-P200 (1642.70 $cm^{-1}$), indicating that the pressure at 200 MPa generated protein hydrolysate with a more ordered structure than the other samples. Upon protease digestion, the spectra exhibited absorption peaks at 1648-1642 $cm^{-1}$ and 1021-1019 $cm^{-1}$ indicating enzymolysis promoting the degradation of cellulose and oligosaccharide in rice bran [34]. The characteristic peak of the stretching vibration of C–N shifted slightly in all treatments from the control (1549 $cm^{-1}$). The saccharides region was indicated by peaks between 1150 and 1018 $cm^{-1}$. Supawong et al. [37] reported that covalent bonds were formed between proteins and polysaccharides in RBH via the Maillard reaction, generating Amadori compounds (C═O), Schiff base (C═N), and pyrazines (C–N), resulting in brown color. The shifting of the saccharide region in the FTIR spectrum of RBH-P200 corresponded to the Maillard reaction products, resulting in a significantly darker color. In comparison to the control sample, the secondary structure of the irradiated and pressurized sample changed from ordered to disordered, and the environment of the functional groups changed, indicating that the conformation of RBH changed after the pre-treatment process.

## 4. Conclusions

By comparing the results of using high pressure and gamma radiation together with enzymatic extraction on physicochemical properties, it can be concluded that the amount of yield product increased by using gamma rays; the protein content was highest in the control sample, followed by the pressurized sample. Also, both technologies still retained the amino acids. SEM images showed that the particles became smaller when subjected to irradiation and pressure, and FTIR spectra showed changes in the protein's secondary structure. Structural changes may affect the functional properties and sensory characteristics of hydrolysate powders; therefore, further studies are required. Another benefit of using radiation and high pressure in combination with protease enzyme is that small peptides can be easily absorbed by the body. Even so, there are some drawbacks because these processes necessarily require investments in both financial and human resources. The author believes that this work will be useful to those who are interested in further research and development in the field of protein extraction from defatted rice bran.

## Data availability statement

All data used for this study are available within this article/supporting material and reference.

## CRediT authorship contribution statement

**Sikarin Masamran:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Supattra Supawong:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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