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Unlocking hidden potential of rice bran: Enzymatic treatment for enhancing techno-functional properties

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ABSTRACT

Rice bran (RB) is a by-product with limited application due to technological constraints. Enhancing its technological functionality as potential food ingredient will improve the sustainability of rice production. The aim was to study the impact of enzymatic and thermal treatments on defatted rice bran using six distinct commercial enzymes (carbohydrases and proteases) and dry heating by evaluating its technological, nutritional and functional properties. Enzymatic treatment increased up to 208% the soluble dietary fiber content (8.19 g/100 g) of defatted RB. Moreover, the solvent retention capacity, including water, oil, sodium carbonate, and sucrose, exhibited a noteworthy increase across all treatments ($p < 0.05$). Bran color changed after treatments, increasing its luminosity (L^*) and decreasing the value of a^* in all cases, but b^* decreased when treated with protein-acting enzymes while increased with carbohydrate-acting enzymes. Proteases played a pivotal role in reducing particle size and forming gels requiring minimal force for application. Microscopic analysis revealed that carbohydrases-treated samples exhibited prominent cell wall breakage, while protease-treated ones showed a gel-like surface with less distinct protein bodies and layered walls. These comprehensive study sheds new transformations brought about by these enzymatic interventions, offering valuable insights into the optimization of rice bran functionality.

1. Introduction

In 2022, a total of 726 million metric tons of unprocessed paddy rice was harvested, which generate 72.6 million metric tons of rice bran (RB) (FAO, 2024). Rice bran has attracted the interest of the cosmetic industry due to its high oil content, rich in bioactive compounds like γ-oryzanol (Garba, Singanusong, Jiamyangyeun, & Thongsook, 2019). However, after fat extraction the remaining secondary by-product is often employed in animal feed, resulting in an underutilization of its nutritional potential (Gul, Yousuf, Singh, Singh, & Wani, 2015; Spaggiari, Dall'Asta, Galaverna, & delCastillo Bilbao, 2021).

To fully harness the many benefits of defatted RB, some research has been dedicated to enhancing the extraction of its components, protein and fibers, or modifying their properties. Physical treatments such as dry heat, parboiling, and microwave treatment have been applied to modify the properties of the protein isolates derived from RB for enhancing oil absorption capacity, emulsifying properties, and gelling abilities of (Khan et al., 2011). Freeze-thaw, sonication or high hydrostatic pressure have been applied to increase the extractability of proteins from defatted RB, either individually or combining sonication with amylase and protease treatment (Tang, Hettiarachchy & Shellhammer, 2002). Other authors opted for using different carbohydrases, singly or combined, to improve the protein extraction (Scarabattoli et al., 2023), and the emulsifying and foaming properties of the protein concentrates improved when cellulase was added to extruded defatted RB (Leal, Senna, Kupski, Mendes, & Badiale-Furlong, 2021). Even those protein concentrates have been further hydrolyzed with proteases to improve their physico-chemical properties (Tang, Hettiarachchy, Horax, & Eswaranandam, 2003). Likewise, Hamada, (2000) successfully created a highly soluble protein hydrolysate with superior emulsifying activity and stability using commercial proteases.

Lately, more attention has been paid to the dietary fraction that could be extracted from defatted RB. With that purpose, dietary fiber extracted from defatted RB, with the aid of amylase, alcalase and glucoamylase, was treated with cellulase to modify its properties, particularly the water binding capacity (Liu, Zhang, Yi, Quan, & Lin, 2021).

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Similarly, the combination of cellulase and xylanase has been very effective to hydrolyze the intramolecular hydrogen bonds in the hemicellulose and cellulose, although water and oil binding capacities were reduced likely due to the low molecular weight of the hydrolysis products (Wen, Niu, Zhang, Zhao, & Xiong, 2017).

Previous studies have highlighted the interest on proteins and dietary fiber extracted from rice bran. However, using one or the other, results in a by-product, without fully utilizing all bran constituents. Nevertheless, the inclusion of RB as ingredient has been challenging owing to the structural disruption and the impact on hydration due to the cellulose content, particularly in bakery products (Doan, Lai, Vo & Nguyen, 2021; Majzoobi, Sharifi, Imani, & Farahnaky, 2013). Enzymatic treatments have proven effective in improving proteins or dietary fiber fractions, but there has been less exploration of modifying rice bran as a food ingredient. Hence, the aim of this study is to evaluate the effect of six different enzymatic treatments, including carbohydrate or protein acting hydrolases, on technological and functional attributes. Since the thermal treatment of bran rice has been the most reported one (Khan et al., 2011; Sharma, Chauhan & Agrawal, 2004; Tang, Hettiarachchy & Shellhammer, 2002), thermal treatment was employed in the present study for comparison purposes. This approach will unlock the full nutritional and functional potential of RB in a more efficient and sustainable manner.

2. Materials and methods

Rice bran from Japonica rice type was procured from Arrocería Pons (Valencia, Spain). The bran was sieved, using a 1000 μm sieve, to obtain a homogenous sample. Enzymes were donated by Novozymes (Bagsvaerd, Denmark). Enzymes selected were: Novozym® (NO) (xylanase, EC 3.2.1.8), Celluclast® (CE) (cellulase, EC 3.2.1.4; 700 U/g), Ultimase® BWL 40 (UL) (cellulase and xylanase; 300 AGU/mL), Shearzyme® Plus 2x (SH) (xylanase, cellulase and β-glucanase, EC 3.2.1.6; 280 U/mL) Alcalase® 2.4 L FG (AL) (endoprotease, EC 3.4.21.62; 2.4 AU-A/g) and Flavourzyme® (FL) (peptidase preparation containing EC 3.2.1.1, EC 3.4.11, EC 3.4.14, EC 3.4.21.63,EC 3.4.24, EC 3.4.24.39; ≥500 U/g).

2.1. Defatting process

RB was defatted following Mohammadi et al. (2021) methodology with some modifications. RB was suspended in hexane (1:3, w:v) and shake for 2 h. The mixture was centrifugated at 4000×*g*, 20 °C for 10 min, in a Beckman centrifuge (Beckman Instruments, USA). Supernatant was decanted and sediment was resuspended in the same amount of hexane. To increase efficiency this step was repeated three times leading to the defatted rice bran (DRB). The supernatants were pooled together and evaporated using a rotary evaporator (Heidolph, Afora, Barcelona, Spain) to get crude RB oil. DRB were kept frozen (−20 °C) till further analysis.

2.2. Enzymatic and thermal treatment

DRB was subjected to six different enzymatic treatments, two of the enzymes mainly protein-acting enzymes and the other four carbohydrate-acting enzymes. RB was suspended in water keeping the ratio 1: 6.5 (w:v). Proteases were added at 1 g/100 mL of the protein content of DRB (Vallabha, Indira, Jyothi Lakshmi & Tiku, 2015), and treatment occurred at optimal pH (6.1) and temperature (50 °C) for 120 min. Carbohydrases were incorporated at a concentration of 1% relative to the DRB fiber content, under identical pH, temperature, and time conditions as the proteases. Then, enzymes were inactivated by raising temperature to 90 °C for 10 min.

The heat treatment (dry) consisted of heating the RB placed in a stainless-steel tray for 30 min at 130 °C using a lab oven (J.P Selecta S. A., Barcelona, Spain).

After enzymatic and thermal treatments, samples were kept at −80 °C and then were freeze-dried and the obtained powders were collected for further analysis.

2.3. Proximate composition

Standard methods were used to determine the proximate composition of the samples. Moisture (ISO 712:2009) and total nitrogen (ISO 16634–2:2016), applying 6.25 as a nitrogen to protein conversion factor, were quantified following ISO methods. Total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) contents were estimated following the method 37–02 (AACC, 2000). Neutral detergent fiber (NDF) content was analyzed following the method proposed by Van Soest, Robertson & Lewis, (1991). Acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined following the procedures outlined in AOAC (973.18). For ADL analysis, samples were treated with 72% sulfuric acid for 3 h. The hemicellulose fraction was calculated by ADF - NDF, and cellulose was calculated by subtracting ADL from ADF.

2.4. Physical properties

2.4.1. Particle size distribution

The particle size distribution was evaluated with a Malvern Mastersizer equipment (Mastersizer Scirocco 2000; Malvern Instruments Ltd., Worcestershire, U.K.). The parameter recorded was volume-weighted mean diameter ($d_{(4.3)}$). The determination was done three times for each sample.

2.4.2. Scanning electron microscopy

The structures of all different samples were examinate using scanning electron microscopy (SEM) (Hitachi S-4800, Tokyo, Japan). All RB samples were coated with gold using a vacuum evaporator (JEE 400; JEOL, Tokyo, Japan). Observation was done an accelerating voltage of 10 kW and 2000x magnification.

2.4.3. Color

The color of the all-study samples was evaluated utilizing a Minolta colorimeter (Chroma Meter CR-400/410, Konica Minolta, Tokyo, Japan). The CIE-$L^*a^*b^*$ values of the samples were captured, including L^* (representing [+] lightness/[−] darkness), a^* (indicating [+] redness/[−] greenness), and b^* (denoting [+] yellowness/[−] blueness). The measurements were conducted at three distinct points on the sample's surface and in different replicates. The ΔE was calculated by using the following equation (1) (Islam, Saha, Monalisa, & Hoque, 2019):

$$\Delta E = \sqrt{(L^* - L^*_{DRB})^2 + (a^* - a^*_{DRB})^2 + (b^* - b^*_{DRB})^2} \quad (1)$$

where L^*_{DRB}, a^*_{DRB}, b^*_{DRB}, are the values of L^*, a^*, b^* of DRB.

2.4.4. Water binding capacity

Water binding capacity (WBC) was analyzed following the method described by Cornejo and Rosell (2015). Briefly, distilled water (1 mL) was added to the DRB (100.0 mg ± 0.5 mg), vortexed for 5 min and centrifuged in Eppendorf centrifuge (Eppendorf AG, Hamburg, Germany) at 2000×*g* for 10 min at room temperature (25 °C). WBC was expressed following the formula given by Cornejo and Rosell (2015).

2.4.5. Oil binding capacity

Oil binding capacity (OBC) was quantified following the method described by Boulemkahel, Betoret, Benatallah, and Rosell (2021) with some modification. DRB (100.0 mg ± 0.5 mg) and vegetal oil (1.0 mL) was vortexed for 5 min and then centrifuged (Eppendorf centrifuge 5430 R, Hamburg, Germany) at 3000×*g*, 4 °C for 10 min. After removing the supernatant, the tubes were inverted for 25 min to drain oil residues.

OBC was calculated by the equation explain by Boulemkahel et al. (2021).

2.4.6. Solvent retention capacity

The Solvent Retention Capacity (SRC) for all samples was determined by adapting the AACC 56-11 method. The SRC values were determined using the following solvents: deionized water, 50 g/100 g sucrose solution, and 5 g/100 g sodium carbonate solution as solvents. SRC was assessed by quantifying the solvent retained by the samples following a 20-min immersion in the solvent, subsequent centrifugation, and a 10-min gel drainage period. The SRC value was expressed as percent of bran weight, on a 14% moisture basis.

2.4.7. Force for penetration

To evaluate the force of penetration of the samples, a 1:5 ratio of bran to water (w:v) was used to ensure optimal hydration of all components, accounting for the moisture content of each sample. Each sample was prepared and analyzed three times. Suspensions and subsequent measurements were performed in 50 mL beakers. Compression was applied at a 10 mm distance from the probe's contact with the sample. A TA-XT plus texture analyzer (Stable Microsystems, Godalming, UK), equipped with a 5 kg load cell and a 25 mm diameter cylindrical aluminum probe was used. The test speed was set at 1.0 mm/s, trigger type auto and the trigger force was 5.0 g. The maximum force required to penetrate the suspensions was recorded. The analysis was conducted in triplicate.

2.5. Statistical analysis

A descriptive statistical analysis (mean ± standard deviation) was applied to the data for the physico-chemical properties. The significant differences associated with the different treatments were evaluated by an analysis of the variance (ANOVA). Fisher's least significant differences test was the method used to present the results. Significant differences were considered as $p < 0.05$. Moreover, Pearson correlation analysis was applied to experimental values obtained from enzymatically treated samples (including the soaked control) to discern potential relationships within analytical parameters.

All analyses were run with the OriginPro versión 2022b, (Origin Lab Corporation, Northampton, MA, USA). All experiments were carried out in triplicate. Principal Component Analysis (PCA) was performed to discern differences among samples. The input matrix comprised 7 rows and 18 columns. PCA analysis was automatically scaled using the statistical program OriginPro.

3. Results and discussion

To better understand the impact of enzymatic and thermal treatments on the rice bran features, rice bran and defatted rice bran were evaluated, although those are not usually included in most of the reported studies. Likewise, to give more forceful results that can be attributed to the treatments or the process itself, a control sample exposed to soaking without enzyme addition (DRB-C) has been considered. Also, a sample subjected to the thermic treatment and subsequently to soaking without enzyme (DRB T-C) was carried out to explore potential synergistic effects arising from these two treatments.

3.1. Proximate composition

The results of the proximate composition and the different dietary fiber fractions of RB, DRB, different enzymatic and heat treatments, and their respective control, are listed in Table 1. As expected, freeze-dried samples showed significant lower moisture content than non-freeze-dried samples. All treatments promoted a significant increase in protein content compared to the control sample RB. Among the enzymatically treated samples, the most substantial increase occurred in the DRB-FL sample, reaching 21.11 g protein/100 g, although no significant differences were detected. The highest protein content was recorded in the defatted, heat-treated and soaked sample (DRB-T-C), with 21.91 g protein/100 g. As expected, defatting increase the TDF, particularly the IDF that was the predominant fraction. Furthermore, heat and enzymatic treatments had a minimal impact on TDF content of the DRB, but

Table 1
Effect of treatment on proximate composition (g/100 g, D.M.) of RB and composition of TDF (%).

Proximate composition					
Treatment	Moisture	Protein	TDF	IDF	SDF (%) db
RB	11.48 ± 0.04 ^{b}	16.06 ± 0.14 ^{d}	33.52 ± 3.53 ^{c}	30.30 ± 4.53 ^{b}	3.22 ^{k}
DRB	11.97 ± 0.06 ^{a}	20.79 ± 0.43 ^{bc}	39.75 ± 0.39 ^{ab}	35.82 ± 2.06 ^{a}	3.94 ^{j}
DRB-T	11.31 ± 0.35 ^{b}	20.82 ± 0.03 ^{bc}	38.19 ± 1.70 ^{b}	32.84 ± 0.82 ^{ab}	5.34 ^{i}
DRB-T-C	6.89 ± 0.09 ^{g}	21.91 ± 0.47 ^{a}	39.20 ± 1.70 ^{ab}	31.99 ± 1.45 ^{ab}	7.21 ^{d}
DRB-C	7.65 ± 0.14 ^{f}	20.55 ± 0.01 ^{c}	42.36 ± 3.34 ^{a}	32.79 ± 4.77 ^{ab}	9.57 ^{a}
DRB-NO	8.00 ± 0.09 ^{e}	20.74 ± 0.16 ^{bc}	39.40 ± 0.64 ^{ab}	32.98 ± 0.56 ^{ab}	6.43 ^{g}
DRB-CE	9.05 ± 0.04 ^{d}	21.10 ± 0.05 ^{bc}	39.74 ± 0.42 ^{ab}	33.53 ± 1.62 ^{ab}	6.21 ^{h}
DRB-UL	7.59 ± 0.05 ^{f}	20.84 ± 0.22 ^{bc}	39.13 ± 1.54 ^{ab}	32.69 ± 0.89 ^{ab}	6.45 ^{f}
DRB-SH	9.47 ± 0.12 ^{c}	20.71 ± 0.27 ^{bc}	42.29 ± 1.02 ^{ab}	34.88 ± 0.18 ^{ab}	7.41 ^{c}
DRB-AL	9.52 ± 0.33 ^{c}	21.00 ± 0.34 ^{bc}	41.36 ± 0.17 ^{ab}	34.00 ± 1.18 ^{ab}	8.19 ^{b}
DRB-FL	9.04 ± 0.28 ^{d}	21.11 ± 0 ^{b}	41.03 ± 1.65 ^{ab}	37.58 ± 0.44 ^{a}	7.16 ^{e}

Treatment	**TDF**		**Fibers**		
	IDF (%)	**SDF (%)**	**Hemicellulose (%)**	**Cellulose (%)**	**Lignin (%)**
RB	82.73 ± 3.56 ^{b}	9.61 ^{k}	44.41 ^{c}	27.56 ± 2.93 ^{ab}	15.12 ± 0.99 ^{ab}
DRB	90.09 ± 5.18 ^{a}	9.91 ^{j}	46.30 ^{b}	27.99 ± 1.02 ^{a}	14.35 ± 1.40 ^{ab}
DRB-T	86.01 ± 2.15 ^{ab}	13.98 ^{i}	59.55 ^{a}	28.30 ± 1.87 ^{a}	14.30 ± 0.51 ^{ab}
DRB-T-C	81.60 ± 3.71 ^{b}	18.39 ^{c}	37.16 ^{g}	27.40 ± 0.89 ^{abcd}	14.31 ± 0.54 ^{b}
DRB-C	72.03 ± 4,13 ^{c}	22.59 ^{a}	23.59 ^{j}	25.53 ± 1.87 ^{ab}	11.30 ± 1.21 ^{ab}
DRB-NO	83.69 ± 1.42 ^{b}	16.32 ^{g}	43.47 ^{d}	20.77 ± 1.98 ^{d}	15.73 ± 2.47 ^{a}
DRB-CE	84.38 ± 4.07 ^{ab}	15.63 ^{h}	41.29 ^{e}	26.27 ± 2.83 ^{abc}	12.69 ± 0.39 ^{ab}
DRB-UL	83.52 ± 2.27 ^{b}	16.48 ^{f}	30.58 ^{i}	27.37 ± 3.72 ^{ab}	13.49 ± 0.36 ^{ab}
DRB-SH	82,48 ± 0.42 ^{b}	17.52 ^{d}	37.85 ^{f}	23.10 ± 1.90 ^{bcd}	12.91 ± 2.81 ^{ab}
DRB-AL	79.81 ± 2.75 ^{b}	19.80 ^{b}	11.04 ^{k}	21.77 ± 3.74 ^{cd}	14.10 ± 2.40 ^{ab}
DRB-FL	82.56 ± 2.60 ^{b}	17.45 ^{e}	31.54 ^{h}	26.63 ± 3.36 ^{ab}	14.32 ± 0.86 ^{ab}

Means with different letters within a column were significantly different ($p < 0.05$).
Abbreviations: RB: rice bran; DRB: defatted rice bran; DRB-T: defatted rice bran thermally treated; DRB-T-C: defatted rice bran thermally treated and soaked; DRB-C: defatted rice bran soaked; DRB-NO: defatted rice bran treated with Novozym® (xylanase); DRB-CE: defatted rice bran treated with Celluclast® (cellulase); DRB-UL: defatted rice bran treated with Ultimase® BWL 40 (cellulase and xylanase); DRB-SH: defatted rice bran treated with Shearzyme® Plus 2x (xylanase, cellulase and β-glucanase); DRB-AL: defatted rice bran treated with Alcalase® 2.4 L FG (endoprotease); DRB_FL: defatted rice bran treated with Flavourzyme® (peptidase preparation).

there was a significant increase trend in SDF content. Although significant differences were found in the SDF content of the enzymatically treated samples, soaking had the greatest impact and no trend regarding the type of enzymatic activities could be envisaged based on dietary fibers solubility. A deeper analysis of the TDF was performed quantifying its composition (Table 1). The fiber of RB was composed of hemicellulose (44.41%), followed by cellulose (27.56%) and lignin (15.12%), being 82.7% IDF and approx. 10% SDF. Thermal treatment significantly increased the amount of hemicellulose, likely water-soluble, based on the enhancement of SDF observed in that sample. Again, soaking affected the fiber distribution. In the samples that underwent soaking (DRB-T-C, DRB-C, and enzymatically-treated samples) SDF were significantly higher. After soaking, enzymatic treatments increased the amount of hemicellulose when treated with xylanase (DRB-NO) or cellulase (DRB-CE) but the combination of those (DRB-UL) did not intensify the effect. This observation could be attributed to the higher enzymatic activity of CE (700 U/g) compared to the enzymatic preparation UL (300 AGU/g). Likewise, cellulose was significantly reduced after treating with xylanase (DRB-NO) or xylanase, cellulase and β-glucanase mixture (DRB-SH), but no after cellulase treatment (DRB-CE). Those results agree with the research conducted by Coda, Rizzello, Curiel, Poutanen, and Katina (2014) in wheat bran treated with xylanase, obtaining an enhancement of the water extractable arabinoxylans and SDF content. It must highlight that the sample treated with endoprotease (DRB-AL) displayed the highest hydrolysis of hemicellulose and extensive hydrolysis of cellulose. These results suggest the existence of side-enzymatic activities in the commercial preparations.

Enzymatic treatment of rice bran has been previously reported and even combined with other physical treatments like micronization (Wen et al. (2017) or high-pressure homogenization (Xie et al. (2019). Those studies reported a substantial increase of SDF content in rice bran and provide significant physicochemical data. However, present study provides information about the various fiber fractions and how enzymatic treatments specifically impact the amounts of each fraction.

3.2. Particle size distribution and microstructure of treated rice bran

Fig. 1 displays the particle size distribution obtained for the different samples, indicating that all treatments reduced the particle size compared to RB. This reduction was cumulative, as it is evident that particle size decreases progressively after each treatment (DRB > DRB-T > DRB-T-C > DRB-C > all enzymatically treated samples). The mean diameter ($d_{(4,3)}$) ranged from 237 to 434 μm (Table 2), showing significant differences among the samples. As noted by Guillon and Champ (2000), the reduction in particle size in samples without enzymatic treatment may be due to the solubilization of some RB components, such as fiber, during processing, or resulting from aggregates dispersion, as mentioned by Rosell, Santos, and Collar (2009). The initial monomodal distribution shifted to bimodal one, particularly after treatment with DRB-AL or DRB-UL. In addition, the sample treated with endoprotease (DRB-AL) has the smallest mean particle size of all the samples. In contrast, the DRB-SH sample, among the group of enzymatically-treated samples, exhibited the largest mean particle size. Therefore, changes in the fiber composition promoted differences in the particle size distribution. Chen, Gao, Yang, and Gao (2013) arrived to similar conclusion when oat samples were subjected to microfluidization process for reducing their particle size, observing a redistribution of IDF and SDF.

At microstructure level, differences associated to the treatments could be envisaged (Fig. 2). Depending on the treatments applied, different proportions of small spherical substances were observed. Starch was predominantly associated with more polygonal spheres, while proteins corresponded to more rounded spheres (Chittapalo & Noomhorm, 2009). Both structures were observable in RB and DRB. In the treated samples, only proteins were observed. The treatment involving cellulases, xylanases, and β-glucanases (DRB-NO, DRB-CE, DRB-UL, and DRB-SH) effectively led to the breakdown of cell walls, as observed in Fig. 2. This finding aligns with Mishra, Ray, Rosell, and Panda (2017) compilation of cell wall degrading enzymes, which includes the aforementioned enzymes. Likewise, Chittapalo and Noomhorm (2009) identified starch and proteins on the surface of the DRB and observed changes in the cell wall structure when applied physical treatments for the DRB proteins extraction. Visibly, samples without enzymatic treatment (RB, DRB, DRB-T, DRB-T-C, DRB-C) exhibited greater disaggregation of all compounds, in contrast to enzyme-treated samples, which displayed a more gel-like structure in their surface. The protease-treated samples displayed noticeable structural changes compared to other enzymatically treated samples. Firstly, these samples exhibited tighter binding, resulting in less distinct differentiation of protein bodies and the layered walls. Moreover, in the sample treated with the endoprotease (DRB-AL), the protein bodies still maintained a spherical shape, while in the sample treated with the peptidase preparation (DRB-FL) the protein bodies acquired a film shape structure,

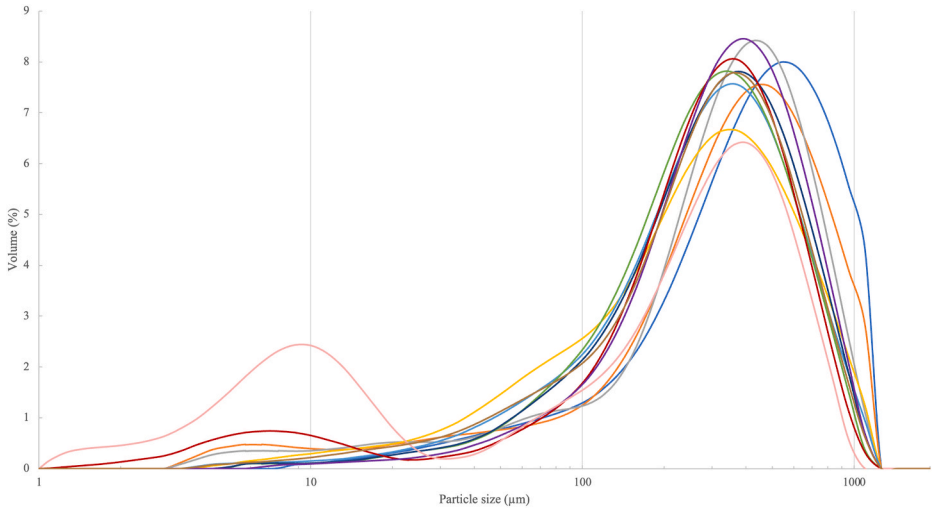

Fig. 1. Particle size distribution of rice bran subjected to different treatments. Abbreviations: RB: rice bran (●); DRB: defatted rice bran (●); DRB-T: defatted rice bran thermally treated (●); DRB-T-C: defatted rice bran thermally treated and soaked (●); DRB-C: defatted rice bran soaked (●); DRB-NO: defatted rice bran treated with Novozym® (xylanase) (●); DRB-CE: defatted rice bran treated with Celluclast® (cellulase) (●); DRB-UL: defatted rice bran treated with Ultimase® BWL 40 (cellulase and xylanase) (●); DRB-SH: defatted rice bran treated with Shearzyme® Plus 2x (xylanase, cellulase and β-glucanase) (●); DRB-AL: defatted rice bran treated with Alcalase® 2.4 L FG (endoprotease) (●); DRB_FL: defatted rice bran treated with Flavourzyme® (peptidase preparation) (●).

Table 2
Technological characteristics of RB with different treatments.

Technological properties						
Treatment	d $_{(4.3)}$ (μm)	L^*	a^*	b^*	ΔE	
RB	434.06 ± 2.48 ^{a}	67.79 ± 0.02 ^{a}	1.78 ± 0.05 ^{a}	18.88 ± 0.01 ^{e}		
DRB	379.33 ± 2.57 ^{b}	80.32 ± 0.01 ^{k}	0.49 ± 0.01 ^{h}	13.79 ± 0.01 ^{i}	13.59 ± 0.01	
DRB-T	358.89 ± 15.19 ^{bc}	77.13 ± 0.02 ^{b}	1.00 ± 0.02 ^{f}	16.31 ± 0.60 ^{h}	4.12 ± 0.33 ^{h}	
DRB-T-C	306.33 ± 10.44 ^{ef}	74.87 ± 0.05 ^{c}	1.35 ± 0.01 ^{d}	20.07 ± 0.03 ^{b}	8.36 ± 0.06 ^{f}	
DRB-C	316.03 ± 11.16 ^{ef}	71.28 ± 0.06 ^{i}	1.51 ± 0.01 ^{b}	19.45 ± 0.01 ^{cd}	10.72 ± 0.06 ^{c}	
DRB-NO	311.62 ± 6.67 ^{ef}	72.40 ± 0.02 ^{f}	1.19 ± 0.03 ^{e}	19.30 ± 0.03 ^{d}	9.68 ± 0.04 ^{de}	
DRB-CE	328.35 ± 8.99 ^{de}	72.59 ± 0.03 ^{e}	1.23 ± 0.03 ^{e}	19.72 ± 0.02 ^{c}	9.77 ± 0.02 ^{d}	
DRB-UL	301.56 ± 33.08 ^{f}	70.90 ± 0.05 ^{j}	1.43 ± 0.01 ^{c}	20.24 ± 0.02 ^{b}	11.46 ± 0.04 ^{a}	
DRB-SH	349.19 ± 8.67 ^{cd}	71.63 ± 0.01 ^{h}	1.48 ± 0.02 ^{b}	20.59 ± 0.04 ^{a}	11.08 ± 0.02 ^{b}	
DRB-AL	237.42 ± 12.30 ^{g}	73.57 ± 0.02 ^{d}	0.66 ± 0.07 ^{g}	17.98 ± 0.01 ^{g}	7.95 ± 0.02 ^{g}	
DRB-FL	314.28 ± 9.23 ^{ef}	72.07 ± 0.08 ^{g}	0.96 ± 0.01 ^{f}	18.54 ± 0.04 ^{f}	9.53 ± 0.05 ^{e}	
Treatment	**WBC (g water/g DW)**	**OBC (g oil/g DW)**	**Deionized Water (g/100 g DW)**	**Sucrose (g/100 g DW)**	**Sodium Carbonate (g/100 g DW)**	**Force (g)**
RB	2.93 ± 0.10 ^{e}	1.93 ± 0.04 ^{g}	258 ± 4 ^{g}	512 ± 16 ^{d}	263 ± 2 ^{f}	4131 ± 224 ^{a}
DRB	3.22 ± 0.11 ^{d}	2.62 ± 0.11 ^{f}	339 ± 12 ^{e}	591 ± 16 ^{a}	362 ± 11 ^{de}	742 ± 166 ^{fg}
DRB-T	3.13 ± 0.21 ^{de}	2.91 ± 0.07 ^{f}	337.27 ± 8.16 ^{e}	591 ± 19 ^{a}	355 ± 9 ^{de}	1022 ± 446 ^{ef}
DRB-T-C	2.96 ± 0.31 ^{de}	4.69 ± 0.07 ^{cd}	361 ± 10 ^{cd}	587 ± 12 ^{a}	384 ± 4 ^{bc}	2524 ± 255 ^{b}
DRB-C	3.54 ± 0.07 ^{c}	4.32 ± 0.36 ^{e}	312 ± 8 ^{f}	564 ± 8 ^{bc}	346 ± 3 ^{e}	2737 ± 267 ^{b}
DRB-NO	4.02 ± 0.12 ^{b}	4.55 ± 0.15 ^{de}	345 ± 8 ^{de}	552 ± 16 ^{c}	354 ± 17 ^{de}	1667 ± 26 ^{c}
DRB-CE	3.67 ± 0.17 ^{c}	5.30 ± 0.45 ^{b}	344 ± 13 ^{de}	585 ± 14 ^{ab}	372 ± 18 ^{cd}	1110 ± 112 ^{def}
DRB-UL	3.69 ± 0.26 ^{c}	5.03 ± 0.22 ^{bc}	341 ± 10 ^{e}	507 ± 7 ^{de}	393 ± 14 ^{ab}	1435 ± 131 ^{c}
DRB-SH	4.00 ± 0.14 ^{b}	4.48 ± 0.16 ^{de}	365 ± 18 ^{c}	508 ± 5 ^{de}	401 ± 7 ^{ab}	1180 ± 57 ^{de}
DRB-AL	3.77 ± 0.16 ^{bc}	4.91 ± 0.16 ^{c}	383 ± 15 ^{b}	486 ± 15 ^{ef}	390 ± 7 ^{b}	255 ± 39 ^{h}
DRB-FL	4.54 ± 0.15 ^{a}	5.72 ± 0.11 ^{a}	416 ± 4 ^{a}	483 ± 14 ^{f}	409 ± 12 ^{a}	376 ± 85 ^{gh}

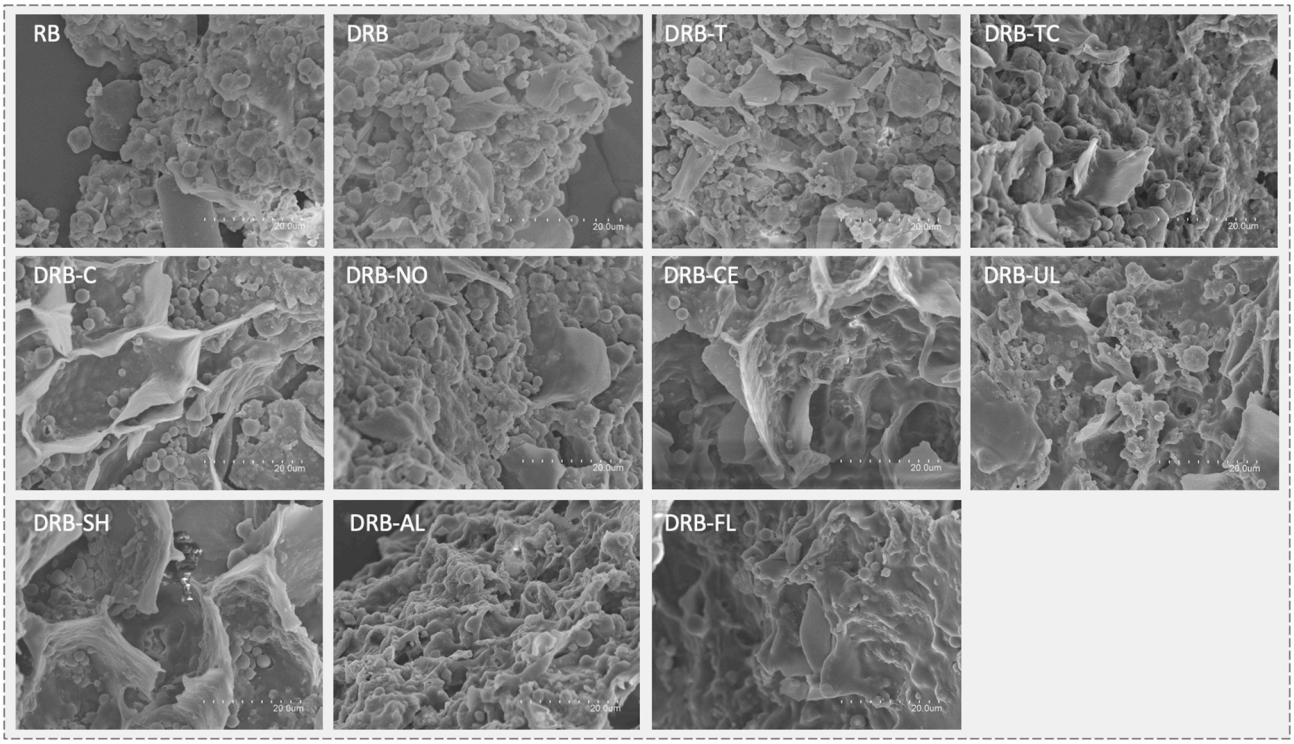

Fig. 2. Scanning electron microscopy analysis of rice bran subjected to different treatments. Abbreviations: RB: rice bran; DRB: defatted rice bran; DRB-T: defatted rice bran thermally treated; DRB-T-C: defatted rice bran thermally treated and soaked; DRB-C: defatted rice bran soaked; DRB-NO: defatted rice bran treated with Novozym® (xylanase); DRB-CE: defatted rice bran treated with Celluclast® (cellulase); DRB-UL: defatted rice bran treated with Ultimase® BWL 40 (cellulase and xylanase); DRB-SH: defatted rice bran treated with Shearzyme® Plus 2x (xylanase, cellulase and β-glucanase); DRB-AL: defatted rice bran treated with Alcalase® 2.4 L FG (endoprotease); DRB-FL: defatted rice bran treated with Flavourzyme® (peptidase preparation).

likely due to small peptides fragments released.

3.3. Impact of rice bran treatment on color

Although only visual differences were perceived after defatting, the results of the instrumental color characterization showed slight but statistically significant changes ($p < 0.05$) for all the treatments performed. The most notable change was in the DRB sample. The L* parameter significantly increase, up to 15.60% when comparing RB with DRB, while the a^* parameter showed a reduction of 72.47% in those samples, and the b^* parameter varied by 33.03% compared DRB sample to DRB-SH. Defatting increases the L^* indicating the lightning of the

bran when removing the fat. Regarding the a^* parameter (representing red-green tones), all treatments decreased it, shifting to more greenish. The b^* parameter (representing yellow-blue tones) increased in the samples treated with carbohydrate-acting enzymes, compared to RB, while samples treated with proteins-acting enzymes showed a decrease in this parameter. The overall color change was determined as the disparity between each treatment and the DRB. The most significant color change was observed in samples subjected to soaking, particularly those treated with carbohydrases, especially Ultimase treatment (DRB-UL), which exhibited the highest difference compared to the DRB, at 11.46. Samples treated with proteases displayed lower and more homogeneous values, akin to the sample subjected to heat treatment and soaking, while the heat-treated sample (DRB-T) showed the least overall color variation relative to the DRB.

3.4. Hydration properties of treated rice bran

The enzymatic treatments yielded noticeable increases in both WBC (Water Binding Capacity) and OBC (Oil Binding Capacity) values when compared to the non-enzymatically treated sample (Table 2). The most remarkable enhancement was observed in the case of DRB-FL, where the WBC and the OBC showed an increase of 1.55 and 2.96 times, respectively, in comparison to the untreated RB. Likely, the action of the peptidase preparation modified the proteins' structure and their affinity for water and oil. Notably, the OBC exhibited a more pronounced increase than the WBC, a trend that was consistently observed in all the samples that had soaking in the presence and absence of enzymes, but no other distinguishable pattern was envisaged due to the type of enzyme added.

However, it is worth noting that water binding exhibited a significative positive correlation ($r = 0.69$) with insoluble fiber content, and oil binding capacity was related ($p < 0.05$) with SDF. These results align with findings from Zhu, Huang, Peng, Qian, and Zhou (2010), where insoluble fiber led to higher WBC, but unlike them OBC was positively correlated to soluble fiber. Similarly, a negative relationship has been reported between particle size and hydration capacity (Chau, Wang & Wen, 2007; Zhao, Yang, Gai, & Yang, 2009), because smaller particles expose more surface area, facilitating greater interaction with surrounding solvents. This concept is consistent with results in the current study, where all treatments resulted in a particle size reduction, with a simultaneous increase in the WBC and OBC values. This accentuates a robust and statistically significant negative correlation between particle size and OBC ($r = -0.80$). This observation underscores the relevance of particle size in influencing hydration properties.

Similarly, SRC in three distinct solvents was conducted (Table 2). Specifically, the SRC of sodium carbonate correlates with the levels of damaged starch, while that of sucrose is associated with pentosan characteristics. Deionized water, on the other hand, serves as a control solvent in this context. This method has been adopted to provide a comprehensive understanding of how the various compounds present in rice bran influence its behavior when employed as an ingredient in a food matrix. The treatments significantly increased SRC, except SRC in sucrose for the samples treated with protein-acting enzymes (DRB-AL and DRB-FL), that decreased those values. All SRC values obtained in the analysis ranged from 100 to nearly 400 times higher than those observed in wheat flour, confirming the remarkable solvent retention capacity of rice bran. The starch content in the RB used in this study was 12.99%, falling within the reported range of 10–20% (Sharma, Chauhan & Agrawal, 2004). The increase observed in the SRC in sodium carbonate, was indicative of enhanced amount of damaged starch. The highest value observed at 409%, corresponded to DRB-FL, which also had the highest SRC in deionized water, while yielding the lowest value in sucrose. Therefore, defatted rice bran with carbohydrate-acting enzymes increased SRC values in sucrose, water, and carbonate solvents. DRB-SH had the most pronounced effect on water and carbonate, while DRB-CE on the sucrose. Again, a notable negative correlation was evident between particle size and the retention of deionized water ($r = -0.74$) as well as the retention of sodium carbonate ($r = -0.71$). Therefore, the SRC in different solvents could be used to discriminate among enzymatic treatments performed on rice bran.

3.5. Textural properties of treated rice bran

The texture of the rice bran samples, after hydrating them by adding five times water, was evaluated (Table 2). Treatments applied to RB led to a notable reduction in the force needed to penetrate the sample. A positive correlation with the particle size of each sample was observed, although it did not reach statistical significance. RB without any treatment exhibited the highest penetration force, 4131 g. Within the group treated with enzymes targeting carbohydrates, no statistically significant differences were observed neither between the DRB-NO and DRB-UL, or the pair DRB-CE and DRB-SH. In contrast, defatted bran treated with proteases, particularly DRB-AL, gave the softest samples.

3.6. Overall features of treated rice bran

A principal components analysis (PCA) (Fig. 3) was conducted to discern potential clusters based on enzymatic treatment (carbohydrate-acting enzymes, and protein-acting enzymes), using the proximate composition and technological properties as studied factors. This analysis accounts for 63.39% of the variances observed among the treated samples. Principal component 1 (PC1) accounted for 43.70% of the variability, while principal component 2 (PC2) explained 19.69%. In Fig. 3, variables that are closely located indicate stronger positive correlations, whereas variables positioned in opposite locations demonstrate inverse correlations. In this sense, PC1 is primarily influenced by the solvent retention capacity in deionized water, proteins, and insoluble fiber, while penetration force and a^* value have an opposite influence. In PC2, hemicellulose, d (4.3), and the b^* represent the driving variables while and an opposite effect was observed for soluble fiber. Fig. 3 clearly showed that PC1 discriminated between samples treated with protein-acting enzymes, positioning them on the positive axis, and the DRB-C sample (as control sample) was placed on the negative axis, confirming the efficiency of the enzymatic treatment in modifying the physico-chemical properties of the DRB. Meanwhile, PC2 discriminated samples treated with carbohydrate-acting enzymes, situating them on the positive axis, with the exception of the DRB-NO sample, which was positioned on the negative axis. This placement of DRB-NO was associated with its high TDF and SDF content. The PCA validates the findings presented in Tables 1 and 2 by grouping the control sample (DRB-C) with penetration force, soluble fiber, and total fiber (the latter being shared with DRB-NO). Hemicellulose and cellulose content, particle size, and color, effectively clustered the samples treated with carbohydrate-acting enzymes. The majority of technological properties successfully clustered and differentiated the DRB-FL sample, while the DRB-Al sample exhibited an opposite trend in relation to particle size, which stands out as one of its most distinct characteristics.

4. Conclusions

Enzymatic treatments can effectively modify the defatted rice bran composition and functionality. Nevertheless, the extension of the impact was highly dependent on the enzymes used for the treatment. Carbohydrate and protein degrading enzymes augmented the proportion of SDF, which was not obtained with the heat treatment. Those variations in the proportion of the fiber composition allowed reducing the particle size distribution with the subsequent impact on the solvent retention capacity of the defatted rice bran, and on the texture of the hydrated bran. The analysis of the solvent retention ability of the rice in different solvents gave indications about the constituents that were modified, starch and proteins, which were also confirmed by SEM micrographs. Modifications were also observed in the chromatic parameters,

Fig. 3. Graph showing the set of defatted rice bran samples treated by soaking (DRB-C) and with enzymatic treatment, and the variables studied by principal component analysis (PCA). The three different circles group the samples depending on the treatment to which they have been subjected, (●) sample treated by soaking, (●) samples treated with carbohydrate-acting enzymes and (●) samples treated with protein-acting enzymes.

particularly in brightness, which increased by 100% in all instances. Given the interest in increasing the fiber content of foods and beverages, enzymatically treated rice bran shows promise for better integration into food matrices due to its smaller particle size and improved hydration performance. Nevertheless, considering the complexity of the bran matrix, additional studies will be needed to explain the mechanisms behind each enzymatic treatment. Thermal and mechanical analysis of the isolated constituents will be undertaken in future studies.

CRediT authorship contribution statement

Eva Grau-Fuentes: Writing – original draft, Investigation, Formal analysis. **Raquel Garzón:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Dolores Rodrigo:** Writing – review & editing, Supervision. **Cristina M. Rosell:** Writing – review & editing, Methodology, 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.

On behalf of all the authors I declare that none have conflict of interest.

Data availability

Data will be made available on request.

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