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High pressure processing at different hydration levels as a tool to enhance rice bran stability and techno-functionality

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ABSTRACT

High-pressure processing (HPP) enhances food safety and shelf life by inactivating microorganisms and preserving food quality, yet its effectiveness in low-humidity environments has not been evaluated. This study investigated the effects of HPP at 500 MPa for 15 min across varying hydration levels (15, 30, 60, 77 %) on rice bran (RB), aiming to identify microbial effectiveness, besides techno-functional and physicochemical properties. HPP effectively reduced mesophilic bacteria, molds and yeast of RB at > 15 % hydration level, achieving reductions of up to 4 logarithmic cycles in the latter, nearing the detection limit of the method. However, it did not significantly impact spore inactivation. HPP treatment of ≥ 30 % hydrated RB induced particles aggregation and a honeycomb formation. The interaction between hydration and HPP treatment significantly affected the distribution of total dietary fibers, with an increase in soluble dietary fiber from 8.73 g/100 g to 11.03 g/100 g after HPP treatment at 15 % hydration level. Protein solubility was enhanced by hydration (15, 30 and 60 %), and peroxide values decreased after HPP treatment at low hydration (≤30 %) but increased when applied to high hydrated (>30 %) RB. Emulsifying activity decreased upon HPP treatment of highly hydrated RB (≥60 %), but more stable emulsions were achieved after HPP, regardless of the hydration level. Therefore, this study highlights the potential of HPP as a sustainable approach to enhance the utilization of rice bran in food applications, addressing existing knowledge gaps regarding its processing under different moisture conditions.

1. Introduction

Rice bran (RB), still considered a by-product of rice milling processes, has substantial potential for its content in lipids, proteins and dietary fiber (Sapwarobol et al., 2021; Wang et al., 2022). Actually, fiber content has been related to water and oil-binding capacities, and the protein fraction demonstrates promising emulsifying and foam stabilizing abilities (Liu et al., 2022; Spaggiari et al., 2021). However, the high microbial load of the rice bran, besides its tendency to rancidity due to the high fat content, limits the use of RB in food applications.

High-pressure processing (HPP) is widely employed as non-thermal technology for enhancing food safety (Allai et al., 2023). In fact, it has been applied for reducing the viable counts in milk, cold brew tea, sauces, meat and seafood (Considine et al., 2008; Hurtado et al., 2019; Song et al., 2021; Stratakos et al., 2019). Those applications require the optimization of HPP conditions because microbial load reduction varies depending on the target microorganism and food matrix, being the most commonly used 600 MPa for 5–6.5 min (Daryaei & Balasubramaniam, 2012; Hiperbaric, 2022). This preservation technique aligns with nowadays consumer preferences for fresh and safe products, extending shelf life by inactivating enzymes as well as microorganisms, and preserving the sensory and nutritional integrity of foods (Bello et al., 2014; Cappa et al., 2016; Roobab & Aadil, 2023).

Although the initial applications of HPP were as a preservative non-thermal treatment, lately, HPP has been applied to modify the physicochemical properties of raw materials (Liu et al., 2022). For instance, Cappa et al., (2016) subjected corn starch, rice flour, and waxy rice flour suspensions (40 %) to HPP treatments at 400 and 600 MPa (5 and 10 min) enhancing their paste behavior and solvent retention capacity. Likewise, Ahmed et al., (2007) studied the impact of HPP (350–650 MPa; 7.5–15 min) on rice slurries containing 16.5 %, 22.9 %, or 33 % rice flour to understand the pressure susceptibility of the main rice components. Authors reported lower starch gelatinization temperature and modifications in the secondary structure of the proteins after treatment. Similarly, changes in proteins have been observed when applying HPP (from 100 to 500 MPa) to 1 % (w/v) rice bran (RB) protein

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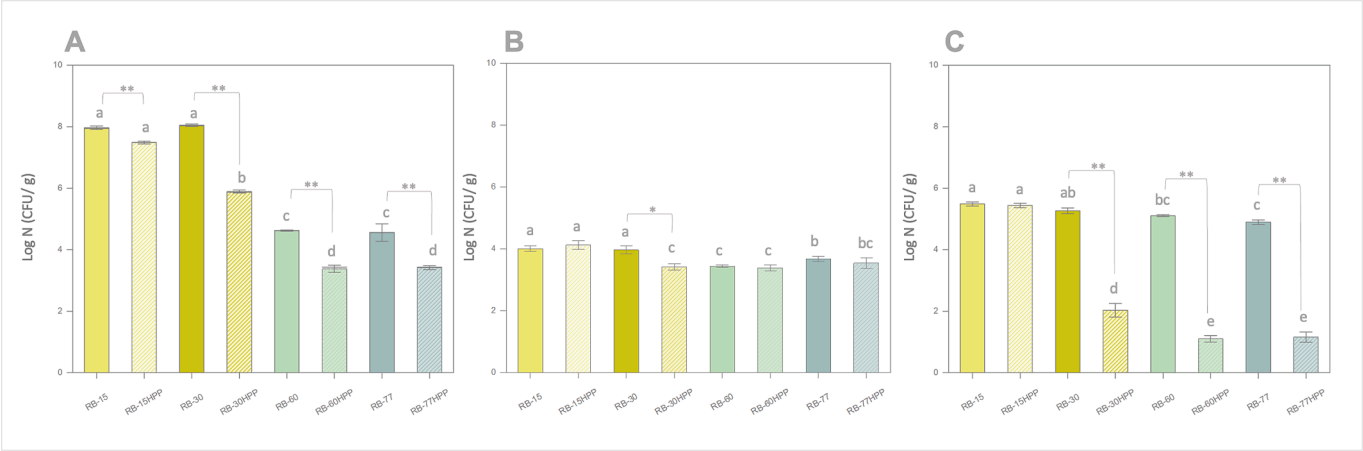

Fig. 1. Graph showing the effect of HPP and hydration level on different microbiological analysis: (**A**) mesophilic bacteria; (**B**) spore-forming; (**C**) molds and yeast. Letters on the bars indicate significant differences ($p \leq 0.05$) between all samples. "Asterisk" indicates significant differences between pair with the same hydrations, * indicates *p value* between 0.05 and 0.01, and ** indicates $p \leq 0.01$.

fractions (Zhu et al., 2017). In addition, Wang et al., (2022) reported significant improvements in solubility, emulsifying properties, and foaming properties in 1 % (w/v) RB protein dispersions after HPP treatment at 100, 200 and 300 MPa for 30 min. However, those treatments were applied to very diluted systems that require posterior dehydration for powder applications.

HPP treatments are typically conducted under high moisture conditions, particularly for microbial inactivation, that requires at least 40 % of free water (Muntean et al., 2016), but its effectiveness in low-moisture content environments has been scarcely studied. Recently, Seo et al. (2023) indicated that HPP (400–600 MPa for 10 min) were effective enhancing the water absorption, solubility, swelling power of rice flour using hydrations as low as 35, 45 or 55 %. Therefore, an understanding of the impact of HPP across different moisture levels as the possibility of successfully processing RB samples with a very high sample-to-water concentration level is important, given its crucial role in the food industry, closely linked with the transformation and formulation processes of food products.

This research aims to study the impact of high-pressure treatment on different hydration levels of rice bran, with a dual focus on improving bran safety and modifying its technological and functional properties, addressing the knowledge gaps concerning the application of high pressures under conditions of low hydration. By adopting a comprehensive approach that considers rice bran as a whole, rather than focusing solely on isolated fractions as commonly observed in the literature, this research was made to promote more sustainable practices within the food industry.

2. Materials and methods

2.1. Rice bran pretreatment

The RB from Arrocería Pons (Valencia, Spain) was sieved to eliminate foreign matter (>1mm). Moisture content was determined using the standard method ISO 712:2009. Rice bran (150 g) was adjusted to different moisture levels (15 %, 30 %, 60 % and 77 %) following the ISO procedure (ISO 27971:2023) designed for tempering cereals, obtaining values of water activity (a_w) of 0.772, 0.951, 0.977, and 0.993, respectively. Samples were coded using RB for rice bran followed with the moisture levels used for pretreatment (RB-15, RB-30, RB-60, RB-77).

2.2. High-pressure treatment

The tempered samples were sealed in polyethylene bags (MULTIVAC Thermosealer, Switzerland) and placed into the HPP unit (High Pressure Food Processor; EPSI NV, Belgium). HPP samples were subjected at 500 MPa for 15 min, excluding rise and fall times. Pressure and time was selected based on previous studies (Muntean et al., 2016; Zhu et al., 2017). Unpressurized samples for each hydration level were kept as references. Microbiology analysis were carried out using 10 g of each sample and the remaining sample was frozen at −80 °C for subsequent freeze-drying. HPP treated samples were coded with HPP.

2.3. Microbiology

Samples for microbiological analysis were collected immediately post HPP and from those without treatment. RB samples (10 g) were homogenized in 0.1 % (w/v) sterile peptone water (Scharlab Chemie S. A., Barcelona, Spain) using a Stomacher (Scharlab, S.L, Barcelona, Spain) before culturing. Total mesophilic bacteria were enumerated following ISO 4833–1:2013 on plate count agar (PCA, Scharlab Chemie S. A., Barcelona, Spain) under aerobic conditions at 30 °C for 48 h. To analyze molds and yeasts, a_w of each sample were taken into account. Since the 15 % a_w was lower than 0.95, the dichloran-glycerol agar (DG18) (Scharlab S.A., Barcelona, Spain) culture medium was used, as detailed in the ISO 21527–2:2008. Following the same protocol but part 1 (ISO 21527–1:2008), samples RB-30, RB-60, and RB-77, were cultured using the dichloran rose bengal chloramphenicol (DRBC) (Scharlab S.A., Barcelona, Spain) medium due to their a_w exceeding 0.95. The samples were incubated under aerobic conditions at 25 °C for five days. Bacterial spore counts were determined following a Katina et al. (2012) protocol with some modifications. Five mL of the homogenized samples were heated at 80 °C in a water bath for 15 min to inactivate vegetative cells. Samples were decimally diluted in 0.1 % (w/v) sterile peptone water and aerobic spore-forming bacteria were quantified on tryptone soy agar (TSA) (Scharlab S.A., Barcelona, Spain) plates, which were incubated at 30 °C for 48 h. All microbiological counts were reported as colony-forming units per gram (CFU/g).

2.4. Physical characterization

Particle size distribution and microstructure of the RB were evaluated in the freeze-dried samples. The Malvern Mastersizer equipment (Mastersizer Scirocco 2000; Malvern Instruments Ltd., Worcestershire, U.K.) was used for assessing particle size distribution. The volume-weighted mean diameter ($d_{(4.3)}$) was recorded from three replicates for each sample.

Scanning electron microscopy (SEM) was used to examine the

Table 1
Technological characteristics of RB with different hydrations and treatments.

Techno-functional characteristics			
	d (4.3) (μm)	**WBC (g water/ g) db**	**OBC (g oil/ g) db**
RB-15	361 ± 2^{b}	2.65 ± 0.23 ^{cd}	2.08 ± 0.13 ^{cd}
RB-15HPP	376 ± 9^{b}	2.82 ± 0.08 ^{bc}	1.91 ± 0.12 ^{d}
RB-30	426 ± 10 ^{a}	2.67 ± 0.11 ^{cd}	2.12 ± 0.11^{c}
RB-30HPP	415 ± 17 ^{a}	2.53 ± 0.18 ^{d}	1.99 ± 0.04 ^{cd}
RB-60	309 ± 9^{cd}	2.87 ± 0.20 ^{abc}	2.69 ± 0.09^{b}
RB-60HPP	318 ± 7^{c}	2.68 ± 0.21 ^{cd}	2.95 ± 0.16 ^{a}
RB-77	308 ± 5^{cd}	3.14 ± 0.03 ^{a}	3.08 ± 0.05 ^{a}
RB-77HPP	299 ± 5 ^{d}	2.96 ± 0.08 ^{ab}	3.11 ± 0.10 ^{a}
Hydration *p*-value	***0.0000***	***0.0012***	***0.0000***
HPP *p*-value	*0.7859*	*0.1977*	*0.9547*
Interaction between hydration and HPP *p*-value	*0.0875*	*0.1867*	***0.0108***

	Foaming capacity (%) db	**Foaming Stability (%) db**	**Emulsifying Activity (AU)**	**Emulsifying Stability (%)**
RB-15	23.59 ± 2.24 ^{a}	22.54 ± 1.88 ^{a}	0.240 ± 0.06 ^{ab}	42.85 ± 3.30^{b}
RB-15HPP	23.21 ± 0.22 ^{a}	22.10 ± 1.08 ^{a}	0.232 ± 0.05 ^{ab}	60.50 ± 4.13 ^{a}
RB-30	20.51 ± 0.18^{b}	18.80 ± 0.16^{b}	0.210 ± 0.02 ^{bc}	48.30 ± 3.42^{b}
RB-30HPP	18.97 ± 0.00^{c}	16.95 ± 1.78^{c}	0.168 ± 0.02 ^{cd}	67.47 ± 0.80 ^{a}
RB-60	0 ± 0 ^{e}	0 ± 0 ^{e}	0.279 ± 0.07 ^{a}	48.32 ± 5.40^{b}
RB-60HPP	13.34 ± 1.07 ^{d}	10.91 ± 0.20 ^{d}	0.159 ± 0.02 ^{cd}	60.51 ± 7.99 ^{a}
RB-77	0 ± 0 ^{e}	0 ± 0 ^{e}	0.275 ± 0.04 ^{a}	44.02 ± 1.82^{b}
RB-77HPP	0 ± 0 ^{e}	0 ± 0 ^{e}	0.147 ± 0.02 ^{d}	60.26 ± 4.48 ^{a}
Hydration *p*-value	***0.0000***	***0.0000***	*0.1520*	*0.1001*
HPP *p*-value	***0.0001***	***0.0001***	***0.0000***	***0.0000***
Interaction between Hydration and HPP *p*-value	***0.0000***	***0.0000***	***0.0128***	*0.5719*

Means with different letters within a column were significantly different ($p < 0.05$) Abbreviations: RB-15; RB-30; RB-60; RB-77: rice bran tempered at 15, 30, 60 and 77 % moisture levels used for pretreatment; RB-15HPP; RB-30HPP; RB-60HPP; RB-77HPP: rice bran tempered at 15, 30, 60 and 77 % moisture levels and treated with HPP.

microstructure. RB samples were coated during 300 s with gold using a vacuum evaporator (JEE 400; JEOL, Tokyo, Japan). Observation was done in SEM (Hitachi S-4800, Tokyo, Japan) at an accelerating voltage of 10 kW at low (600×) and high (1000×) magnification.

2.5. Chemical composition

Moisture was quantified following ISO methodology (ISO 712:2009). The estimation of Total Dietary Fiber (TDF), Insoluble Dietary Fiber (IDF), and Soluble Dietary Fiber (SDF) contents was carried out by following method 32–07 (AACC International, 1999). The Neutral Detergent Fiber (NDF) content was assessed following the methodology proposed by Van Soest et al. (1991). For Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) determination, the procedures outlined in ISO 13906:2008 were used. The hemicellulose and cellulose fractions were calculated as Equation 1 and 2, respectively.

$$\boldsymbol{Hemicellulose = NDF - ADF}$$

$$\boldsymbol{Cellulose = ADF - ADL}$$

Glucose and soluble protein content were also evaluated. Samples (0.2 g) were suspended in 20 mL of deionized water, mixed vigorously for 3 h using a magnetic stirrer and centrifuged at 16,000 x*g* for 15 min. The supernatant was used to quantify glucose and soluble protein. The glucose content was quantified using a glucose oxidase–peroxidase (GOPOD) kit (Megazyme, Dublin, Ireland). The absorbance was measured at 510 nm using an SPECTROstar Nano microplate reader (BMG LABTECH, Ortenberg, Germany). Protein content was quantified with a Pierce^{TM} bicinchoninic acid (BCA) Protein Assay Kit (Pierce Biotechnology, Rockford, USA). Bovine serum albumin was used as the standard protein, and absorbance was measured at 562 nm. All experiments were conducted in quadruplicate.

Peroxide values were determined using AOCS Cd 8-53 method (AOCS, 2004). The sample was solubilized in a solution composed of acetic acid and chloroform in a 3:2 ratio, followed by treatment with potassium iodide saturated solution. The resulting iodine liberated during the reaction was subsequently titrated using a 0.01 N solution of sodium thiosulfate, in the presence of starch solution as an indicator for endpoint determination. Results were expressed as the average of at least three replicates in meq O_2/kg fat.

2.6. Hydration, foaming and emulsifying properties

Water binding capacity (WBC) analysis was conducted according to the protocol described by Cornejo & Rosell, (2015). Samples (100.0 mg ± 0.5 mg) were suspended in one mL of distilled water, vortexed for 5 min, and then centrifuged at 2,000 × *g* for 10 min at room temperature. WBC was calculated using the formula provided by Cornejo & Rosell (2015).

Oil binding capacity (OBC) determination was analyzed using the procedure defined by Boulemkahel et al. (2021) with slight modifications. Sample (100.0 ± 0.5 mg) was mixed with one mL of vegetable oil, followed by vortex for 5 min and centrifugation at 3,000 x *g*, 4 °C for 10 min. Drained samples were used to calculate OBC as reported (Boulemkahel et al. (2021).

Foaming properties were determined following Martínez et al. (2014) method with slight modifications. RB suspension (4 g/100 mL) was whipped in an Ultra Turrax (IKA T18 basic, Wilmington, NC) at 14,000 rpm for one min at room temperature. The foam volume at 30 s and 20 min was recorded and used to calculate the foam capacity (FC) and foam stability (FS) as follows:

$$\boldsymbol{FC} = \left(\frac{\boldsymbol{ifv}}{\boldsymbol{tsv}}\right)*100$$

$$\boldsymbol{FS} = \left(\frac{\boldsymbol{ffv}}{\boldsymbol{tsv}}\right)*100$$

where *ifv* is the initial foam volume at 30 s, *ffv* is the foam volume after 20 min and *tsv* is the total suspension volume. Results were the average of three determinations.

Emulsifying properties were assessed using the method reported by Rios et al. (2018). The absorbance of the emulsions was measured at 500 nm in a spectrophotometer (UV mini-1240, Shimadzu Corporation, Kyoto, Japan). Emulsifying activity was expressed as the initial absorbance, expressed as absorbance units (AU), and emulsion stability (EA) was calculated using the equation 5 explain by Rios et al. (2018). Values being the average of four replicates.

$$ES(\%) = \left(\frac{Abs_{60min}}{Abs_{0min}}\right)*100$$

2.7. Force of penetration

The force of penetration was evaluated following the methodology

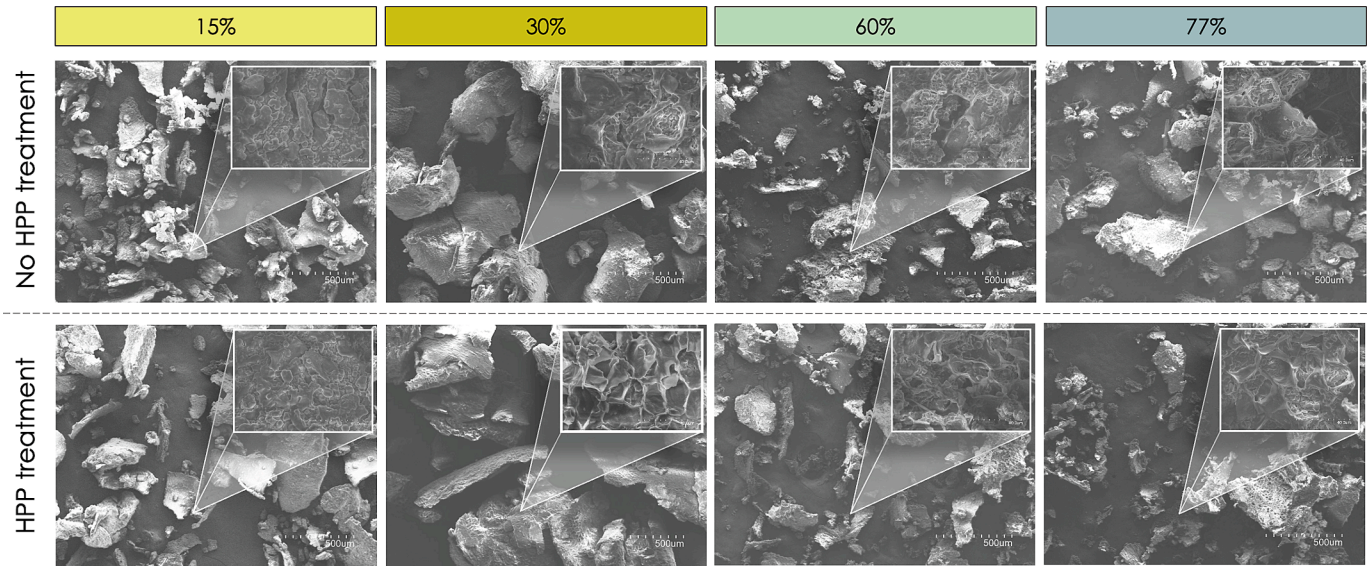

Fig. 2. Scanning electron microscopy analysis of rice bran subjected to different hydrations and HPP treatment. Micrographs of larger scale correspond to lower magnification levels (600x), whereas smaller micrographs had higher magnification (1000x).

described by Grau-Fuentes et al. (2024). 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 to carry out the analysis. A 1:5 bran-to-water ratio (w:v) was used to ensure optimal hydration of all components, accounting for each sample's moisture content. A compression at a 10 mm distance from the probe's contact was used and the maximum force required to penetrate the rice bran suspensions was recorded. Analysis was performed in triplicate.

2.8. Statistical analysis

The proximate composition, physic-chemical properties and microbiology data were analyzed using OriginPro version 2022b (Origin Lab Corporation, Northampton, MA, USA), and each experiment was conducted in triplicate. Distinctions among the multivariate analysis of variance (MANOVA), and Fisher's least significant differences test was employed to present the results, expressed as mean ± standard deviation. Differences of p *value* < 0.05 were considered significant. Additionally, Pearson correlation analysis was conducted on the results to identify probable relationships among experimental parameters. Principal Component Analysis (PCA) was conducted to discriminate between samples. The input dataset consisted of 8 rows and 21 columns. The analysis was performed in the statistical software OriginPro, which applied automatic scaling to the data.

3. Results and discussion

RB was tempered to four different hydration levels before being subjected to HPP. The lowest hydration level (15 %) is aligned with the standard cereal operational moisture content and the highest (77 %) was the water binding capacity of the RB. The intermediate levels were chosen in between of the above range.

3.1. Microbiology

To explain the impact of hydration levels and the efficacy of HPP on microbial inactivation all samples were analyzed. The inactivation of total mesophilic bacteria, aerobic spore-forming bacteria, and molds and yeasts under different hydration levels, both with and without HPP treatment is displayed in Fig. 1. Mesophilic bacteria, spore-forming bacteria and yeast and molds were analyzed in the original RB (non-hydrated, non-HPP treated) obtaining a 7.11, 2.99 and 5.71 Log N (CFU/g), respectively. At 15 and 30 % hydration, higher concentration of mesophilic bacteria was observed. However, at hydration levels of 60 and 77 %, the sample's dilution resulted in a lower bacterial concentration. Remarkably, HPP treatment significantly reduced mesophilic bacteria load by 1 to 2 logarithmic cycles at hydration levels of 30, 60 and 77 %, whereas at 15 % hydration, the effect was minimal due to the low a_w of the sample. Spore-forming bacteria exhibits significant resistance to HPP when in the spore form (Muntean et al., 2016). In fact, in the present study the HPP treatment does not notably decrease spore concentration in samples compared to untreated hydrations, only at 30 % was observed a significative reduction comparing treated and untreated sample, as know bacterial spores are much more resistant to pressure than vegetative cells (Zhang & Mittal, 2008). These results are consistent with those obtained by Roberts & Hoover, (1996), who employed a pressure of 400 MPa at room temperature, obtaining no significant impact on the reduction of viable spores of *Bacillus coagulans*. Molds and yeasts typically require longer incubation period compared to bacteria, which explains the lack of growth observed during the RB tempering phase to reach the desired hydration levels. Furthermore, the progressive dilution of samples leads to a decrease in the initial mold and yeast load across untreated samples as hydration levels rise. Molds and yeasts display a high degree of vulnerability to HPP, leading to significant modifications in cell morphology. Particularly, subjecting them to high pressures such as 500 MPa results in notable alterations and harm to their cell wall integrity (Ogawa et al., 1990; Shimada et al., 1993). In samples with a moisture content of 15 %, no discernible difference between the analogs can be observed due to the low a_w, decreasing the effectiveness of HPP treatment. However, for hydrations of 30, 60 and 77 %, the HPP process proves highly effective, achieving a notable reduction of 2 to 4 logarithmic cycles in mold and yeast load across samples. Notably, the magnitude of reduction increases proportionally with higher hydration levels.

3.2. Physical analysis

Particle size distribution of the RB was evaluated to identify possible particles' aggregation due to the treatments (Grau-Fuentes et al., 2024). The mean diameter ($d_{(4.3)}$) of the RB samples ranged from 299 μm to 426 μm. There was significant impact of the different hydration levels applied for tempering on the particle size distribution (Table 1). Nevertheless, hydration higher than 15 % was required to modify the particles size. Tempering at 30 % hydration led to certain agglomeration

Table 2
Chemical composition of RB with different hydrations and treatments.

Proximate composition	TDF (g/100 g) db	IDF (g/100 g) db	SDF (g/100 g) db	Glucose (g/100 g) db	Protein solubility (g/100 g) db
RB-15	31.64 ± 0.23 ^{cd}	22.92 ± 1.44^{b}	8.73^{b}	1.35 ± 0.08 ^{e}	11.89 ± 4.54 ^{abc}
RB-15HPP	37.95 ± 1.56 ^{a}	26.92 ± 0.53 ^{a}	11.03 ^{a}	1.03 ± 0.02^{f}	9.33 ± 0.25^{c}
RB-30	34.12 ± 1.52^{b}	25.95 ± 2.19 ^{ab}	8.17^{c}	1.68 ± 0.15 ^{d}	13.58 ± 0.57 ^{a}
RB-30HPP	29.84 ± 0.95 ^{de}	24.57 ± 1.74 ^{ab}	5.27^{f}	2.61 ± 0.10^{b}	12.11 ± 0.38 ^{ab}
RB-60	30.76 ± 0.52 ^{de}	26.31 ± 2.13 ^{a}	4.45 ^{g}	0.68 ± 0.02 ^{g}	11.26 ± 1.47 ^{abc}
RB-60HPP	29.01 ± 0.34 ^{e}	26.09 ± 0.77 ^{ab}	2.93 ^{h}	3.10 ± 0.13 ^{a}	11.36 ± 0.28 ^{abc}
RB-77	31.90 ± 0.40 ^{bcd}	25.16 ± 1.75 ^{ab}	6.73 ^{e}	0.48 ± 0.07 ^{h}	9.61 ± 1.86 ^{bc}
RB-77HPP	33.66 ± 0.79 ^{bc}	26.62 ± 0.76 ^{a}	7.04 ^{d}	2.32 ± 0.07^{c}	10.01 ± 0.32 ^{bc}
Hydration *p*-value	***0.0004***	*0.5846*	***0.0000***	***0.0000***	***0.0199***
HPP *p*-value	*0.3297*	*0.2100*	***0.0000***	***0.0000***	*0.1858*
Interaction between hydration and HPP *p*-value	***0.0002***	*0.0988*	***0.0000***	***0.0000***	*0.3519*

	Fibers (% of TDF) **Lignin (g/100 g) db**	**Cellulose (g/100 g) db**	**Hemicellulose (g/100 g) db**
RB-15	13.09 ± 2.81^{b}	26.16 ± 3.17 ^{a}	50.86 ^{e}
RB-15HPP	10.83 ± 1.34^{b}	18.65 ± 0.36 ^{bc}	40.62 ^{h}
RB-30	10.87 ± 0.86^{b}	22.80 ± 2.91 ^{ab}	45.4 ^{g}
RB-30HPP	11.09 ± 0.04^{b}	26.64 ± 0.35 ^{a}	56.58^{b}
RB-60	11.53 ± 0.27^{b}	22.21 ± 2.24 ^{ab}	57.11 ^{a}
RB-60HPP	17.46 ± 0.78 ^{a}	20.20 ± 0.02 ^{bc}	55.29^{c}
RB-77	11.98 ± 2.39^{b}	19.46 ± 2.06 ^{bc}	51.84 ^{d}
RB-77HPP	13.21 ± 1.59^{b}	15.98 ± 2.77^{c}	46.83^{f}
Hydration *p*-value	*0.0537*	***0.0056***	***0.0000***
HPP *p*-value	*0.1220*	*0.0624*	***0.0000***
Interaction between hydration and HPP *p*-value	***0.0252***	***0.0328***	***0.0000***

Means with different letters within a column were significantly different ($p < 0.05$). Abbreviations: RB-15; RB-30; RB-60; RB-77: rice bran tempered at 15, 30, 60 and 77 % moisture levels used for pretreatment; RB-15HPP; RB-30HPP; RB-60HPP; RB-77HPP: rice bran tempered at 15, 30, 60 and 77 % moisture levels and treated with HPP.

increasing the d $_{(4.3)}$ (426 μm for RB-30), but the opposite effect was observed beyond that hydration.

At the microstructural level (Fig. 2), noticeable distinctions associated with the treatments were observed, validating the outcomes of particle size analysis. Micrographs at low magnification (600×) readily show the impact of hydration on the samples surface. At 15 % hydration, HPP treated and untreated samples showed similar size. In contrast, particle aggregation was observed in samples with 30 % hydration, resulting in large particles with a spongelike structure on the surface. This spongelike with deep groves formation was also discernible on the surfaces of samples with higher hydration, besides some fragmentation into smaller particles. Wu et al. (2021) noted that treating insoluble rice bran fiber with HPP resulted in the formation of a rough and porous structure within the fiber. Furthermore, as pressure increased (up to 150 MPa), the fiber microstructure exhibited increased looseness. The HPP treatment employs the hydration present in the samples as a pressure transmission medium, while its powerful mechanical force disrupts the regular fiber structure and creates deep grooves (Floury et al., 2002). For this reason, a more pronounced alteration in the surfaces of the samples could occur at higher hydration levels. The observation at higher magnification (1000x) revealed a cell structure, like a honeycomb, at high hydration levels (60 and 70 %), but also it could be envisaged at lower hydration (30 %). Presumably, the carbohydrates present in the samples, particularly starch, might be responsible for the formation of those structures.

3.3. Chemical composition

The total (TDF) and soluble (SDF) dietary fibers content was significantly affected by the hydration level during tempering, but HPP did not have a statistical effect in TDF (Table 2**)**. However, the interaction between both factors induced a significant impact, depending on the hydration condition. The HPP treated sample containing 15 % hydration (RB-15HPP) showed the highest TDF (37.95 g/100 g) and SDF (11.03 g/100 g) contents, with lower values obtained when hydrating at 60 % (both with and without HPP treatment). The changes observed in IDF were not associated with either hydration or treatment. SDF levels hardly decreased with the hydration level till 30 % but greatly drop at 60 % hydration in the HPP treated and untreated RB, and augmented at the highest hydration (77 %). Similarly, Li et al. (2024) reported a fiber redistribution on insoluble fiber isolates from highland barley bran. They observed a reduction of 4.77 % in IDF and a 68 % increase in SDF after treating twice at 120 MPa. Dietary fiber is commonly categorized into soluble and insoluble fiber, but based on its technological attributes, it can be subdivided into hemicellulose, cellulose, and lignin. Hemicellulose was the main fraction in the RB samples, followed by cellulose and lignin. Hydration level and the interaction between hydration and HPP treatment significantly affected the cellulose content in the RB samples, and the lignin was affected due to the interaction between hydration and HPP treatment. Nevertheless, hemicellulose was significantly affected by both factors and through their interaction. However, no consistent trend in the effect of HPP treatment related with the hydration ratio was detected. The compressibility of air and water might explain the incongruity, resulting in different behaviors among samples with higher hydration due to the greater compressibility of water in those samples. Indeed, the significance of the fluid-sample ratio has been highlighted, affecting the effect of the treatment and the heating behavior (Balasubramaniam et al., 2015).

To provide additional understanding of the changes occurring during tempering and HPP treatments of RB, the glucose content and protein solubility were evaluated (Table 2). The glucose content in the untreated RB, significantly increased when tempering up to 30 % hydration levels, whereas lower values were obtained beyond that hydration. Interestingly, it was observed that HPP played a crucial role in the glucose content of the samples but was significantly dependent on the hydration level. A minimum hydration of 30 % was required to enhance the glucose content after applying HPP. The most substantial increase was observed at the 77 % hydration level, where HPP demonstrated the capability to enhance glucose content by 383 %. Therefore, HPP did induce a significant effect when combined with the right level of hydration, being able to release glucose molecules. Consequently, it can be inferred that HPP influences glucose content by breaking down larger carbohydrates, thereby facilitating their conversion into monomeric glucose forms or facilitating its extraction. The rapid and intense energy generated during HPP, combined with associated forces such as cavitation, friction, impact compression, and the accumulation of

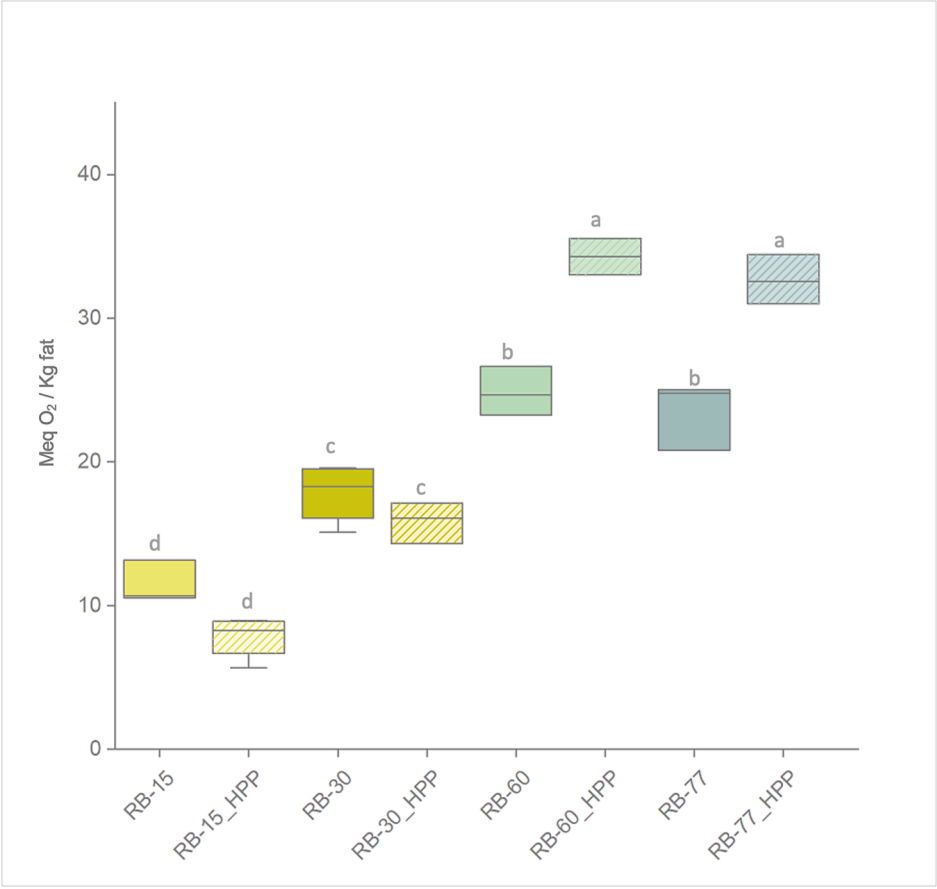

Fig. 3. Analysis of peroxide value in the rice bran (RB) samples subjected to HPP after being tempered at different hydration levels (15 %, 30 %, 60 %, 77 %). Numbers following RB indicated the hydration level of untreated and treated (HPP) samples. Letters on the bars indicate significant differences ($p \leq 0.05$) between samples.

electrostatic charges, is regarded as a key factor driving alterations in food compounds. These effects notably include structural modifications in carbohydrates, such as changes in their branching patterns (Mateos-Aparicio et al., 2010). The samples subjected to hydration or HPP treatment, particularly those with increased soluble fiber content (such as RB-15 treated and untreated), exhibited reduced glucose solubility. RB contained 16.06 ± 0.14 g/100 g protein and to identify possible impact of the treatments, the protein solubility was evaluated. Protein solubility exhibits an overall tendency to increase across all samples, compared to the original RB (9.58 g/100 g). The highest concentration of soluble protein (13.58 g/100 g) was recorded for sample RB-30. The impact of HPP treatment varies depending on hydration levels, although the interactions between those factors was not significant. At lower hydration (15 %), the capacity for protein solubility in the HPP treated samples declines compared to untreated sample, reaching a minimum concentration of 9.33 g/100 g in sample RB-15HPP. However, at higher hydration, the concentration of soluble proteins in HPP-treated samples did not show a significant change. Conversely, Cao et al., (2018) observed an increment in protein solubility after treating a suspension of 10 % pine nuts protein isolate at 200 MPa. Probably, the interaction of proteins with other RB components, such as fibers and lipids, might impede the protein solubilization, which it is facilitated in a diluted system having the pine nuts protein isolate as unique constituent.

To identify the impact of HPP on the fat present in RB the peroxide value (PV) was selected to quantify the concentration of peroxides and hydroperoxides formed in the first's stages of lipid oxidation. Likely, the breakdown of primary oxidative products into secondary oxidative products could be responsible of that reduction at low hydration levels. As displayed in Fig. 3, HPP significantly influences the PV of RB. It must be stressed that at lower hydration levels (15 %), HPP decreased the PV value up to 32 %. Similarly, HPP reduced the PV value of RB-30, although in lower extent. Conversely, opposite effect was induced by HPP at higher hydration levels, resulting in a 38 % increase in the PV for higher hydration levels (60 and 77 %). Hendrickx et al. (1998) reviewed the effect of high pressures on the lipoxygenase enzyme, responsible for increasing PV, concluding that only pressures greater than 700 MPa were able to reduce its activity in fresh vegetables at room temperature. Nevertheless, the present study conducted at 500 MPa reveals for the first time that HPP impact is dependent on the hydration of the material. In low hydrated (15 and 30 %) materials, HPP is effective in reducing PV and decreasing enzyme activity. Conversely, at higher hydration levels, enzyme activity is activated by HPP.

3.4. Technological characteristics

Impact of hydration and HPP on the techno-functional properties was assessed (Table 1). WBC capacity and OBC were significantly influenced by hydration, being OBC also affected by the interaction of both parameters (hydration and HPP). The most remarkable change was observed in RB-77, increasing by 12 % and 48 % the WBC and OBC, respectively, compared to RB-15. Regarding OBC, tempering using different hydration levels induced a steady increase of the OBC, which confirmed structural changes that modify the hydrophobicity of the RB. However, HPP treatment was only able to enhance that effect at high (≥60 %) hydration. Presumably, those results might be related to the different particle size, in fact a negative correlation was found with WBC and OBC ($r = -0.71$ and $r = -0.80$, respectively). Xie et al., (2019) related the higher hydration capacity of RB dietary fiber with the reduction in

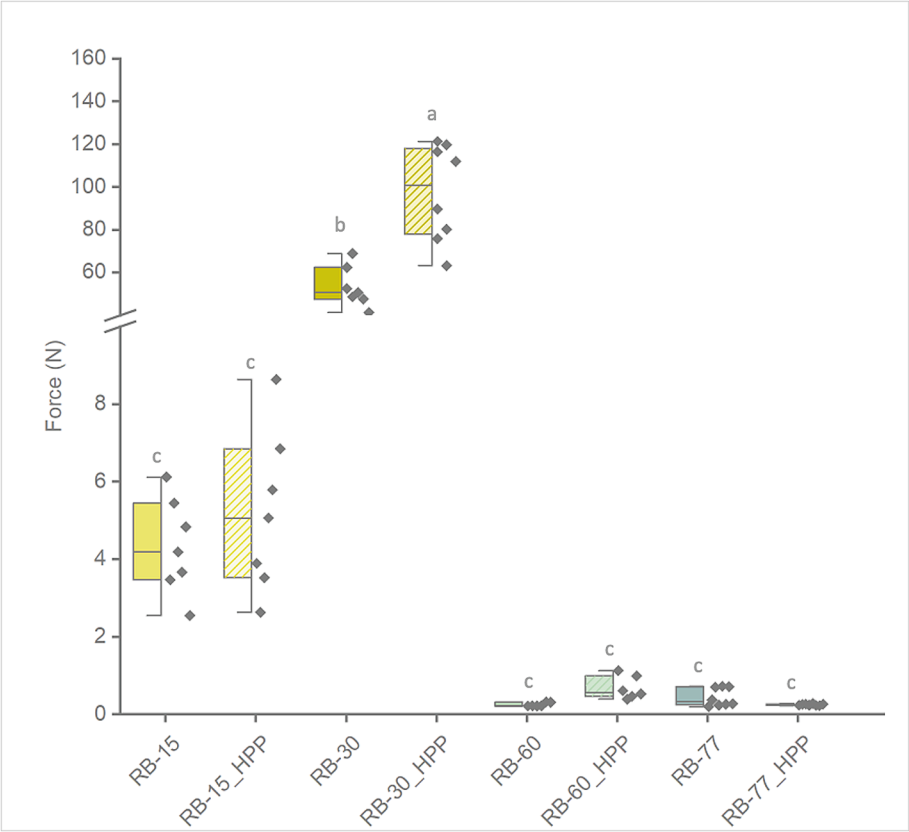

Fig. 4. Analysis of force of penetration in all of samples studied. Rhombuses represents all the determinations considered for the results. Letters on the bars indicate significant differences ($p \leq 0.05$) between samples.

their particle size, which was induced by the combination of enzymatic and HPP treatment. In the present study, no significant impact on the particle size distribution was observed after HPP treatment.

The foaming properties of the RB were significantly affected by HPP and the tempering hydration levels (Table 1**)**. Foaming properties include foaming capacity (FC) and stability (FS). At low hydration level (15 %), the FC increased and also the FS with or without HPP treatment. However, FC and FS decreased when increasing the tempering hydration, being negligible at maximum hydration (77 %). Surprisingly, HPP increased the FC and FS of RB tempered at 60 % hydration level (RB-60HPP). The 60 % hydrated sample initially lacked foaming abilities. Nevertheless, following HPP treatment, a notable improvement in FC and FS was observed, resulting in 13.34 % and 10.91 %, respectively. According to the findings of Zhang et al., (2022), who investigated the impact of humidity on the structural and functional properties of pea protein isolates during extrusion process, FC decreases at moistures higher than 30 %. The foaming properties exhibited a strong negative correlation with WBC and OBC (r > -0.69 in all cases). Considering that FC refers to the volume of foam generated under specific conditions (Raikos et al., 2007) and it is related to the sample hydrophobicity, the foaming capacity increases when enhancing the material hydrophobicity (Sun et al., 2022). However, in the present study, no correlation was observed with OBC, suggesting that foaming is not only dependent on hydrophobicity. Other contributions from molecular-level dynamics such as protein–protein interactions, changes in interfacial tension or orientation of the molecular structures of proteins, and the presence of minor compounds might be affecting the foaming properties.

Concerning the emulsifying properties of the samples, HPP treatment using different tempering hydration levels significantly affected both, emulsifying stability (ES) and activity (EA) (Table 1). The EA was reduced in all cases, with HPP stressing that reduction, although the reduction was only significant with hydration levels beyond 30 %. Moreover, HPP enhances the ES independently of the hydration level of the RB. The sample RB-30HPP showcases the highest stability a 67.47 % value (representing an increment of 40 % compared to its untreated counterpart). Similar impact was reported for RB protein hydrolysates when 1 % dispersion was subjected to 200 MPa (Wang et al., 2022). An increase in emulsion stability and activity was observed, this increase was attributed to the structural unfolding of proteins under pressure, which increased their adsorption at oil–water interfaces and promotes a better balance between hydrophilicity and lipophilicity (Wang et al., 2022). Similarly, in the present study, HPP treatment across different hydration levels consistently enhanced emulsion stability, where no significant differences among all the HPP treated samples were found, aligning with the evidence that controlled structural modification under high pressure optimizes protein functionality for emulsion stabilization. Molecular flexibility of proteins is a prerequisite for emulsion stability (Kato & Nakai, 1980), thus, it seems that HPP treatment confer greater flexibility to protein molecules, resulting in enhanced emulsion stability. Hence, HPP treatment of RB decreases emulsifying activity but they are more stable.

RB pastes were prepared by hydrating samples with five volumes of water (1:5 (w/v)). The force required to penetrate the hydrated bran was used to indicate its ability to expand, with lower force indicating higher swelling. In Fig. 4 could be observed that at 30 % hydration level, there was an increase in penetration force observed in both HPP-treated (97 N) and untreated (53 N) samples. Conversely, samples with higher hydration levels displayed a decrease in the penetration force, and that was independent of the HPP treatment. Hydration exerted a considerable effect on this analysis ($p < 0.05$). These findings agree with previous results reported by Grau-Fuentes et al., (2024), who explored different treatments (thermal and enzymatic) of RB and identified a positive correlation between penetration force and particle size. Results obtained applying tempering with or without HPP treatments also confirmed this

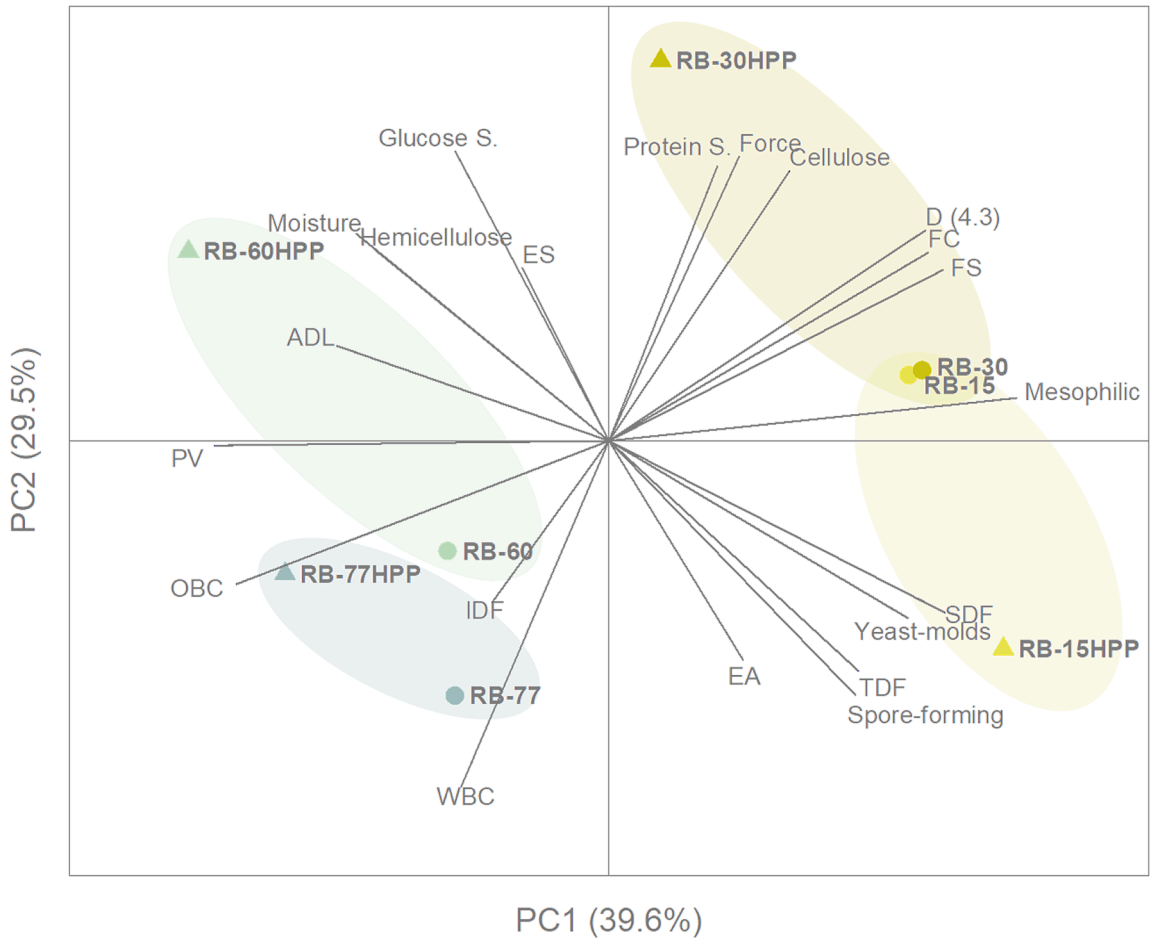

Fig. 5. Principal component analysis the samples hydrated and hydrated + treated with HPP that included all the variables analyzed (proximate composition, techno functional properties and microbiology analysis). Identified clusters based on their hydration levels appeared circled in different colors: 15 % (■), 30 % (■), 60 % (■) and 77 % (■). (●) Indicates samples without HPP treatment and (▲) indicates samples underwent HPP treatment.

significantly positive correlation ($r = 0.80$).

3.5. Principal component analysis

A principal component analysis (PCA) (Fig. 5) was conducted to discern potential groupings among the various hydration levels and HPP treatments applied to the RB, using proximal composition, techno-functional properties, and microbiological analysis. Within the graph, the closer the variables are to each other, the stronger their positive correlation, while variables positioned on opposite sides indicate inverse correlations. This PCA explained 69.1 % of the observed variance among the treated samples, with Principal Component 1 (PC1) explaining 39.6 % of the variability and Principal Component 2 (PC2) explaining 29.5 %. PC1 indicated positive associations with low hydrations (15 % and 30 %) and negative associations with high hydration levels (60 %, 77 %). Specifically, samples with 15 % and 30 % hydration levels were positioned positively along PC1, closely correlated with mesophilic bacteria analysis, yeast-molds and spore-forming, foaming properties, particle size distribution, total and soluble dietary fiber, cellulose content, penetration force, and protein solubility. Conversely, high hydration levels were linked with hydration properties, insoluble dietary fiber, particularly lignin, peroxide value and glucose solubility. HPP discriminated the samples along the y-axis, with RB-30HPP showing the strongest impact followed by RB-60HPP. Conversely, samples treated at 77 % and 15 % hydration were positioned on the negative axis. The least impact of HPP treatment was observed with the highest tested hydration (77 %) compared to its counterpart without treatment; however, samples with 15 % and 60 % hydration were the most significantly affected by HPP treatment positioned in different quartiles than their corresponding untreated samples. In the case of 15 % hydration, as evidenced in the microbiological section, this differentiation was determined by its limited efficacy in reducing microorganisms' post-treatment and its higher content of soluble fiber. For the RB-60HPP sample, the differences compared to the untreated sample were less pronounced but still present. This distinction was particularly evident when comparing the lignin and IDF for RB-60HPP and RB-60, respectively. The results obtained in the PCA demonstrate how the effect of high pressure varied depending on the hydration used in all samples, and the efficiency of using high hydration levels for microbiological purposes, but low hydration when looking for modifying physical–chemical features.

4. Conclusions

HPP treatment represents a versatile approach capable of enhancing its microbiological safety while concurrently changing the composition and functionality of rice bran. Although usually HPP are carried out at high hydration levels, the present study reveals that even at low hydration levels HPP can be effective promoting microstructure and chemical changes. The efficacy of HPP treatment is significantly influenced by the hydration level of the sample. Low hydration levels helped fiber redistribution, resulting in increased SDF content, reduced peroxide value, and enhanced foam capacity and stability. Conversely, samples with higher moisture content were microbiologically safe and

exhibit increased protein solubility post-HPP treatment, besides a reduction in particle size, and improvement in WBC and OBC. Nonetheless, the observed changes in the samples did not demonstrate a linear relationship with the moisture levels utilized during treatment. However, the greater the hydration, the greater the antimicrobial effect of the HPP treatment for molds and yeasts, with 30 % humidity again affecting a greater reduction in mesophilic bacteria. These findings underscore the importance of understanding hydration-dependent effects in optimizing HPP treatment strategies for rice bran, contributing valuable insights for enhancing its functional attributes in various food applications. For the first time HPP is applied at low hydration levels using RB as a model, opening the possibility of fostering technological modifications in raw materials by modulating their hydration.

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:** Conceptualization, Formal analysis, Supervision, Funding acquisition, Writing-review & editing.

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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Data availability

Data will be made available on request.

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