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Physical modification caused by acoustic cavitation improves rice flour bread-making performance

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ABSTRACT

Acoustic cavitation has been shown to cause physical damage and partial starch depolymerization in ultrasoundtreated flours. However, the promising effects of this modification on bread-making performance of gluten-free flour are still unexplored. Based on this hypothesis, sonicated rice flour (2–20 min) was used to replace 30% native flour in the control formulation of gluten-free bread. Breadmaking performance was characterized by doughs' pasting, thermal, and rheological properties, and physical quality of breads. Ultrasonication time presented a direct correlation with particle fragmentation. Doughs' rheology presented reduced $\tan(\delta)_4$ values (up to -11%) and improved recovery after the application of stress (up to +14%), denoting an enhanced elastic behavior with respect to the control dough. Rheo-fermentative tests demonstrated that ultrasonication accelerated the generation of CO₂ and its retention within the dough structure, as consequence of eased accessibility of yeast to simpler sugars after starch depolymerization. The small-size particles ($\sim10 \mu$ m) in ultrasonicated flours seem to have enhanced their Pickering emulsifying ability and led to breads with higher specific volumes (up to 24%), softer crumbs, and delayed hardening during storage. Ultrasonication, a low-cost technology, has been shown to significantly improve the fermentative and viscoelastic behavior of rice flour dough and its breadmaking performance.

1. Introduction

The gluten free (GF) market has been increasingly growing in recent years due to increasing population diagnosed with celiac disease, and a popular trend of reducing gluten ingesta for considering it as a healthy improvement (Witczak, Ziobro, Juszczak, & Korus, 2016). The development of GF products with a comparable quality to the traditional wheat-based products represents a technological challenge to the food industry due to structural issues derived from the natural characteristics and limited baking capacity of GF ingredients. The lack of gluten leads to liquid batters rather than doughs, which are unable to sufficiently retain the gas bubbles generated during fermentation, and result in baked breads with a crumbling texture and poor color (Villanueva, Harasym, Muñoz, & Ronda, 2019). Doughs without gluten can only retain the gas from fermentation by using additives in the formulation. The GF sources greatly depend on starch to provide structure and texture to the products made with them. The proprieties of native starches do not usually fulfill the industry's specific requirements because of limitations such as low shear and thermal resistance, and high tendency towards retrogradation (Singh, Kaur, & McCarthy, 2007). A strategy adopted by the food industry is the application of modifications by different means (i.e., genetic, mechanical, physical, chemical, or enzymatic) to alter the natural physicochemical properties of GF sources (starches and flours) so they can adapt better to specific processing requirements (Yang et al., 2019a; Zhu, 2015). The modification of raw GF ingredients prior the dough making is important to improve the quality of the bread, since it has been demonstrated that the characteristics of starch granules have a strong influence on the quality of the final products, derived from the interaction that starch has with other compounds present in the bread system (Witczak et al., 2016).

Physical modification methods are better perceived in the food industry for being environmentally respectful, regarded as "clean label". These modifications improve the flours' processing performance by altering their water absorption capacity, the gelatinization and thermal properties, and properties related to their utilization such as rheological properties of the doughs and texture of the breads (Qin et al., 2022; Villanueva et al., 2019; Witczak et al., 2016). Among the physical modification methods of starches and flours, ultrasound (US) treatments

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have been recognized as a promising approach, attributed to their higher selectivity and efficiency, and reduced processing time (Amini, Razavi, & Mortazavi, 2015; Yang et al., 2019a). Ultrasounds can be classified into two categories: low intensity (1 W/cm^2) with a frequency of 5–10 MHz, and high intensity $(10-1000 \text{ W/cm}^2)$ with a frequency of 20–100 kHz (Vera, Valenzuela, Yazdani-Pedram, Tapia, & Abugoch, 2019). The energy from US acoustic waves is transformed into a chemically feasible form by the cavitation effect, in which multiple collapsing bubbles induce high pressure gradients and high local velocities of liquid layers, resulting in shear forces and microjets that cause granular damage and alter the molecular structure of starches and proteins (Amini et al., 2015; Jambrak et al., 2010; Vera et al., 2019; Yang et al., 2019a). The extent of modification caused by ultrasonication depends on treatment parameters (frequency, power, time, temperature, and starch/flour concentration in the suspension) and the nature of the treated matter (composition and type of starch). The structural damage induced by ultrasonication modifies the interaction that flours have with water as consequence of increased surface area after particle fragmentation, resulting in modification of the water-dependent properties, such as swelling power, solubility, thermal, pasting, and rheological properties (Amini et al., 2015; Kaur & Gill, 2019; Yang et al., 2019a).

Rice (Oryza sativa L.) flour is one of the leading crops in the world, and the most suitable ingredient in GF bakery formulation given its natural lack of gluten, hypoallergenic properties, bland taste, white color, low protein and sodium content, wide range of amylose/amylopectin ratios, and the presence of easily digested carbohydrates (Qin et al., 2022; Villanueva et al., 2019). Previous studies have shown the marked disruptive effect that US cavitation has on rice flour particle size and surface damage (Vela, Villanueva, & Ronda, 2021; Vela, Villanueva, Solaesa, & Ronda, 2021), as well as molecular fragmentation caused to amylose and amylopectin, resulting in chains with shorter length (Vela, Villanueva, Li, Hamaker, & Ronda, 2023). Furthermore, the modifications caused to starch and proteins in rice flour due to US treatments led to the formation of stronger gels capable of resisting higher stress before the disruption of their structure (Vela, Villanueva, Solaesa, & Ronda, 2021). These changes caused by the action of acoustic cavitation represent a promising strategy to improve the bread-making performance of gluten-free flours. According to our knowledge, the use of US-treated rice flour at different times in bread formulations has not been covered by the available literature. Therefore, the objective of this study was to determine the influence of the incorporation of US-treated rice flour as function of ultrasonication time on the doughs' rheological, thermal, and fermentative properties, and the physical quality of the breads obtained. Treatments were performed on rice flour dispersed on an aqueous medium at a high concentration (25% w/w) and different times (2, 5, 10, and 20 min).

2. Materials and methods

2.1. Rice flour

Indica rice flour used in this study was kindly supplied by Herba Ricemills (Herba Ricemills S.L.U., Valencia, Spain), and presented the following composition: moisture content: 12.62%, proteins: 6.5%, fat: < 1%, ash: < 0.9% (given by the manufacturer). The flour was kept at 4 °C until use.

2.2. Ultrasound treatment

A Hielscher UP400St ultrasonicator (Hielscher Ultrasonics, Teltow, Germany) coupled with a S24d22D titanium tip was used for flour modification. Treatment conditions were a frequency of 24 kHz, on-off pulse of 80%, and maximum output power of 180 W, with the probe placed at 4 cm depth. Rice flour dispersions (400 g) at a concentration of 25% (w/w) were treated by ultrasounds using a glass jacket with recirculating water from a RA12 LAUDA water bath (LAUDA, Lauda-

Königshofen, Germany) to keep the treatment temperature constant (20 °C). Ultrasound treatments were performed for 2, 5, 10, and 20 min. A magnetic stirrer was used during treatments to ensure a homogeneous temperature and ultrasonication effect, and to avoid flour sedimentation. The whole sonicated aqueous dispersion (flour + water) was used in dough formulation. For the particle size distribution analysis (section 2.3), the sonicated dispersion was freeze-dried using a Telstar Lyoquest equipment (Telstar, Terrassa, Spain) to retrieve the ultrasonicated flour, which was subsequently sieved to $< 250 \mu m$. Untreated rice flour was used as control in the study.

2.3. Particle size distribution

Granulometry of the flours was determined using a Mastersizer 2000 coupled with a dry dispersion unit (Malvern Panalytical, Malvern, U.K.). The median diameter (D_{50}) and span values [$(D_{90}-D_{10})/D_{50}$] are reported as indicators of particles' dispersion, according to Abebe, Collar, and Ronda (2015). Samples were measured in triplicate.

2.4. Formulation and elaboration of doughs and breads

For dough and bread formulation, the following recipe based on 100 g rice flour (13% moisture) was used: 90% water, 6% sunflower oil, 5% sugar, 2% hydroxypropyl methylcellulose (HPMC), 1.5% salt, and 3% dry yeast (all amounts are expressed as % flour). Yeast was only included in the preparation of doughs destined to the elaboration of breads and for the proofing test with rheofermentometer; not in those used to study the other properties of the dough. In doughs and breads containing US-treated rice flour, the whole aqueous flour dispersion was incorporated in the formulation after US treatment, which replaced 30% of the native rice flour, and 86.2% of the required water.

Yeast was rehydrated in water and mixed with the previously homogenized dry ingredients (flour, sugar, salt and HPMC) for 2 min using a KitchenAid Professional mixer (KitchenAid, Benton Harbor, MI, U.S. A.) with a K45DH dough hook at speed 2. Sunflower oil was added to the dough and the mixing continued for 8 min at speed 4. The dough rheological and fermentative properties were evaluated right after doughs were prepared, while for the study of the thermal and pasting properties, doughs were freeze-dried using a Telstar Lyoquest equipment (Telstar, Terrassa, Spain) and ground prior measurements.

To prepare the breads, 160 g of dough were placed into an aluminum pan, followed by fermentation at 32 °C and 80% relative humidity for 50 min in a HPP260eco Memmert constant climate chamber (Memmert GmbH, Buechenbach, Germany), and baking at 170 °C for 20 min in a S400 Sveba Dahlen oven (Sveba Dahlen AB, Fristad, Sweden). Breads were left to cool down for 60 min at room temperature before the measurement of the physical characteristics. All bread elaborations were studied in duplicate.

2.5. Dough measurements

2.5.1. Pasting properties

Pasting properties of the doughs were determined using a Rapid Visco Analyzer 4500 equipment (PerkinElmer Inc., Waltham, MA, U.S. A.) according to the AACC International Method 76–21.02 Standard 1 (AACC International Approved Methods, 2017). The freeze-dried dough sample (3.50 g on a moisture basis of 14%) was placed in the RVA canister with 25 mL of distilled water. The paddle speed was set to rotate at 960 rpm for the first 10 s to disperse the sample, and continued at 160 rpm the rest of the essay. The parameters determined were pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV) and setback viscosity (SV), using TCW3 software (PerkinElmer Inc., Waltham, MA, U.S.A.). Samples were measured in duplicate.

2.5.2. Thermal properties

Thermal properties of the doughs were studied using a DSC3 equipment (Mettler Toledo, Barcelona, Spain). Freeze-dried doughs samples (~6 mg) were placed in 40 µL aluminum pans with the corresponding amount of deionized water to reach the original moisture content of the dough (50.4%). The sealed pans were kept at room temperature for 60 min to allow moisture homogenization before measurements. The scan was performed from 0 to 120 °C at 5 °C/min using an empty pan as reference. A first scan was performed to determine the gelatinization enthalpy (ΔH , J/g of dry matter, dm) and temperatures [Onset (T_O), peak (T_P), and conclusion (T_C) temperatures (°C)] of the doughs. The pans were scanned a second time after 7 days of storage at 4 °C to study the retrogradation parameters of the doughs. The dissociation of the amylose-lipid complex was determined in both runs. All doughs were measured in duplicate.

2.5.3. Rheological characterization of doughs

Oscillatory measurements were performed using a Kinexus Pro + rheometer (Malvern Panalytical, Malvern, UK) with a parallel serrated plate geometry (40 mm diameter, 1 mm gap), coupled with a Peltier KNX2002 C25P plate temperature control at 25 °C.

2.5.3.1. Strain and frequency sweeps. Before measurement, the doughs were placed in the plate and left for 5 min to allow relaxation. Strain sweeps were performed from 0.01 to 200% strain at constant frequency of 1 Hz, while frequency sweeps were performed from 10 to 1 Hz, at 0.05% strain, within the linear viscoelastic region (LVR). Frequency sweeps data were fitted to the potential equations indicated by Ronda, Villanueva, and Collar (2014). The coefficients G'₁, G''₁ and tan(δ)₁ obtained from the power law fittings represent the elastic and viscous moduli and the loss tangent, respectively, at a frequency of 1 Hz, while the exponents *a*, *b* and *c* quantify the dependence of these parameters to the oscillation frequency (ω). All doughs were evaluated in duplicate.

2.5.3.2. Creep-recovery assays. Creep-recovery measurements were performed applying a constant stress of 10 Pa (outside of the LVR) to the dough for 60 s, followed by removal of the stress and a strain recovery phase for 180 s. The obtained data were analyzed and fitted to the 4-parameter (creep) and 3-parameter (recovery) Burgers models, as described by Villanueva et al. (2019), using Statgraphics Centurion XVIII software (Statgraphics Technologies Inc., The Plains, VA, U.S.A.). The recovery (%) was calculated as the ratio J_{steady}/J_{max} (elastic recovery) where J_{max} represents the maximum creep compliance obtained at the end of the creep phase, and J_{steady} refers to the steady-state compliance in recovery phase, calculated by subtracting the compliance value at the terminal region of curve (where dough recovery reached equilibrium) from J_{max} . Samples were evaluated in triplicate.

2.5.4. Rheofermentometer

Development of doughs and gas production were continuously measured using a Chopin rheofermentometer F3 (Chopin Technologies, Villenueve-La-Garenne, France). In contrast to the traditional method, the dough's weight was reduced to 160 g and the four 0.5 kg weights were removed due to dough softness. Fermentation was performed at 32 °C for 180 min. The determined parameters were: $H_{\rm m}$, height at the maximum development of the dough (mm); T_1 , time required for the maximum development of the dough (min); Dough tolerance, time in which the dough is stable at a volume beyond 90% of $H_{\rm m}$ (min); $H'_{\rm m}$, maximum heigh of CO_2 production (mm); T'_1 : time corresponding to $H'_{\rm m}$ (min); $V_{\rm T}$, total volume of CO₂ produced (mL); $V_{\rm R}$, total volume of CO_2 retained by the dough (mL); V_{NR} , total volume of CO_2 not retained by the dough (mL); $R_{\rm C}$, CO₂ retention coefficient, calculated as $V_{\rm R}/V_{\rm T}$, (%); T_x , time of appearance of dough's porosity (min), in which all the CO₂ generated is no longer retained by the dough (Villanueva, Mauro, Collar, & Ronda, 2015). All samples were measured in duplicate.

2.6. Evaluation of bread quality

2.6.1. Bread appearance

A PowerShot SX410 IS camera (Canon, Tokyo, Japan) was used to photograph the breads elaborated and the slices obtained from them. Three photographs were taken from each elaboration: one from side point of view, one from the top of the loaf, and one of a bread slice taken from the center of the loaf.

2.6.2. Bake loss

Breads were weighted using a COBOS precision scale (COBOS, Barcelona, Spain). Measurements were done immediately after removing the breads from the pan. The baking loss was stablished as the percentual weight difference between the weight of the bread and the weight of the dough placed in the pan before proofing and baking (160 g). Three different breads were measured in each elaboration.

2.6.3. Volume

The volume of breads was obtained using a Volscan profiler 300 analyzer (Stable Microsystems, Godalming, U.K.). The specific volume was calculated as the ratio between the bread volume and its weight (mL/g). Two different breads were measured in each elaboration (four measurements in total for each formulation).

2.6.4. Color

Color parameters of the crust and crumb were measured using a PCE-CSM 2 colorimeter (PCE Instruments, Spain), controlled with the 3nh Color Quality Controller System software (CQCS3) (Shenzhen ThreeNH Technology Co. Ltd, Shenzhen, China). Results were reported in the CIE L^{*} a^{*} b^{*} and CIE L^{*} C^{*} h coordinates using the D65 standard illuminant and the 10° standard observer. Five measurements were made for the evaluation of the crust, while the crumb was measured in quadruplicate. The color difference (Δ E) of the crust and the crumb of each bread containing US-modified rice flour with respect to the control bread was calculated using the following equation:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$
(1)

2.6.5. Texture

The texture properties of the crumb were determined with a "Texture Profile Analysis" (TPA) double compression test using a TA-XT2 texture analyzer (Stable Microsystems, Godalming, U.K.) connected to the Texture Expert software (Villanueva et al., 2019). Analyses were carried out on bread slices of 20 mm thickness taken from the center of the loaf. A cylindrical aluminum probe with a diameter of 20 mm was used for the test, penetrating 50% depth at a speed of 1 mm/s, and 30 s delay between compressions. Hardness, springiness, cohesiveness, chewiness, and resilience were determined from the TPA graphs. Hardness was also evaluated after 7 days of bread storage at 4 °C in hermetic bags. Samples were evaluated in quadruplicate.

2.6.6. Statistical analysis

Statgraphics Centurion XVIII software (Statgraphics Technologies Inc., The Plains, VA, U.S.A.) was used to model the creep-recovery data into the 4- and 3-parameter Burger models, to generate a Pearson correlation matrix, and to perform analysis of variance (ANOVA) by Least Significant Differences (LSD) test at p-value \leq 0.05 to evaluate statistical differences between samples.

3. Results and discussion

3.1. Particle size of sonicated rice flour

The values determined for median particle diameter (D₅₀) and size

dispersion $[(D_{90}-D_{10})/D_{50}]$ of the control and ultrasonicated flours are presented in Table 1, and the size distribution profiles are shown in Fig. 1. Results showed a direct trend of median size reduction with longer US exposure, increasing the proportion of particles with a diameter <50 µm, leading to greater size dispersion values. Longer ultrasound treatments have been indicated to intensify the damage induced to the treated particles (Cui & Zhu, 2020). Particle fragmentation has been reported to happen due to mechanical collision and high shear forces caused by the cavitation phenomenon, that progressively erode the particles' surface until fragmentation (Bel Haaj, Magnin, Pétrier, & Boufi, 2013; Qin et al., 2022). Besides particle fragmentation, another effect of cavitation's mechanical degradation has been said to be the partial depolymerization of starch (Chemat, Zill-E-Huma, & Khan, 2011), modifying its molecular structure and chain length distributions after ultrasonication (Yang et al., 2019b; Vela et al., 2023). This fine structure modification would consequently lead to changes on the techno-functional properties of the US-treated flours and their performance in starch-based food product development.

3.2. Effect of incorporating US-treated flours in dough properties

3.2.1. Pasting properties

The pasting properties of the studied freeze-dried doughs are presented on Table 2. The structural changes that take place during starch gelatinization in RVA analysis include crystallite melting and doublehelix unwinding, water absorption in the amorphous regions, displacement of amylopectin units and leaching of amylose from the grains (Iida, Tuziuti, Yasui, Towata, & Kozuka, 2008). Results showed that pasting temperature (PT), the temperature at which paste viscosity development starts, was significantly increased by the incorporation of US-treated flours (p < 0.05) at an equal extent with respect to the control. It has been reported that ultrasonication caused a PT delay in rice flour at the same extent independently of exposure time, in the range from 5 to 60 min (Vela, Villanueva, Solaesa, & Ronda, 2021). The results obtained showed that the delay reported for flours can also be determined in the freeze-dried doughs, even in the studied formulations where the US-modified flour only represented 30% of the total flour used, meaning that most of the flour was in the native state exactly as in the control dough. Higher PT values indicate reorganization of starch structures that require a higher temperature for structural disintegration and paste formation, suggesting a denser cross-linking within the starch granules after US treatments (Villanueva et al., 2019).

The viscometric profiles obtained after the incorporation of UStreated flour presented significantly (p < 0.05) lower peak (PV) and trough (TV) viscosities than those determined for the control, while the final (FV), breakdown (BV) and setback (SV) viscosities were not significantly modified by the incorporation of ultrasonicated flours. Ultrasounds can rupture the macromolecular chains of starch, and destroy its crystalline structure, which would decrease the viscous resistance of starch paste, leading to reduced values of PV (Li et al., 2018). The unaffected BV values indicate that doughs containing

Table 1

Median diameter (D_{50}) and size dispersion $\left[(D_{90}\text{-}D_{10})/D_{50}\right]$ of native and sonicated flours.

US time (min)	D ₅₀ (μm)	(D ₉₀ -D ₁₀)/D ₅₀			
0 (Control)	$190\pm 1~\text{e}$	$1.70\pm0.01~\mathrm{a}$			
2	$160 \pm 1 \; d$	$1.99\pm0.01b$			
5	$133\pm3~{ m c}$	$2.35\pm0.05~\mathrm{c}$			
10	$107\pm2~b$	$2.79\pm0.04~d$			
20	56 ± 1 a	$4.30\pm0.04~e$			
Analysis of variance and significance (p-values)					
	***	***			

The presented results correspond to mean \pm standard deviation. Different letters in the same column indicate statistically significant differences between means at p<0.05. Analysis of variance and significance: ***p<0.001.



Fig. 1. Particle size distribution of the native rice flour and flours sonicated at different times. The illustrated curves represent the samples sonicated for 0 (untreated/control) (black), 2 (purple), 5 (blue), 10 (green) and 20 (red) min. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

US-treated flours still presented the same stability to heating and stirring as the control dough, containing exclusively native rice flour. FV illustrates amylose retrogradation, which happens during the cooling phase of the test due to starch molecular reassociation into a more ordered arrangement. FV results showed that the samples containing US-treated flours were able to form an equally viscous structure after amylose retrogradation as the control dough. The behaviors observed in pasting properties are believed to surge from partial depolymerization of starch components by US treatments, as other studies have demonstrated (Amini et al., 2015; Falsafi et al., 2019; Vela et al., 2023), diminishing the starch ability to develop viscosity during gelling, but allowing a significantly equal capacity of amylose retrogradation due to the higher availability of linear chains after the release of side chains of amylopectin and cleavage of amylose chains by cavitation.

3.2.2. Thermal properties

The thermal properties of the studied freeze-dried doughs are presented in Table 2, and the thermograms are presented in Supplementary Fig. 1. Two scans were performed on the freeze-dried doughs to characterize their gelatinization (first) and retrogradation (second) properties. The thermograms obtained from the first scan presented two endothermic peaks: a bigger first one due to starch gelatinization, and a second much smaller peak appearing at higher temperatures, corresponding to the dissociation of the amylose-lipid complex (Vela, Villanueva, & Ronda, 2021). Starch gelatinization enthalpy (ΔH_{gel}) was not significantly affected by the incorporation of ultrasonicated rice flour. ΔH_{gel} values reflect the disruption of hydrogen bonds or double helices in the crystalline and non-crystalline regions of the granules (Li, Hu, Zheng, & Wang, 2019). The fact that no significant modification of ΔH_{gel} was determined after the incorporation of ultrasonicated flours indicates that US treatment did not have an important impact on the number of these ordered structures. It is believed that ultrasonication has a higher impact on linear amylose chains of the amorphous regions, while the highly branched amylopectin molecules are more resistant and require greater energy to be affected, meaning either higher US power or longer sonication exposure (Amini et al., 2015; Kaur & Gill, 2019; Li et al., 2019).

The onset gelatinization temperature ($T_{O.gel}$) represents the melting temperature of the weakest crystallites in starch granules (Wang, Wu, Liu, & Ouyang, 2020). Results showed a significant decrease (p < 0.01) of $T_{O.gel}$ in doughs containing US-treated flours. It was demonstrated by Yu et al. (2013) that $T_{O.gel}$ in rice starch was constantly reduced by increasing sonication times in treatments below 60 min, attributed to breakage of polymeric chains and damage caused to starch granules. In the present study, it is possible that the incorporation of US-treated flours at 30% in the doughs had a dilution effect, hiding the effect of

Table 2

Pasting and thermal properties of the studied doughs.

Parameter	US-0	US-2	US-5	US-10	US-20	ANOVA
Pasting Prope	rties					
PT (°C)	84.0 \pm	85.4 \pm	86.2 \pm	86.2 \pm	86.0 \pm	*
	0.1 a	0.5 b	0.6 b	0.5 b	0.7 b	
PV (cP)	2271	$2126~\pm$	$2125~\pm$	$2167~\pm$	$2172~\pm$	*
	$\pm 2 b$	45 a	8 a	36 a	40 a	
TV (cP)	1998	$1863~\pm$	1876 \pm	$1920~\pm$	$1911~\pm$	*
	\pm 6 b	13 a	2 a	48 ab	50 a	
BV (cP)	$274 \pm$	$264 \pm$	249 ± 7	$265 \pm$	$261~\pm$	ns
	4 a	32 a	а	13 a	11 a	
FV (cP)	3252	$3213~\pm$	$3144 \pm$	$3251~\pm$	$3279~\pm$	ns
	$\pm14~ab$	99 ab	13 a	7 ab	7 b	
SV (cP)	1255	1350 \pm	$1268 \pm$	1295 \pm	$1369 \pm$	ns
	\pm 8 a	99 a	11 a	11 a	45 a	
Thermal prop	erties					
ΔH_{gel} (J/g	8.6 \pm	8.3 \pm	8.2 \pm	8.4 \pm	8.4 \pm	ns
dm)	0.4 a	0.3 a	0.1 a	0.3a	0.2 a	
T _{O-gel} (°C)	67.4 \pm	66.8 \pm	66.7 \pm	$66.9~\pm$	$67.0~\pm$	**
-	0.1 c	0.2 ab	0.2 a	0.1 ab	0.1 b	
T _{P-gel} (°C)	83.1 \pm	83.1 \pm	82.4 \pm	83.7 \pm	83.1 \pm	**
0	0.2 b	0.1 b	0.4 a	0.2 c	0.2 b	
T _{C-gel} (°C)	99.1 \pm	$\textbf{98.8} \pm$	98.6 \pm	98.7 \pm	98.4 \pm	ns
0	0.4 b	0.1 ab	0.4 ab	0.1 ab	0.2 a	
ΔH_{am-lip} (J/	$1.03~\pm$	$1.02 \pm$	0.83 \pm	$0.80~\pm$	$0.93 \pm$	**
g dm)	0.04 d	0.03 cd	0.05 ab	0.09 a	0.05 bc	
T _{P-am-lip} (°C)	113.0	110.7	112.8	112.4	111.3	ns
Ĩ	$\pm 0.2c$	$\pm 0.9a$	± 0.2	± 0.1	± 0.9	
			abc	bc	ab	
$\Delta H_{ret} (J/g)$	$6.7 \pm$	$6.7 \pm$	$6.7 \pm$	$6.4 \pm$	$6.9 \pm$	ns
	0.1 ab	0.4 ab	0.1 ab	0.1 a	0.1 b	
T_{O-ret} (°C)	$35.2~\pm$	35.1 \pm	36.1 \pm	36.5 \pm	$36.9~\pm$	**
	0.2 ab	0.2 a	0.2 bc	0.3 cd	0.7 d	
T _{P-ret} (°C)	49.6 \pm	49.4 \pm	48.8 \pm	49.1 \pm	50.2 \pm	ns
	0.3 ab	0.4 ab	0.5 a	0.8 ab	0.6 b	
T _{C-ret} (°C)	69.8 \pm	69.2 \pm	69.5 \pm	69.5 \pm	69.1 \pm	*
	0.2 b	0.2 a	0.3 ab	0.3 ab	0.1 a	
$\Delta H_{am-lip-ret}$	$0.6 \pm$	$0.6 \pm$	$0.8~\pm$	$0.8 \pm$	$0.8 \pm$	ns
(J/g dm)	0.2 a	0.1 a	0.1 a	0.1 a	0.1 a	
T _{P-am-lip-ret}	108.0	106.4	106.2	105.9	106.4	**
(°C)	\pm 0.2 b	\pm 0.5 a	\pm 0.6 a	\pm 0.9 a	\pm 0.6 a	

 $\begin{array}{l} PT = Pasting \ Temperature. \ PV = Peak \ Viscosity. \ TV = Trough \ Viscosity. \ BV = Breakdown \ Viscosity. \ FV = Final \ Viscosity. \ SV = Setback \ Viscosity. \ \Delta H_{gel} = Enthalpy \ of \ dough \ gelatinization. \ T_{O^*gel}, \ T_{P\cdotgel}, \ T_{C\cdotgel} = Onset, \ peak \ and \ conclusion \ temperatures \ of \ gelatinization. \ \Delta H_{am-lip} = Enthalpy \ of \ the \ amylose-lipid \ dissociation \ at \ the \ first \ scan. \ \Delta H_{ret} = Enthalpy \ of \ melting \ of \ retrograded \ dough. \ T_{O\cdotret}, \ T_{P\cdotret}, \ T_{C\cdotret} = Onset, \ peak \ temperature \ of \ the \ amylose-lipid \ dissociation \ at \ the \ scan. \ \Delta H_{ret} = Enthalpy \ of \ melting \ of \ retrograded \ dough. \ T_{O\cdotret}, \ T_{P\cdotret}, \ T_{C\cdotret} = Onset, \ peak \ and \ conclusion \ temperatures \ of \ melting \ of \ retrograded \ amyloge-lipid \ dissociation \ at \ the \ second \ scan. \ \Delta H_{am-lip-ret} = Enthalpy \ of \ the \ amylose-lipid \ dissociation \ at \ the \ second \ scan. \ \Delta H_{gel}, \ \Delta H_{am-lip}, \ \Delta H_{ret}, \ and \ \Delta H_{am-lip}, \ \Delta H_{ret}, \ and \ \Delta H_{am-lip-ret} \ are \ referred \ to \ dry \ matter. \ \$

The presented results correspond to mean \pm standard deviation. Different letters in the same row indicate statistically significant differences between means at p < 0.05. Analysis of variance and significance: ***p < 0.001. **p < 0.01. *p < 0.05. ns: not significant.

longer US exposure on leading to a greater modification of $T_{O.gel}$. Lower $T_{O.gel}$ values could result from a change in starch matrix, allowing greater mobility of starch polymers after US treatments, promoting water accessing to the interior of the starch granules and accelerating gelatinization (Hu, Li, & Zheng, 2019). The conclusion temperature $(T_{C.gel})$ was not significantly modified by US treatments.

The enthalpy determined for the second peak obtained in the first scan (ΔH_{am-lip}), corresponding to the amylose-lipid complex dissociation, was found to be significantly reduced (p < 0.01) by the incorporation of US-treated flours at 5, 10 and 20 min in the doughs. These results indicate that ultrasonication led to the formation of less stable amylose-lipid complexes, and probably also lower amounts, as has been determined in chemically modified starches (Eliasson, 1994). The amylose-lipid complex is described as a helical inclusion complex with amylose forming a helix around the hydrophobic chain of the ligand

(Eliasson, 1994). It was indicated by Eliasson (1994) that each monoacyl chain would require a helix composed of at least 18 glucosyl residues to form the amylose-lipid complex. In the present study, it could be possible that the fragmentation of linear chains by cavitation would lead to short chains that were unable to form a complex, resulting in lower total amount of complexes, hence reducing the ΔH_{am-lip} values in doughs incorporating sonicated flour.

The second scan performed after 7 days of sample storage at 4 °C allowed the analysis of amylopectin recrystallization. The melting enthalpy of retrograded amylopectin (ΔH_{ret}) was not modified by the incorporation of US-treated flours, suggesting that the crystalline regions of starch were not significantly affected by ultrasonication, in agreement with the results obtained in ΔH_{gel} . However, the onset melting temperature of retrograded amylopectin (T_{O-ret}) was significantly increased (p < 0.01) in US-10 and US-20, in agreement with the results reported by Yu et al. (2013) after ultrasonication of rice starch. The conclusion melting temperature of retrograded samples (T_{C-ret}) was significantly reduced (p < 0.05) in US-2 and US-20. Both T_{O-ret} and T_{C-ret} results illustrate a lower degree of heterogeneity in the retrograded structure when US-treated flour was incorporated in the doughs.

3.2.3. Doughs' rheological properties

3.2.3.1. Dynamic oscillatory rheology. The rheological properties derived from the dynamic oscillatory tests performed on the studied doughs are presented in Table 3 and Fig. 2 (A & B). Strain sweep tests allowed the determination of the maximum stress that the doughs were able to resist before the collapse of the structure (τ_{max}), which marks the end of the linear viscoelastic region (LVR), and the cross over point, where the elastic modulus matches the viscous modulus (G' = G''). Results showed that τ_{max} and the cross over point of the doughs were not significantly changed by the ultrasonicated flour, indicative that the structural damage caused to starch molecular structure by cavitation was faded due to the high amount of native flour (70%) in the formulations, resulting in an equal resistance to deformation as that presented by the control dough.

Frequency sweep tests were performed to determine the frequency dependence of the viscoelastic moduli and the loss tangent of the studied doughs within the LVR. The parameters determined after fitting the frequency sweep data to the power-law model were: the coefficients G'1, G''_1 , and $tan(\delta)_1 = G''_1/G'_1$, that represent the elastic and viscous moduli, and the loss tangent at 1 Hz, respectively, which are used to quantify the strength of the doughs (Amini et al., 2015), and the exponents *a*, *b*, and *c* that quantify the variation of G', G'', and $tan(\delta)$ to oscillation frequency, respectively, and inform about the stability of dough structure versus the rate of deformation. For all the samples studied, the elastic modulus was above the viscous modulus, resulting in $tan(\delta)_1 < 1$, denoting the predominant solid/elastic behavior in the doughs (Villanueva et al., 2019). Although no clear differences were found in G'_1 and G''_1 with the incorporation of US-treated flours, $tan(\delta)_1$ was systematically reduced with prolonged ultrasound exposure, suggesting a greater structuring effect with increasing treatment time. The $tan(\delta)_1$ value determined in the dough containing rice flour sonicated by 20 min (0.574) was 11% lower than that determined for the control (0.647), which indicates an important effect of ultrasonication, considering that the substitution in the dough was 30%. Lower values of tan $(\delta)_1$ have also been reported by Qin et al. (2022) in batters containing 15% of rice flour ultrasonicated for 10 min, which indicates that this effect can also be observed at lower substitutions. In the rheological study of gels entirely made with US-treated rice flours, it was reported by Vela, Villanueva, Solaesa, and Ronda (2021) that ultrasonication led to lower values of $tan(\delta)_1$ for longer treatment times. In both matrices (doughs and gels), the lowering of $tan(\delta)_1$ values could be related to the damaged caused to starch macromolecules by ultrasonication, promoting polymeric fragmentation to shorter linear chains capable of

Table 3

Rheological and proofing properties obtained for the studied doughs.

Parameter	US-0	US-2	US-5	US-10	US-20	ANOVA
Oscilatory tes	ts					
τ _{max} (Pa)	1.4 \pm	1.5 \pm	1.7 \pm	$1.8~\pm$	$1.9 \ \pm$	ns
	0.1 a	0.5 a	0.3 a	0.1 a	0.2 a	
Cross over (Pa)	7 ± 1 a	6 ± 2 a	8 ± 1 a	7 ± 1 a	8 ± 1 a	ns
G'1 (Pa)	908 ±	767 ±	903 ±	$787 \pm$	919 ±	ns
	40 a	54 a	67 a	61 a	69 a	*
а	0.341 ± 0.002	0.336 ± 0.007	0.313 ± 0.002	0.318	0.317	~
	± 0.002	± 0.007	⊥ 0.002 a	0.002	0.003	
			-	ab	ab	
G''₁ (Pa)	564 \pm	$436~\pm$	514 \pm	406 \pm	497 \pm	*
	24 c	76 ab	24 bc	29 a	20 abc	
b	0.450	0.431	0.442	0.437	0.426	ns
	±	±	±	±	± 0.005	
	0.009 b	0.001	0.009	0.007	а	
ton (8)	0.647	ad 0.629	ad 0 596	aD 0 591	0 574	**
tan (0)1	0.647	0.638	0.580	0.581	0.574	~ ~
	⊥ 0.009.b	⊥ 0.008 b	± 0.000	± 0.009	± 0.004	
с	0.124	0.115	0.129	0.139	0.114	ns
c	±	±	±	±	± 0.009	10
	0.004	0.006	0.009	0.009 b	a	
	ab	ab	ab			
Creep-recover	y tests					
J_{0c} (10 ⁻⁴	31.8 \pm	$\textbf{28.5}~\pm$	$25.5~\pm$	$26.3~\pm$	$\textbf{22.4} \pm$	**
Pa^{-1})	1.5 d	0.7 c	1.0 b	0.5 bc	1.0 a	
$J_{1c} (10^{-3})$	34 ± 2	33 ± 2	26 ± 1	22 ± 2	24 ± 2	**
Pa^{-1})	b	b	а	а	а	
λ _c	5.3 ±	6.5 ±	$6.1 \pm$	$6.0 \pm$	$6.1 \pm$	*
(10 ³ D-	0.3 a	0.3 b	0.1 b	0.2 b	0.1 b	*
$\mu_0 (10^\circ \text{ Pa} \cdot$	2.1 ±	2.0 ±	2.6 ±	2.8 ±	2.8 ±	*
(L + L)/	0.5 a 51 ⊥ 1	0.1 a 56 ⊥ 1	0.1 D 57 ⊥ 1	0.1 D 56 ⊥ 1	0.2 D 57 ⊥ 1	**
$(J_{0c} + J_{1c})$	31 ± 1 a	50 ⊥ 1 b	57 ⊥ 1 h	50 ⊥ 1 b	57 ⊥ 1 h	
J_{0r} (10 ⁻⁴	42 + 3	40 ± 1	34 ± 1	34 ± 1	33 ± 1	**
Pa^{-1})	b	b	a	a	a	
$J_{1r} (10^{-3})$	5.66 \pm	5.25 \pm	$4.92~\pm$	$4.80~\pm$	4.38 \pm	***
Pa ⁻¹)	0.09 d	0.03 c	0.02 b	0.08 b	0.08 a	
λ_r (s)	18.4 \pm	19.9 \pm	$21.5~\pm$	$22.2~\pm$	$23.3~\pm$	***
	0.6 a	0.2 b	0.7 c	0.5 cd	0.2 d	
Recovery	$15.1 \pm$	$15.5 \pm$	16.6 ±	$17.2 \pm$	$17.0 \pm$	ns
(%)	0.6 a	0.6 ab	0.6 ab	0.5 b	0.9 b	
Proofing tests	(2) + 1	E0 0	E7 1	(1 + 1)	64 + 0	*
n _m (iiiii)	$b_{2} \pm 1$	30 ± 2	37 ± 1	01 ± 1 abc	04 ± 2	
T_1 (min)	113 +	110 +	105 ± 1	110 +	112 +	ns
11 ()	110 ±	3 ab	a 100 ± 1	1 ab	5 b	10
Dough	50.0 \pm	53.3 \pm	54.8 \pm	55.5 \pm	$60.8 \pm$	**
tolerance	0.7 a	0.9 ab	0.9 b	0.9 b	0.9 c	
(min)						
H' _m (mm)	72.8 \pm	74.5 \pm	75.6 \pm	74.5 \pm	78.1 \pm	*
	0.3 a	0.9 ab	0.4 bc	0.3 ab	0.9 c	
T'1 (min)	70 ± 1	65 ± 1	63 ± 1	64 ± 1	59 ± 3	**
	C I C C C	b	ab	b	a	
V _T (mL)	1282 ± 20 o	$12/9 \pm 20$ a	$1284 \pm$	1278 ±	1303 ±	ns
V _n (mI)	20 a 1170 +	22a 1190 ⊥	22 a 1203 +	⊿ча 1201 ⊥	12 a 1198 +	*
AK (mp)	7a	8 h	1203 ±	9 h	11 b	
V _{NR} (mL)	101.5	94.5 +	95.0 +	87.5 +	85.0 +	***
INR (-III)	± 0.7 d	0.7 c	0.1 c	0.7 b	0.1 a	
R _C (%)	92.15	92.55	92.60	93.15	93.45	***
	± 0.07	± 0.09	± 0.09	± 0.07	± 0.07	
	а	b	b	с	с	
T _x (min)	71 ± 1	56 ± 2	51 ± 6	62 ± 3	51 ± 7	ns
	b	ab	а	ab	а	

 τ_{max} and the cross over (G'=G'') were obtained from strain sweeps. The power law model was fitted to experimental results from frequency sweeps. $G'=G'_{1} \cdot \omega^{a}$; $G''=G''_{1} \cdot \omega^{b}$; $tan(\delta)=tan(\delta)_{1} \cdot \omega^{c}$. J_{0} and J_{1} indicate the instantaneous and retarded elastic compliances. λ is the retardation time and μ_{0} the steady state viscosity. Subscript "c" refers to parameters in the creep phase; Subscript "r" refers to parameters in the recovery phase. Recovery is the elastic recovery obtained in the recovery phase expressed as percentage of the maximum compliance. H_{m} : Height at the maximum development of the dough. T_{1} : time

corresponding to $H_m.$ $H'_m:$ Maximum height of CO_2 production. $T'_1:$ time corresponding to $H'_m.$ $V_T:$ total volume of CO_2 produced. $V_R:$ Total volume of CO_2 retained by the dough. $V_{NR}:$ Total volume of CO_2 not retained by the dough. $R_C:$ CO_2 retention coefficient. $T_x:$ time of appearance of dough's porosity. The presented results correspond to mean \pm standard deviation. Different letters in the same row indicate statistically significant differences between means at p < 0.05. Analysis of variance and significance: ***p < 0.001. **p < 0.01. *p < 0.05. ns: not significant.



Fig. 2. Rheological properties of the studied bread doughs. (A) Strain sweeps, (B) Frequency sweeps, and (C) creep-recovery tests. The illustrated curves represent the doughs containing flour sonicated for 0 (untreated/control) (black), 2 (purple), 5 (blue), 10 (green) and 20 (red) min. In (A) and (B) G' is represented by full symbols and G'' by empty symbols. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

reassociating to form a more consolidated network (Monroy, Rivero, & García, 2018; Qin et al., 2022). In the case of doughs, however, the changes caused to the molecular structure of starch in rice flours by ultrasonication would also alter its interactions with the rest of the components present in the matrix (i.e., proteins, sugar, oil, and HPMC), so the lower values of $\tan(\delta)_1$ cannot be exclusively attributed to the fragmentation of starch macromolecules. The exponents *a*, *b* and *c* indicated that the dependencies of the moduli and the loss tangent on angular frequency were not particularly affected by the incorporation of ultrasonicated flour.

3.2.3.2. Creep-recovery tests. The creep-recovery tests were performed at 10 Pa, outside of the LVR, where the stress applied exceeded the maximum stress the doughs could withstand without breaking their structure. Illustrative graphs are presented in Fig. 2C. Different stages of the baking process (i.e., mixing, molding, fermentation, baking) involve high stresses in the doughs, so the study of conditions outside of the LVR are useful to predict the deformation that doughs will experience during processing (Villanueva, Pérez-Quirce, Collar, & Ronda, 2018). The parameters determined after adjusting the creep-recovery tests data to the Burgers models are presented in Table 3. The studied doughs showed a typical viscoelastic behavior (data not shown), as previously reported for rice flour doughs (Perez-Quirce et al., 2018; Villanueva et al., 2019). The instantaneous compliance (J_0) is related to the elastic stretching energy of the bonds, when stress is applied, and vanishes immediately after its removal, while the retarded elastic compliance (J1) is related to the disruption and conversion of the bonds (Witczak, Juszczak, Ziobro,

& Korus, 2012). Results showed that the incorporation of ultrasonicated flours in doughs led to a significant decrease (p < 0.01) of J₀ and J₁ values with respect to the control, in both creep and recovery phases, associated to stiffer doughs with lower dough deformation when submitted to a constant stress, and a higher recovery when stress was removed (Perez-Quirce et al., 2018; Ronda, Villanueva, & Collar, 2014). This effect of sonication on dough elastic compliances was increased by extended sonication exposure, where J_{0c}, J_{1c}, J_{0r} and J_{1r} determined for US-20 showed a reduction of 30%, 29%, 21% and 23%, respectively, compared to the values presented by the control dough. The $[(J_{0c} +$ J_{1c})/J_{max}] values represent the elastic (instantaneous + retarded) to total (elastic + viscous) compliance ratio (Villanueva et al., 2018). A significant increase of the $(J_{0c} + J_{1c})/J_{max}$ values was determined with the incorporation of US-treated flours. These results indicate a higher elastic deformation with respect to the total (elastic + viscous) deformation, and confirm the higher elasticity of the doughs made with 30% sonicated rice flour compared to the control dough (100% untreated rice flour). This is important, since the gas-holding capacity of a dough requires a well-structured matrix with enough elastic behavior (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007).

Higher retardation times were determined in both creep (λ_c) and recovery (λ_r) phases of tests performed on doughs incorporating sonicated flour. Higher λ values indicate more time needed to obtain a viscoelastic deformation on the dough (Villanueva et al., 2019). Retardation times in the creep tests (λ_c) were equally improved by the incorporation of ultrasonicated flours, regardless of the treatment time. The increased retardation times were probably due to the increased swelling ability derived from particle fragmentation which allows a higher interaction with water (see section 3.1) (Vela, Villanueva, Solaesa, & Ronda, 2021; Witczak et al., 2012). A major factor in doughs' elasticity is water hydration capacity, which depends on the flour particle size and number of damaged starch granules (Witczak et al., 2012), which according to previous research is greatly modified by US treatments due to cavitation (Vela, Villanueva, & Ronda, 2021; Vela, Villanueva, Solaesa, & Ronda, 2021). Changes in water binding ability lead to changes in dough structure because hydration of starch granules determines their shape and interconnectivity (Witczak et al., 2012).

The highest values of the steady state viscosity (μ_0) were determined for US-10 and US-20, statistically equal to that presented by US-5, while the value determined for US-2 was not significantly different to the control. A proper consistency in gluten-free doughs, with high enough μ_0 , also helps to better retain the carbon dioxide produced during fermentation (Ronda, Villanueva, & Collar, 2014). A dough viscosity increase might be attributed to higher water retention capacity (Perez-Quirce et al., 2018), associated to particle rupture and disintegration of starch intermolecular bonds by ultrasonication, which allows water molecules to bind with the free hydroxyl groups of amylose and amylopectin (Kaur & Gill, 2019).

The recovery capacity of the doughs after applying the stress is related to the contribution of the elastic deformation with respect to the total deformation (Villanueva et al., 2019). Results indicated that recovery capacity was improved by the incorporation of rice flour ultrasonicated for 10 and 20 min. The increase in recovery values reflect a better bonding between the structural elements of the dough, resulting from higher elastic behavior, as reflected by a strong negative correlation with $\tan(\delta)_1$ (p < 0.001; r = -0.9799).

3.2.4. Fermentative properties of the doughs

The fermentative properties of the studied doughs were evaluated using a rheofermentometer. The results obtained are presented in Table 3. During the tests, the volume increase (related to the development of the dough) was measured, as well as the gas produced and retained by the dough (see Figs. 3 and 4, respectively). H_m and T_1 indicate the height at maximum dough development, and the time at which it is reached, respectively. Results showed that both properties were not particularly affected by the incorporation of ultrasonicated



Fig. 3. Development of the studied doughs during fermentation. The curves presented correspond to US-0 (black), US-2 (purple), US-5 (blue), US-10 (green) and US-20 (red). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



Fig. 4. Gas production (discontinuous line) and gas retention (continuous line) in the studied doughs during fermentation. The curves presented correspond to US-0 (black), US-2 (purple), US-5 (blue), US-10 (green) and US-20 (red). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

flours. The dough tolerance, however, showed a positive correlation with sonication time, reaching a value of 60.8 min in US-20, which represents an increase of 21.6% with respect to the value determined for the control dough (50.0 min). This property indicates the time that dough remains stable at a volume beyond 90% of $H_{\rm m}$. Dough stability during fermentation depends on the amount of CO₂ produced and the rheological properties of the dough, which both seemed to be improved by the incorporation of US-treated flours.

Regarding the gas generated, results showed that the use of UStreated flours in the doughs increased the maximum heights of CO_2 produced during fermentation (H'_m), and that they were reached at shorter times (T'_1) compared to the control dough. It is believed that this improved CO_2 production derives from starch molecule fragmentation caused by cavitation (Czechowska-Biskup, Rokita, Lotfy, Ulanski, & Rosiak, 2005; Vela et al., 2023). The fragmentation of linear chains would result in the generation of simpler sugar in the US-modified rice flours, facilitating their accessibility to yeast during proofing, accelerating the generation of CO_2 and achieving a higher amount of CO_2 produced. The total volume of CO_2 produced (V_T), on the other hand, was not altered by the incorporation of ultrasonicated flours, while the total volume of CO₂ retained (V_R) was significantly increased, resulting in increased values of the retention coefficient ($R_C = V_R/V_T$). R_C measures the fraction of CO₂ generated and retained by the dough; therefore, it is related to the porosity of the dough (Villanueva et al., 2015). These greater R_C values obtained in doughs containing US-treated flours result from the faster generation of CO₂ [correlation with D₅₀ (p < 0.01, r = -0.9753)] and the improved elastic behavior and increased viscosity in those doughs [correlation with τ_{max} (p < 0.01, r = 0.9473)], allowing them to better retain the gas generated during proofing. The time of appearance of dough porosity (T_x) was shortened in doughs containing US-treated flours, also related to the improved generation of CO₂ in these flours, resulting in an accelerated profile compared to the control dough.

3.3. Effect of incorporating US-treated flours in bread physical properties

3.3.1. Bake loss and specific volume of bread

The values determined for bake loss and specific volume of the breads studied are presented in Table 4. Results showed that the incorporation of US-treated flour in the doughs led to significantly higher values of bake loss and specific volume in the breads obtained, for all the evaluated ultrasonication times. A higher specific volume in rice bread after the incorporation of US-treated rice flour at a lower dose (15%) and 10 min ultrasonication had previously been reported by Qin et al. (2022). These authors reported a volume increase of 15%, while in the present study the incorporation of 30% US-treated rice flour led to volume increases of 14% (US-2), 22% (US-5), 25% (US-10) and 21% (US-20), compared to the control bread, which suggests higher results for higher substitution of the native flour by ultrasonicated flour. The higher volumes achieved by doughs containing US-treated flours could be related to the partial starch depolymerization due to cavitation, leading to improved fermentation and higher CO2 production, better retained by doughs with US-treated flours (see section 3.2.4) thanks to their enhanced viscoelastic properties, as demonstrated by the correlation with $tan(\delta)_1$ (p < 0.05, r = -0.8862) and recovery capacity (p < 0.05, r = 0.9176). It was indicated by Aoki, Kataoka, and Nishiba (2020) that amylose plays an important role in achieving a high loaf volume in rice breads, after comparing the volumes obtained in breads using rice flours with amylose contents ranging from 9% to 22%. These authors determined that the amylose content was positively correlated with the specific volume of the rice breads. The linear chain fragmentation induced by ultrasonication (Czechowska-Biskup et al., 2005; Vela et al., 2023) would result in the generation of amylose-like structures that could have shown a similar behavior as that of increasing amylose content indicated by Aoki et al. (2020). Results seem to indicate that when the US doughs containing a higher amount of gas in their structure and presenting a higher development were baked, an easier evaporation of water was obtained probably due to a greater surface area exposed to dryness in the oven (Villanueva et al., 2019). The upper and side photographs of these breads, as well as a photograph of a slice are presented in Fig. 5; significantly bigger pieces are clearly observed after the incorporation of ultrasonicated flours. The highest specific volume was determined for US-10, which represents a volume increase of 24% with respect to the control bread. The pasting properties of the dough are also related to the bread volume and baking loss [as indicated by the strong correlation with PT (p < 0.001, r = 0.9938), and PV (p < 0.05, r =-0.9120]. The higher pasting temperatures (see section 3.2.1) in doughs with US-treated flours in their formulation would allow a greater development of the doughs during baking before the fixation of the crumb structure upon baking, also allowing longer time for water to evaporate during proofing (Villanueva et al., 2019).

3.3.2. Color

The color parameters determined for the crust and crumb of the breads studied are presented in Table 4. Results showed that lightness (L_{crust}) , redness (a_{crust}) and yellowness (b_{crust}) of the crust were

Table 4

Effect of incorporation of 30% US-treated rice flour at different times on rice flour bread quality properties.

Bread	08-0	US-2	08-5	US-10	08-20	ANOVA
parameter						
Bake loss (%)	19.0 \pm	$21.4 \pm$	$20.8 \pm$	$20.3 \pm$	19.7 \pm	***
	0.2 a	0.7 d	0.5 cd	0.5 bc	0.3 ab	
Specific	3.71 +	4.23 +	4.52 +	4.63 +	4.49 +	***
volume	0.04 a	0.09 b	0.01 c	0.03 c	0.09 c	
(mL/g)	010 1 4	0.05 0	0.01 0	0.00 0	0.03 C	
L*arriet	$62.3 \pm$	$61.3 \pm$	60.9 +	$61.5 \pm$	$61.3 \pm$	*
- crust	0.3 c	0.4 ab	0.8 a	0.5 ab	0.7 ab	
a* .	15.6 +	153+	14 8 +	14.6 +	$147 \pm$	***
a crust	0.6.0	0.4 bc	0 3 ab	05a	032	
b*	317+	31.4 +	30 9 +	30 4 +	$30.2 \pm$	***
D crust	05d	0.4 cd	0.4 bc	0.1 ab	032	
C*	35 5 ±	34 9 ±	34 4 ±	33 5 ±	0.5 a 33 6 ±	***
C crust	$0.5 \pm$	0.4 bc	03b	0.62	052	
h	627	62.9	642	64.4 L	0.5 a	***
¹¹ crust	$03.7 \pm$	$03.0 \pm$	$04.3 \pm$	04.4 ±	$04.1 \pm$	
A E	0.5 a	0.5 ab	1.0	1.0 1	0.7 DC	
ΔE_{crust}	-	1.1 ±	1.8 ±	1.8 ±	2.0 ±	-
*	(0.0.)	0.2 a	0.4 ab	0.3 ab	0.4 D	
L*crumb	$68.9 \pm$	69.4 ±	$69.4 \pm$	$68.9 \pm$	67.8 ±	**
	0.9 D	0.4 b	0.5 D	0.5 D	0.6 a	
a* _{crumb}	$0.41 \pm$	$0.35 \pm$	$0.26 \pm$	$0.20 \pm$	$0.30 \pm$	***
	0.09 c	0.09 bc	0.09 ab	0.05 a	0.06 b	
b* _{crumb}	$5.56 \pm$	$5.55 \pm$	5.40 \pm	5.17 \pm	$4.93 \pm$	***
	0.09 c	0.05 c	0.09 c	0.06 b	0.05 a	
C*crumb	5.57 \pm	5.55 \pm	5.40 \pm	5.18 \pm	$4.95 \pm$	***
	0.09 c	0.05 c	0.09 c	0.05 b	0.05 a	
h _{crumb}	85.8 \pm	86.0 \pm	87.4 \pm	$\textbf{88.0}~\pm$	86.9 \pm	***
	0.8 a	0.9 a	0.9 b	0.9 b	0.8 b	
ΔE_{crumb}	-	$0.50 \pm$	$0.55~\pm$	0.44 \pm	1.27 \pm	-
		0.05 ab	0.04 b	0.03 a	0.06 c	
Hardness (N)	$1.00~\pm$	$0.69~\pm$	0.75 \pm	$0.79~\pm$	0.74 \pm	***
	0.08 c	0.08 a	0.06 ab	0.06 b	0.03 ab	
Springiness	0.91 \pm	$0.89 \pm$	0.91 \pm	$0.93 \pm$	0.92 \pm	ns
	0.01 ab	0.04 a	0.01 ab	0.01 b	0.01 ab	
Cohesiveness	0.536	0.599	0.581	0.589	0.619	***
	±	±	±	±	±	
	0.008 a	0.005 c	0.009 b	0.007	0.009 d	
				bc		
Chewiness (N)	$0.51 \pm$	$0.37 \pm$	0.40 \pm	$0.43 \pm$	$0.43 \pm$	***
	0.03 c	0.05 a	0.04 ab	0.01 b	0.01 b	
Resilience	0.228	0.275	0.261	0.268	0.285	***
	±	±	±	±	±	
	0.005 a	0.006	0.009 b	0.004	b 000.0	
	21000 1	cd		bc		
Hardness-7	$3.21 \pm$	3.19 +	$2.38 \pm$	2.85 +	2.47 +	***
d (N)	0.09 c	0.01 c	0.01 a	0.01 h	0.09 2	
2 (11)	5.05 C	0.01 C	0.01 u	0.01 0	5.07 u	

L*, a*, b*: CIELAB color coordinates, C*: chroma; h: hue; ΔE : Difference of color between each sample and the control.

The presented results correspond to mean \pm standard deviation. Different letters in the same row indicate statistically significant differences between means at p<0.05. Analysis of variance and significance: ***p<0.001. **p<0.01. *p<0.05. ns: not significant.

reduced by the incorporation of ultrasonicated flours, where significant differences (p < 0.05) were found at treatment times \geq 5 min with respect to control values. The color of the crust results from the Maillard reaction and caramelization of sugars during baking, which depends directly on the available water and the concentration of carbonyl groups from reducing sugars (Villanueva et al., 2019). The lower L*_{crust} values in breads containing US-treated flours indicated a darker crust as consequence of Maillard reaction to a greater extent. Particle fragmentation and partial starch depolymerization caused by cavitation may be the precursors of increased Maillard reaction due to higher availability of simpler sugars that increase the reaction potential. Lower values of a*crust and b*crust and the corresponding reduction in C*crust indicate less vivid colors in the crust of the breads containing ultrasonicated rice flours. Park et al. (2014) reported a decrease of a*crust and b*crust values in breads made using fine rice flour fractions, in agreement with the results of the present study, suggesting that the change of these color



Fig. 5. Effect of US-treated rice flour addition on the external appearance and internal structure of gluten-free breads depending on sonication time. 1) US-0, 2) US-2, 3) US-5, 4) US-10, 5) US-20. A) Upper surface, B) Side surface, C) Bread crumb structure.

properties could be related to the reduced particle sizes after US treatments (see section 3.1). In fact, C*_{crust} was directly correlated with the median particle size of the flours (p < 0.05, r = 0.9199). The values determined for L*_{crust} a*_{crust} and b*_{crust} were very similar among breads containing US-treated flours, despite the different applied sonication times, which could be due to the presence of 70% native flour in these breads, hiding the possible effect of increasing ultrasonication time, resulting in very uniform values. ΔE_{crust} , which combines these three parameters (L*, a* and b*), showed an increasing trend with increasing sonication time. However, none of the determined differences represented a significant difference from a sensory point of view, since a

difference of at least 5 would be required to be sensory noticeable (Gutiérrez, Rico, Ronda, Martín-Diana, & Caballero, 2022). ΔE_{crust} demonstrated that there was not a linear trend with treatment time, but rather an increase that was faster at short times (+0.714 when going from US-2 to US-5) and slowed down at longer times (+0.190 when going from US-10 to US-20). The hue of the crust (h_{crust}) was increased by the incorporation of ultrasonicated flours, which indicates a slight increase towards more yellow tones.

The color parameters determined for the crumb were also significantly modified by the use of ultrasonicated flours, mainly by longer treatment times. Since the color of the crumb is mainly related to the color of the ingredients (Pérez-Quirce, Collar, & Ronda, 2014), the differences found could be related to alterations caused by US treatment on rice flour. L*_{crumb} values were not particularly affected (only in US-20), while in C*_{crumb} a significant decrease (p < 0.001) was found at US-10 and US-20, resulting from the reduction of a*_{crumb} and b*_{crumb} values, indicative of less vivid colors for the crumb of breads containing flours exposed to longer US times. C*_{crumb} was strongly correlated with D₅₀ (p < 0.01, r = 0.9768), evidencing that the effect originated from ultrasonication. The values of h_{crumb} were significantly increased starting at 5 min of ultrasonication (p < 0.001), indicative of more yellowish hues. The highest value of ΔE_{crumb} was determined for US-20, as well as in ΔE_{crust} , which was also not relevant at a sensory point of view.

3.3.3. Texture

The use of US-treated rice flour led to breads with a softer crumb (see Table 4). The lack of gluten in rice breads increases the role of starch in providing structure and texture (Witczak et al., 2016). Crumb hardness decreased from 1.00 N in the control to values ranging between 0.69 N and 0.79 N in breads containing ultrasonicated flours. The effect of sonication time could not be observed in this property, since all crumb hardness of the breads containing US-treated flours was the same. Lower hardness values were associated with higher bread volume, due to a greater amount of air retained in the dough structure during proofing and baking (Pérez-Quirce et al., 2014; Villanueva et al., 2019), as evidenced by the strong correlation with the total volume of CO₂ retained (V_R) (p < 0.05, r = -0.9265). A similar, although more modest reduction in hardness has been reported in rice breads incorporating 15% US-treated rice flour in the formulation (Qin et al., 2022), suggesting a higher crumb softness with increasing incorporation of US-treated flour. The same trend was obtained in chewiness, probably because this parameter mainly depends on hardness. It is believed that the linear molecules obtained after ultrasound treatments were more tightly bound to form a new ordered structure, which facilitated the generation and maintenance of the network structure of the doughs and breads (Qin et al., 2022). Flour granulation and uniformity of particle size are also important factors that affect the processing performance of the flours by determining their hydration and pasting properties (Abebe et al., 2015). As it was mentioned before (section 3.1), ultrasonication led to particle fragmentation, so doughs made with 30% US-treated flour and 70% native flour contained a mixture of normal-size and small-size particles. It is believed that the presence of particles of smaller size (both starch and proteins) had a Pickering stabilization effect that helped the doughs to better retain the gas generated during proofing, hence reducing the bread's hardness. Nanoscale particles have been indicated to perform good as stabilizing droplets and gas bubbles in food applications (Dickinson, 2012). Resilience and cohesiveness increased significantly (p < 0.001) in the breads incorporating sonicated flour, which demonstrates the higher recovery capacity (instantaneous or retarded, respectively) after a compression of their crumbs with respect to the control bread, indicative of freshness and a higher elastic behavior (Ronda, Quilez, et al., 2014). Springiness was not found to be affected by ultrasonication.

Hardness determined after 7 days of bread storage showed that the use of sonicated flour delayed the hardening of bread during storage (see Table 4). Crumb hardening is a complex phenomenon involving multiple mechanisms, including starch recrystallization and moisture loss (Pérez-Quirce et al., 2014; Villanueva et al., 2015). Considering the hardness stablished for the fresh crumbs, the values determined after storage represented an increase of 2.21 N, 2.50 N, 1.63 N, 2.06 N and 1.73 N, for the control, US-2, US-5, US-10, and US-20, respectively. This retarded hardening could derive from particle fragmentation caused by US treatments, enhancing interaction with water and favoring crumb moist over time.

4. Conclusion

Ultrasound treatments demonstrated to be a technology capable of modifying the physicochemical properties of rice flour and influence its breadmaking performance, even at treatments performed at short times, of 5-10 min, and high flour concentration, of 25%. US treatments led to particle fragmentation and molecular reorganization of starch by the action of cavitation, that improved dough elasticity when the US-treated flours were incorporated in dough formulations to replace 30% of the native rice flour. It is believed that the linear chain fragmentation allowed an improved fermentation by easier accessibility of simpler sugars to yeast, accelerating the generation of CO2 and achieving a higher production of CO2. The partial depolymerization and its consecutive increased generation of CO2 were also confirmed in the breads obtained after baking of the doughs, where the incorporation of ultrasonicated flours led to higher volumes, softer crumbs, and lower L*crust values, all indicative of higher availability of simpler sugars when US-treated flours were used. The breads containing ultrasonicated flours presented a crumb with a lower tendency towards staling possibly due to improved interaction of smaller particles with water, better preserving their moist. The main limitation in the present study was the excess water during US treatment (75%), resulting in 30% being a constant substitution level of US-treated flour in the dough/bread formulation. Freeze-drying is an approach that would allow reaching any desired substitution, however, it also has an inconvenient economic impact on the final product that would make the strategy interesting only at laboratory-scale. Further studies are needed to investigate the effect that higher substitutions of ultrasonicated flours would have on the physical quality and digestive properties of breads, as well as the incorporation of different gluten-free flours to produce breads with improved technological, nutritional, and sensory qualities.

CRediT authorship contribution statement

Antonio J. Vela: Formal analysis, Writing – original draft, conceived and designed the experiments, Formal analysis, Data curation, Writing – review & editing, Writing – original draft. Marina Villanueva: Formal analysis, Writing – review & editing, Formal analysis, Writing – review & editing. Felicidad Ronda: Formal analysis, conceived and designed the experiments, Data curation, contributed reagents, materials, analysis tools or data, Writing – original draft, Funding acquisition, Conceptualization, Methodology, Resources, Investigation, Visualization, Supervision, Writing – review & editing, Project administration.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.114950.

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