Flours from microwave-treated buckwheat grains improve the physical properties and nutritional quality of gluten-free bread

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Abstract

Microwave (MW)-assisted hydrothermal treatment of buckwheat grains was explored to improve the physical properties and nutritional quality of gluten-free (GF) bread. A mixture of 80% rice flour and 20% corn starch was used as control recipe (CR), whereas for fortification, 50% of the rice flour was replaced with native buckwheat flour (BN) or buckwheat flour from grains treated with several MW cycles (exposure/rest cycles of 10/50 s, BT1, 20/40 s, BT2, or 30/30 s, BT3, at 30% moisture content and 8 min MW exposure). The BN fortified dough showed increased consistency and elastic response, compared to CR, with the MW treatment further enhancing these effects. Due to the enormous increase in complex modulus (G_1^*) (from 1060 Pa for CR to 10679 Pa for BT3), the hydration of doughs was subsequently adjusted to obtain similar G_1^*. The inclusion of MW-treated flours led to higher consistency and elastic recovery. The lower specific volume (SV) and higher crumb hardness encountered for BN (3.88 mL/g and 1.45N) were alleviated by the inclusion of MW-treated flours (4.61 mL/g and 0.90N for BT1, 4.39 mL/g and 0.85N for BT3), resulting in similar SV and lower staling than CR. Moreover, compared to BN and CR, the BT2 and BT3 breads showed a significant reduction in glucose release during *in vitro* starch digestion (up to –25%), and an increase in protein digestibility (up to +23%). Overall, the experimental findings pointed to the feasibility of using MW to improve the physical and nutritional quality of buckwheat flour-enriched GF bread.

Keywords: Gluten-free bread, Buckwheat, Microwave treatment, Dough rheology, *In vitro* digestion

1 Introduction

Gluten-free (GF) bread is a staple food product with growing demand due to the increasing number of people, both celiac and non-celiac, following GF diets (Aguiar, Santos, Centeno & Capriles, 2021; Xhakollari, Canavari & Osman, 2019). However, the sensory and nutritional quality and shelf life remain insufficient to meet consumer needs. In general, commercial GF breads tend to be lower in protein, vitamins, and minerals and higher in fat than gluten-containing breads, and they often have a high glycaemic index since refined rice flour and starch blends are mostly employed as the main ingredients in their formulations (Aguiar, Santos, Krupa-Kozak & Capriles, 2023). In terms of sensory quality, textural changes (e.g., quick crumb hardening) remain as main problems of GF breads, which is usually addressed by the addition of fats and emulsifiers. The latter ingredients improve texture and palatability, and reduce the bread staling rate, but worsen its nutritional quality (Aguiar et al., 2023; Houben, Höchstötter & Becker, 2012).

There is a trend to study the inclusion of alternative nutrient-rich flours, such as pseudocereals, as raw materials in GF breads to improve their nutritional quality (Aguiar et al., 2021; Burešová et al., 2017; Hager et al., 2012). Buckwheat has been proposed as an applicable functional grain for the production of GF breads because it is rich in vitamins, minerals, and antioxidants, and contains proteins with a well-balanced amino acid profile, dietary fibre, and resistant starch (Torbica, Hadnađev & Dapčević, 2010; Vicente, Villanueva, Caballero, Muñoz & Ronda, 2023; Wronkowska, Haros & Soral-Śmietana, 2013). Buckwheat is already included in small quantities in commercial GF bread formulations. However, recent studies are underway to assess the conditions under which larger quantities can be incorporated to overcome the problems of functionality, appearance, and taste that have been encountered in some studies including this pseudocereal in GF bread (Aguiar et al., 2021).

An interesting approach to improve the functionality of cereal/pseudocereal flours and make them more suitable for the production of GF bread is their physical modification using heat treatments. In particular, heat-moisture treatment (HMT) of starch, in which starch granules with restricted moisture content (< 30%) are heated to high temperatures, above the glass transition temperature (85–130 °C), for a fixed period of time, has proven to be of great interest for applications of pretreated starch containing flours in the food industry, including bread production (Wang, Li & Zheng, 2021; Zavareze & Dias, 2011). These treatments can modify not only the functional properties of flours, fulfilling requirements such as the desired rheological behaviour of the bread dough during proofing and baking, but also modifying the nutritional properties such as starch digestibility (Ronda, Pérez-Quirce & Villanueva, 2023; Wang et al., 2021). However, the use of HMT can be challenging, as it usually requires the application of high temperatures for long periods of time (Ronda et al., 2023). In this context, the use of alternative technologies, such as microwave (MW) irradiation, to perform HMT is gaining interest; actually, MW heating is a quite rapid process for food items, with reduced treatment times and energy consumption, resulting in a more efficient and less costly method to modify the starch/protein matrix in cereal-based products (Rosell, Aalami & Mahdavi, 2020).

Previous studies investigated the effect of MW treatment at 20% and 30% initial moisture content on rice flour properties (Villanueva, Harasym, Muñoz & Ronda, 2018a) and its application at 30% and 50% substitution levels in production of GF rice doughs and breads (Villanueva, Harasym, Muñoz & Ronda, 2019). The incorporation of MW-treated rice flour resulted in enhanced viscoelasticity and elastic behaviour of the dough, as well as increased resistance to deformation. The bread-making performance was also improved, with breads exhibiting higher specific volume, softer crumb, and delayed staling; the best visual features for the end-products were obtained with MW-treated flour at 30% moisture. Recently, we have also studied the application of MW for modification of buckwheat grains (Vicente et al., 2023), and found a change in the thermal behaviour of the amylose-lipid complex as well as an increased V-type crystallinity; this was attributed to the formation of polyphenol-starch complexes and type II amylose-lipid complexes, especially when treatment was at high moisture content (30%). These modifications have the potential to reduce the glycaemic index of products made from MW-treated buckwheat, as increases in resistant starch have been reported in buckwheat flours as well as for starches treated with other types of hydrothermal processing methods (Zhu, 2016).

Based on the literature reviewed, there is still limited knowledge about the impact of various temperature-moisture-time microwave treatment conditions on bread-making performance. In addition, no studies have been conducted to establish if the observed potential nutritional enhancement in microwave-treated buckwheat flour results in an improvement in bread's nutritional value. Therefore, the present study aimed to evaluate the effects of substituting GF flour mixtures with native buckwheat flour and buckwheat flour obtained from MW-treated grains on gluten-free dough and bread properties. For this purpose, three different treatments were carried out at the same initial grain moisture and MW energy applied (same total MW heating time), optimised in preliminary studies, but evaluating different microwave on/off patterns in order to obtain treatments with shorter total duration but at higher temperature, or alternatively longer times at lower temperature. The effect on dough rheology was first evaluated at constant hydration levels, and then upon adjustment of the moisture content, rheological tests were carried out at doughs with similar consistencies, to lessen the pronounced microwave effects on dough viscosity/elasticity in bread making. In addition, bread quality was measured and the effects on *in vitro* starch and protein digestibility were evaluated. This study provided an insight on the impact of MW-treatment conditions of buckwheat grains on rheology of GF composite doughs and its relationship with GF bread quality, as well as their effect on starch and protein digestibility.

2 Materials and methods

2.1 Raw materials

Dehulled buckwheat (*Fagopyrum esculentum* Moench) grains of the Kora variety (14.0% moisture, 2.3% ash, 12.3% protein, 2.8% fat, 10.5% fibre and 58.1% carbohydrates) were obtained from the Grupa Producentów Ekologicznych Dolina Gryki Sp ZOO (Miedzylesie, Poland). An indica rice flour (12.6% moisture, 0.7% ash, 7.1% protein, 0.5% fat, 2.7% fibre and 76.4% carbohydrates) with coarse particle size was provided by Herba Ricemills, S.L.U. (Sevilla, Spain) and maize starch (> 98% purity, 23% amylose content) by Ferrer Alimentación S.A. (Barcelona, Spain). Sunflower oil, sugar, and salt were purchased from local stores, and hydroxy-propyl-methyl-cellulose (HPMC, Methocel K4M food grade) was provided freely by Dow Chemical (Midland, USA). Water from the local water supply was used in dough and bread making. The water absorption capacity (WAC) of the flours (g water/g flour dry matter) was 1.22, 1.30, and 0.99 for buckwheat, rice, and maize, respectively, determined as described by Vicente et al. (2023).

2.2 Microwave treatment

A customized microwave oven R342INW (SHARP, Sakai, Japan) at 900 W and 2450 MHz was used to perform the treatments, as described by Vicente et al. (2023). The treatment parameters and conditions were chosen after preliminary trials. Buckwheat grain moisture content (MC) was set to 30% by adding distilled water to the grain. Portions of

200 ± 0.05 g of grain was placed for processing in a 2 L non-hermetic Teflon container. Although using the same microwave energy (8 min of total microwave heating), heat treatment was applied under three different microwave cycles (s exposure / s rest) with different durations: 10/50 (48 cycles, 48 min total), 20/40 (24 cycles, 24 min total), and 30/30 (16 cycles, 16 min total).

The maximum temperature reached at each treatment was measured with Testoterm® temperature strips from TESTO (Barcelona, Spain), placed in the container in permanent contact with the sample. The highest temperatures reached were 101 ± 3 °C, 107 ± 5 °C and 113 ± 3 °C for the 10/50, 20/40, and 30/30 cycle processes, respectively. The final MC of the samples, measured according to AACC official method 44–19.01 (AACC, 2010), was 25 ± 1%, 10 ± 1%, and 8 ± 3% for treatment with the 10/50, 20/40, and 30/30 cycles, respectively. The WAC (g/g) of the treated flours was also determined as for the raw materials (see section 2.1).

Based on their final MC, the treated samples were subsequently adjusted to 14 % MC after each treatment by either moistening (with the addition of distilled water) or drying (in an incubation chamber at 35ºC). Two batches were prepared by mixing three treatments in each batch. All batches of treated buckwheat grains, as well as the native grains, were ground (to < 500 µm particle size) in a LM 3100 hammer mill (Perten Instruments, Sweden) and stored at 4 °C until further use.

2.3 Dough preparation

Five different mixes of flour were used for dough preparation. A commercial recipe made with 80% rice flour and 20% maize starch was used as the control formulation (CR). Then, 50% native buckwheat flour (BN), treated buckwheat flour at cycle 10/50 (BT1), cycle 20/40 (BT2), or cycle 30/30 (BT3) were included in the formula, by replacing a respective amount of rice flour in the composite flour mixture.

For dough making, the following formula (additional ingredients) was used on a 100 g total flour basis (13% moisture): 1.5% salt, 2% HPMC, 5% sucrose, and 6% oil. The water added to the dough was first set at 90% for preliminary rheological testing (Table 1), and then adjusted for breadmaking in such a way to achieve the same dough consistency, as determined by the complex moduli G_1^*, with varying amounts of water, as specified in Table 2. The doughs were prepared using an Auto Bakery equipment (Funaj, China). First, the solid ingredients were mixed with water for 2 min, first min at low speed and second at fast speed. Then oil was added, and the dough ingredients were further mixed for an additional period of 8 min at fast speed.

Table 1. Rheological properties of gluten-free bread doughs at constant dough hydration (90% flour basis).

	CR	BN	BT1	BT2	BT3	SE
Dough hydration (g water/100 g flour)	90	90	90	90	90	
Frequency sweeps						
G_1' (Pa)	877 a	2145 b	7812 c	9876 d	9909 d	507
a	0.370 c	0.241 b	0.233 ab	0.224 a	0.221 a	0.004
G_1'' (Pa)	595 a	999 b	3248 c	4040 d	3981 d	198
b	0.41 c	0.26 b	0.23 ab	0.21 a	0.22 a	0.01
$(\tan \delta)_1$	0.68 c	0.47 b	0.42 a	0.41 a	0.40 a	0.01
c	0.035 c	0.018 bc	-0.001 ab	-0.015 a	-0.006 ab	0.008
G_1^* (Pa)	1060 a	2366 b	8460 c	10670 d	10679 d	544
Strain sweeps						
Crosspoint (Pa)	12 a	60 b	189 c	281 d	259 d	14
τ_{max} (Pa)	3 a	4 a	20 b	33 c	31 c	1
Creep-recovery						
$J_{0c} + J_{1c}$ (10^{-4} Pa^{-1})	94 c	68 b	10 a	6 a	7 a	4
λ_{1c} (s)	0.2 a	3.9 b	6.5 d	5.5 c	6.1 d	0.2
μ_0 (10^{+3} Pa·s)	0.2 a	23.6 b	38.7 c	74.9 e	62.6 d	3.0
J_{max} (10^{-4} Pa^{-1})	3295 c	323 b	25 a	14 a	16 a	31
$J_{0r} + J_{1r}$ (10^{-4} Pa^{-1})	102 c	58 b	11 a	7 a	7 a	3
λ_{1r} (s)	2.2 a	6.8 b	10.2 c	16.4 d	17.6 d	0.8
Recovery (%)	3 a	19 b	43 c	51 e	48 d	1

CR: dough control recipe with 80% rice flour and 20% maize starch. BN: dough obtained by replacing 50% of rice flour with native buckwheat flour. BT1, BT2 and BT3: doughs obtained by replacing 50% of rice flour with flours of the MW-treated buckwheat grains. The power law model was fitted to the experimental frequency sweep data, i.e., $G' = G_1' \cdot \omega^a$; $G'' = G_1'' \cdot \omega^b$; $\tan \delta = (\tan \delta)_1 \cdot \omega^c$; the 'a', 'b', and 'c' coefficients were obtained by fitting the elastic moduli, viscous moduli and loss tangent at a frequency of 1 Hz in the power law model for each parameter. These coefficients (exponent values) specify the dependence of the rheological parameters on the oscillation frequency. G_1^*: complex modulus at a frequency of 1 Hz. Crosspoint: stress value for $G'=G''$. τ_{max}: maximum stress that the samples could withstand in the LVR. The subscript "c" in the creep-recovery data corresponds to the creep phase and the subscript "r" to the recovery phase. J_{0c} and J_{0r}: instantaneous compliances. J_{1c} and J_{1r}: retarded compliances. J_{max}: maximum creep compliance at the end of the creep step. λ_{1c} and λ_{1r}: retardation times. μ_0: steady-state viscosity in the creep phase. SE: pooled standard error obtained from ANOVA. Means with different letters for the same parameter indicate significant differences between means, at $p < 0.05$.

2.4 *Oscillatory and creep–recovery tests*

Dynamic oscillatory and creep-recovery tests were performed at least in duplicate on a Kinexus Pro + rheometer (Malvern Instruments, UK) equipped with a parallel plate geometry (40 mm) with a serrated surface and a 1 mm gap. The measurements were performed on doughs prepared with the same hydration (90%) to compare the effect of the different flours included, and with different hydration level to achieve similar consistencies (verified by the complex modulus G_1^* values of the composite doughs), as adopted for the breadmaking process. For rheological evaluation of the doughs, measurements started 10 min after dough preparation, and for each test, the sample was placed between the plates and allowed to relax for 5 min. The temperature was maintained at 25 °C using a Peltier plate controller.

Strain sweeps were performed in duplicate from 0.01 to 1000% at 1 Hz frequency. The linear viscoelastic region (LVR) was thus established, and the maximum stress (τ_{max}) beyond which the dough structural integrity is lost as well as the stress at the crosspoint (G′=G″) were determined. Frequency sweeps were performed in duplicate from 1 to 10 Hz in the LVR at a constant deformation of 0.05%. The frequency sweep data were fitted to the power law model, as reported elsewhere (Ronda, Pérez-Quirce, Angioloni & Collar, 2013):

$$G'(\omega) = G'_1 \cdot \omega^a \tag{1}$$

$$G''(\omega) = G''_1 \cdot \omega^b \tag{2}$$

$$\tan\delta\ (\omega) = \frac{G''(\omega)}{G'(\omega)} = \left(\frac{G''}{G'}\right)_1 \cdot w^{b-a} = (\tan\delta)_1 \cdot w^c \tag{3}$$

$$G_1^* = \left({G_1'}^2 + {G_1''}^2\right)^{\frac{1}{2}} \tag{4}$$

The rheological parameters G_1', G_1'', and $(\tan \delta)_1$ represent the elastic and viscous moduli, and the loss tangent, respectively, at a frequency of 1 Hz. The exponents ‘a’, ‘b’, and ‘c’ quantitatively describe the dependence of the dynamic moduli and loss tangent on the oscillation frequency, ω. G_1^* represents the complex moduli.

Creep-recovery tests were performed in triplicate outside the LVR (OLVR) by applying a constant shear stress of 50 Pa for 60 s, after which the stress was removed, and the sample was left to recover for 180 s. The data from the creep phase were modelled to the 4-parameter Burgers model, and for the recovery phase, as there is no viscous flow, to the 3-parameter Burgers model, as specified in previous studies (Villanueva et al., 2019):

$$J_c(t) = J_{0c} + J_{1c}\left(1 - \exp\left(-\frac{t}{\lambda_{1c}}\right)\right) + \frac{t}{\mu_0} \tag{5}$$

$$J_r(t) = J_{max} - J_{0r} - J_{1r}\left(1 - \exp\left(-\frac{t}{\lambda_{1r}}\right)\right) \tag{6}$$

The coefficients J_c (t) and J_r (t) are the creep and recovery compliances, respectively. J_0 is the instantaneous compliance, J_1 is the retarded elastic compliance, λ_1 is the retardation time, and μ_0 is the steady state viscosity in the creep phase. The subscript "c" corresponds to data from the creep phase and the subscript "r" to the recovery phase data. The ratio J_{steady}/J_{max} (elastic recovery) was calculated and expressed as recovery (%), where J_{max} is the maximum creep compliance obtained at the end of the creep step, and J_{steady} is the steady-state compliance in the recovery step.

2.5 Bread-making

The five recipes with the composition described in the section 2.3, with adjusted hydration (specified in Table 2) and an additional 3% of dried yeast dispersed in water were used for the bread-making process. Bread-making was performed using a Kitchen-Aid professional mixer 5KPM50 (St. Joseph, MI, USA). First, the solid ingredients were mixed with water for 2 min at a speed of 2. The oil was then added, and the dough was mixed for 8 min at a speed of 4. Portions of 160 g of dough were placed in aluminium pans and proofed in a Memmert HPP 260 eco chamber (Schwabach, Germany) for 50 min at 32°C and 85% relative humidity. Baking was then performed in a Sveba Dahlen S200 oven (Fristad, Sweden) for 20 min at 170 °C with 7 s of steam injection. After baking, breads were removed from the pan and stored for 1 h at room temperature before further analysis.

2.6 Bread proximal composition

The protein, fat, ash and moisture contents were determined according to the AACC official methods 46–19.01, 30–10.01, 08–01.01, and 44–19.01, respectively (AACC, 2010). Total, soluble, and insoluble dietary fibre were determined as previously described (Kotsiou, Sacharidis, Matsakidou, Biliaderis & Lazaridou, 2021) using the Total Dietary Fiber Assay kit of Megazyme (Wicklow, Ireland), with the provided thermostable α-amylase, protease, and amyloglucosidase preparations, and by following the AACC official method 32–05 (AACC, 2010). The FibreBags filtration system from Gerhardt Analytical Systems (Konigswinter, Germany) was used to separate soluble and insoluble dietary fibre fractions. Starch content was measured using the rapid method for total starch, as described by Englyst, Hudson & Englyst (2006). All analyses were performed at least twice.

2.7 *Bread physical properties*

Baking loss was determined by weighing the breads one hour after breadmaking. The volume of all prepared breads was determined using a Volscan profiler 300 (Stable Microsystems, Surrey, UK) analyser.

The crumb texture was analysed using a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) equipped with a 20 mm cylindrical aluminium probe. The two central slides cut of 20 mm thickness were used for “Texture Profile Analysis” (TPA), with a double compression-decompression cycle test (deformation at 50% depth and a speed of 1 mm/s, with a 30 s delay period between the two cycles). From the TPA graph, firmness (N), cohesiveness, springiness, and resilience were calculated using the “Texture Expert” software (Stable Microsystems, Surrey, UK). For each bread-making, two breads were analysed fresh, and another was analysed after 7 days of storage (evaluation of Δ Firmness – 7 d) in hermetic bags at 4 °C to determine the staling index.

Photographs of the slices and whole loaves were taken using a PowerShot SX410 IS camera (Canon, Japan). Image analysis of the crumb grain characteristics was performed with the ImageJ software (National Institutes of Health, USA) using 25 mm × 40 mm area sections taken from the centre of slices; cell density (number of cells per cm^2) and mean cell area (mm^2) were thus calculated.

The colour of the bread crust and crumb was determined using a PCE-CSM5 colorimeter (PCE Instruments, UK) and CQCS3 software to obtain the CIE L*C*h coordinates (D65 standard illuminant and a 10° standard observer); each sample was measured at five different points.

2.8 *In vitro digestibility of bread*

The *in vitro* enzymatic digestibility of starch was assessed by measuring the release of reducing sugars from the bread crumbs through a dialysis tube, mimicking the epithelium of the small intestine, as previously described by Lazaridou, Marinopoulou, Matsoukas & Biliaderis (2014). Briefly, 5 g of bread crumb were crumbled to a size of approximately 0.5 cm^3, suspended in 35 mL of sodium phosphate buffer (20 mM, pH 6.9), and adjusted to pH 1.5 for the first digestion step with pepsin (575 units/g starch, Sigma-Aldrich) for 30 min at 37 °C under mild stirring in a Memmert WNB 7-45 water bath shaker

(Schwabach, Germany) to simulate the gastric environment. Then, the pH was re-adjusted to 6.9, and 30 mL of sodium phosphate buffer (20 mM, pH 6.9) and porcine pancreatic α-amylase (110 units/g starch, Megazyme) were added. The suspension was transferred to a Sigma-Aldrich dialysis tube cellulose membrane D9402-100FT (St. Louis, MO, USA) and placed in a screw-cap glass bottle containing 435 mL phosphate buffer; incubation was carried out for 5 h at 37 °C under mild stirring in a water bath, simulating the intestinal digestion phase. Aliquots (1 mL) of dialysates were collected at 20, 60, 120, 180, 240, and 300 min in duplicate. Aliquots were incubated with amyloglucosidase from Rhizopus sp. mold (Sigma-Aldrich), and the released glucose was measured using the GOPOD reagent. The area under the curve (AUC) of glucose released over a 300 min of digestion period was calculated to evaluate *in vitro* starch digestibility; the analysis was performed in triplicate.

In vitro protein digestibility was measured using the pepsin-pancreatin digestion method described by Fasuan, Gbadamosi & Omobuwajo (2018) with minor modifications. Lyophilised crumb samples containing 250 mg of protein were suspended in 25 ml of 0.1 M HCl containing 1.5 mg of pepsin. The mixture was stirred at 30°C for 1h and the resulting suspension was neutralised with 0.5 M NaOH solution. Then 7.5 ml of phosphate buffer (0.2 M, pH 8.0) containing 4.0 mg of dissolved pancreatin was added. The mixture was stirred for 24 h at 30°C and then filtered through a Whatman No. 1 filter paper. The residue was washed twice with distilled water, oven-dried, and used for protein determination using the AACC official method 46–19.01 (AACC, 2010). Protein digestibility (%) was calculated as the difference between the protein content of the sample before and after digestion, relative to the content before digestion.

2.9 *Statistical analysis*

The Statgraphics Centurion 19 software (Bitstream, Cambridge, MN, USA) was used for the statistical analysis. Significant differences ($p < 0.05$) between samples were assessed using analysis of variance (ANOVA) with the least significant difference (LSD) test. Results were presented as mean values of different replicates, and the pooled standard error (SE) obtained from ANOVA was also presented for each parameter. Differences were indicated by different letters in the tables and figures.

3 Results and discussion

3.1 *Dough rheology*

3.1.1 Dynamic oscillatory and creep-recovery test at constant dough hydration

The effect of replacing 50% of rice flour with flours from native or MW-treated buckwheat grains in a control recipe made with 80% rice flour and 20% maize starch on dough rheology was first studied at a dough hydration of 90% (flour basis). Table 1 shows the rheological parameters and Figure 1 the frequency sweeps, strain sweeps, and creep-recovery plots. Sample BT2 was not plotted in the frequency and strain sweeps graphs because the data overlapped with those of BT3.

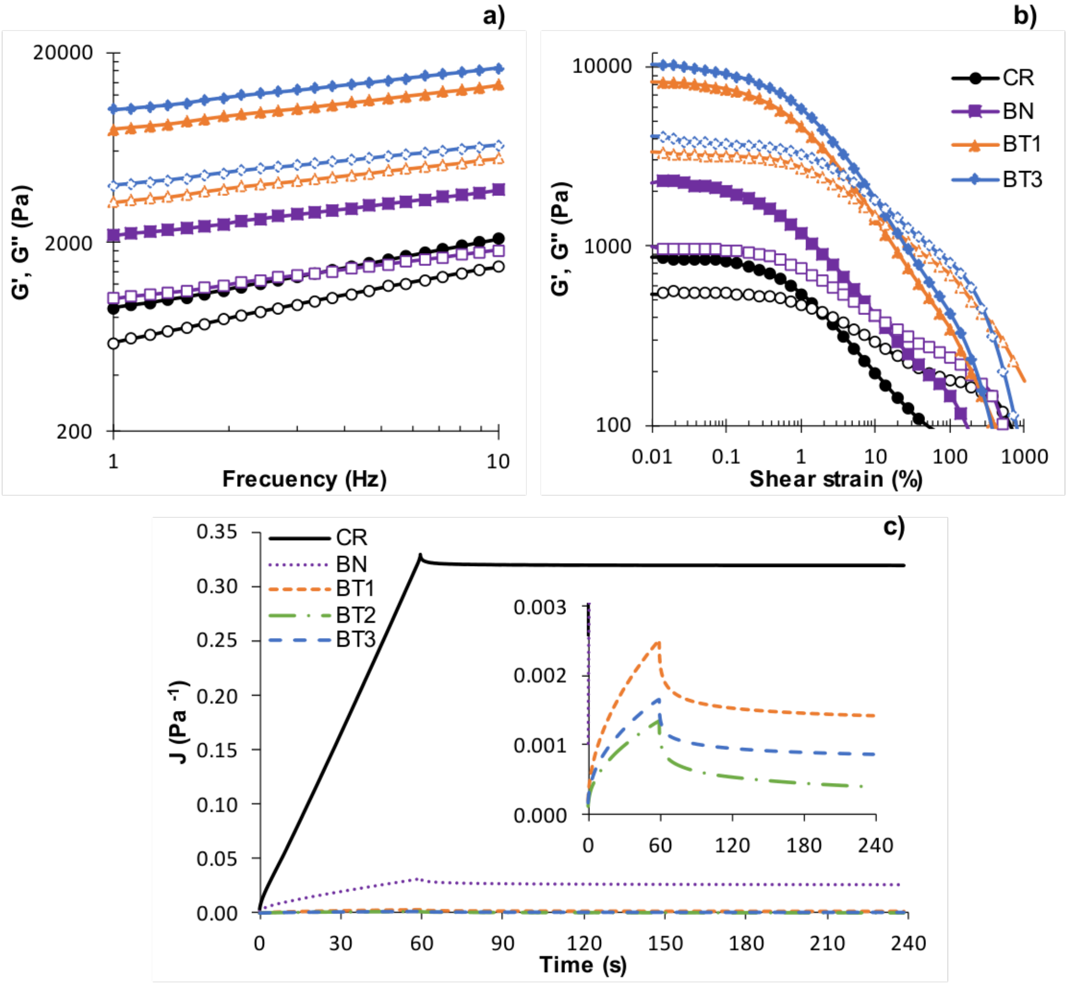


Figure 1. Dough rheology at constant hydration (90% flour basis) of doughs obtained by replacing 50% of rice flour in the control recipe (CR) with flours from either native buckwheat (BN) or microwave-treated buckwheat (BT1, BT2, and BT3) grains. (a) Frequency sweeps, (b) strain sweeps, and (c) creep-recovery test outside the linear viscoelastic region. In a and b plots G' is

presented by solid symbols and G" by empty symbols. BT2 is not presented in (a) and (b) because the responses overlap with those of BT3.

The addition of native buckwheat flour (BN) to the control formulation (CR) resulted in doughs with enhanced elastic behaviour, as evidenced by the –31% reduction in the loss tangent, $(\tan \delta)_1$, values, with the doughs having higher consistency, as shown by the higher elastic, G_1', and viscous, G_1'', moduli, +144% and +68%, respectively. The BN fortified doughs also presented a lower frequency dependence, with a reduction in the values of the exponents 'a' and 'b' by –35% and –36%, respectively. However, the stress resistance before failure of the dough structures, i.e., τ_{max}, did not increase significantly for the fortified preparation. Instead, the stress at the crosspoint, where the dough behaviour changes from predominantly elastic to predominantly viscous, increased from 12 to 60 Pa. When microwave-treated flours were included in the formulations, the same effects were noted in the rheological responses, as with the inclusion of native buckwheat, although to a greater extent. The doughs were of higher consistency compared to those fortified with the native buckwheat flour, showing a complex modulus, G_1^*, for BT1 3.6 times that of BN, and for BT2 and BT3 4.5 times higher. Moreover, they also showed reductions in $(\tan \delta)_1$ of up to –14% for BT3, thus implying a greater elastic behaviour and denoting more stable dough structure matrices (Collar, Villanueva & Ronda, 2020). Doughs with MW-treated flours also showed significantly higher τ_{max} and stress values at the crosspoint than BN and CR; i.e., they were able to withstand higher stresses before failure of their structures, thus denoting a structuring impact of MW treatment, as reported previously for gels made with flour of MW-treated buckwheat (Vicente et al., 2023). Villanueva et al. (2019) observed lesser variations in consistency and resistance to stress when included MW-treated rice flour in breads (with similar treatment conditions to BT2). For example, they obtained a 2.3-fold increase in G_1', whereas in the present study there was a 4.6-fold rise in the modulus values. The different conditions adopted during the MW treatment of buckwheat grain compared to rice flour, as well as its composition with higher protein and fibre contents, may be responsible for these differences. MW heating of the whole grain can have a different impact on the kinetics of heat transfer and water mobility in comparison to treating only the flour, which ultimately affects the properties of the resulting flour (Vicente et al., 2023).

The creep-recovery test was performed OLVR (outside the linear viscoelastic region) at 50 Pa, as this situation is similar to the stresses that the dough is subjected to in real bread making processes (Ronda et al., 2023). A typical viscoelastic behaviour, combining both viscous-fluid and elastic components, was observed in the creep recovery curves of the GF doughs (Figure 1), similar to those of other GF doughs studied (Lazaridou, Duta, Papageorgiou, Belc & Biliaderis, 2007; Perez-Quirce, Caballero, Vela, Villanueva & Ronda, 2018; Villanueva et al., 2019). The BN showed a decrease in elastic compliance in the creep (J_{0c} + J_{1c}) and in the recovery (J_{0r} + J_{1r}) phases compared to CR. It also exhibited a clear increase in the steady-state viscosity (μ_0), implying dough resistance to deformation and reducing the maximum creep compliance at the end of the creep phase (J_{max}). The μ_0 was proven to increase with the addition of protein (Villanueva, Pérez-Quirce, Collar & Ronda, 2018b) and insoluble fibre components (Perez-Quirce et al., 2018) in GF doughs. The use of microwave-treated flour further accentuated these effects, leading to a large reduction in J_{max} and increase in μ_0. In addition, the elastic compliance values were reduced, and both creep and recovery retardation times (λ_c and λ_r) increased. The elastic recovery, which is represented by the ratio of the steady-state compliance in the recovery step (J_{steady}) to J_{max}, also increased for the fortified doughs with the microwave-treated buckwheat flours (from 19% for BN up to 51% for BT2), despite the reduction in the value of their elastic compliance (from $68\cdot10^{-4}$ Pa^{-1} for BN up to $6\cdot10^{-4}$ Pa^{-1} for BT2). This effect is attributable to the large differences in consistency and viscosity of the doughs, which had a greater impact on the recovery capacity than the decrease in the elastic component. The elastic compliance was only 21% of the total shear compliance in the creep phase for BN, whereas it was 40% for BT1, 43% for BT2, and 44% for BT3. A similar effect, but to a lesser extent, was observed for GF doughs when ultrasonicated rice flour was included in the formulation (Vela, Villanueva & Ronda, 2023). These authors also reported that the doughs exhibited reduced elastic compliance and J_{max} values, and increased μ_0, which resulted in higher elastic recovery; these responses were in turn related with improvements in the specific volume of GF bread.

3.1.2 Dynamic oscillatory and creep-recovery test at adapted dough hydration

The large increases in consistency noted for the fortified doughs (Table 1) at a constant water hydration level (90 %) made it unfeasible to perform bread-making under these conditions and prompted us to examine the various dough formulations for their rheological properties and baking performance at different hydration levels for comparison purposes. It has been shown that the consistency of the dough has a great influence on the stability of gas bubbles during fermentation, and large differences in this parameter could mask the impact of flour component alterations caused by pretreatment of a flour with a physical method (Föste, Jekle & Becker, 2017), as in the present study, using the flours from microwaved buckwheat in GF dough formulations. Therefore, a complementary rheological study was carried out on the doughs, adapted at a constant consistency, as measured by G_1^*, and selected from preliminary baking trials; the results of this study and the adjusted hydration of each dough are given in Table 2, whereas the strain sweep and creep-recovery plots are presented in Figure 2.

Table 2. Rheological properties of gluten-free bread doughs at various hydration levels for adjustment of similar consistency.

	CR	BN	BT1	BT2	BT3	SE
Dough hydration (g water/100 g flour)	80	90	105	110	110	
Frequency sweeps						
G_1' (Pa)	2225 a	2145 a	2216 a	2427 a	2388 a	134
a	0.324 c	0.241 a	0.266 b	0.245 a	0.251 a	0.003
G_1'' (Pa)	1302 b	999 a	1084 ab	1098 ab	1111 ab	66
b	0.34 b	0.26 a	0.29 a	0.26 a	0.26 a	0.01
$(\tan\delta)_1$	0.585 c	0.466 a	0.489 b	0.453 a	0.465 a	0.005
c	0.02 a	0.02 a	0.02 a	0.01 a	0.01 a	0.01
G_1^* (Pa)	2577 a	2366 a	2467 a	2663 a	2634 a	149
Strain sweeps						
Crosspoint (Pa)	33 a	60 b	70 bc	87 c	79 c	5
τ_{max} (Pa)	6 ab	4 a	8 bc	9 c	9 c	1
Creep-recovery						
$J_{0c} + J_{1c}$ (10^{-4} Pa^{-1})	72 b	72 b	63 ab	65 ab	57 a	4
λ_c (s)	2.9 a	3.9 b	5.6 c	5.2 c	5.1 c	0.2
μ_0 (Pa·s)	941 a	2362 b	3671 cd	3515 c	4247 d	227
J_{max} (10^{-4} Pa^{-1})	703 d	323 c	224 b	232 b	196 a	10
$J_{0r} + J_{1r}$ (10^{-4} Pa^{-1})	61 b	58 b	52 a	56 ab	52 a	2
λ_r (s)	5.9 a	6.8 a	11.6 b	10.6 b	10.5 b	0.5
Recovery (%)	9 a	19 b	24 c	26 c	28 d	1

CR: dough control recipe with 80% rice flour and 20% maize starch. BN: dough obtained by replacing 50% of rice flour with native buckwheat flour. BT1, BT2 and BT3: doughs obtained by replacing 50% of rice flour with flours of the MW-treated buckwheat grains. The power law model was fitted to the experimental frequency sweep data, i.e., $G' = G_1' \cdot \omega^a$; $G'' = G_1'' \cdot \omega^b$; $\tan\delta = (\tan\delta)_1 \cdot \omega^c$; the 'a', 'b', and 'c' coefficients were obtained by fitting the elastic moduli, viscous moduli and loss tangent at a frequency of 1 Hz, in the power law model for each parameter. These coefficients (exponent values) specify the dependence of the rheological parameters on the oscillation frequency. G_1^*: complex modulus at a frequency of 1 Hz. Crosspoint: stress value for $G'=G''$. τ_{max}: maximum stress that the samples could withstand in the LVR. The subscript “c” in the creep-recovery data corresponds to the creep phase and the subscript “r” to the recovery phase. J_{0c} and J_{0r}: instantaneous compliances. J_{1c} and J_{1r}: retarded compliances. J_{max}: maximum creep compliance at the end of the creep step. λ_c and λ_r: retardation times. μ_0: steady-state viscosity in the creep phase. SE: pooled standard error obtained from ANOVA. Means with different letters for the same parameter indicate significant differences between means, at $p < 0.05$.

The replacement of rice flour with native buckwheat flour (BN) required a 10% increase in hydration compared to CR to yield a similar dough consistency value. Although the water absorption capacity (WAC) of rice flour (1.30 ± 0.03 g/g) was higher than that of buckwheat (1.22 ± 0.05 g/g), the higher fibre content of buckwheat may be responsible for the more viscous doughs. In particular, the increase in insoluble fibre content has been associated with stiffer doughs, thus requiring an increased amount of water in the formulations to moderate the consistency enhancement of the composite dough system (Perez-Quirce et al., 2018). When flour from microwaved buckwheat was introduced, the dough hydration had to be raised from 90% on a flour basis for BN to 105% for BT1, and 110% for BT2 and BT3 to achieve the same G_1^*. In this case, as the doughs similar proximal composition (see 3.2), the observed differences can be related to the increased WAC of the MW-treated flours, which were 1.76 ± 0.05 g/g, 1.95 ± 0.04 g/g, and 1.99 ± 0.01 g/g for the flours used in the BT1, BT2, and BT3 formulations, respectively, compared to the native buckwheat flour (1.22 ± 0.05 g/g). The relation between a higher WAC and an increase in dough consistency has been previously observed for GF doughs with ultrasonicated rice (Vela et al., 2023) and high hydrostatic pressure-treated starches (Rahman, Zhang, Sun & Mu, 2022). These authors suggest that a change in water binding capacity alters the structure of dough since hydration affects both the conformation and molecular interactions among its constituents.

For the frequency sweep tests, performed in the LVR, little differences were noted, following the adjustment of consistency (i.e., similar G_1^*) for all composite doughs. The inclusion of MW-treated flours had no relevant effect on the behaviour of the dough, compared to BN, when the hydration was adjusted to obtain similar G_1^*. However, the replacement of rice flour with the buckwheat flours in the formulations seemed to reduce the viscous modulus, which resulted in lower loss tangent values (Table 2). Additionally, a reduction in the frequency dependence of the elastic and viscous moduli (‘a’ and ‘b’ exponents) was also noted. Föste et al. (2017) showed similar reduction in the ‘a’ exponent with the addition of increasing contents of quinoa bran in a GF dough.

Therefore, the observed rheological changes can be associated, at least in part, to the inclusion of the buckwheat bran components.

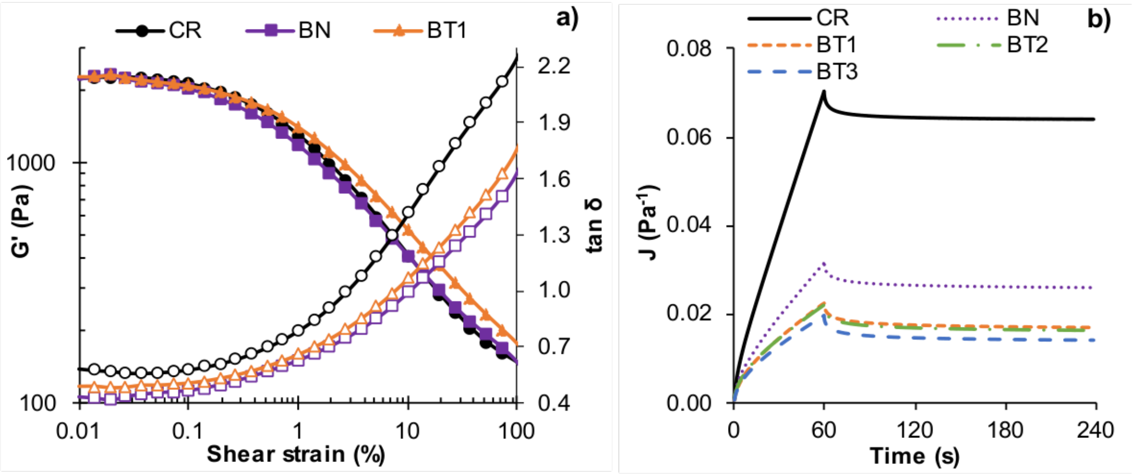


Figure 2. Rheological responses of doughs at adjusted hydration levels in order to obtain a constant consistency for all samples; doughs were obtained by replacing 50% of rice flour in the control recipe (CR) with flours from either native buckwheat (BN) or microwave-treated buckwheat grains (BT1, BT2, and BT3). (a) strain sweeps and (b) creep-recovery test outside the linear viscoelastic region. In (a), the plot of G' is presented by solid symbols and tan δ by empty symbols; only CR, BN and BT1 are presented to help visualization of the data.

Regarding the strain sweep rheological responses, in Figure 2a, the G' and tan δ are shown for the doughs made with the control formulation (CR), the untreated buckwheat flour (BN) and the MW-treated flour (BT1), as an example (BT2 and BT3 exhibited similar behaviour to that BT1). The data for viscous and elastic moduli, as indicated by the tan δ values, showed very different behaviour in the case of CR than for doughs fortified with the buckwheat flours. Moreover, the tan δ increased more rapidly with increasing deformation (% strain), presenting the crosspoint (G'=G") at a strain of ~ 3% for the CR. Instead, the tangent values for all buckwheat flour-fortified doughs increased in a slower way with increasing strain level, exhibiting the crosspoint at a strain of 9% (apparently, no significant differences between the different MW treatments of the grains were found). A faster increase in tan δ of the dough with deformation indicates that the elastic modulus decreases faster than the viscous modulus, i.e., more pronounced weakening of its solid-like character (Vicente et al., 2023). All doughs presented similar G' in the LVR (Table 2). Nevertheless, at higher strains, the doughs with the treated flours were of higher consistency and, therefore, withstood higher stresses, as evidenced by the higher τ_{max} and crosspoint stresses.

The results of creep recovery test also showed much smaller differences among the doughs when the ‘adjusted - constant consistency (for G_1^*)’ experimental scheme was adopted, in comparison with the data obtained at the constant 90% hydration level (Table 1), but with a similar trend. It should be noted that the consistency measurements were in the LVR, whereas the creep-recovery was performed in the OLVR. Therefore, the 50 Pa stress applied to all samples corresponded, in the strain sweep experimental mode, to strains between 4% and 6%. This point is between the LVR and the crosspoint for the samples containing the buckwheat flours and after the crosspoint for CR, as depicted in Figure 2a and discussed previously. The samples exhibited differences in their consistency at a 50 Pa applied stress level, leading to differences in their viscoelastic response in the creep-recovery test. There were relatively small differences in the elastic compliance in the creep and recovery phases, indicating small reductions for the BT1, BT2 and BT3 formulations. However, the calculated retardation times, λ_c and λ_r, slightly increased for BN and even more for the fortified doughs with the microwaved flours of buckwheat, compared to CR, indicating that more time is needed to obtain the viscoelastic deformation on the dough (Vela et al., 2023). The viscosity increased by 150% with the inclusion of native buckwheat and up to 350% with the use of treated buckwheat flours, whereas the J_{max} was reduced by –54% and up to –68%, respectively. These results, as in the case of doughs with constant hydration (Table 1), led to an increase in the % recovery from 9% for CR to 19% for BN and up to 28% for BT3. The higher consistency of the treated doughs in the OLVR found in the strain sweep seemed to be related to the increased viscosity and recovery found in the creep-recovery test.

3.2 Bread proximal composition

The composition on dry basis (db) of CR bread was 2.2 ± 0.2% ash, 6.7 ± 0.1% protein, 6.3 ± 0.1% fat, 5 ± 2% fibre (2 ± 1% insoluble fibre and 3 ± 1% soluble fibre), and 80 ± 3% starch. The microwave treatment did not alter the proximal composition of the bread, thus the proximal compositions (db) of BN, BT1, BT2 and BT3 were the same: 3.0 ± 0.2% ash, 9.2 ± 0.1% protein, 7.5 ± 0.1% fat, 9 ± 2% fibre (4 ± 1% insoluble fibre and 5 ± 1% soluble fibre), and 71 ± 3% starch. The crumb moisture (g water / 100 g bread) was 44.3 ± 0.2, 47.6 ± 0.5, 51.6 ± 0.3, 53.4 ± 0.2, and 53.2 ± 0.2 for CR, BN, BT1, BT2, and BT3, respectively.

The inclusion of buckwheat into the GF products resulted in nutritional improvement over the GF bread made with the standard recipe of rice flour and maize starch (CR). The protein content of the buckwheat-fortified breads increased by ~ 2.5%, contributing also to a more balanced amino acid profile than that with rice flour alone (Bhinder, Kaur, Singh, Yadav & Singh, 2020). The fibre content also rose by 4%, with both the insoluble and the soluble fractions, being ~ 2% higher. The mineral content also increased, as indicated by the higher ash content. The increase in macro- and micronutrients is considered an important aspect for improving the health of individuals following a GF diet (Aguiar et al., 2023). There was an increase in fat content as well, from 5% in CR to 9% in BN, but it should be pointed out that buckwheat has a better fatty acid profile than cereal grains, with around 80% being unsaturated fatty acids (and more than 40% polyunsaturated lipids) (Wronkowska et al., 2013).

The MW treatment did not seem to greatly modify the proximal composition of the breads formulated with the various buckwheat flour preparations, although, as noted in the present study, it did change the bread-making performance and the *in vitro* digestibility of starch and protein of the fortified products. Other studies on heat treatments have shown a reduction in some components, when analysing the proximal composition, and this was attributed to either the diffusion of the water used during the thermal treatment (chemical alterations in some components) or to increased interactions among the flour components, which make their quantification more difficult with the conventional analytical techniques used for determination of proximate composition (Deng, Padilla-Zakour, Zhao & Tao, 2015; Liu et al., 2015).

3.3 Bread physical properties

The physical properties of breads are presented in Table 3, whereas the respective photographs of representative samples are shown in Figure 3. The evaluated breads were those made with adjusted amount of water to achieve a similar dough consistency (see Table 2), which can allow us to confirm that the true impact of buckwheat flour fortification on the bread properties by negating the effect of varying dough consistency levels (Perez-Quirce et al., 2018). The bake loss slightly increased with the inclusion of native BW (+4% compared with CR), but there were no differences among the products made with the various MW treated buckwheat flour preparations. The inclusion of native buckwheat also resulted in a reduction in specific volume (SV) from 4.65 mL/g CR to 3.88 mL/g BN. Doughs made with 50% replacement of the rice flour by native buckwheat flour were less able to maintain a shelf-supported hydrated network during fermentation, and particularly during baking (data not shown), showing a structural collapse in the centre of the bread (see Figure 3) and thus a lower final bread SV. Sciarini et al. (2020) also observed a reduction in SV when replacing 30% of rice flour with buckwheat flour fractions milled in a hammer mill, with the reduction being greater when a flour of coarser particle size and enriched with the bran fraction was involved. Therefore, the reduction in SV could be related to the weakening effect bran components exert in the composite dough structure, as well as to the coarse particle size obtained with a hammer mill with a 500 μm mesh (Föste et al., 2017; Sciarini et al., 2020).

Table 3. Gluten-free bread physical properties: volume, texture, colour, and crumb grain characteristics.

Sample	CR	BN	BT1	BT2	BT3	SE
SV (mL/g)	4.65 c	3.88 a	4.61 bc	3.79 a	4.39 b	0.07
Bake loss (%)	18.9 a	19.6 b	19.8 b	19.6 b	19.5 ab	0.2
Firmness (N)	0.90 b	1.47 d	0.72 a	1.14 c	0.85 ab	0.04
Springiness	0.91 a	0.96 c	0.96 c	0.95 bc	0.94 ab	0.01
Cohesiveness	0.64 d	0.58 ab	0.63 cd	0.56 a	0.60 bc	0.01
Resilience	0.29 c	0.25 ab	0.28 c	0.23 a	0.27 bc	0.01
L^*_{crust}	68.1 c	57.5 a	59.4 b	57.4 a	58.5 ab	0.5
h_{crust}	64.7 c	61.3 b	60.3 a	60.4 a	60.6 a	0.1
C^*_{crust}	34.6 c	31.1 b	31.6 b	30.1 a	31.6 b	0.3
L^*_{crumb}	67.9 c	53.6 a	57.9 b	58.3 b	57.7 b	0.4
h_{crumb}	83.0 b	64.9 a	64.8 a	64.3 a	65.1 a	0.4
C^*_{crumb}	5.6 a	8.1 b	8.9 c	9.4 d	10.6 e	0.1
ΔFirmness-7d (N)	2.6 b	2.4 b	1.1 a	2.9 b	1.0 a	0.3
Cell density (nº cell/cm^2)	45 c	34 a	52 d	40 b	45 c	2
Mean cell area (mm^2)	0.89 ab	1.29 c	0.77 a	0.98 b	0.87 ab	0.04

CR: dough control recipe with 80% rice flour and 20% maize starch. BN: dough obtained by replacing 50% of rice flour with native buckwheat flour. BT1, BT2 and BT3: doughs obtained by replacing 50% of rice flour with flours of the MW-treated buckwheat grains. SV: specific volume, L*: luminosity, h: hue, C*: chroma. SE: pooled standard error obtained from ANOVA. Mean values with different letters for the same parameter indicate significant differences between the means, at $p < 0.05$.

The replacement of rice flour with the MW-treated flours resulted in an improvement of SV, compared to BN, with the exception of BT2, which showed no statistically significant differences for this quality parameter ($p > 0.05$). The best results were noted with the BT1 treatment, reaching the SV of the CR reference bread. The BT3 gave only 5% less SV than BT1 with the same energy cost, but being a much faster treatment (48 min vs. 16

min), thus making it an interesting alternative in MW processing of buckwheat grains. The combination of time, temperature, and duration of MW treatment are all important parameters affecting the SV of the fortified GF breads, as well as the moisture during MW treatment and the level of flour replacement included in the formulations, as demonstrated by Villanueva et al. (2019) for GF breads with microwaved rice flour.

The crumb fine structure was strongly affected by the formulation. Crumb grain, as illustrated in Figure 3D, was greatly modified by the use of native or the MW-treated buckwheat flours. The BN had the largest alveoli and the lowest cell density. With the inclusion of MW-treated buckwheat flours, the cell density increased in the order of BT1>BT3>BT2, showing the formation of more alveoli with smaller size. This could be related to improved viscoelastic behaviour of the dough matrix, which helps to effectively wrap the bubbles and prevent coalescence phenomena (Perez-Quirce et al., 2018). This observation agrees also with the dough rheology results, which indicated that the MW treatment improved the consistency and elastic recovery of stresses in the OLVR.

Bread texture was highly affected by the replacement of rice flour with either native or MW-treated buckwheat flours. The BN bread exhibited a harder crumb than CR, similar to observations made by Aguiar et al. (2021) when buckwheat was included at increasing levels in GF bread formulations containing rice flour and potato starch. The inclusion of fibres, particularly soluble fibres, can lead to a thickening of gas cell (alveolar) walls in the crumb, leading to an increase in firmness (Brites, Rebellato, Meinhart, Godoy & Steel, 2022). However, the use of MW-treated flours resulted in a reduction of crumb hardness, with BT1 exhibiting even a softer crumb than CR, possibly due to the higher MC and the less compact crumb fine structure of breads fortified with the MW-treated BW flours. The MW treatment of buckwheat grains is therefore quite effective in mitigating the crumb hardening defect associated with the use of this pseudocereal flour in a GF bread formulation. Moreover, for the BT1 and BT3 treatments, the reduction of the initial hardness of bread was also accompanied by a decline of the staling phenomena, as demonstrated by the changes in hardness values after 7 days of storage; this implies an extension of shelf life of these breads. The processes associated with crumb staling are related to the recrystallisation of amylopectin, but mainly to the fine structure of crumb (open vs. compact), the moisture water, and the time-dependent redistribution of water among the components (starch-protein-fibre) of crumb and crust in the composite bread matrix (Biliaderis, 2009; Hager et al., 2012). As observed by our group's previous studies using differential scanning calorimetry, the microwave treatment of buckwheat grains resulted in increased amylopectin retrogradation (Vicente et al., 2023), thus having an opposite effect. The lower staling may hence be caused by the increased volume of air trapped in the crumb, as reported by Villanueva et al. (2019) for rice breads with the microwave-treated flour; a higher volume of cells in the crumb structure would result in softer texture and a lower progress of the staling events. Moreover, small changes in moisture content can also influence crumb hardness, in view of the strong plasticizing action water has on the starch-protein matrix, even when small differences in MC exist among the specimens (Biliaderis, 2009). Cohesiveness and resilience were slightly reduced with the inclusion of most of the buckwheat flours, compared to CR, but recovered to CR values for breads with BT1 treated flour. This demonstrates the improvement of the recovery capacity of the crumb (both instantaneous and retarded) after a compression is applied to breads with the treated flours, which is indicative of freshness and an improved elastic response (Vela et al., 2023).

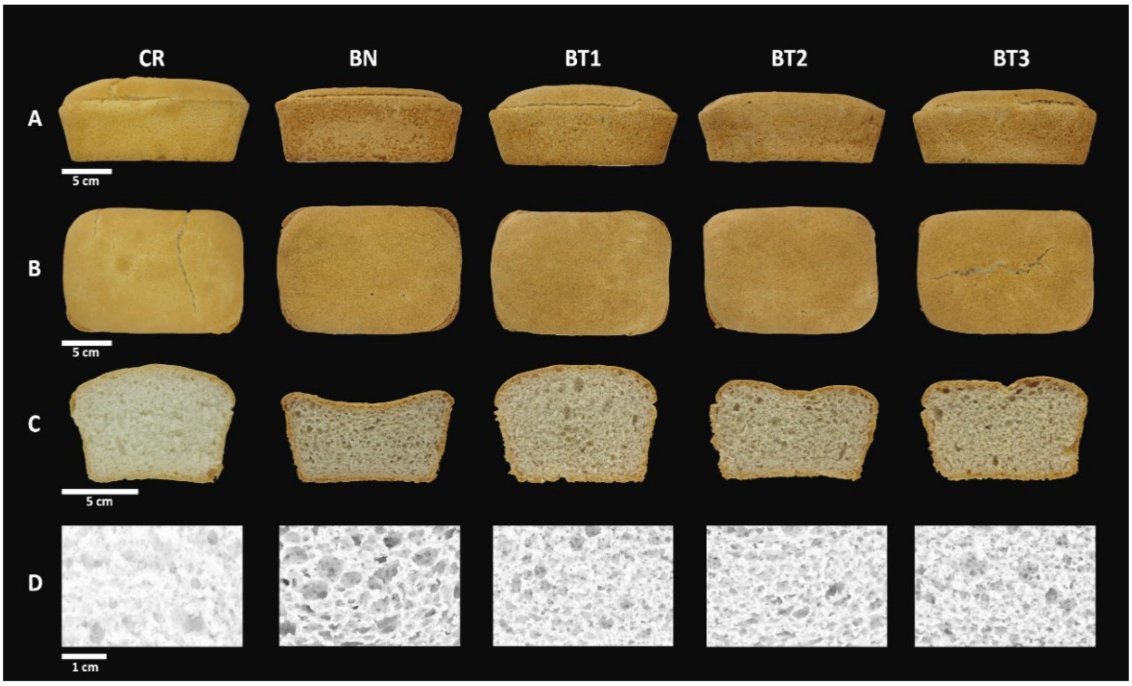


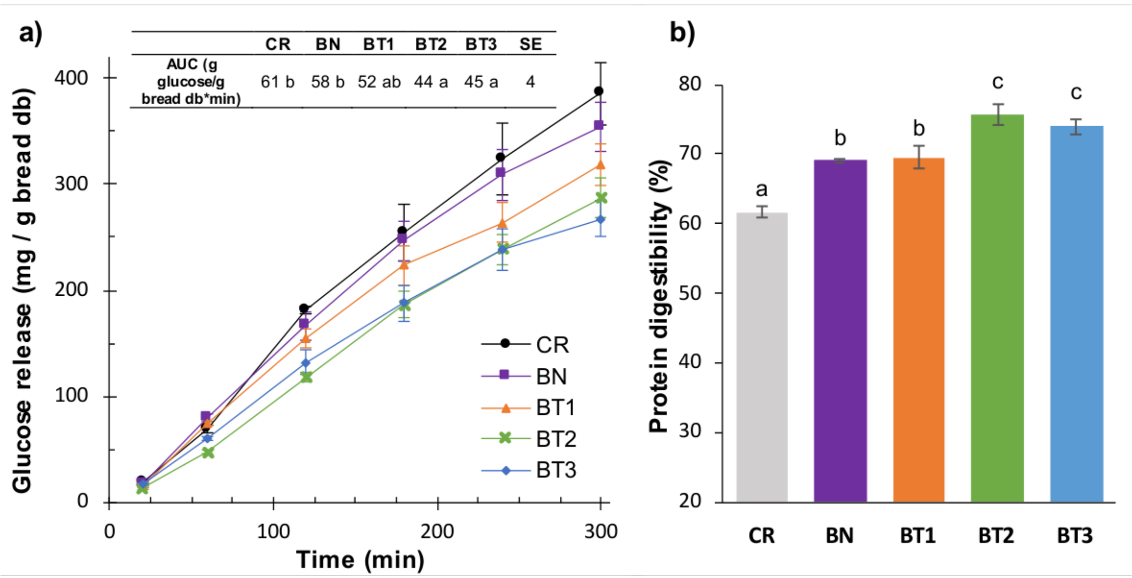
Figure 3. Photographs (top & side view) of bread loaves (A, B), slices (C) and crumb detail (D) obtained by replacing 50% of rice flour in the control recipe (CR) with flours from either native buckwheat (BN) or microwave-treated buckwheat (BT1, BT2 and BT3) grains.

Crust and crumb colours were significantly affected by buckwheat inclusion and the microwave treatment of the flours ($p < 0.05$). The crust of buckwheat-containing breads showed significantly lower lightness (L^*), hue (h), and chroma (C^*) values than those of CR, resulting in darker, reddish and less vivid colour. However, the use of different MW-treated flours resulted in only slight differences in crust colour, compared to BN, with no clear trend and no significant differences from a sensory point of view, because the colour difference, ΔE, was less than 5 in all cases of the fortified samples (2.3 BT1, 1.4 BT2, and 1.4 BT3) (Vela et al., 2023). Villanueva et al. (2019) indicated that heat treatments on flours may enhance the concentration of Maillard reaction products (coloured compounds) and precursors, thus changing the colour of the crust of breads made with MW-treated flours. However, this finding was not confirmed for breads made with the MW-treated buckwheat vs. the native buckwheat flour, probably because of the lower maximum temperature reached during the MW treatment.

The crumb of buckwheat-containing breads was darker with a reddish hue and had a higher C^* than CR. The use of the MW-treated flour resulted in an increase in L^* and no change in h, compared to the native buckwheat flour, whereas C^* was the only parameter affected by the treatment cycles applied, increasing more when a shorter microwave cycle was employed (BT3>BT2>BT1). Apparently, the colour of the crumb is primarily related to the colour of the ingredients used in fortified GF doughs (Villanueva et al., 2019). A higher C^* has been previously reported for MW-treatment of buckwheat grains at 30% MC (Vicente et al., 2023). The increase in brightness may additionally be related to the inhibitory effect of microwave treatment on oxidative enzymes such as polyphenol oxidases (PPO). The PPOs are normally involved in enzymatic browning reactions of the crumb during bread-making, as oxygen is incorporated into the dough during kneading, favouring the activity of PPOs (Every, Simmons & Ross, 2006). On the other hand, the activity of PPO in buckwheat bran has been shown to be reduced by heat treatments such as steam explosion (Li et al., 2022). Therefore, in BN, which has more active enzymes, enzymatic oxidation may occur more extensively during bread making, resulting in darker colour of the end-product. Instead, for breads with MW-treated flours, as the enzymes were at least partially inactivated, enzymatic oxidation reactions are expected to occur at a lower extent, resulting in a brighter crumb.

3.4 In vitro digestibility of bread

The glucose release kinetics during *in vitro* enzymatic starch digestion of breads was measured to determine the potential impact of GF bread fortification with the buckwheat flours in reducing the glycemic response of the baked products (Kotsiou et al., 2021). The inclusion of native buckwheat flour in the formulation had no significant effect on glucose release responses from BN bread; the area under the curve (AUC), calculated over 300 min of digestion, was not statistically different from that of the CR (Figure 4a). However, MW modification of buckwheat grains resulted in a decrease in glucose release during *in vitro* starch digestion of the fortified breads with the respective flours. This decrease was slight, not statistically significant ($p > 0.05$), for BT1, but more pronounced and with a significant reduction ($p < 0.05$) for BT2 and BT3, as can be seen in Figure 4a by the graphs and the calculated values for the areas under the curve (AUC) (–11% BT1, –25% BT2, and –23% BT3, compared to BN). In the case of BT1 bread, the reduction in digestibility is not statistically significant, probably due to the larger loaf volume, relative to BN, giving a more open crumb structure that is more accessible to the digestive enzymes, and thereby resulting in greater starch digestibility (Kotsiou et al., 2021; Lazaridou et al., 2014). According to the available literature, microwave treatments generally lead to increased levels of RDS and decreased RS as discussed for pure starch preparations in the review by Brasoveanu & Nemtanu (2014), and in studies of rice flour treatments (Solaesa, Villanueva, Vela & Ronda, 2022) and millet kernels (Zhi et al., 2022). In general, heat treatments can increase starch digestibility due to the creation of wide cracks and deep cavities on the starch granule surface, facilitating the access of starch hydrolysing enzymes (Deng et al., 2022). On the other hand, there are other processes that can be enhanced by hydrothermal treatments, e.g., by MW treatment, which can impair starch digestibility. Structural changes may occur in the starch component (chain reorganization) as well as interactions between starch and other flour constituents (e.g., lipids, proteins and polyphenols), that can hinder the accessibility of the digestive enzymes to the substrate and thus slow down starch digestion (Deng et al., 2022; Iuga & Mironeasa, 2020). Reduced digestibility was also reported by Xiao, Liu, Wei, Shen & Wang (2017) for HMT of buckwheat starch and flour, and was related to increased resistant starch (RS) fraction and reduction of the rapidly digestible starch (RDS) components of the cooked flours. In the present study, a number of processes could be responsible for the observed reduction in starch digestibility of the MW buckwheat - fortified breads. The MW treatment may cause unfolding of proteins, which can form a denser protein network structure around the starch granules, as has been demonstrated for the MW-treated buckwheat grains (Vicente et al., 2023), and this effect would prevent amylolytic enzymes to easily access the surface of starch granules (Chen, He, Fu & Huan, 2015; Iuga & Mironeasa, 2020). The same result can also occur with the creation of molecular complexes of starch with lipids and/or polyphenols, forming different types of resistant starch entities (Chen et al., 2015; Zhao et al., 2019). Evidence supporting the formation of such complexes was recently provided in our study on MW treatment of buckwheat grains (Vicente et al., 2023).



	CR	BN	BT1	BT2	BT3	SE
AUC (g glucose/g bread db*min)	61 b	58 b	52 ab	44 a	45 a	4

Figure 4. Degree of starch enzymatic degradation of the fresh bread crumb during *in vitro* digestion (a), and protein *in vitro* digestibility of lyophilised bread crumb (b). The fortified breads were made by replacing 50% of rice flour in the control recipe (CR) with flours from either native buckwheat (BN) or buckwheat-treated grains (BT1, BT2 and BT3). AUC: Area under the curve. Error bars indicate standard deviation. Mean values with different letters for the same parameter indicate significant differences between the means, at $p < 0.05$.

The *in vitro* protein digestibility of lyophilised crumbs was also determined using pepsin-pancreatin enzyme systems, and the results are presented in Figure 4b. Replacement of rice flour with buckwheat flours has led to higher protein digestibility. The BT1 bread, containing buckwheat flour obtained by a longer duration of MW treatment of the grains, showed no differences compared to the BN bread. However, the BT2 and BT3 breads, made with flours of grains treated with MW for a shorter time, but reached at a higher maximum temperature, showed an increase in protein digestibility. Both positive and negative effects on protein digestibility have been demonstrated by microwave treatments, depending on the conditions applied (time, temperature, power, etc.) and their effects on proteins (molecular structure and physical state) (Deng et al., 2022; Hafez, Mohamed, Hewedt & Singh, 1985). For example, Deng et al. (2015) microwaved buckwheat grains in excess of water (1:10 w/v) and measured the *in vitro* protein digestibility; they have reported a significant increase in digestibility from 73.6% in native flour to 76.2% in the MW-treated flour. An enhancement in protein digestion by MW radiation has generally been attributed to changes in protein conformation, leading to increased exposure of protein binding sites that are susceptible to enzymic hydrolysis (Huang, Ruan, Qin, Li & Zheng, 2017). This increased exposure may result from heat-mediated protein unfolding - denaturation, and/or reduced particle size due to structural modifications of the protein matrix (Deng et al., 2022; Hafez et al., 1985). Moreover, microwave treatment has been found to be more effective than conventional heat treatment in modifying the structure of proteins, since the absorption of microwave energy could favour the breaking of non-covalent bonds of proteins, thus modifying their secondary and tertiary structures (Zheng, Li, Zhang, Zheng & Tian., 2020). Conversely, an excessive duration of MW treatments may lead to cross-linking between protein molecules, resulting in insoluble aggregates and reducing the accessibility of proteases (Deng et al., 2022). These effects altogether may explain why there was an increase in digestibility for the breads fortified with microwaved flours obtained by the shorter duration treatments of the buckwheat grains (16 min BT3 and 24 min BT2), whereas for the longest duration (48 min BT1) no differences were noted compared with the untreated control sample. At short durations of MW treatment, the proteins may unfold, facilitating their greater access to proteases, whereas at longer times of thermal processing, cross-linking and aggregation events of protein chains may take place, counteracting the initial unfolding effects and thereby reducing the accessibility of the substrate to proteases.

4 Conclusions

This study demonstrated the feasibility of using flours obtained from MW-treated buckwheat grains for the production of GF breads with improved physical and nutritional qualities. The use of MW-treated buckwheat flour resulted in a large increase in dough consistency, which had to be moderated by adding more water until a similar consistency was achieved among all the GF fortified doughs in the LVR to permit comparisons among the samples. Even when the consistency was adjusted (similar G_1^*), differences in the OLVR were observed for doughs fortified with the various MW-treated buckwheat flours. The fortified doughs exhibited higher consistency and withstood higher stresses for the same level of deformation (strain), showing also higher viscosity and elastic recovery responses. The combination of MW treatment time and temperature reached in the buckwheat grains during heating also had a major influence on flour functionality and the properties of the fortified breads. Depending on the choice of MW parameters underlying a specific temperature-time processing protocol, the nutritional or structural modifications of the buckwheat-supplemented GF breads can be favourably maximised. Compared to the native-untreated buckwheat flour (BN), the BT1 flour preparation seemed to be optimal for the physical quality attributes of the fortified GF breads (in particular specific volume and crumb hardness), although it did not show significant improvements for starch and protein digestion using the adopted *in vitro* assay protocols. Instead, the buckwheat flour preparations obtained by the BT2 and BT3 processing schemes resulted in nutritional improvements of the baked products, with a moderation in glucose release kinetics by an *in vitro* starch digestion assay as well as an increase in protein digestibility, as also assessed by an *in vitro* test. Overall, there is a need to further investigate all the important processing parameters involved in the MW-treatments of buckwheat and other pseudocereal grains (tempering at certain moisture content, temperature reached in the grains upon MW treatment, cycling of the applied MW energy using different time periods of the on-off stages, etc.) in order to maximize the functionality of the resultant flours in GF dough formulations and ultimately accomplish the desired physical and nutritional properties for the baked products.

Author contributions

Ainhoa Vicente: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. Marina Villanueva: Conceptualization, Validation, Writing - review & editing. Pedro A. Caballero: Conceptualization, Supervision, Visualization, Writing - review & editing. Athina Lazaridou: Supervision, Validation, Resources, Writing - review & editing. Costas G. Biliaderis: Resources, Writing - review & editing. Felicidad Ronda: Conceptualization, Supervision, Resources, Writing - review & editing, Project administration, Funding acquisition.

Acknowledgements

The authors thank the Spanish Ministerio de Ciencia e Innovación (PID2019-110809RB-I00/AEI/10.1303/501100011033) and the Junta de Castilla y León/FEDER (VA195P20) for their financial support. The authors thank Mr. Agustín Martín for the design and construction of Teflon® containers and María Mate Correa for helping in the experimental measurements. A. Vicente thanks the Spanish Ministerio de Ciencia, Innovación y Universidades for her FPU doctorate grant.

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