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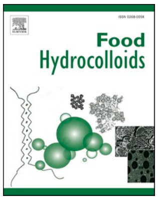

Microwave treatment enhances the physical and sensory quality of quinoa-enriched gluten-free bread

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ABSTRACT

The suitability of microwave-assisted hydrothermally treated (MWT) quinoa flour was investigated as an ingredient for enhancing the quality of gluten-free (GF) bread fortified with quinoa. Different levels (0%, 25%, 37.5%, 50%, and 75%) of native/untreated (N) or MWT quinoa flour (30% moisture content, 9 W/g, total 8 min of microwave exposure) were evaluated to replace maize starch in a starch-based GF recipe. The hydration of the dough was adjusted to obtain similar consistency in terms of complex modulus, G_1*, to compensate its increase due to quinoa addition and treatment (up to +737%). The incorporation of increasing amounts of native quinoa flour reduced dough viscosimetric profiles, delayed its gelatinization peak in DSC scans (up to +3.4 °C), and decreased the dough's development and stability during the fermentation test (the final height decreased from 88 mm for 0% quinoa to 4 mm for 75%). When substituting native by MWT quinoa flour at a certain substitution level, a reduction in the breakdown viscosity, an increase in pasting temperature, and an increase in dough development and stability during the fermentation test were noted. As a result, the MWT quinoa flour-fortified breads exhibited a higher specific volume, lower hardness, and retarded staling. In addition, sensory evaluation of the breads showed a reduction in the herbaceous off-flavour associated with the quinoa (untreated) flour, and no effect on bitterness. The experimental findings indicate the feasibility of using microwave hydrothermal treatment to improve the physical properties and sensory quality of quinoa-enriched GF bread.

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is one of the most important pseudocereals in terms of global production, presenting a significant agronomic potential, capable of maintaining good yields under adverse soil and drought conditions (Romano & Ferranti, 2023). In recent years, this crop has received considerable attention for its potential to meet the emerging specific nutritional needs while being an efficient crop (Yadav, Gore, Gupta, Saurabh, & Siddique, 2023). On the one hand, there is a continuous rise in the demand for plant proteins with good nutritional and functional properties for the production of plant-based foods to meet the greater expectations for food supply in feeding a growing global population, without causing adverse effects on the environment (Ghumman et al., 2021). On the other hand, there has been an increase in the incidence of celiac disease and the adoption of gluten-free (GF) diets, resulting in a demand for GF products with good nutritional and sensory qualities (Aguiar, Santos, Krupa-Kozak, & Capriles, 2023). The inclusion of quinoa in these products is appealing as a source of plant proteins due to its high protein content of the crop (12–23%) that has a balanced amino acid profile, being particularly rich in lysine, methionine, and threonine, exhibiting a high apparent digestibility and protein efficiency ratio (Abugoch, 2009). Quinoa is also a valuable source of dietary fibre (7–14%), lipids (1.8–9.5%, of which 80–90% are unsaturated fatty acids), phenolic compounds, minerals, and vitamins (Abugoch, 2009; Li & Zhu, 2017).

Quinoa has been the subject of numerous studies exploring its potential use as ingredient in bakery products, mainly enriched wheat-based goods, but also GF alternatives (Romano & Ferranti, 2023). Regarding GF bread, Elgeti et al. (2014) found an enhancement in the physical and sensory attributes of GF breads through the incorporation

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of 40–100% refined quinoa flour into a base flour consisting of a mixture of rice flour and corn starch, demonstrating the potential of the quinoa flour in GF baked items. However, these authors did not report any nutritional improvements, probably due to bran removal for production of the quinoa refined flour. The use of wholemeal flours, though presenting a considerable technological challenge, represents an opportunity for significant improvement in nutritional quality of composite bakery products. Previous research has assessed the impact of incorporating whole quinoa flour into GF breads by using varying ratios of different flour and starch blends (Aguiar, Santos, Centeno, & Capriles, 2021; Burešová et al., 2017; Hager et al., 2012; Turkut, Cakmak, Kumcuoglu, & Tavman, 2016), quinoa milling fractions with different chemical compositions (Sciarini, Steffolani, Fernández, Paesani, & Pérez, 2020), or different hydrocolloids (Encina-Zelada, Cadavez, Teixeira, & Gonzales-Barron, 2019). These experimental works showed that incorporating quinoa flour into GF formulations can enhance the nutritional value of GF breads. However, the technological and sensory qualities of quinoa-based GF bakery products remained inferior to their gluten-containing counterparts. Quinoa, due to its characteristic odour and taste, usually causes lower sensory acceptability than other seed-based matrices like buckwheat, rice, maize, sorghum, or teff when included in GF recipes (Burešová et al., 2017; Hager et al., 2012). From a technological point of view, proteins, lipids, and insoluble fibre (as found in whole quinoa flour) generally have an adverse effect on the physical quality of GF breads (Sciarini et al., 2020). Nevertheless, there are several strategies that can be used to improve the performance of wholemeal flours in GF breadmaking, such as the use of different flour blends, hydrocolloids, hydration levels, flour pretreatments or flour particle sizes (Bourekoua, Benatallah, Zidoune, & Rosell, 2016; Encina-Zelada, Cadavez, Monteiro, Teixeira, & Gonzales-Barron, 2018; Föste, Jekle, & Becker, 2017; Ronda, Pérez-Quirce, & Villanueva, 2023; Sciarini et al., 2020).

In the present study, our approach to improve the suitability of quinoa flour for production of GF bread was focused on physical modification of the flour by microwave (MW) hydrothermal treatment. MW treatment has been proposed as a rapid and cost-efficient method for performing heat-moisture treatment of cereals and pseudocereals (Rosell, Aalami, & Mahdavi, 2020; Vicente, Villanueva, Caballero, Muñoz, & Ronda, 2023). Hydrothermal treatments have proven effective in modifying the functional properties of several flours, including quinoa, affecting both protein and starch, and thus influencing the rheological behaviour of composite bread doughs during proofing and baking, as well as breadmaking performance (Bourekoua et al., 2016; Ronda et al., 2023; Vicente et al., 2023). Previous studies investigated the effect of MWT rice (Villanueva, Harasym, Muñoz, & Ronda, 2019; Villanueva, Vicente, Náthia-Neves, & Ronda, 2024) and buckwheat (Vicente et al., 2024) on GF dough characteristics and bread quality. These studies revealed differences in dough and bread characteristics depending on the treatment conditions, i.e., temperature, moisture, and time. It is also worth noting that the ideal conditions for a desired improvement of one characteristic may not necessarily align and be ideal for another. MWT flour with adequate hydrothermal treatment can enhance dough viscoelasticity, elastic behaviour, and resistance to deformation, improve bread physical quality through increased specific volume, softer crumb, and delayed staling, and/or upgrade the nutritional quality of the end-product, as evidenced by *in vitro* assays revealing a modulation on glucose release kinetics (starch amylolysis) and increased protein digestibility. However, a deeper understanding of the effects of native vs. treated flours from pseudocereals or other non-wheat flours (e.g., legumes) used in GF formulations, over a wider range of fortification for the composite flour mixes, on the rheological and thermal properties of the dough, and their relationships with the physical properties, is still lacking. Sensory attributes of GF breads are another important quality factor that largely influences consumer preference – acceptability of these products. While no literature has been found on the effect of MWT flour on the sensory properties of GF bread, other thermal treatments like roasting have shown a reduction in the “green, grass-like” off-flavour of yellow split pea (Kotsiou, Sacharidis, Matsakidou, Biliaderis, & Lazaridou, 2021) and chickpea (Kotsiou, Sacharidis, Matsakidou, Biliaderis, & Lazaridou, 2022) incorporated in wheat-flour breads. Hence, it is of interest to investigate whether such a flavour improvement can be also realized at the lower temperatures adopted in MW treatment.

Therefore, this study aimed to evaluate the effect of replacing maize starch by native (untreated) or MWT quinoa flour in GF dough and bread characteristics. For this purpose, different quinoa flour substitution levels were investigated (0%, 25%, 37.5%, 50% and 75%). The rheological, pasting, thermal, and fermentative properties of the doughs were evaluated. In addition, bread physical properties and proximate composition were measured, and a sensory evaluation was performed.

2. Materials and methods

2.1. Raw materials

Quinoa grains (*Chenopodium quinoa* Willd.) cv. Titicaca were procured from Extremeña de Arroces (Cáceres, Spain) with saponins being removed via abrasion polishing. Wholemeal quinoa flour was obtained by milling the decorticated grains in a hammer mill with 800 μm mesh (LM 3100, Perten Instruments, Sweden). Maize starch was provided by Ferrer Alimentación S.A. (Barcelona, Spain). The proximate composition (g/100 g dry basis) of quinoa flour was 15.6% protein, 6.2% fat, 11.7% dietary fibre, 2.4% ash, and 64.1% carbohydrates. Maize starch had 0.6% protein, 0.1% fat, 1.1% dietary fibre, 0.2% ash, and 98.1% carbohydrates. AACC official methods 46–19.01, 30–10.01, 08–01.01, and 32-05 were used for protein, fat, ash, and dietary fibre analysis, respectively (AACC, 2010). Carbohydrates were calculated by difference. Hydroxy-propyl-methyl-cellulose (HPMC, Methocel K4M Food Grade) was generously supplied by Dow Chemical (Midland, USA). Salt, sugar, and sunflower oil were purchased from the local market, while tap water from the general water supply (drinking water quality) was used to prepare the dough and bread.

2.2. Microwave treatment

Quinoa flour was moistened to 30% moisture content (MC). The required amount of water was calculated based on the initial MC of the flour. The required quantity of distilled water was slowly sprayed while mixing in a Kitchen-Aid professional mixer model 5KPM50 (St. Joseph, MI, USA). The moisturized samples were stored at −35 °C to prevent microbial growth and allow longer preservation. Prior to use, samples were defrosted for 1 h to condition them to room temperature. MW treatments were performed in a customised microwave oven R342INW (SHARP, Sakai, Japan) at a power of 900 W and a frequency of 2450 MHz. Portions of 100.00 ± 0.05 g of quinoa flour at 30% MC and room temperature were placed in a non-hermetic customized Teflon® container. MW treatment was performed in cycles, comprising 10 s of microwave exposure followed by 50 s of rest, resulting in total 8 min of applied microwave radiation (48 cycles). The conditions were selected based on preliminary trials and previous studies (Villanueva et al., 2019). The maximum temperature recorded was 102 ± 3 °C, as measured using Testoterm® temperature strips from TESTO (Barcelona, Spain). The MC of the flour after MW treatment was 19 ± 2%. The treated samples were dried to 11% MC (natural MC of native flour) in an incubation chamber (Memmert ICP260, Schwabach, Germany) at 35 °C.

2.3. Dough preparation and bread-making

Nine flour mixes were prepared by combining different amounts of native or MWT quinoa flour with maize starch. Sample identification codes were adopted by denoting the type of quinoa flour used (N for native and MW for microwave-treated), followed by the percentage of quinoa flour included per 100 g of flour-starch blend (0%, 25%, 37.5%, 50%, or 75 %). Thus, the resulting samples were named N-0, N-25, MW-25, N-37.5, MW-37.5, N-50, MW-50, N-75, and MW-75.

For dough rheological properties, the doughs were prepared according to the following formula based on 100 g of flour-starch mixture (13% moisture): 1.5% salt, 2% HPMC, 5% sucrose, and 6% oil. First, 85 g of water per each 100 g of flour mix were added to explore the impact of quinoa flour addition level and the MW treatment on dough rheological properties. Then, a complementing test was conducted to determine the precise amount of water necessary to attain a similar dough consistency, in terms of the complex moduli G^*_1 (refer to Table 1), to eliminate this secondary effect of the studied factors on bread properties. This adapted dough hydration was used for the rest of assays. The doughs were prepared using an Auto Bakery (Funaj, China) as described by Vicente et al. (2024). DSC and RVA analysis were performed on freeze-dried dough samples (Telstar Lyoquest equipment, Terrassa, Spain) as described below.

The recipes described previously at adjusted hydration levels and supplemented with 3% dried yeast dispersed in water, were used for the breadmaking and proofing tests. The doughs were prepared with a Kitchen-Aid professional mixer 5KPM50 (St. Joseph, MI, USA) as described by Vicente et al. (2024). Three 160 g dough portions were placed into aluminium pans and proofed for 40 min at 35 °C and 90% relative humidity in a Memmert HPP 260 eco-chamber (Schwabach, Germany), and baked in a Sveba Dahlen S200 oven (Fristad, Sweden) for 15 min at 180 °C, with steam injected for 7 s. The loaves of bread were allowed to cool for 1 h prior to analysis. Breadmaking was performed in duplicate to obtain 6 loaves for each formulation.

2.4. Dough oscillatory and creep–recovery tests

Dough rheology was studied by means of dynamic oscillatory and creep-recovery tests performed on a Kinexus Pro + rheometer (Malvern Instruments, UK) that featured a parallel plate geometry (40 mm) with a serrated surface and a 1 mm gap. In order to assess the effect of the different flour blends studied, all the tests were carried out on doughs formulated with identical hydration (85%) at least in duplicate. In addition, frequency sweeps were also performed varying hydration levels to achieve similar consistency values, in terms of G_1*, in all the samples. Before analysis, the dough rested for 10 min in a hermetic container, followed by 5 min of relaxation on the equipment plates at 25 °C. Strain sweeps were performed from 0.01 to 1000% at 1 Hz frequency to determine the linear viscoelastic region (LVR). The data of the frequency sweep test were fitted to the power law model, as it was previously reported by Ronda, Pérez-Quirce, Angioloni, and Collar (2013). Creep-recovery tests were also conducted outside the linear viscoelastic region (OLVR) by applying a constant shear stress of 50 Pa for 60 s, then releasing the stress and allowing the sample to recover for 180 s. The creep phase data was modelled using the 4-parameter Burgers model, while the recovery phase was modelled using the 3-parameter Burgers model, as defined in previous studies (Villanueva et al., 2019).

2.5. Dough pasting properties

The lyophilized doughs' pasting properties were evaluated in duplicate using a Rapid Visco Analyser (RVA) model 4500 (Perten Instruments, Australia). 2.5 g of dough (dry basis) was mixed with 25 mL of distilled water and the Standard 1 temperature profile of the official method 76–21.01 (AACC, 2010) was applied. The parameters of pasting temperature (PT), peak viscosity (PV), peak time (Pt), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV), and setback viscosity (SV) were determined from the pasting curves.

2.6. Dough thermal properties

The thermal properties related to the gelatinization and

Table 1

Rheological properties of gluten-free bread doughs at constant dough hydration (85% flour basis) and adjusted hydration.

	N-0	N-25	MW-25	N-37.5	MW-37.5	N-50	MW-50	N-75	MW-75	SE
Constant dough hydration	**85**	**85**	**85**	**85**	**85**	**85**	**85**	**85**	**85**	
Oscillatory tests										
τ_{max} (Pa)	1.6 a	2.2 a	4.2 b	4.2 b	8.9 d	4.2 b	9.3 d	7.9 c	24.1 e	0.3
Crosspoint (Pa)	19 a	39 a	84 b	84 b	120 c	115 c	190 d	255 e	434 f	7
G_1' (Pa)	1009 a	2256 b	2173 b	3095 c	2394 b	4075 d	3469 c	6980 e	9285 f	140
a	0.29 g	0.25 f	0.24 ef	0.22 de	0.22 de	0.19 bc	0.21 cd	0.18 b	0.15 a	0.01
G_1'' (Pa)	578 a	1095 b	996 b	1364 c	1033 b	1577 d	1402 c	2398 e	2910 f	51
b	0.27 e	0.26 de	0.25 cd	0.23 b	0.25 c	0.23 b	0.22 b	0.20 a	0.18 a	0.01
$(\tan \delta)_1$	0.57 f	0.49 e	0.46 de	0.44 d	0.43 cd	0.39 b	0.40 bc	0.34 a	0.32 a	0.01
c	−0.02 a	0.02 bc	0.01 b	0.01 b	0.02 bc	0.03 c	0.01 b	0.02 bc	0.02 bc	0.01
G_1^* (Pa)	1163 a	2507 b	2391 b	3382 c	2607 b	4369 d	3741 c	7380 e	9730 f	147
Creep-recovery tests										
J_{0c} (10^{-4} Pa^{-1})	11.9 g	11.4 g	9.5 f	8.9 f	7.8 e	6.5 d	5.4 c	3.1 b	1.6 a	0.3
J_{1c} (10^{-4} Pa^{-1})	53 f	73 g	45 e	58 f	32 d	28 d	20 c	9 b	3 a	3
λ_c (s)	1.9 a	5.7 d	4.1 b	7.1 e	5.0 c	7.5 e	6.1 d	6.0 d	4.8 c	0.2
μ_0 (10^{-3} Pa s)	0.4 a	1.6 c	1.2 b	2.6 d	2.6 d	4.3 e	8.3 f	34.1 g	128.4 h	1.4
J_{max} (10^{-4} Pa^{-1})	1455 g	453 f	528 f	291 e	271 e	173 d	98 c	29 b	7 a	17
J_{0r} (10^{-4} Pa^{-1})	7.6 f	3.6 c	7.2 f	5.6 e	6.2 e	4.7 d	4.9 d	2.3 b	1.3 a	0.2
J_{1r} (10^{-4} Pa^{-1})	43 f	28 d	33 e	24 c	31 de	22 c	23 c	9 b	3 a	1
λ_r (s)	4.7 a	4.8 a	6.7 b	9.1 c	8.6 c	9 c	11.9 e	10.5 d	10.6 de	0.4
Recovery (%)	4 a	8 b	7 b	11 c	15 d	16 d	33 e	42 f	47 g	1
Adjusted dough hydration	**73**	**82**	**78**	**85**	**80**	**86**	**85**	**94**	**100**	
G_1^* (Pa)	3541 ab	3413 a	3471 a	3382 a	3464 a	3692 b	3741 b	3644 b	3543 ab	70

Samples are identified as native quinoa flour (N) or microwave-treated quinoa flour (MW) and the level of maize starch replacement (0%, 25%, 37.5%, 50% or 75%) by quinoa flour. Dough hydration is expressed as grams of water per 100 g of flour mix. Crosspoint: the stress at which $G' = G''''$. τ_{max}: maximum stress that the samples can withstand (end of the linear viscoelastic region). The power law model was fitted to the experimental frequency sweep data ($G' = G_1' \cdot \omega^a$; $G'' = G_1'' \cdot \omega^b$; $\tan \delta = (\tan \delta)_1 \cdot \cdot \omega^c$). G_1', G_1'' and $\tan(\delta)_1$: represent the elastic modulus, viscous modulus, and loss tangent at a frequency of 1 Hz, respectively. a, b, and c: exponents quantifying the degree of dependence of G′, G″, and tan δ with the oscillation frequency. G_1*: complex modulus at a frequency of 1 Hz. The subscript "c" refers 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 time. μ_0: steady-state viscosity. SE: pooled standard error obtained from ANOVA. Means with different letters for the same parameter indicate significant statistical differences between means at a significance level of $p < 0.05$.

retrogradation transitions of the dough samples were determined using a differential scanning calorimeter DSC3 (STARe-System, Mettler-Toledo, Switzerland). Approximately 10 mg of freeze-dried dough was weighed into aluminium pans (40 μL). Distilled water was added to achieve the level of hydration of the fresh dough used for breadmaking. The samples were scanned from 0 to 120 °C at 5 °C/min using an empty pan as a reference. The samples were subsequently stored at 4 °C for 7 days before being rescanned. The enthalpy (ΔH), expressed in J/g dm, and the peak temperature (T_p), expressed in °C, were obtained from the thermograms of both the gelatinization (fresh samples) and the retrogradation thermal scans (7 d-stored samples).

2.7. Dough fermentative properties

Dough development and gas production were monitored during proofing of freshly prepared doughs using a Chopin Rheofermentometer F3 (Chopin Technologies, Villeneuve-La-Garenne Cedex, France). To adapt the traditional method to GF doughs, the weight of dough was reduced to 180 g, the four weights (0.5 kg/each) were removed, and fermentation was carried out at 35 °C for 3 h. For dough development, the following parameters were registered: H_m (mm), the height of the dough at the maximum development time; h (mm), the height of the dough at the end of the test; and T_1 (min), the time corresponding to H_m. Regarding the gaseous release, the parameters were: H'_m (mm), the maximum height of CO_2 production; T'_1 (min), the time of the maximum gas production; V_T (mL), the total volume of CO_2 produced over the 3-h fermentation; V_R (mL), the total volume of the CO_2 retained within the dough; and RC (%), the CO_2 retention coefficient calculated as V_R/V_T.

2.8. Bread physical properties

The bread specific volume was measured using a Volscan Profiler 300 (Stable Microsystems, Surrey, UK). A TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) equipped with a 20 mm cylindrical aluminium probe was used to assess the texture of the crumb by the "Texture Profile Analysis" double compression test (TPA). The test was conducted on the centre of the two central slices, each 20 mm thick, with a penetration depth of 50% at a speed of 1 mm/s and a delay of 30 s between compressions. The resultant graph was analysed using the "Texture Expert" software (Stable Microsystems, Surrey, UK) to determine hardness (N), cohesiveness, springiness, and resilience. For each batch of bread, two loaves were analysed after baking (8 tests), while the other was analysed after 7 days of storage in a hermetic bag at 4 °C to determine the staling index under refrigerated condition (ΔHardness – 7 d) (4 tests). The colour of both the bread crust and crumb were assessed with a PCE-CSM5 colorimeter (PCE Instruments, UK) and using the CQCS3 software to obtain the CIE L*C*h coordinates (D65 standard illuminant and a 10° standard observer). Five distinct points were measured for each sample. The bread slices and loaves were photographed using a PowerShot SX410 IS camera (Canon, Japan). The crumb grain characteristics were evaluated with ImageJ software (National Institutes of Health, USA). Eight pictures per sample were processed to determine the cell density (number of cells per cm^2), mean cell area (mm^2), and void fraction (%).

2.9. Bread nutritional aspects

AACC official methods 46–19.01, 30–10.01, 08–01.01, and 44–19.01 were used for protein, fat, ash, and moisture analyses, respectively (AACC, 2010). Total, soluble, and insoluble dietary fibre were analysed according to the method described by Kotsiou et al. (2021). The Fibre-Bags filtration system from Gerhardt Analytical Systems (Konigswinter, Germany) was used in conjunction with the Total Dietary Fiber Assay kit from Megazyme (Wicklow, Ireland), following the official AACC method 32–05 (AACC, 2010).

Digestible and resistant starch in bread crumb was determined using the Megazyme procedure (K-DSTRS), based on a modified method of Englyst, Kingman, and Cummings (1992), as described by Kotsiou et al. (2021). Briefly, 0.5 g of sample was incubated with a mixture of pancreatic α-amylase and amyloglucosidase at 37 °C for 4 h. Aliquots of 1 mL were removed at 20, 120, and 240 min and added to acetic acid (50 mM) to stop the enzymic hydrolysis. Rapidly (RDS), slowly (SDS) and total digestible starch (TDS) were determined as the starch fractions digested from 0 to 20, 20–120, and 0–240 min, respectively. The starch remaining after 240 min of digestion, defined as resistant starch (RS), was dissolved in sodium hydroxide (1.7 N). The RDS, SDS, TDS and RS were determined using the Megazyme reagent for glucose determination (glucose oxidase/peroxidase, GOPOD) after incubation of the digested fractions with amyloglucosidase to hydrolyse the remaining maltose to glucose. The analysis was performed in triplicate.

2.9.1. Bread sensory analysis

A sensory analysis of the breads was conducted by a panel of 13 trained assessors from the Food Technology Area of the University of Valladolid, consisting of eight females and five males aged between 25 and 63 years. The analysis aimed to assess the effect of dose and MW treatment on the sensory properties of breads containing 25%, 37.5%, and 50% native or treated quinoa. The breads with 75% and 0% quinoa levels were excluded as they were not considered acceptable formulations due to their poor physical quality. The tested attributes were selected in the training sessions according to the panel's perceptions on bread quality and previous literature reports. The perceived/described characteristics included two odour attributes and four of taste. The odour attributes were "herbaceous", associated with quinoa grassy, plant-like smell, and "toasted", associated with a baked product. The taste attributes were "bitter", related to various compounds such as saponins, polyphenols, and flavonoids present in quinoa, "herbaceous", associated with quinoa plant-like taste, "toasted", associated with a baked product, and "aftertaste", defined as the persistency of the predominant taste. The panel evaluated the intensity of these characteristics in a structured 9-point scale (scale 1: very low to 9: very high). For evaluation, slices of bread samples containing crust and crumb were assigned a three-digit number and presented in a random order to panellists at room temperature. To decrease the possibility of carryover, panelists rested between samples and cleared their palate by drinking water.

2.10. Statistical analysis

Statistical analysis was conducted using the Statgraphics Centurion 19 software (Bitstream, Cambridge, MN, USA). The least significant difference (LSD) analysis of variance (ANOVA) was used to assess significant differences ($p < 0.05$) of means among samples; different mean values were specified by discrete letters in the tables and figures. Mean values of different replicates were presented, and the pooled standard error (SE) obtained from ANOVA was reported for each measured parameter. The Pearson correlations between certain properties were also evaluated.

3. Results and discussion

3.1. Dough oscillatory and creep–recovery tests

A rheological study of the doughs at the same hydration level (85% flour basis) was conducted to assess the influence of incorporating quinoa flour at different doses and the impact of the physical treatment (MW treatment) on the rheological characteristics (Table 1 and Fig. 1).

The results of the strain sweep test showed a progressive increment in the maximum stress that the dough could withstand before suffering irreversible deformations, τ_{max}, and the stress at the crosspoint with quinoa dosage (from 1.6 Pa τ_{max} and 19 Pa crosspoint for N-0 to 7.9 Pa τ_{max} and 255 Pa crosspoint for N-75). As depicted in Fig. 1 (B1 and B2)

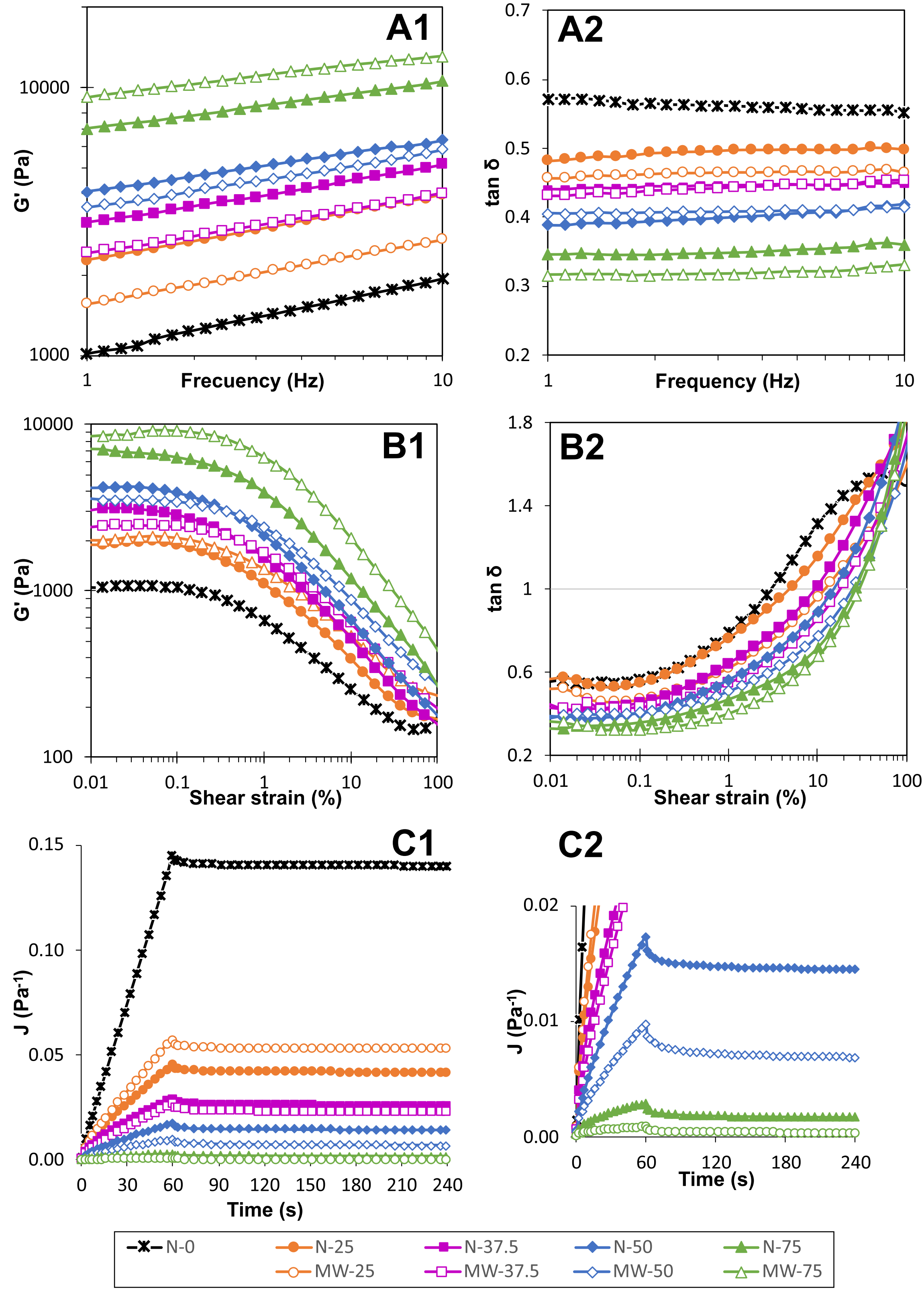

Fig. 1. Rheological measurements at constant dough hydration (85% flour basis) for doughs produced by replacing different levels (25%, 37.5%, 50% or 75%) of maize starch with either native (N) or microwave-treated (MW) quinoa flour in the control recipe, containing 100% maize starch (N-0). The graphs A1 and A2 present the evolution of elastic modulus (G′) and loss tangent (tan δ) with frequency, obtained from frequency sweeps; the graphs B1 and B2 present the evolution of elastic modulus (G′) and loss tangent (tan δ) with increasing shear strain (i.e., strain sweep test). Graph C1 presents the evolution of compliance with time obtained from Creep-Recovery tests, whereas, the graph C2 shows the same evolution but with an amplified scale for better visualization of samples with the weaker responses in compliance values.

and Table 1, this behaviour was further enhanced by the use of treated flour (up to 24.1 Pa τ_{max} and 434 Pa crosspoint for MW-75) at increasing levels of maize starch substitution, indicating a structuring effect of MW treatment on the composite GF bread dough (Villanueva et al., 2019). Similar observations were made when native buckwheat was substituted by MWT buckwheat in a GF recipe (Vicente et al., 2024). Power law fitting of the frequency sweep data showed that the consistency of the dough, in terms of G_1^*, gradually increased with native quinoa flour addition, from 1163 Pa for N-0 to 7380 Pa for N-75. Both the elastic, G_1', and viscous, G_1'', moduli at 1 Hz also evolved with the same trend. However, G_1' increased more than G_1'', which led to a reduction of the loss tangent at 1 Hz, $(\tan \delta)_1$, from 0.57 to 0.34 for the maximum addition of untreated quinoa flour in the mixed blends, thereby denoting a greater strengthening of the dough's elastic behaviour (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007). Furthermore, the exponents "a" and "b" of the power law relationships decreased, indicating a weaker dependence of G' and G'' on frequency. Increases in G_1^* values and reductions in frequency dependence ("a" exponent) have been also observed with increasing levels of quinoa bran incorporated in a maize starch-based dough (Föste et al., 2017). The greater elastic behaviour and lower frequency dependence, together with the higher resistance to deformation, have been linked to a more stable dough structure (Collar, Villanueva, & Ronda, 2020). When treated flour was used at the same level as the native quinoa flour, there were no significant differences in the values of $(\tan \delta)_1$. In contrast, G_1^*, G_1', and G_1'' varied significantly (excluding the lowest quinoa flour substitution level, 25%). A reduction in these moduli was observed for 37.5% and 50%, while an increase was obtained for 75%, highlighting the different evolution patterns of dough consistency with the fortification level and type of quinoa flour added (native vs. MWT flour). However, it is worth noting that the reductions in the viscoelastic moduli observed for MW-37.5 and MW-50 samples, only occurred at low shear strain values (in the LVR); at higher stresses these moduli were always higher for the doughs made with MWT flour (Fig. 1, graph B1). The same behaviour can be concluded from the creep-recovery assays, performed outside the LVR.

The creep-recovery curves (Fig. 1, graphs C1 and C2) revealed a typical viscoelastic behaviour, similar to that of other GF doughs (Lazaridou et al., 2007; Ronda et al., 2023; Vela, Villanueva, & Ronda, 2023), with a combination of viscous-fluid and elastic components mainly in doughs fortified with a quinoa flour above 37.5% maize starch substitution. In the less consistent doughs (those made with 25% quinoa flours and the control dough) the viscous component was clearly predominant (see an almost linear evolution of compliance with time in the creep phase). A decrease in the instantaneous and retarded compliances (J_{0c} and J_{1c}, respectively) in the creep phase was noted with increased levels of quinoa flour added, which was further accentuated by the use of the treated flour (Table 1). J_{0c} and J_{1c} were reduced −87% and −95% for MW-75, and −74% and −84% for N-75, compared to N-0. This denotes a lower deformation for a same stress, which is coherent with a dough of higher consistency (higher viscoelastic moduli). The retardation time for both the creep, λ_c, and recovery, λ_r, phases showed a gradual increment with increasing quinoa content (excluding 75%), suggesting that longer time is required to achieve the viscoelastic deformation on the dough (Vela et al., 2023). When MWT flour was used, instead of the untreated flour, λ_c was reduced and λ_r was slightly higher for the doughs fortified at the same level of quinoa flour. The steady-state viscosity, μ_0, largely increased with the proportion of native quinoa flour in the mixed GF formulations, ranging from $0.4 \cdot 10^{-3}$ Pa s for N-0 to $34.1 \cdot 10^{-3}$ Pa s for N-75. Similarly, μ_0 increased with MWT quinoa dose (up to $128.4 \cdot 10^{-3}$ Pa s for MW-75), although only the MW-50 and MW-75 fortifications showed a significant increase compared to the use of native flour at the same level. The maximum compliance at the end of the creep phase, J_{max}, decreased and the elastic recovery of the samples increased with the fortification level of quinoa flours. Improvements in the elastic recovery capacity were also reported in doughs with MWT and ultrasonicated rice flour in GF dough systems, and were associated with a strengthening of the extent of bonding between the structural elements, which could be beneficial for the gas-holding capacity of the dough (Vela et al., 2023; Villanueva et al., 2019).

Dough consistency has been suggested to largely affect bubble stability during fermentation (Föste et al., 2017), and could mask any additional effect caused by using different amounts of native or thermally treated quinoa flours in the composite GF formulations. Considering the strong influence of the amount of quinoa flour added and the MW treatment on dough consistency (data presented in Table 1 and Fig. 1), as well as the preliminary baking trials conducted, it was concluded that using the same hydration level for all doughs was not a proper approach for assessment of changes in dough rheological responses due to fortification of the GF mixes with the quinoa flours. Hence, the amount of water incorporated into the doughs was adjusted accordingly to obtain uniform consistencies in terms of G_1^*, and this was selected based on preliminary baking trials. The dough hydration, expressed as grams of water per 100 g of flour mix, and corresponding G_1^* values derived from this adjustment process are presented in Table 1. Accordingly, these doughs with adjusted hydration were used in the fermentative properties and for breadmaking.

3.2. Dough pasting properties

The pasting parameters of the freeze-dried doughs are presented in Table 2 and the pasting curves are plotted in Supplementary Fig. 1A. The viscometric profile of a dough is linked to that of the flour/starch that comprise it. Nevertheless, the dough usually exhibits lower viscosity than the corresponding flour/starch due to the presence of the other non-starch ingredients added to the recipe, mainly HPMC and lipids, which dilute the dough, reduce water availability, and restrict starch swelling and gelatinization during cooking (Villanueva et al., 2019; Witczak, Chmielewska, Ziobro, Korus, & Juszczak, 2021). Increasing amounts of native quinoa flour to maize starch-based GF dough resulted in a gradual reduction in the viscosimetric profile up to −76% and −52% in PV and FV respectively, and from 1099 mPa s (N-0) to 2 mPa·(N-75) in BV. Furthermore, an increase of 9.7 °C in PT and a delay of 2.5 min in Pt, were recorded. These effects can be explained by the lower viscosimetric profile of quinoa flour, displaying lower PV and BV, and a delayed pasting compared to maize starch (Supplementary Fig. 1B).

The incorporation of thermally treated quinoa flour resulted in a decrease in BV and an increase in PT, compared to samples fortified with the same levels of native quinoa flour. Previous studies on MW treatment of various matrices, including quinoa (Vicente et al., 2023) and millet (Zhi et al., 2022), indicated similar reductions in BV and increments in PT, which were also observed in this study (refer to Supplementary Fig. 1B). The reduction in BV reveals an improvement in starch stability under heat and shear conditions (Schafranski, Ito, & Lacerda, 2021). The delayed PT has been also related to intermolecular rearrangements in starch granules brought about by the MW treatment, resulting in enhanced resistance to swelling of the starch granules (Iuga & Mironeasa, 2020). The pasting profile of MWT flour was significantly below than that of native quinoa flour (Supplementary Fig. 1B). However, such a response was only reflected in the PV, TV and FV of the dough made with the highest addition of quinoa flour (75%), where the values of these parameters were significantly lower in the case of doughs fortified with the MWT flour. This was not observed for lower proportions of maize starch substitution by the quinoa flours, probably because of the diluting effect of maize starch on dough properties.

3.3. Dough thermal properties

The thermal properties of freeze-dried doughs studied by differential scanning calorimetry are given in Table 2 and the respective thermograms are shown in Supplementary Fig. 2. The dough powders were initially rehydrated to achieve the original hydration level of the dough,

Table 2
Pasting, thermal, and fermentative properties of gluten-free bread doughs.

	N-0	N-25	MW-25	N-37.5	MW-37.5	N-50	MW-50	N-75	MW-75	SE
Pasting properties										
PT (°C)	75.85 a	77.48 b	78.23 c	77.45 b	80.68 d	83.15 e	83.23 e	85.50 f	88.00 g	0.04
PV (mPa·s)	2862 h	1648 g	1654 g	1479 f	1328 e	953 c	1035 d	688 b	522 a	20
Pt (s)	4.77 a	5.44 b	5.44 b	5.57 b	5.77 c	6.04 d	7.14 e	7.27 e	7.27 e	0.06
TV (mPa·s)	1764 h	1190 f	1276 g	1089 e	1164 f	908 c	1014 d	686 b	522 a	20
BV (mPa·s)	1099 f	458 e	378 d	391 d	165 c	46 b	21 ab	2 a	0 a	9
FV (mPa·s)	2735 f	2335 e	2275 e	2191 d	2297 e	1988 c	1974 c	1326 b	1138 a	22
SV (mPa·s)	971 b	1145 c	999 b	1103 c	1133 c	1081 c	982 b	668 a	686 a	22
Thermal properties										
ΔH_{gel} (J/g db)	11.2 cd	11 bcd	11.2 cd	10.6 bc	11.5 d	10.4 ab	10.8 bcd	10.3 ab	9.9 a	0.3
$T_{o\text{-}gel}$ (°C)	68.0 a	68.8 bc	68.5 ab	68.6 ab	69.3 c	68.8 bc	68.8 bc	70.1 d	68.7 bc	0.2
$T_{p\text{-}gel}$ (°C)	73.5 a	74.3 b	73.4 a	74.7 b	74.7 b	75.3 c	74.7 b	76.9 d	75.8 e	0.2
ΔH_{ret} (J/g db)	6.7 f	5.3 d	5.9 e	4.8 c	5.3 d	4.5 c	5.4 d	2.8 a	3.5 b	0.1
$T_{p\text{-}ret}$ (°C)	50 a	51 a	51 a	51 a	50 a	51 a	50 a	51 a	50 a	1
Fermentative properties										
H_m (mm)	88 d	102 e	104 e	57 c	99 e	33 b	56 c	18 a	28 b	2
h (mm)	88 d	23 b	104 e	21 b	67 c	12 a	25 b	4 a	5 a	3
T_1 (min)	180 d	129 c	179 d	84 b	126 c	56 a	97 b	44 a	57 a	7
H'_m (mm)	68 a	91 cd	90 cd	85 bc	92 d	82 b	85 bc	71 a	81 b	3
T'_1 (min)	71 ab	89 d	67 a	80 c	78 bc	70 a	80 c	90 d	72 ab	2
V_T (ml)	1021 a	1625 c	1345 b	1660 cd	1417 b	1733 d	1374 b	1597 c	1418 b	33
RC (%)	100 e	93 cd	98 e	80 a	97 de	79 a	89 bc	87 b	86 b	2

Samples are identified as native quinoa flour (N) or microwave-treated quinoa flour (MW) and the level of maize starch replacement (0%, 25%, 37.5%, 50% or 75%). PT: pasting temperature, PV: peak viscosity, Pt: peak time, TV: trough viscosity, BV: breakdown viscosity, FV: final viscosity, SV: setback viscosity, ΔH_{gel}: starch gelatinization associated enthalpy; $T_{o\text{-}gel}$ and $T_{p\text{-}gel}$: onset and peak temperatures for gelatinization peak; ΔH_{ret}: enthalpy associated with the melting of recrystallized amylopectin, $T_{p\text{-}ret}$: peak temperature of melting of recrystallized amylopectin, %DR: degree, H_m: height of the dough at the maximum development time, h: height of the dough at the end of the test, T_1: time corresponding to H_m, H'_m: maximum height of CO_2 production, T'_1: time of the maximum gas production, V_T: total volume of CO_2 produced over the 3-h fermentation, RC: CO_2 retention coefficient. SE: pooled standard error obtained from ANOVA. Means with different letters for the same parameter indicate significant statistical differences between means at a significance level of $p < 0.05$.

the same as used in breadmaking. The first scan, corresponding to the gelatinization profile of the dough samples, shows a main endothermic peak with a long tail (see Supplementary Fig. 2A). This shape denotes gelatinization events with water restriction, as would be expected from the moderate water content used (46%–51%), in addition to the presence of the hydrocolloid HPMC and quinoa fibre, which compete with starch for water (Biliaderis, 2009; Föste et al., 2017; Sabanis & Tzia, 2011). Moreover, the peak shape can be influenced by the presence of two types of starch, from quinoa and maize, due to their difference in gelatinization temperature range (Witczak et al., 2021). As can be seen in scans performed on pure maize starch and quinoa flours in excess water (Supplementary Fig. 3), the maize starch exhibited a narrower gelatinization peak, with a peak temperature 4 °C lower than that of quinoa flour (Supplementary Fig. 3). Additionally, in the first thermal scans of doughs, the second peak/shoulder around 90- 100 °C might be attributed, at least in part, to the amylose-lipid complex dissociation (Biliaderis, 2009; Sabanis & Tzia, 2011). However, considering the difficulty in quantifying the individual contribution of this second endothermic transition (amylose-lipid dissociation) from the main peak, the whole peak area was calculated and reported in terms of an overall starch gelatinization enthalpy, ΔH_{gel}, in this study. The addition of quinoa resulted in only a gradual decrease in ΔH_{gel} (Table 2). The incorporation of MWT flour led to comparable ΔH_{gel} at equivalent levels of maize starch substitution in the GF mixed flours. The peak temperature, $T_{p\text{-}gel}$, showed a progressive increase with the addition of quinoa flour (from 73.5 °C for N-0 to 76.9 °C for N-75), while the onset temperature, $T_{o\text{-}gel}$, was similar for all quinoa flour-containing doughs (~69 °C), except for that with the highest addition (70.1 °C for N-75). The doughs containing MWT quinoa flour showed slightly lower $T_{p\text{-}gel}$ values (except for MW-37.5) than those made with native quinoa flour at the same level of maize starch replacement. Föste et al. (2017) have demonstrated that with increasing amounts of added water the onset of gelatinization endotherm of GF doughs containing quinoa bran and maize starch was reduced, and this effect was accentuated at a higher proportion of quinoa bran. Therefore, the observed differences in the gelatinization temperatures among the various GF dough formulations examined in the present study, can be attributed to the combined effect of dough hydration level and the proportion of native or treated quinoa flour used for maize starch replacement.

The second scan, performed on dough samples after seven days of storage at 4 °C, presented one peak corresponding to the melting of the retrograded amylopectin component in the various dough preparations (Biliaderis, 2009). This peak appeared at a similar temperature, $T_{p\text{-}ret}$, for all samples (~50 °C), but varied in enthalpy values, ΔH_{ret}. The ΔH_{ret} was progressively reduced with the inclusion of higher amounts of quinoa flours added in the fortified mixtures (up to −58% for N-75, compared to N-0). The quinoa flour showed a much lower extent of amylopectin retrogradation, as illustrated in Supplementary Fig. 3 and confirmed by relevant literature information (Li & Zhu, 2017); as a result, there is a reduction in the retrogradation endotherm of the mixed dough formulations by increasing the level of quinoa flour incorporation. With inclusion of MWT flour, the ΔH_{ret} was raised for all levels of GF dough fortification by quinoa flour (up to +24% for MW-75 compared to N-75). This effect is consistent with the increase in the extent of retrogradation observed in quinoa flour derived from MWT quinoa grains thermally treated at 30% water content (Vicente et al., 2023).

3.4. Dough fermentative properties

The behaviour of the dough during fermentation was also examined using a rheofermentometer to monitor the dough development (Fig. 2), as well as the gas produced and retained by the dough (Fig. 3). The obtained parameters from the curves are summarized in Table 2. The maximum height, H_m, and the height at the end of the test, h_m, are parameters that measure dough development. As the amount of native quinoa increased in the GF formulation, there was a noticeable decrease in these two parameters. N-25 reached the maximum H_m among doughs including native quinoa, 104 mm, but was comparatively weaker than N-0. After 140 min, N-25 exhibited a drop in height and was unable to withstand its height during the rest of the experiment (h_m of 23 mm). However, the N-0 endured throughout the complete period of the

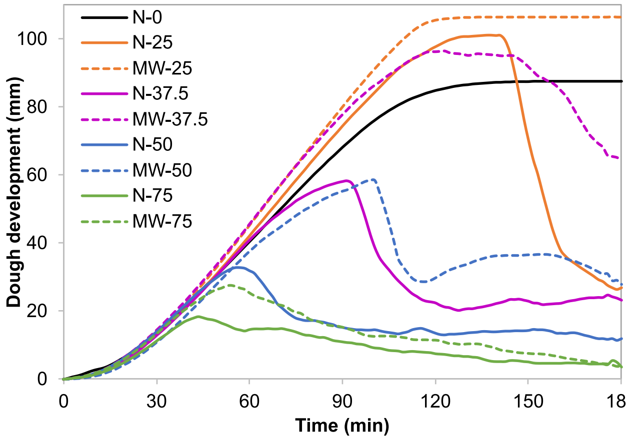

Fig. 2. Dough development during fermentation for doughs produced by replacing maize starch in different proportions (25%, 37.5%, 50% or 75%) with either native (N) or microwave-treated (MW) quinoa flour in the control recipe containing 100% maize starch (N-0).

experiment without any drop in dough height ($H_m = h_m = 88$ mm). This pattern was observed in all native quinoa doughs, which progressively reached a lower H_m at an earlier time, T_1, and fell earlier as the quinoa dose increased. These findings indicate that in the presence of quinoa flour the dough's ability to maintain a shelf-supported network during fermentation is reduced. This may be linked to the detrimental impact of bran constituents on the structure of the composite dough, disrupting the continuity of the hydrated network (Föste et al., 2017; Hager et al., 2012). However, the addition of MWT quinoa flour, mitigated partially this undesirable effect. As can be seen in Fig. 2, the dough development profiles obtained for MW-25 and MW-37.5 doughs were analogous to those corresponding to N-0 and N-25, respectively.

Fig. 3 shows the changes in the gas production profiles over time when the level of added native or MWT quinoa flour varied. The use of native quinoa led to a rise in the total volume of gas produced, V_T, from 1021 ml for N-0 to 1597 (N-75) - 1733 (N-50) ml. As the quinoa level for dough fortification was raised, the gas production profile varied, showing a lower maximum peak height, H'_m, and higher gas production at the end of the test (180 min). In contrast, the use of MWT flour led to a significant decrease in V_T (1345–1418 ml) compared to doughs made with native quinoa, regardless of the dosage, but with a very high gas retention capacity (RC). The gas production profile was comparable to that of N-0, albeit with a higher H'_m and V_T. The high gas production profiles obtained for doughs containing native or treated quinoa flour could be explained by the presence of high endogenous activity of α-amylase and α-glucosidase in quinoa, that is nearly undetectable in maize starch (Elgeti et al., 2014). The amylolytic enzymes of native quinoa produced more available fermentable sugars for the yeast, increasing the gas production at longer times (particularly with higher quinoa addition). In the MWT flour these enzymes could be partially inactivated by the heating treatment, resulting in less abundant simple sugars for yeast fermentation and thus lower CO_2 production. However, the high RC of doughs made with the MWT flour can compensate for the decreased gas production in these systems.

The greater ability of doughs with MWT flour to retain gas and maintain a shelf-supported hydrated network during fermentation, as evidenced by higher RC, H_m and H, is consistent with the structuring effect displayed by the dough rheological tests in this study (see section

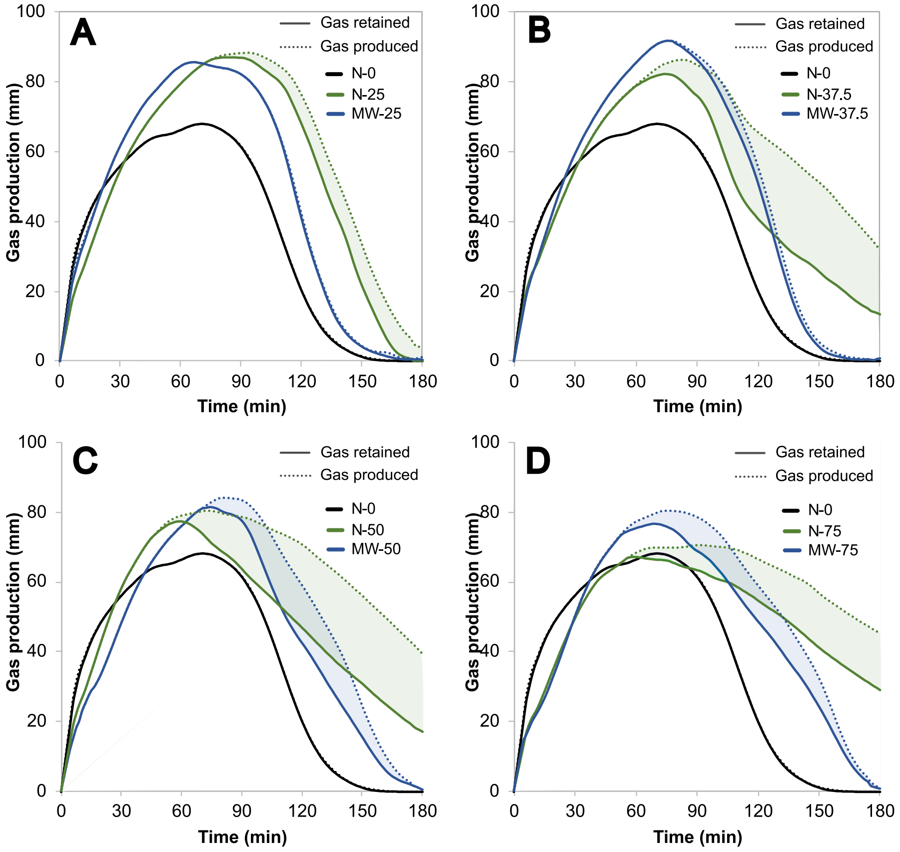

Fig. 3. Gas production (discontinuous line) and gas retention (continuous line) during dough fermentation for GF doughs obtained by replacing maize starch in different proportions: 25% (A), 37.5% (B), 50% (C) or 75% (D) with either native (N) or microwave-treated (MW) quinoa flour. The control recipe containing 100% maize starch (N-0) is also included in all graphics.

3.1). Doughs made with the MWT quinoa flour showed a higher recovery response in the creep-recovery tests, denoting a greater elasticity, i.e., being able to withstand higher stresses, while preserving their structure, compared to doughs made with native flour, as it was also observed in previous studies with other MWT flours (Vicente et al., 2024; Villanueva et al., 2024). This behaviour could explain the greater ability of doughs made with MWT quinoa flour to retain the gas formed, without structure collapse, as it happened in doughs made with untreated flour (see next section).

3.5. Bread physical properties

Table 3 presents a summary of the physical properties of breads made with various GF flour formulations, while Fig. 4 displays images of the respective bread loaves and slices. The physical properties were evaluated for correlations with dough properties. The N-0 and MW-25 breads were considered undesirable due to formation of large holes in the crumb.

The specific volume of the breads decreased gradually with increasing quinoa addition in both treated and native quinoa breads. Aguiar et al. (2021) also observed highest bread specific volume with the inclusion of low quinoa additions in GF breads (17% and 33%). This is a behaviour commonly reported for other pseudocereals, although it depends on the formulation and process used (Alvarez-Jubete, Auty, Arendt, & Gallagher, 2010; Hager et al., 2012; Turkut et al., 2016). However, when the MWT quinoa was used, the specific volume increased compared to the baked products made with native quinoa at the same concentration level, except for the MW-75 formulations where there were no significant differences. The specific volume correlated positively with the fermentation parameters h ($r = 0.951$, $p < 0.001$), T_1 ($r = 0.923$, $p < 0.001$), and RC ($r = 0.81$, $p < 0.01$), and negatively with the gelatinization temperature $T_{p\text{-}gel}$ ($r = -0.81$, $p < 0.01$). Breads containing untreated quinoa, particularly at high levels, partially collapsed in the first stages of baking, contributing to their reduced specific volume (see Fig. 4C). However, the doughs made with MWT quinoa flour mitigated such a structure collapse compared to those with the same substitution level of native quinoa flour, resulting in breads with higher specific volume. This improvement was probably related to the ability of doughs made with MWT flour to tolerate longer fermentation time before structural failure, to retain more gas, and to maintain the network structure, minimizing bubble coalescence and gas escape. Furthermore, MWT was also effective in reducing the cracks and holes in the crust for formulations with 37.5% and 50% of quinoa, as evidenced in Fig. 4B.

The crumb grain characteristics were also studied excluding the breads N-0 and MW-25. The cell density decreased with the use of MWT flour compared with native quinoa flour at the same fortification level, while the mean cell area increased (Table 3). In addition, the void fraction (proportion of the slice surface occupied by cells) increased with the use of MWT flour instead of native quinoa for 37.5% and 50% substitution (+50 and + 26%, respectively). The higher void fraction indicates a more open structure, which has been related to a higher specific volume (Hager et al., 2012). In the present study the void fraction also correlated positively with the specific volume of the bread ($r = 0.87$, $p < 0.01$), as well as with the gas retention capacity of the dough, RC ($r = 0.941$, $p < 0.001$). Breads with a more open crumb granulometry and larger mean cell size, as was obtained with MWT flour, seems to be preferred by consumers over those having a more compact and denser crumb of smaller pores in quinoa breads (Encina-Zelada et al., 2019).

Bread texture was greatly affected by the level of quinoa added and MW treatment. Bread crumb hardness increased with the amount of native quinoa, up to a maximum hardness for N-50 bread. Meanwhile, incorporating MWT flour up to 50% resulted in a reduction of hardness by over 50%, compared to the same amount of untreated flour, and with a less pronounced effect at the 75% addition level. The hardness negatively correlated with the void fraction ($r = -0.86$, $p < 0.01$) and the specific volume ($r = -0.81$, $p < 0.01$). The greater amount of air trapped in the structure, the less dense it becomes and consequently, a softer texture of the bread is acquired (Villanueva et al., 2019). Cohesiveness and resilience increased with the use of treated flour for low fortification (25% and 37.5%), but decreased for higher levels (50% and 75%). However, there was no difference in springiness between native and MWT flour at the same level of fortification, except for the lowest quinoa addition (25%). The increase in crumb hardness after seven days of storage at 4 °C followed the same trend as the initial hardness, with reductions being noted upon use of MWT flour compared to native flour (up to − 60% for 37.5% quinoa). Therefore, the fortification with MWT quinoa flour up to 50% maize starch substitution proved effective in reducing both the initial crumb hardness and the staling phenomena. This was an interesting positive outcome as quinoa has been found to produce GF breads with a harder crumb than wheat flour breads, particularly at high substitution levels (Aguiar et al., 2021; Turkut et al., 2016).

The colour of bread crust originates from the coloured pigments present in the flours and the products from sugar caramelization and the Maillard reactions, which are favoured by the high protein content

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

	N-0	N-25	MW-25	N-37.5	MW-37.5	N-50	MW-50	N-75	MW-75	SE
Specific volume (ml/g)	7.20 g	3.18 d	6.81 f	2.77 b	3.97 e	2.45 a	3.02 c	2.32 a	2.44 a	0.05
Texture										
Hardness (N)	1.1 b	2.4 d	0.8 a	3.3 e	1.7 c	6.5 h	3.6 e	5.5 g	5.0 f	0.1
Springiness	0.89 ab	0.99 cd	0.87 a	0.99 cd	1.00 cd	0.97 d	0.98 cd	0.94 bc	0.96 cd	0.02
Cohesiveness	0.83 f	0.70 e	0.83 f	0.58 b	0.70 de	0.66 d	0.62 bc	0.65 cd	0.52 a	0.02
Chewiness	0.8 b	1.6 d	0.6 a	1.9 e	1.2 c	4.3 i	2.2 f	3.4 h	2.5 g	0.1
Resilience	0.39 cd	0.39 de	0.40 de	0.26 a	0.42 e	0.37 cd	0.34 b	0.35 bc	0.24 a	0.01
ΔHardness-7d (N)	5.3 b	7.5 c	3.4 a	8.8 c	3.5 ab	15.6 e	8.6 c	12.7 d	11.3 d	0.8
Colour										
L^*_{crust}	81.3 h	60.2 f	61.9 g	48.1 e	49.6 e	46.1 d	44.3 c	36.4 a	40.9 b	0.8
h_{crust}	69.5 f	63.0 e	61.5 d	60.8 d	56.7 b	61.2 d	58.9 c	53 a	57.1 b	0.5
C^*_{crust}	23.7 a	33.4 e	36.1 g	31.7 d	34.9 f	31.6 d	31.4 d	27.8 b	30.1 c	0.5
L^*_{crumb}	76.6 g	58.8 e	67.5 f	56.4 d	54.6 cd	54.0 bc	51.6 a	51.8 ab	50.3 a	0.8
h_{crumb}	92.1 g	78.0 f	75.1 d	76.4 e	74.9 d	74.6 d	73.6 c	72.3 b	69.8 a	0.3
C^*_{crumb}	4.9 a	11.9 b	13.0 c	14.7 d	15.8 e	16.6 f	17.4 g	17.9 g	21.5 h	0.3
Crumb grain characteristics										
Cell density (cells/cm^2)	nd	28 d	nd	31 e	26 cd	24 c	16 a	27 cd	20 b	2.9
Void fraction (%)	nd	35 d	nd	24 a	36 d	27 b	34 d	29 bc	31 c	1.0
Mean cell area (mm^2)	nd	1.4 ab	nd	1.2 a	1.5 bc	1.2 a	2.4 d	1.2 a	1.7 c	0.1

Samples are identified as native quinoa flour (N) or microwave-treated quinoa flour (MW) and the level of maize starch replacement (0%, 25%, 37.5%, 50% or 75%). L*: luminosity, h: hue, C*: chroma, nd: not-determined. SE: pooled standard error obtained from ANOVA. Means with different letters for the same parameter indicate significant statistical differences between means at a significance level of $p < 0.05$.

Fig. 4. Photographs depicting GF bread loaves (A, B) and slices (C) produced by replacing in different proportions (25%, 37.5%, 50% or 75%) maize starch with either native (N) or microwave-treated (MW) quinoa flour. The control recipe containing 100% maize starch (N-0) is also included for comparison.

(Aguiar et al., 2021). Crust colour was clearly impacted by the amount of quinoa flour used, with lightness, hue, and chroma decreasing as its concentration increased (excluding hue for N-0), resulting in darker, reddish, and less vivid crust colour. However, no clear trend was observed for any impact of the MW treatment, revealing minor differences between the colour coordinates of breads made with MWT flour, compared to breads made with the native flour at the same fortification level. In contrast, there was a clear effect of quinoa addition and MW treatment on the bread crumb. Lightness and hue decreased and chroma increased significantly with quinoa addition, denoting a darker, reddish and more vivid crumb colour. Similarly, the breads made with MWT flour showed a decrease in hue and an increase in chroma compared to the untreated flour at the same amounts used, thus resulting in a more reddish and vivid crumb colour. Villanueva et al. (2019) explained that the colour of the crumb is mainly related to the colour of the ingredients. This observation is in agreement with previous findings on the colour changes observed for MWT quinoa at 30% water content (Vicente et al., 2023).

3.6. *Bread nutritional aspects*

There were no significant differences in proximate composition between breads fortified with the MWT flour and the native flour (Table 4), as previously reported for bread supplemented with MWT buckwheat (Vicente et al., 2024). The modifications in bread composition can only be attributed to the proportion of quinoa flour used in the formulation. Incorporation of quinoa resulted in an increase in protein, fibre, fat, and ash contents at the expense of a reduction in the carbohydrate content. Bread fortified with 75% of quinoa resulted in a composition (g/100 g bread) of 6.5% protein, 6.1% fibre (3.4% insoluble and 2.7 soluble), and 1.9% ash. The increase in macro- and micronutrients, as obtained with quinoa flour fortification, is recognized as an important factor in improving the health of people following a typical GF diet (Aguiar et al., 2023). However, the physical and sensory properties of the fortified baked products would be the ultimate determinant for the highest acceptable level of GF dough supplementation with quinoa flours at a commercial scale.

The *in vitro* digestible (TDS) and resistant starch (RS) of the freeze-dried crumbs, expressed as g/100 g of fresh bread crumb, are reported in Table 4. The rapid digestible starch (RDS) was ~95% of the TDS (no significant differences between samples were noted). The high proportion of RDS aligns with the findings of the analysis of starch-based GF breads, where the starch is highly gelatinized and the product structure is very porous, resulting in rapid digestion of the starch (Matos Segura & Rosell, 2011). The addition of higher amounts of quinoa flour led to a decrease in the total digestible starch (TDS) and resistant starch (RS), as a result of the reduction in the total amount of carbohydrates present in the fortified bread ($r = 0.995$, $p < 0.001$). Previous studies have also shown that the use of whole quinoa flour can lead to lower predicted glycaemic load in products due to its low carbohydrate content (Wolter, Hager, Zannini, & Arendt, 2013). In this study, the use of MWT flour did not seem to significantly affect the starch digestibility of the bread crumb compared to the use of untreated flour.

3.7. *Bread sensory analysis*

The results of the sensory analysis of the odour and taste of the bread, performed by a trained panel, are presented in Fig. 5. The panel identified a distinct odour and taste for quinoa, defined as “herbaceous”, and described as an off-flavour. The intensity of these attributes clearly increased with amount of quinoa flour added in the GF mixtures. Although some studies have reported good acceptability of quinoa taste in GF bread (Aguiar et al., 2021), other studies indicated a reduced acceptability of bread fortified with quinoa flour due to its typical aroma and taste (Burešová et al., 2017; Hager et al., 2012). The use of MWT quinoa flour notably reduced the intensity of such “herbaceous” odour and flavour perception. This moderation in off-flavour/taste notes could potentially increase quinoa acceptability by consumers. Reduction/masking of “green”, “beany”, and “earthy” off-flavours, associated with legume flour fortification of breads, was also noted by thermal treatment of the flour, i.e., roasting, and was linked with better consumer acceptability (Kotsiou et al., 2021, 2022). In the present study, the adopted milder MW hydrothermal treatment (performed at a lower temperature and higher moisture content) was also found effective in reducing off-flavour notes for quinoa flour.

With increased quinoa content in the GF breads there was also an increase of a characteristic odour and taste of backed products, defined as “toasted” and usually perceived positively by the panel. The use of MWT flour resulted in a slight increase in these attributes, although not statistically significant ($p > 0.05$). Bitterness also increased with the addition of quinoa; however, no differences were observed in most cases with the use of MWT flour in the formulations. A similar behaviour was also noted for the aftertaste, as the bitter taste predominated and lasted longer. Other studies did not highlight the bitterness taste and aftertaste when incorporating whole quinoa flour (Hager et al., 2012). The bitter taste observed in this study may be attributed to the presence of higher polyphenol and flavonoid contents in the variety of quinoa used. Recent studies have shown that these compounds are the primary contributors to quinoa’s bitter taste, even at low concentrations, rather than saponins (Song et al., 2024).

4. Conclusions

The findings from the present experimental study provided information on the suitability of quinoa flour as an ingredient in GF bread formulations and demonstrated the feasibility of using MW treatment to improve the physical and sensory quality of baked GF bread. The experimental data demonstrated a significant impact of quinoa fortification level and the use of MWT flour on dough properties and bread quality attributes. The progressive addition of higher levels of untreated quinoa in the GF flour blends resulted in breads with reduced specific volume and harder crumb. However, the inclusion of MWT quinoa flour, instead of its native counterpart, proved effective in enhancing bread specific volume and reducing crumb hardness, allowing a greater

Table 4
Proximate composition and starch digestibility of gluten-free breads.

	N-0	N-25	MW-25	N-37.5	MW-37.5	N-50	MW-50	N-75	MW-75
Proximate composition (g/100g bread)									
Ash	1.2 a	1.4 a	1.5 abc	1.5 abcd	1.5 abc	1.7 bcd	1.6 bcd	1.9 d	1.8 cd
Protein	1.1 a	3 b	3.2 b	4 c	4 c	4.9 d	4.9 d	6.6 e	6.4 e
Fat	4.2 a	4.6 b	4.8 c	5 de	4.9 cd	5.3 f	5.2 e	5.9 g	5.6 h
TDF	2.5 a	3.7 ab	3.9 ab	4.4 abc	4.4 abc	5 bc	5 bc	6.1 c	6.1 c
IDF	0.4 a	1.5 b	1.6 b	2.1 bc	2 bc	2.6 cde	2.5 bcd	3.5 e	3.3 de
SDF	2.1 a	2.2 a	2.4 a	2.3 a	2.4 a	2.4 a	2.6 a	2.6 a	2.8 a
Carbohydrates	59.8 g	50.5 f	52.8 e	48.4 d	47.2 cd	45.5 bc	44.2 b	39.4 a	37.8 a
Starch digestibility (g/100 g bread crumb)									
RDS	47.9 f	40 de	41.7 e	38.8 cd	37.5 cd	37.1 bc	34.5 b	30.6 a	28.4 a
SDS	1.9 a	1.5 a	1.4 a	1.2 a	1.4 a	1.1 a	1.7 a	0.6 a	1 a
TDS	50.4 f	43.5 e	44.5 e	40.8 d	39.3 cd	38.4 bc	35.7 b	31.6 a	29.7 a
RS	1.6 d	1.4 cd	1.3 c	1.2 c	0.9 b	0.8 b	0.7 b	0.4 a	0.4 a

Samples are identified as native quinoa flour (N) or microwave-treated quinoa flour (MW) and the level of maize starch replacement (0%, 25%, 37.5%, 50% or 75%). TDF: total dietary fiber; IDF: insoluble dietary fibre, SDF: soluble dietary fiber; RDS: rapidly digestible starch; SDS: slowly digestible starch; TDS: total digestible starch; RS: resistant starch. Means with different letters for the same parameter indicate significant statistical differences between means at a significance level of $p < 0.05$.

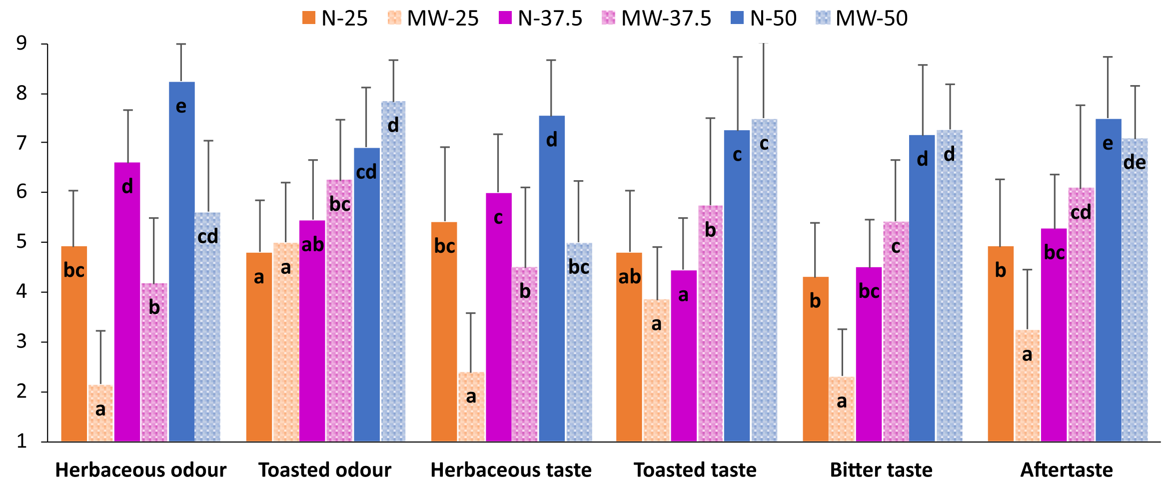

Fig. 5. Sensory analysis of breads produced by replacing different proportions of maize starch (25%, 37.5%, 50% or 75%) with either native (N) or microwave-treated (MW) quinoa flours in the control recipe consisting of 100% maize starch (N-0).

fortification level, while maintaining adequate physical quality. The improvements in specific volume and texture were related to proper composite dough network structuring. This hydrothermal treatment increased the GF dough resistance to deformation during fermentation, allowing greater gas retention and proper pore development during baking, preventing collapse of the network structure. The MW treatment also had a positive impact on reducing the herbaceous off-flavour associated with quinoa, thus improving the sensory quality of bread. Overall, the use of MWT quinoa flour has the potential to improve the physical and sensory qualities of GF bread, and also lead to significant up-grading of the nutritional value of the baked product.

CRediT authorship contribution statement

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

Declaration of competing interest

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

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.foodhyd.2024.110244.

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