International Journal of Food Science and Technology, 2025, **60(1)**, vvae084
https://doi.org/10.1093/ijfood/vvae084
Advance access publication 8 January 2025
Original Article

Gluten-free bread made with non-defatted ground walnuts: microstructural, technological, and nutritional properties

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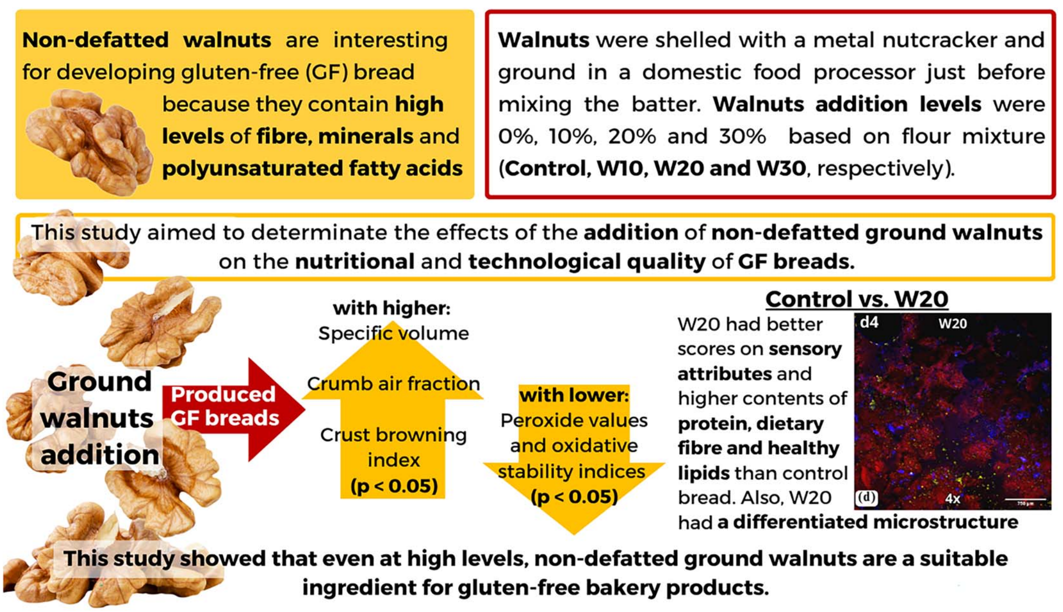
Abstract

Non-defatted walnuts are interesting for developing gluten-free (GF) bread because they contain bioactive compounds and high concentrations of fibre and polyunsaturated fatty acids (PUFAs). This study aimed to determine the effects of the addition of non-defatted ground walnuts (10%, 20%, and 30%) on the nutritional and technological quality of GF breads. Furthermore, control bread (formulation without ground walnuts) and bread with 20% walnuts were selected for microstructure and sensory evaluation. The addition of walnuts led to a significant increase in specific volume, affected crumb and crust colour and produced harder and crumblier crumbs. Despite these changes, the walnuts improved the nutritional and sensory properties of breads without affecting the starch gelatinisation or protein stabilising role (on air bubbles and lipid droplets). Lipid oxidation did not increase in breads containing walnuts at 10% and 20% level. These findings suggest that, even at high levels, non-defatted ground walnuts are a suitable ingredient for GF bakery products.

Keywords: texture, confocal microscopy, oxidative stability index, bread quality, sensory evaluation, *Juglans regia* L.

Graphical abstract



Received: 28 August 2024. **Accepted:** 13 December 2024

Introduction

Gluten-free (GF) breads are usually obtained from mixtures of different refined flours (rich in starch) and present lower nutritional value than the same type of product from wheat. Thus, people who undergo GF diets could suffer a deficiency of different nutrients after a long-term adherence to a GF diet (Kreutz et al., 2020).

Daily consumption of nuts and dried fruits is recommended because of their health benefits (Chang et al., 2016), such as reducing the risk of chronic non-communicable diseases. However, for most of the population, the daily intake of dried fruits is lower than the recommended intake (Carughi et al., 2016). Therefore, it was proposed that an appropriate way to promote dried fruit (hazelnut) consumption would be by delivering them in breads. In addition to encouraging their consumption, this strategy showed that the breads were healthier, resulting in improved postprandial glycaemia and diet profiles of subjects who participated in a crossover study (Devi et al., 2016).

In this context, walnuts (*Juglans regia* L.) represent an interesting alternative to the development of healthier foods. Though walnuts are an energy-dense food, containing up to 70% fat by weight, it has been found that they do not increase the risk of obesity when replacing other foods in the diet. Walnuts contain mostly polyunsaturated fatty acids (PUFAs) esterified as triacylglycerols and minor amounts of other fat constituents. The major fatty acids found in walnut oil are oleic (18:1), linoleic (18:2) (the most abundant), and linolenic (18:3) acids. Particularly, it is the only nut that contains a relatively high percentage of the omega-3 alpha-linolenic acid (ALA), which is beneficial for heart health and improving blood fat profile (Tabasum et al., 2018). Walnuts contain bioactive components like polyphenols (~20 mg gallic acid g^{-1}), flavonoids (4 mg catechin g^{-1}) and present a high content of proteins (~45%), fibre (~17%), and minerals (~5%) (Santos et al., 2018). Walnut soluble fibre has been reported to be prebiotic thus modulating intestinal microbiota (Wang et al., 2021). Thus, the addition of walnuts in GF formulations could be a good strategy to increase the nutritional quality of this kind of product.

From a technological point of view, the employment of walnuts is sometimes difficult because of the susceptibility to oxidation of its lipids, caused by the major presence of PUFA (Burbano & Correa, 2021).

Though the benefits of walnuts as an ingredient for GF breads are recognised (Šmídová & Rysová, 2022), few studies focussed on the effect of this ingredient on the product properties. Burbano et al. (2022a, 2022b) reported the characteristics of GF batters and cakes made with rice flour, corn starch, cassava starch and partially defatted walnut flour (subproduct of oil extraction). Wójcik et al. (2023) investigated the effect of different walnut flour levels on GF breads based on white buckwheat flour, flaxseed flour, and partially defatted walnut flour. The aim of this study was to determine the effects of the addition of non-defatted ground walnuts on the nutritional and technological quality of GF breads.

Materials and methods

The ingredients employed in formulations were rice flour, and cassava and corn starches (Santa María, Argentina), commercial sodium chloride (Celusal, Argentina), walnuts in shell (*J. regia* L.) and commercial sunflower oil acquired from the local market. The information given by the flours producers was (on wet basis), for rice flour: carbohydrates, 80%, proteins, 6%, lipids, 1.4%, fibre, 2.4%; for cassava starch: carbohydrates, 89%, proteins, 0.2%, fibre, 0.9%; for corn starch, carbohydrates, 91%, proteins, 0.3%, fibre, 0.9%. Supplementary protein source was dehydrated egg white (Ovobrand, Argentina) and leavening agent was active dry yeast (Calsa, Argentina). Hydroxypropylcellulose—HPMC F4M- with 29.3% substitution with methoxyl groups and 6.0% substitution with hydroxypropyl groups was used as a structuring agent (Dow Chemical, USA). Distilled water was used.

Methods

Formulation, preparation, and baking

Rice flour and cassava and corn starches (50%, 25%, and 25%, respectively) were used as a flour mixture (FM). In addition, 15 g of HPMC, 20 g of salt, 25 g of dry yeast, 70 g of dehydrated egg white, 75 g of sunflower oil and 1,250 g of water were added for each kg of FM. Three levels of walnuts were employed: 100, 200, and 300 $g \cdot kg^{-1}$ of FM. Walnuts were shelled with a metal nutcracker and ground in a domestic food processor (Atma, Argentina) just before mixing the batter. Particle sizing of ground walnut ranged from 0.0006 to 0.057 cm^2 (median = 0.009 cm^2).

A planetary kneader for batters was used (Kenwood, Italy). Dry ingredients were pre-mixed for 1 min (speed 1) and afterwards the yeast dissolved in a part of the total water amount was added. The rest of water and the sunflower oil were subsequently added. Batters were mixed for 5 min (speed 2) and then fermented in the mixer bowl for 20 min in a chamber at 30 °C (Brito Hnos, Argentina). Next, ground walnuts were added (except for control) and batters were mixed for 1 min (speed 1). Each batter (≈1 kg) was divided in portions of 46 ± 2 g into individual silicone moulds, fermented for 30 min at 30 °C and baked in a convection oven for 60 min at 210 °C. Breads were let to rest 2 hr before the measurements. Each formulation was prepared in duplicate.

Bread quality

Specific volume

Eight breads were measured in each breadmaking. All bread pieces were weighed and then each volume was determined by rapeseed displacement. The specific volume was calculated as bread volume/piece weight (Correa et al., 2021).

Bread crumb and crust properties

Appendix S1 shows a detailed description of the methods for determining some properties of bread crumb (moisture; water activity; pH; colour; structural porosity; texture profile analysis) and bread crust (colour, hardness evaluated by puncture).

Lipid oxidation in bread crumb

Crumb oxidation stability: The effect of the walnut addition on the oxidative stability of GF breads was determined with a 743 Rancimat (Metrohm, Herisau, Switzerland). Crumb samples were frozen with liquid nitrogen and immediately milled to obtain a fine powder. Then, in each reaction vessel an amount of 0.50 ± 0.05 g of the powdered sample was weighed in order to cover only the bottom of the reaction vessel ($n = 3$). This procedure is recommended with those samples that do not melt with the purpose of avoiding a temperature gradient within the sample (Metrohm, application bulletin 408/1e). All samples were submitted to an accelerated oxidation at 120 °C and with an air flow rate of 20 $L \cdot hr^{-1}$. The induction times (obtained from Rancimat's curves) were taken as crumb oxidative stability index (OSI) values.

Crumb peroxide value and Kreiss assay: Peroxides were extracted from crumb sample with $CHCl_3/CH_3OH$ (2:1) according to the method described by Chapman et al. (1996). Peroxides were determined in the chloroform phase in accord with the Acetic Acid-Chloroform method 58-16.01 (AACC, 2000) ($n = 3$). Kreis assay is indicative of incipient rancidity. In this assay, phloroglucinol reacts with epihydrinaldehyde under acidic conditions to form a product of pink–red colour. Epihydrinaldehyde is a secondary oxidation product formed from decomposition of peroxides. In a tube were added: 1 g of crumb, 2 ml of ether, and 2 ml of HCl concentrated. This tube was shaken for 20 s, and 1 ml of phloroglucinol was added. Subsequently, it was vortexed (20 s) and let to rest for 10 min. After this period, the tube was observed ($n = 3$).

Crumb free fatty acid: The assay was performed in accordance with the AACC 58-15.01 method (AACC, 2000) with minor modifications. A fine power of crumb samples was obtained as in the case of oxidation assay. Samples were put in an Erlenmeyer flask containing 50 ml of neutralised ethanol/ether (1:1) mix and shacked during 30 min. Then, samples were centrifuged at 1,800 rpm (Rolco SRL, Argentina). The supernatant was titrated with NaOH 0.01 N ($n = 3$). The results were expressed as mg KOH · g^{-1} of crumb.

Crumb microstructure

Crumb was analysed by confocal scanning laser microscopy (CSLM) following Burbano et al. (2022a) methodology ($n = 4$). In addition, in order to evaluate the affinity of the fluorophores for dehydrated egg white after heat treatment (simulating cooking). The egg white was dissolved in distilled water (1:1 wt/wt). The beaker with the mixture was heated in boiling water for 5 min. The sample was allowed to cool and stained with a mixture of Rhodamine B (Biopack) and Calcofluor white (Sigma) following the same methodology as for the crumbs.

Proximal composition

Proximate composition of GF breads was measured following AACC methods (2000): Protein (46-12.01, factor = 5.3), lipid (30-25.01), ash (08-01.01), and total dietary fibre (32-05.01). The available carbohydrates content was obtained by difference. Samples energy content was calculated based on Atwater factors (four for protein and available carbohydrates, two for dietary fibre, and nine for fat). All the assays were done at least in duplicate.

Sensory evaluation

For sensory evaluation W20 formulation was compared with the control sample. A descriptive test was performed with a non-trained panel consisted of 20 male and 20 female evaluators (aged between 20 and 50 years old). The evaluators judged GF breads in terms of overall acceptability, appearance, colour, texture, taste, and crunchiness using a nine-point hedonic scale (1 = dislike very much, 9 = liked very much). Breads were cooled for 2 hr at room temperature then breads were cut by the middle and coded with random three-digit numbers. The order in which the samples were evaluated was randomly assigned to each evaluator.

Statistical analysis

Statgraphics Plus software was used to performed ANOVA and comparison among mean values. To discriminate among means Bonferroni's multiple comparison procedure was applied at 95% confidence level.

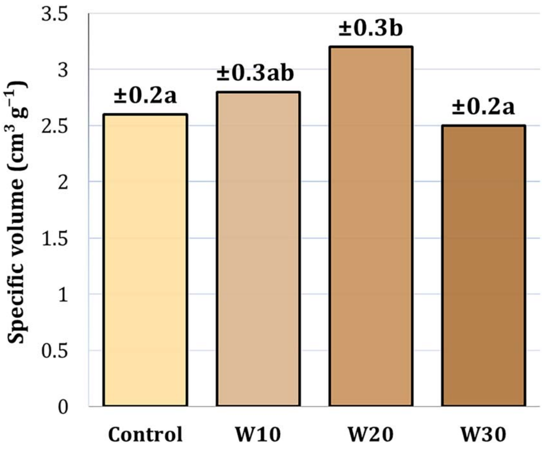


Figure 1. Specific volume of gluten-free bread made with non-defatted ground walnuts. Mean ± standard deviation (SD). Different letters indicate significant differences ($p < .05$). Walnut addition levels were 0, 100, 200, and 300 g kg^{-1} of flour mixture (Control, W10, W20, and W30, respectively).

Results and discussion

Bread quality

The specific volume is a determining parameter for bread quality. GF baked goods with low volume are an indication that the structure of the batter is weak and is not capable of retaining the gases either produced during fermentation or incorporated during the mixing process (Burbano et al., 2022a; Gularte et al., 2012; Moscoso Ospina et al., 2023). Figure 1 shows the specific volume of bread, the addition of ground walnuts produced an increase in the specific volume ($p < .05$), except W30 which showed a decrease which was possibly related to the excess weight due to the 30% of walnut on the batter. Regarding the behaviour of water in the crumb, both the moisture content and *aw* decreased with the addition of ground walnuts, in the case of moisture there was only a significant difference with 20% and 30% of addition (Table 1). Also, even with the decrease of *aw*, all crumb values were around 0.98 (Table 1). These results could be because the amount of water in the formulations was kept constant and the moisture content of the walnuts is less than 5% (Pereira et al., 2008). Table 1 shows the pH values of the crumb, an average decrease of 3% was observed in all samples with walnuts when compared to the control, however, no significant differences were observed between W10, W20, and W30.

The crumb and crust colour were significantly affected by the addition of ground walnuts (Table 1 and Supplementary Table 1). Crumb and crust luminosity (L) progressively decreased as the walnut concentration increased, while at the same time, an increase in crumb ΔE values was observed ($p < .05$). In the case of crumb, values of a and b increased in the breads with walnut compared to the control bread. These differences in the crumb of the different formulations are clearly seen in Supplementary Table 2. Because the temperatures inside the bread during baking do not exceed 100 °C, this change in the crumb colour is due to the inherent colour of the walnut integuments. On the other hand, the browning index values of the crust (Supplementary Table 1) increased compared to the control ($p < .05$). This was probably due to a combination of the colour provided by the walnuts and the Maillard reactions occurring during baking (Purlis, 2010).

Moreover, the crumb porosity parameters are also shown in Supplementary Table 2. The values of air fraction were highly

Table 1. Physicochemical and colour parameters of the crumb of gluten-free bread made with non-defatted ground walnuts.

	Moisture (%)	aw	pH	L	*a*	*b*	Variation of colour—ΔE
Control	50.9 ± 0.6^b	0.9824^b	6.17 ± 0.07^b	69 ± 2^c	−1.6 ± 0.1^a	7.9 ± 0.8^a	–
W10	50.3 ± 0.2^b	0.9826^b	5.96 ± 0.06^a	55 ± 5^b	1.8 ± 0.4^b	11 ± 2^b	14 ± 4^a
W20	46 ± 1^a	0.9795^{ab}	5.99 ± 0.02^a	53 ± 4^a	2.5 ± 0.6^c	11 ± 2^b	17 ± 3^b
W30	47.2 ± 0.7^a	0.9775^a	6.1 ± 0.1^a	52 ± 3^a	2.8 ± 0.5^d	14.5 ± 0.9^c	19 ± 3^c

Note. Mean ± *SD*. Walnut addition levels were 0, 100, 200, and 300 g · kg^{-1} of flour mixture (Control, W10, W20, and W30, respectively). Different superscript letters in the same column indicate significant differences ($p < .05$).

correlated with the specific volume, demonstrating that the spongiest bread had a better alveolar structure. On the other hand, in terms of cell density, the addition of 10% and 20% of walnuts produced larger alveoli ($p < .05$) but significantly lower cell density (number of alveoli cm^{-2}) The maximum air fraction was obtained at 20% level of ground walnut. A higher level (30%) led to similar values of air fraction, and cell density than control crumb. At the same time, only the 10% of walnut addition affected the alveoli form, which was reflected in the values of circularity. Finally, the fractal dimension values serve as an index that summarises the complexity of the alveoli in the bread (Burbano et al., 2022b). The addition of walnut led to a slight but non-significant increase in these values with respect to the control. Overall, the results suggest that, at the 20% level in batter, walnuts tend to improve bread specific volume and crumb structure. GF bread properties, including volume, would also benefit from lipids released from ground walnuts. It has been reported that lipids, particularly emulsifiers, can improve bubble stabilisation in GF breads and as a consequence, render a better volume (Elgeti et al., 2015). On the other hand, the presence of walnut particles in the batter constitutes a load that makes leavening difficult, which could explain volume diminution when 30% level of ground walnuts was employed.

Wójcik et al. (2023) studied GF bread formulated with white buckwheat flour, flaxseed flour and partially defatted walnut flour, the latter in a range from 5% to 20% of replacement. These authors found that the replacement with 20% walnut flour led to an increased volume compared to GF bread without walnut. In the present study, although the basic components were different (flour mixture: rice flour, and corn and cassava starches), the specific volume also increased even with the 20% addition of ground walnuts (on 100 g flour mixture basis). Specific volumes found in the present study were higher than the respective specific volumes reported by Wójcik et al. (2023). These trends might be explained by differences in the bread formulations, walnut specifications (ground walnut; walnut flour), and baking procedures.

Crumb texture profile analysis

Table 2 shows the effect of the addition of ground walnuts on the hardness (*N*), cohesiveness, springiness, resilience, and chewiness (*N*) of the crumb. An increase in crumb hardness for the formulations with walnuts was observed ($p < .05$), except W10 which showed no significant differences with the control in this parameter. Wójcik et al. (2023) also found that hardness of GF breads increased with walnut flour level, suggesting that walnut flour strengthens the dough structure. Lodi and Vodovotz (2008) also reported an increase in crumb hardness in soy bread with almonds. This increase in hardness could be because these dried fruits (walnuts and almonds) have protein and fibre with the capacity to retain water and oil (Burbano & Correa, 2021, 2024). On the other hand, cohesiveness, springiness and resilience, which are related to the crumb integrity and the ability to recover after a deformation (Baixauli et al., 2008; Bourne, 2002), decreased with the addition of walnuts. In the case of cohesiveness and resilience, no change was observed at 10% addition, contrary data obtained with 20% or 30% ground walnuts. This could be because, to some degree, the irregular walnut fragments interfere with the setting of the crumb or due to the relative reduction of gelatinisable starch when the walnuts were added (Arp et al., 2018; Baixauli et al., 2008). Finally, the chewiness of the crumb increased slightly but showed no significant difference between the control, W20 and W30.

Crust hardness by puncture

When defining the "ideal crust" of a baked product, it is necessary to consider the type of product. For instance, crispy crusts are a desired attribute for consumers in salted bread (French type) with and without gluten (Cauvain, 2003). Supplementary Table 2 shows the crust hardness of the GF breads in general did not change in comparison with the crust of control GF bread.

Lipid oxidation in bread crumb

In this type of product, breads with high levels of unsaturated fatty acids (provided by non-defatted ground walnuts), the study of oxidative stability of bread is relevant. Thus, with the aim to evaluate the state of lipids after baking the peroxide index, the qualitative Kreiss assay, the oxidative stability index (OSI) and the free fatty acid value were determined (Table 3). Regarding the peroxide assay (mEq O_2 · kg^{-1} of crumb), the values decreased from 5.9 ± 0.9 (Control) to 1.9 ± 0.5 (W30). These values could reflect two opposite situations. On the one hand, the fragments of ground walnut could provide thermal protection to their lipids during baking due to the high level of natural antioxidants present in them (Labuckas et al., 2008; Vinson & Cai, 2012). On the other hand, these peroxide values could show that the oxidation of lipids is more advanced in formulations with walnuts. In favour of the first, Pycia and Ivanišová (2020) found that the amount of antioxidants was significantly increased by adding walnuts to a wheat bread, e.g., in the bread with 9% addition the total polyphenols were 4.75 times higher than the control bread. In addition, in our samples, Kreiss assay was negative for rancidity even for the sample with 30% of addition of ground walnuts. Respect to the accelerated assay, the OSI values obtained for the crumb ranged from 2.33 ± 0.03 (Control) to 1.34 ± 0.01 (W30). As shown in Table 3, the addition of the walnut at 10% and 20% did not affect the oxidative stability of the bread but at 30% it would be more prone to oxidate. Finally, the free fatty acid values (mg of KOH · g^{-1} of crumb) increased from 0.49 ± 0.07 (Control) to 1.67 ± 0.06 (W30). These results showed that as the level of ground walnuts increases, the amount of free fatty acids also increases. These results are in accord with the OSI since the free fatty acids are more prone to oxidation.

Given that the bread with the addition of 20% walnuts obtained the highest specific volume and air fraction, and that the textural

Table 2. Textural parameters of the crumb of gluten-free bread made with non-defatted ground walnuts.

	Hardness (N)	Cohesiveness	Springiness	Resilience	Chewiness (N)
Control	4.6 ± 0.7^{a}	0.67 ± 0.01^{c}	0.94 ± 0.02^{b}	0.39 ± 0.02^{b}	2.9 ± 0.5^{ab}
W10	3.8 ± 0.8^{a}	0.64 ± 0.01^{b}	0.90 ± 0.02^{a}	0.37 ± 0.01^{b}	2.2 ± 0.5^{a}
W20	6.7 ± 1.0^{b}	0.56 ± 0.01^{a}	0.88 ± 0.02^{a}	0.33 ± 0.01^{a}	3.2 ± 0.6^{b}
W30	7.6 ± 1.2^{b}	0.54 ± 0.02^{a}	0.87 ± 0.02^{a}	0.32 ± 0.02^{a}	3.6 ± 0.6^{b}

Note. Mean ± SD. Walnut addition levels were 0, 100, 200 and 300 $g \cdot kg^{-1}$ of flour mixture (Control, W10, W20, and W30, respectively). Different superscript letters in the same column indicate significant differences ($p < .05$).

Table 3. Values of lipid oxidation assays of the crumb of gluten free bread made with non-defatted ground walnuts.

	Peroxide assay values (mEq $O_2 \cdot kg^{-1}$ of crumb)	OSI (h)	Free fatty acid values (mg of KOH $\cdot g^{-1}$ of crumb)
Control	5.9 ± 0.9^{b}	2.33 ± 0.03^{b}	0.49 ± 0.07^{a}
W10	2.4 ± 0.4^{a}	2.33 ± 0.1^{b}	0.83 ± 0.09^{b}
W20	1.8 ± 0.4^{a}	2.18 ± 0.01^{b}	1.05 ± 0.13^{c}
W30	1.9 ± 0.5^{a}	1.34 ± 0.01^{a}	1.67 ± 0.06^{d}

Note. Mean ± SD. Walnut addition levels were 0, 100, 200 and 300 $g \cdot kg^{-1}$ of flour mixture (Control, W10, W20, and W30, respectively). Different superscript letters in the same column indicate significant differences ($p < .05$). OSI = oxidation stability index.

(crumb and crust), visual, structural porosity and oxidative stability parameters are within the desired quality standards; it was decided to select this sample (W20) for the rest of the analyses (CSLM, proximal composition, and sensory analysis).

Microstructure of crumb by CSLM

Confocal laser microscopy allows the differentiation of major food components by using different fluorophores. Each fluorophore targets a particular component. Thus, CSLM was used to evaluate the microstructure of the samples. Fluorophore fluorescein isothiocyanate (FITC) attaches to proteins and starch granules and emits a bright green colour. Rhodamine B (RB) is a red dye that prefers to attach to proteins. Calcofluor white (CF) strongly attaches to cellulose-containing structures, giving the fibre a blue colour, and Nile red (NR) attaches to lipids, staining them green (Burbano et al., 2022a). In addition, FITC and Nile red were not used together, as they absorb and emit at the same wavelength, so their signal could not be distinguished.

Figure 2 shows CSLM micrographs of the heat-coagulated egg white (the same dehydrated egg white used as an ingredient in all formulations) and the crumb of both breads (Control and W20). In the micrographs, the fluorophore channels were selectively switched on and off to facilitate the differentiation of the components. Ground walnut fragments were not visualised in the micrographs since their size places them high over the scale used. The micrographs of both crumbs (B to I) show a blue film with different intensity levels. Part of the blue colouration could be because of the presence of HPMC in the formulations. However, the concentration of HPMC is low for the level of colouration, suggesting another type of interaction by the CF. Albani and Plancke (1999) found that CF can interact with glycoproteins such as α1-acid glycoprotein (α1-ac GLY) as it can interact with different glycans, mainly those containing galactose, glucose and mannose. In addition, the spatial location of the glycans in the protein seems to play an important role. This is observed in micrograph (A) where CF + RB (which binds to proteins) were used. In image (a1) where both fluorophore channels are on, a violet colouration was observed, while in (a2) leaving only the CF channel on showed that the ovalbumin that was previously heat coagulated turned blue. At 4x magnification, the control (f4) and W20 (h4) crumbs had the starch granules (greenish-yellow) embedded in a matrix formed by egg white protein. The latter is more evident when only the RB and CF channels were left on (f1 and h1 for control and W20, respectively). While, when looking at the crumbs at higher magnification (G and I for control and W20, respectively), it was observed that the starch granules were almost fully gelatinised. This shows that the addition of 20% ground walnuts in the formulation did not affect the gelatinisation process. Furthermore, in almost all the micrographs (B, D, and F to I), it can be seen how the egg white protein was located surrounding the alveoli, thus helping to stabilise them. This protein matrix also stabilised the lipid droplets in both formulations (Control: B and c4. W20: D and e4). Finally, in W20 (e5) it was observed that some droplets were slightly larger than in the control crumb (c5), although this did not affect the oxidative stability measured by Rancimat.

Proximal composition of breads

The proximal composition (dry basis) of control bread was: 17.79 ± 0.05 g of protein, 9.6 ± 0.3 g of lipids, 6.17 ± 0.01 g of total dietary fibre, 4.91 ± 0.02 g of ashes, and 61.5 ± 0.4 g of carbohydrates (calculated by difference). For the W20 bread, the respective values were: 19.4 ± 0.5 g of proteins, 24.7 ± 0.1 g of lipids, 11.6 ± 0.1 g of total dietary fibre, 4.03 ± 0.01 g of ashes, and 40.3 ± 0.6 g of carbohydrates (calculated by difference). All these values were significantly different ($p < .05$) from those of the control values. As a result, the W20 bread had a lipid content that is 2.5 times greater than that of the control sample. This contributed to a higher calorie content of 484 kcal per 100 g, compared to 416 kcal per 100 g for the control bread. Despite the higher amount of lipids, they presented better technological quality than the control sample. Since walnuts are rich in unsaturated fatty acids and present a ratio $\omega6/\omega3$ around 4 which is considered as optimal for consumption in moderation. Meanwhile, a high $\omega6/\omega3$ is related to cardiovascular disease, cancer, and inflammatory and autoimmune diseases (Tapia et al., 2013). Furthermore, although in sample W20 the proportion of unsaturated lipids probably increased, these did not affect the OSI values as previously discussed.

Sensory evaluation

In some cases, non-compliance with the GF diet is mainly due to sensory dissatisfaction with GF foods (Pagliarini et al., 2010).

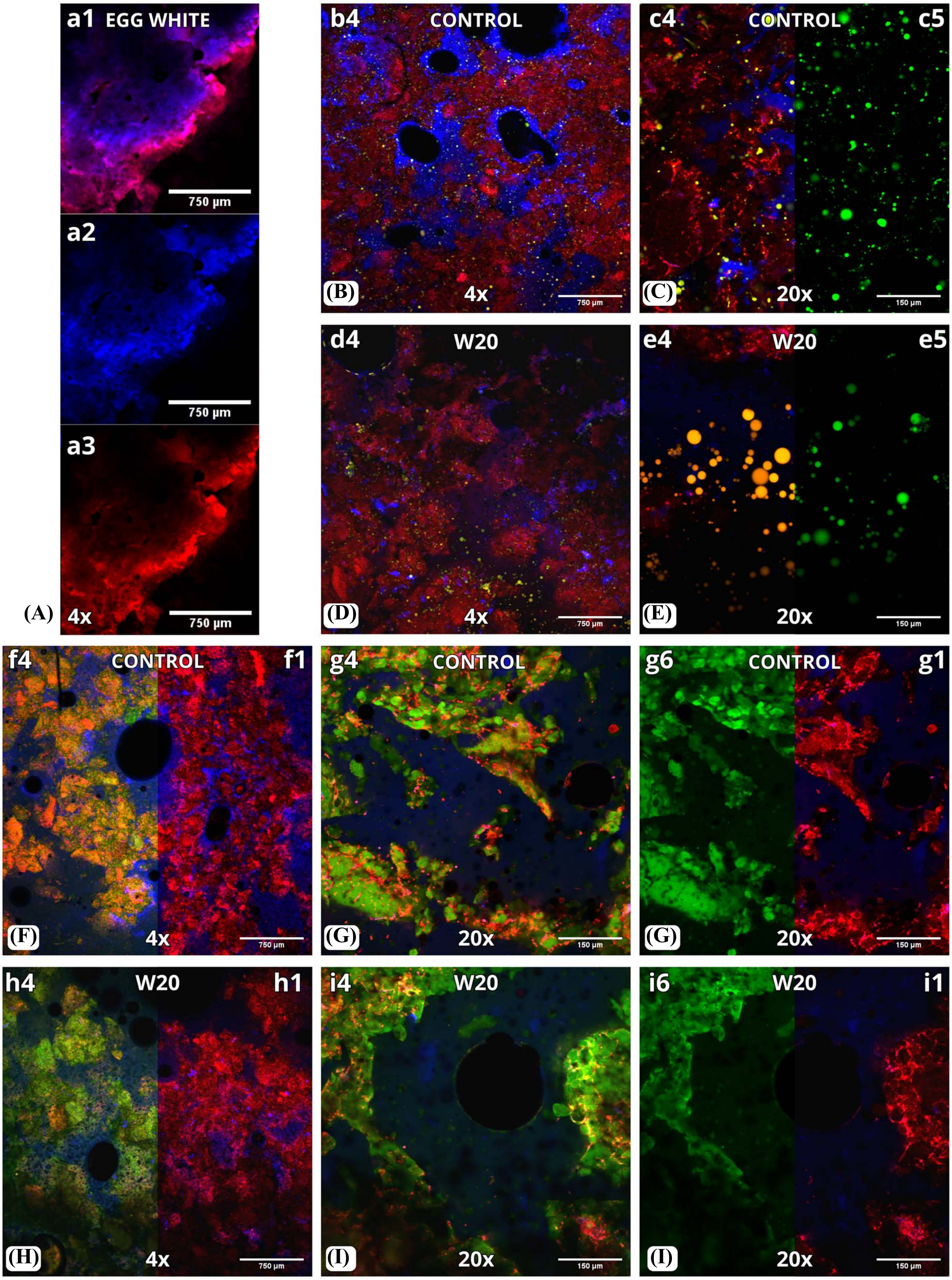


Figure 2. Micrographs of heat coagulated egg white and gluten-free crumbs by confocal scanning laser microscopy. Fluorophores mixes used were: Mix A: rodamine B (RB) + calcofluor white (CF), mix B: FITC + RB + CF and mix C: Nile red (NR) + RB + CF. samples were dyed as follows: (a) with mix A, (B–E) with mix B and (F–J) with mix C. The micrographs numbered as 1 showed RB and CF channels on, 2 showed CF channel on, 3 showed RB channel on, 4 showed all their corresponding channels on, 5 showed NR channel on, and 6 showed FITC channel on. Walnut addition levels were 0 and 200 g kg^{-1} of flour mixture (Control and W20, respectively).

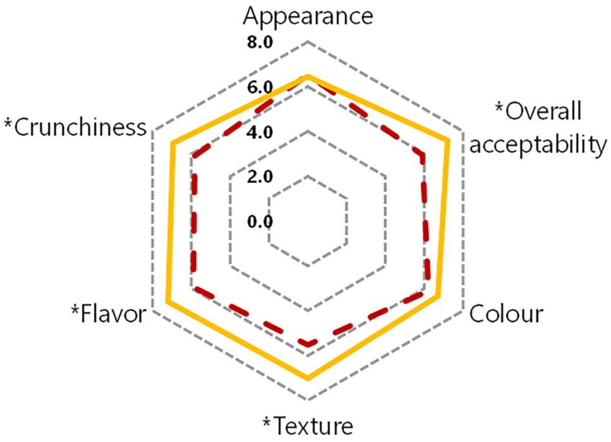


Figure 3. Sensory analysis of gluten-free breads (Control: dotted line and W20: solid line). Walnut addition levels were 0 and 200 g kg^{-1} of flour mixture (Control and W20, respectively). *Attributes with significant differences ($p < .05$, $n = 40$).

Figure 3 shows the results obtained in the sensory evaluation of bread with 20% of ground walnuts (W20) with respect to the control sample. The overall acceptability of bread with walnuts was significantly higher than the acceptability of control bread. In the same sense, the crunchiness, taste and crumb texture of bread get a significantly higher score in these attributes than the control sample ($p < .05$). Despite this, there were no significant differences between both samples in relation to appearance and colour. Thus, the overall acceptability would be highly influenced by the parameters connected with taste in the mouth and not by the ones related to visual perception.

Conclusion

The present work has demonstrated the feasibility of using non-defatted ground walnuts in the production of GF bread, even in high concentrations. The GF bread made with non-defatted ground walnuts showed a similar or better technological quality than control GF bread. Lipid oxidation did not increase in bread containing 10% and 20% walnuts; more assays are needed on lipid oxidation after bread storage. Furthermore, the addition of ground walnuts did not affect the gelatinisation of the starch nor the stabilising role of the protein in the crumb and the walnut-enriched products showed better sensory and nutritional characteristics than the control bread. Thus, GF bread made with non-defatted ground walnuts is a tasty and nutritive alternative for celiac individuals.

Supplementary material

Supplementary material is available at *International Journal of Food Science and Technology* online.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Author contributions

Juan José Burbano (Formal analysis [equal], Writing—original draft [equal], Writing—review & editing [equal]), Antonela David Gara (Formal analysis [equal], Investigation [equal]), Cristina Ferrero (Conceptualisation [equal], Data curation [equal], Funding acquisition [equal], Project administration [equal], Writing—original draft [equal]), and María Jimena Correa (Conceptualisation [equal], Data curation [equal], Formal analysis [equal], Funding acquisition [equal], Investigation [equal], Supervision [equal], Writing—original draft [equal])

Funding

Universidad Nacional de La Plata - UNLP (X1002) and Ministerio de Ciencia, Tecnología e Innovación de la Nación Argentina - MINCYT (PICT-2020-SERIEA-02634 and PICT 2019–00877).

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors wish to thank the kind cooperation of Fernanda Hamet and Enrique Portiansky in textural analysis, and CSLM, respectively.

Ethical guidelines

Ethics approval was not required for this research.

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International Journal of Food Science and Technology, 2025, **60(1)**, vvae084
https://doi.org/10.1093/ijfood/vvae084
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