

Research Article

Sensory and Nutritional Improvement of Gluten-Free Bread Using Sourdough and Seeds

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Abstract

Gluten-free (GF) bread making faces significant sensory, technological, and nutritional challenges. GF breads, typically formulated with commercial premixes, often exhibit deficiencies in texture, flavor, and nutritional value compared to wheat-based breads, leading to dissatisfaction among consumers with celiac disease or similar conditions. Although sourdough (SD) improves technological and sensory attributes, its production is laborious and complex. Starter cultures of selected lactic acid bacteria (LAB) strains are used to prepare alternative sourdoughs (type II), allowing for process standardization and shortening. The aim of this study was to obtain an improved GF bread formulation by using an SD starter culture and seed fortification to counteract the technological and sensory limitations inherent to GF bakery products. Therefore, commercial premixes were used to elaborate GF breads



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fermented with type II SD prepared with *Weissella confusa* 20 (W20) and fortified with seeds. Two commercial premixes (C and D) were selected for the elaboration of experimental breads (15% or 50% w/w replacement with SD, and 10% w/w addition of ground seeds). The formulation was optimized using a multidisciplinary approach, characterizing its mechanical behavior via TPA (texture profile analysis), its sensory profile through QDA (quantitative descriptive analysis), and its macro-and micronutrient composition via theoretical assessment.

Keywords

Sourdough; lactic acid starter; *Weissella confusa*; gluten-free breads; gluten-free premixes; seed breads; nutritional improvement

1. Introduction

Argentina ranks among the countries with the highest bread consumption, with an annual average of 72 kg per capita [1]. Interest in sourdough (SD) baked products, from both consumers and the food industry, is continuously growing [2], as is the demand for gluten-free foods. Bread remains the most sought-after product by celiac consumers [3].

However, gluten-free bread making poses a technological challenge, as gluten provides the viscoelastic structure characteristic of dough, and its replacement with starches and refined flours often results in products with inferior flavor and textural attributes.

Some studies indicate that SD can modify dough rheology, enabling the production of higher-quality gluten-free (GF) breads [4]. Its incorporation was shown to notably improve organoleptic characteristics, making it a relevant factor in bread acceptability [5]. Improvements in nutritional quality were also reported, as it increases mineral bioavailability and significantly reduces the bread's glycemic index [6].

Traditional SD is a natural starter culture established over time through the spontaneous fermentation of flour and water, mediated by lactic acid bacteria (LAB) and yeasts. Consequently, its standardization is complex and difficult to achieve. Furthermore, its preparation requires considerable time, skilled personnel, and precise control of processing parameters. One method to simplify the procedure while maintaining the positive outcomes of SD baking is the use of type II SD. This involves inoculating a flour-and-water mixture with selected microorganisms (a defined starter culture) and subjecting it to a single, prolonged fermentation at a controlled temperature. Among LAB of interest as starter cultures are several genera of lactobacilli, as well as some species from the genus *Weissella* [7]. Specifically, some strains belonging to *Weissella cibaria* and *Weissella confusa*, which are common in SD, were characterized by their high production of exopolysaccharides (EPS), primarily dextrans, in the presence of sucrose. These polymers have generated interest due to their prebiotic potential and wide range of industrial applications, especially in bakery products [8].

Most commercial GF premixes, commonly used by consumers to prepare their products [3], are composed of refined flours and starches, which are fiber-deficient and low in proteins, vitamins, minerals, and antioxidants. In contrast, many gluten-containing baked goods and flours possess up to three times more protein and a significantly lower energy and lipid content, particularly saturated

fatty acids. Furthermore, surveyed participants cited flavor, price, and nutritional content or added nutritional value as the main factors considered when selecting a GF product [3].

One strategy to enhance the nutritional profile of baked products is adding seeds, which can increase fiber, protein, and polyunsaturated fatty acid content [9-11]. It is worth noting that increased fiber intake is associated with improved gastrointestinal health, better body weight control, and the prevention of colon cancer, cardiovascular disease, and type 2 diabetes [12].

In a previous work, we selected strain *Weissella confusa* 20 (W20) as a defined lactic starter to prepare type II SD to further elaborate GF breads. Strain W20 produces high amounts of EPS in the presence of sucrose and demonstrated notable improvements in the sensory properties of GF sliced bread made from a commercial GF premix [13]. In this context, the present study aimed to extend the use of W20 as a lactic starter culture in other commercial premixes and to improve the nutritional profile of the resulting breads through the incorporation of seeds.

2. Materials and Methods

2.1 Preparation of Type II Sourdough with Commercial Premixes

To prepare type II SD, five commercial GF premixes were used: (A) Santa María (Ana Hernández Productos Alimenticios S.R.L., Buenos Aires, Argentina), (B) Dimax (DIMAX S.R.L., Córdoba, Argentina), (C) Celidarina (Agroalimentos PRAGA, Córdoba, Argentina), (D) Delicel (Compañía Argentina de Alimentos Saludables S.R.L., Buenos Aires, Argentina), and (E) Las 3 Harinas (Oscar Santomero, Santa Fe, Argentina).

The elaboration of type II SD was carried out by thawing a concentrated frozen culture (10^{10} CFU/ml, -20°C) of strain *Weissella confusa* 20 (W20). Strain W20 had been previously isolated, characterized, and identified at the Instituto de Lactología Industrial (INLAIN, UNL-CONICET) [14]. The lactic starter W20 was directly incorporated into the GF premixes (10^6 - 10^7 CFU/g final concentration), after the addition of table sugar (Chango, Ingenio y Refinería San Martín del Tabacal, Salta, Argentina) and powdered or fluid milk (San Regim, SanCor, Santa Fe, Argentina), according to each corresponding recipe. The high initial concentration of W20 allowed strain dominance in the matrix, given that the fermentation substrate is neither sterile nor feasible to sterilize. An additional amount of sugar (1.1%-1.5% w/w), based on the amount of premix, was added to promote the production of EPS by the strain. Each inoculated dough was incubated at 30°C for 24 h.

2.2 Bread Making

Control and experimental breads were prepared as follows. For the former preparation, doughs were prepared following each manufacturer's recipe, which included inoculation with commercial baker's yeast (*Saccharomyces cerevisiae*, 10^7 CFU/g final concentration) (Compañía Argentina de Levaduras S.A., CALSA, Lanús, Buenos Aires, Argentina). These doughs lacked LAB fermentation. On the other hand, experimental doughs required two fermentation steps: (i) first fermentation, to obtain type II SD by inoculating the dough with lactic ferment W20 (W20-SD) as detailed previously (section 2.1), and (ii) second fermentation (leavening step), in which the SD was added with the commercial baker's yeast (CALSA). Prior to inoculation with yeast, type II SD obtained with each commercial premix was added to the remaining ingredients (egg, salt, oil, and/or baking powder, depending on the specific recipe) to continue with bread elaboration. Then, both control and

experimental dough samples were placed in greased disposable aluminum molds, covered with plastic wrap, and incubated at 30°C for leavening until they doubled in volume. Baking was performed in a convection electric oven (Ultracomb Uc-70acn, Buenos Aires, Argentina) at 180°C until the crust was golden brown, followed by cooling at room temperature (20°C ± 2°C) for 1 h and packaging in polyethylene bags.

Determination of pH values, viable cell counts of W20 (MRS agar, 24 h at 30°C), total aerobic mesophilic bacteria (TAM; agar plate count, APC, 48 h at 30°C), and molds and yeasts (MY; Chloramphenicol Yeast Glucose, GCY agar, 3-5 d at 25°C) were performed at the required steps: before (t1) and after (t2) type II SD preparation (pH, TAM and W20 cell counts), and before (t3) and after (t4) the leavening step (pH, W20 and MY cell counts). Dough samples were homogenized in sterile saline, serially diluted, and incubated as previously stated.

2.3 Optimization of Sourdough Replacement Level

For the following assays, two premixes were selected based on preliminary sensory screening of breads (see section 2.6.1) and because of their nutritional composition. With each selected premix, three experimental breads were prepared by replacing 15%, 50%, or 100% (w/w) of the original dough with type II SD prepared with W20 (sourdough inoculum). For comparison, control breads were prepared following each manufacturer's recipe.

2.4 Selection and Incorporation of Seeds

In order to fortify the GF formulations, a commercial seed mix containing chia, sesame, and flaxseed (Genser, Mix Desayuno, Industrias Químicas y Mineras Timbo S.A., Buenos Aires, Argentina) was selected based on the following criteria:

- Fiber and protein content. Dietary fiber contribution was a determining factor, as all blends from the brand displayed the same protein content. The selected mix provides 3.3 g/100 g, higher than other blends (2.7 g/100 g). Although this difference may appear marginal, any increment was deemed valuable given that GF products generally exhibit low fiber content.
- Seed type. The selected brand offers three combinations, two of which include sunflower seeds. The blend without sunflower seeds was chosen because this seed imparts a strong flavor that could dominate the sensory profile of the breads. This choice aimed to achieve a more balanced combination of seed flavors in the mix.
- Presentation format. A packaged mix was preferred over bulk seeds to ensure consistency in the proportion of each seed type, thereby guaranteeing the standardization of the resulting breads. Furthermore, bulk seeds are typically more exposed to contamination and oxidation, which could negatively affect the quality of the final product.

Preliminary trials were conducted to determine the optimal percentage of seeds to be incorporated. The required quantity was calculated to ensure the final breads met the "source of fiber" claim defined by the Argentine Food Code (Código Alimentario Argentino, CAA; [15]), considering the fiber content of the selected mix. We initially tested a 30% (w/w) seed addition. Based on these results, a second trial used a 10% (w/w) level. In both trials, a control bread without seeds was prepared following the manufacturer's recipe. Breads were manufactured using a commercially known GF premix (Padoan, Tahin S.A., San Javier, Santa Fe, Argentina), as it had been repeatedly utilized in previous studies by the research group [16].

Seeds were incorporated by proportionally replacing a part of the premix with the calculated amount of the selected seed mix. The incorporation was performed at the final stage of dough preparation for control breads or after bulk fermentation, i.e., after SD-W20 preparation, for experimental breads, mixing them into the dough with a spatula before placing the dough into the mold.

Additionally, two incorporation methods were evaluated: (i) crushing using a stone mortar and (ii) grinding using a laboratory blade mill (Dalvo, Santa Fe, Argentina) to produce medium-ground flour.

Following the selection of the appropriate percentage of seeds for the breads, additional trials were carried out with the selected GF premixes and the adequate type II SD replacement (see section 2.3 Optimization of Sourdough Replacement Level).

2.5 Preparation of GF Sourdough Breads with Seed Addition

Based on preliminary results, three types of bread were prepared for each selected premix: (i) with 15% or 50% (w/w) of the dough replaced by W20-SD (breads W15 and W50, respectively), (ii) according to the original recipe with the addition of 10% (w/w) of seed mix (bread S) and (iii) with 15% or 50% w/w of W20-SD and 10% w/w of seeds (breads SW15 and SW50, respectively). To evaluate the proper performance of type II W20-SD and the baker's yeast, pH measurements (SA 720 pH meter, Orion, Beverly, MA, USA) and microbial counts were performed at critical points throughout the bread manufacturing process. W20 viable cells were counted using de Man, Rogosa and Sharpe agar (MRS, Biokar, Beauvais, France; 24 h at 30°C), total aerobic mesophilic microorganisms (TAM) on agar plate count (APC, Biokar; 48 h at 30°C), and baker's yeast on chloramphenicol glucose agar (CGA, Biokar; 5 d at 25°C) (Figure 1).

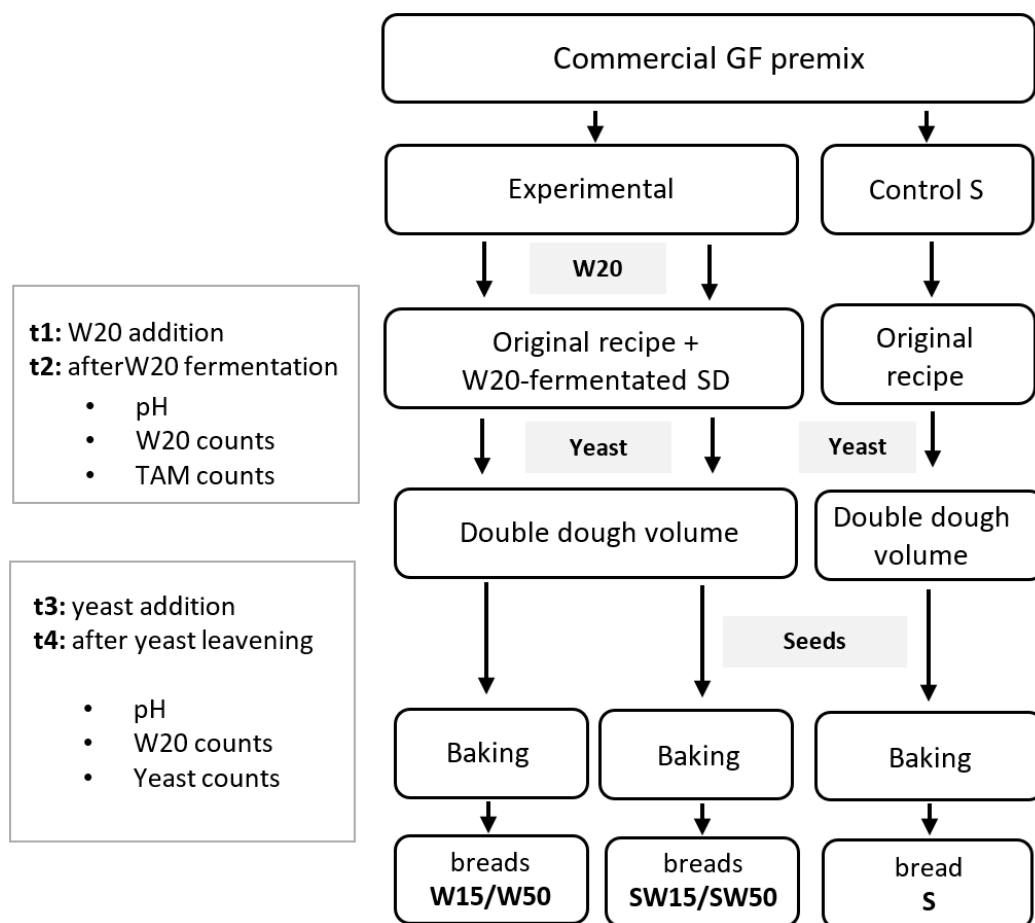


Figure 1 Schematic representation of gluten-free (GF) breads manufacturing using commercial GF premixes (C and D). Experimental bread was made with a percentage of W20-fermented sourdough (15% and 50% w/w for premix C and D, respectively), added (SW15/SW50) or not (W15/W50) with ground seeds (10% w/w) and baker's yeast. Control bread (S) was elaborated following the manufacturer's instructions, using baker's yeast and the addition of ground seeds (10% w/w). Times t1 to t4 correspond to W20 addition (t1), after W20 fermentation (t2), baker's yeast addition (t3), and after yeast leavening (t4).

2.6 Bread Evaluation

2.6.1 Sensory Analysis

A preliminary sensory screening was conducted before the descriptive sensory analysis to reduce the number of formulations included in the Quantitative Descriptive Analysis (QDA). The aim of this screening was to identify those formulations exhibiting the most desirable sensory characteristics and to exclude samples presenting evident sensory defects or poor overall performance. Five assessors with previous sensory evaluation experience evaluated the commercial premixes (A, B, C, D and E). During this step, appearance, aroma, flavor, texture, and overall sensory quality were assessed. Qualitative observations were recorded to select those premixes with the most balanced sensory profile and fewest sensory defects based on the overall consensus among the evaluators.

For the selected formulations, experimental breads (Eb-C and Eb-D, with seeds and fermented by W20-SD) and their corresponding control breads (Cb-C and Cb-D, original recipe without seeds, or lactic fermentation) were subsequently characterized by QDA using a trained sensory panel. For this purpose, a panel of eight trained assessors (two men and six women between 34 and 59 years old) from UNRaf (Universidad Nacional de Rafaela, Rafaela, Santa Fe, Argentina), with previous experience in quantitative descriptive sensory analysis (QDA), was convened. Before the sensory evaluation, training sessions were conducted using experimental GF breads made from commercial premixes C and D. During these sessions, the assessors developed, by consensus, a list of sensory descriptors related to appearance, aroma, flavor, taste, and texture that characterized the samples and established the definition of each descriptor, the evaluation procedure, and the scale anchors (Table 1). Additionally, GF breads made from different commercial premixes-distinct from the previous ones-and commercial sliced bread (white, whole wheat, and toasted) were used as scale references. Subsequently, the tested samples were evaluated in groups of two during individual sessions. The samples were presented at room temperature, in identical containers, and coded with random three-digit numbers. For each sample, a 15 mm thick slice of bread was served. The presentation order was balanced. The intensity of each attribute was measured on a 10 cm unstructured scale anchored at the extremes (1 and 9, representing the minimum and maximum perceived intensity, respectively). The sensory evaluation was carried out in standardized sensory booths according to ISO 8589:2007 [17], and water was provided as a palate cleanser.

Table 1 List of sensory descriptors related to appearance, aroma, flavor, taste and texture to characterize the bread samples, their definition, and scale anchors.

Descriptor	Definition	Anchor points	
		Minimum	Maximum
Crumb color	Visual intensity of crumb color.	1 = very light (sliced bread crumb)	9 = very dark (rye bread crumb)
Crumb porosity	Visual perception of the number, size, and distribution of air cells within the crumb.	1 = very weak	9 = very strong
Seed odor	Intensity of the characteristic odor of edible seeds.	1 = very weak	9 = very strong
Crumb firmness	Force required to compress the bread crumb.	1 = very soft	9 = very firm
Elasticity	Degree to which the bread crumb returns to its original shape after compression.	1 = slightly elastic	9 = highly elastic
Crumbliness	Degree to which the bread crumb breaks into small fragments.	1 = slightly crumbly	9 = highly crumbly
Salty taste	Intensity of the salty basic taste.	1 = very weak	9 = very strong
Seed flavor	Intensity of the characteristic flavor of edible seeds.	1 = very weak	9 = very strong
Pastiness	Degree to which the crumb forms a paste-like mass during chewing.	1 = slightly pasty	9 = highly pasty
Aftertaste	Persistence of flavor sensations after swallowing.	1 = very weak	9 = very strong

2.6.2 Texture Profile Analysis (TPA)

Texture profile analysis (TPA) was performed on cylindrical bread crumb specimens obtained from the center of 26-mm-thick bread slices using a 30-mm-diameter cork borer. Measurements were carried out using a Brookfield CTX texture analyzer (Brookfield Engineering Laboratories, Middleboro, MA, USA) equipped with a 50-kgf load cell (approximately 500 N), a TA-CTP rectangular flat compression platen (150 cm²), and a flat support platform of the same area.

Before each test, 1-2 drops of mineral oil were applied to both compression surfaces to minimize friction between the probe and the sample. Subsequently, the sample was positioned at the center of the lower compression plate, and the test was initiated. The applied load (force, N) exerted by the compression plate was recorded as a function of time (s) and distance (gel height) until 50% compression was achieved. The compression cycle was repeated twice. Ten replicates were performed for each sample.

2.6.3 Crumb Structure Evaluation

Three central bread slices of identical thickness were obtained from each loaf and scanned at high resolution (HP Scanjet 300, USA), and the images were analyzed with the ImageJ software (version 1.54k, September 2024; <https://imagej.net/ij/index.html>). Crumb structural parameters, including porosity (%), mean alveolar area (mm²), and alveolar density (number of alveoli/cm²), were determined from the scanned images. The total number of alveoli, individual alveolar area, and mean alveolar area were then determined. The remaining structural parameters were calculated from these measurements and the selected area. Porosity and alveolar density were calculated according to the following equations:

$$\text{Porosity (\%)} = (\text{Total alveolar area} / \text{Total cross-sectional area}) \times 100$$

$$\text{Alveolar density (alveoli/cm}^2\text{)} = \text{Total number of alveoli} / \text{Total cross-sectional area (cm}^2\text{)}$$

2.6.4 Bread Moisture and Bake Loss

Total moisture content of the sliced bread was determined using the two-stage official method according to AACC Method 62-05.01 of the Cereals & Grains Association (formerly the American Association of Cereal Chemists). This method provides a protocol for determining total moisture in fresh bread slices by air-drying samples to equilibrium, weighing, grinding to a homogeneous state, and performing final oven-drying. Total moisture content is calculated by combining the percentage loss from the air-drying stage with the subsequent oven-drying stage [18].

Bake loss was determined to account the amount of water lost during the baking process. The loaves were weighed on a digital balance (OHAUS Scout Pro SP2001, Ohaus Corporation, USA) prior to baking. Once baked and cooled, a second weighing was performed. The bake loss of four loaves per batch was analyzed and calculated according to the following formulas [19]:

$$\text{Moisture Lost (g)} = W_{\text{dough}} - W_{\text{bread}}$$

$$\text{Baking Loss (\%)} = [\text{Moisture Lost} / W_{\text{dough}}] \times 100$$

Where:

W_{dough} = weight of the dough before baking (g)

W_{bread} = weight of the baked and cooled bread (g)

2.7 Nutritional Composition

The nutritional composition of the breads was theoretically calculated based on the information declared on the nutritional labels of the ingredients used. For ingredients lacking commercial packaging or labeling, nutritional tables available on Nutrinfo [20] were consulted. This method was selected for its accessibility and feasibility, providing a reasonable approximation of the nutritional profile of the final products.

Nutritional labels were generated according to the specifications of the CAA. This involved calculating the contribution per gram of each ingredient in terms of energy value, carbohydrates, protein, total fat, saturated fat, trans fat, dietary fiber, and sodium. Subsequently, these values were multiplied by the specific quantities of ingredients used in each formulation and summed to obtain the total amounts corresponding to each loaf. Finally, the contents were expressed per serving. The portion size for the nutritional calculation was defined as 60 g, given that the CAA does not include specific references for homemade or GF breads. The 50 g reference applies exclusively to packaged breads [15]. The rationale was that artisanal breads tend to be denser than commercial ones because they lack typical industrial additives that lighten the product's texture. Furthermore, during the trials, the slices obtained typically weighed between 60 and 70 g, making the 60 g portion the most representative.

2.8 Statistical Analysis

Data are expressed as mean $\pm$ standard deviation (SD). Statistical analyses were performed using Statgraphics Centurion (Statgraphics Inc., Rockville, MD, USA). Differences among samples were evaluated by one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test when appropriate ($p < 0.05$). Crumb structure parameters, bread moisture, and bake loss were compared using Student's t-test ($p < 0.05$).

3. Results and Discussion

Based on the preliminary sensory screening, two premixes-designated as C and D-were selected for subsequent experimental trials. The remaining premixes were discarded as they did not show noticeable improvements.

3.1 Sourdough Replacement Level

Optimal incorporation levels of W20-SD were 15% w/w for premix C and 50% w/w for premix D. These formulations exhibited notable improvements in sensory attributes of the resulting breads.

For premix C, the use of a lower SD proportion helped to moderate the excessive acidity observed at higher levels, thus promoting a more balanced overall organoleptic profile.

For premix D, the most relevant improvements were associated with reduced floury flavor, odor, and mouthfeel linked to the presence of rice flour and starches in the commercial formulation.

These characteristics were negatively perceived in preliminary evaluation as palate fatigue following repeated exposure during preliminary trials.

3.2 Seed Fortification

The addition of 30% (w/w) of seeds resulted in bread classified as a “source” of dietary fiber, providing 4.0 g of fiber per serving. However, this proportion negatively affected the organoleptic properties of the product, reducing loaf volume by half (Figure 2A) and producing a markedly bitter taste. Consequently, this level was discarded.

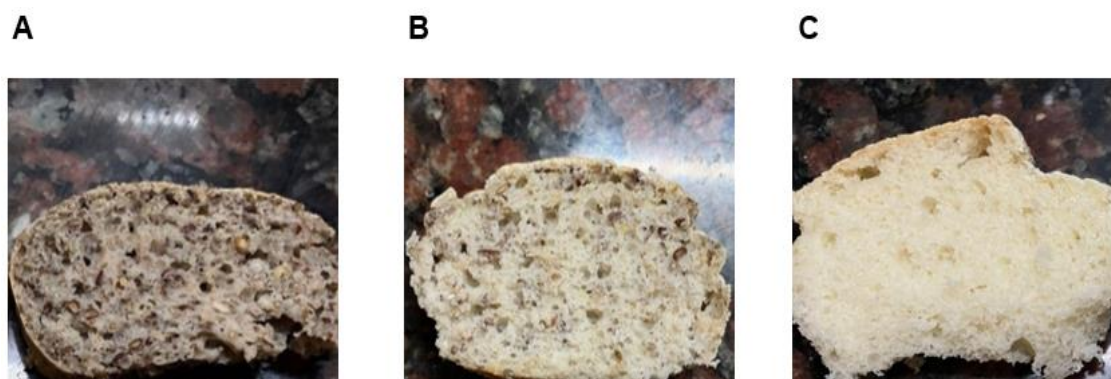


Figure 2 Breads from preliminary trials prepared with Padoan premix, containing (A) 30% w/w and (B) 10% w/w of seeds; (C) control bread with no seeds (original recipe).

The incorporation of 10% (w/w) of seeds yielded a bread with an acceptable fiber content (1.3 g per serving), causing only a moderate reduction in volume (Figure 2B) and contributing a pleasant flavor characteristic of the seeds. Based on these results, we selected this proportion for the final formulation. Figure 3 illustrates an experimental bread prepared using premix C with 10% w/w of seeds and 15% w/w of W20-SD.



Figure 3 Breads prepared using premix C. Control (left; original recipe) and experimental (right; containing 10% w/w of seeds and 15% w/w of W20-fermented SD).

Regarding seed processing, using seeds in flour form promoted more homogeneous incorporation into the dough. Conversely, seeds crushed using a stone mortar formed small agglomerates that detached during slicing, together with portions of crumb exhibiting higher moisture content. This behavior can be attributed to the grinding method, as the mortar produced

a considerably smaller particle-size reduction than the laboratory blade mill (Figure 4). Additionally, the mill not only improved uniformity but also streamlined the grinding process.



Figure 4 Ground seeds obtained using a stone mortar (top) and a laboratory blade mill (bottom).

3.3 Evaluation of the Fermentation Process

To assess the fermentation progress and validate the performance of the W20 starter, microbial counts were conducted at critical points (Figure 1) of the process using classical microbiological techniques. The high initial concentration of the W20 starter (approximately 10^6 - 10^7 CFU/g) in the doughs prepared with the selected GF premixes ensured the strain's dominance within the fermentation matrix. After the prolonged fermentation period (t1 to t2), a decrease in dough pH was observed, consistent with the increase in viable cell counts of W20 reaching approximately 10^9 CFU/g (Table 2). This behavior helped to limit the growth of the native microorganisms present in the ingredients, as reflected by the maintenance or reduction of TAM microorganisms (data not shown) relative to their initial levels (t1). Overall, these microbial counts confirmed the appropriate growth and effective predominance of the W20 starter.

Table 2 Microbiological counts and pH values determined during bread making using premixes C and D. Experimental breads (Eb) were elaborated with the total dough fermented with lactic starter W20 (100% w/w SD-W20); control breads (Cb) were made following each manufacturer's premix recipe.

Time ^a	pH		Viable cell counts (log CFU/g)					
			W20 ^b		TAM ^b		MY ^b	
	Cb	Eb	Cb	Eb	Cb	Eb	Cb	Eb
Premix C								
t1	nd	6.58	nd	5.8	nd	3.3	nd	nd
t2	nd	4.51	nd	8.3	nd	3.5	nd	nd
t3	6.15	4.43	nd	8.1	nd	3.2	5.9	5.9
t4	6.23	4.40	nd	8.1	nd	3.0	6.1	6.9
Premix D								
t1	nd	5.99	nd	7.1	nd	3.0	nd	nd
t2	nd	4.10	nd	8.8	nd	3.7	nd	nd
t3	6.38	5.09	nd	8.6	nd	2.3	8.1	8.0
t4	5.99	4.96	nd	9.0	nd	1.8	8.3	8.2

^a Sampling times: t1, immediately after the addition of lactic starter W20; t2, after W20 fermentation (30°C, 24 h); t3, immediately after baker's yeast addition; t4, after yeast's leavening (30°C until double dough volume).

^b Viable cell counts: strain W20 (MRS agar, 30°C, 24 h), total aerobic mesophilic bacteria (TAM; APC, 30°C, 48 h), and molds and yeasts (MY; CGY agar, 25°C, 5-7 d).

nd: not determined.

The proper performance of the added baker's yeast was verified. In the experimental breads, yeast counts at times t3 and t4 were comparable to those obtained in the control breads (differences of approximately 0.1-0.8 log units CFU/g), demonstrating that W20 exerted no inhibitory effect on baker's yeast. Furthermore, for each premix, both the control and the experimental bread, the time required to double dough volume during yeast fermentation was. All breads doubled their volume, reaching the same final height. The same effect was observed in breads prepared by partially replacing the dough with W20-SD (see section 3.1) and fortified with seeds.

3.4 Bread Evaluation

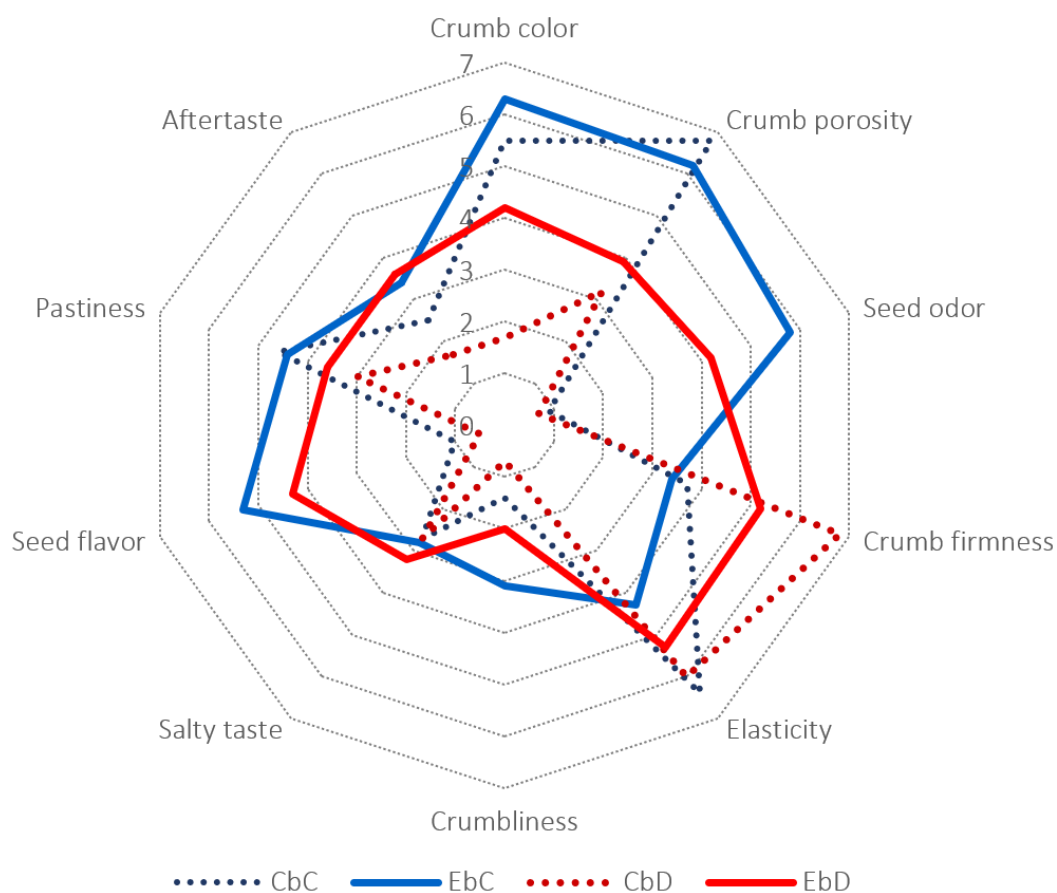
3.4.1 Sensory Analysis

Table 3 presents the mean intensity scores $\pm$ standard deviation obtained for each sensory descriptor evaluated in the GF sliced breads. Significant differences ($p < 0.05$) were observed among samples for all sensory descriptors, except for salty taste. Figure 5 shows the radar chart obtained from the QDA of GF sliced breads prepared with premixes C and D. Control breads (Cb-C and Cb-D) were formulated without W20-SD substitution. In contrast, experimental breads (Eb-C and Eb-D) contained 15% and 50% (w/w) W20-SD, respectively. All formulations contained 10% (w/w) ground seeds.

Table 3 Sensory descriptors evaluated in gluten-free sliced breads (mean intensity scores $\pm$ standard deviation) and the corresponding statistical analysis.

Descriptor	Premix C		Premix D		<i>p</i> -value	F-value
	Cb-C	Eb-C	Cb-D	Eb-D		
Crumb color	5.5 $\pm$ 0.8 ^c	6.3 $\pm$ 0.8 ^d	1.7 $\pm$ 0.4 ^a	4.2 $\pm$ 0.9 ^b	<0.0001	58.61
Crumb porosity	6.8 $\pm$ 0.8 ^b	6.2 $\pm$ 0.8 ^b	3.2 $\pm$ 0.8 ^a	3.9 $\pm$ 1.0 ^a	<0.0001	33.04
Seed odor	0.9 $\pm$ 0.6 ^a	5.8 $\pm$ 0.7 ^c	0.7 $\pm$ 0.9 ^a	4.2 $\pm$ 0.9 ^b	<0.0001	79.67
Crumb firmness	3.7 $\pm$ 0.6 ^a	3.4 $\pm$ 0.6 ^a	6.8 $\pm$ 0.8 ^c	5.2 $\pm$ 0.7 ^b	<0.0001	46.14
Elasticity	6.4 $\pm$ 0.7 ^c	4.3 $\pm$ 0.7 ^a	6.0 $\pm$ 0.8 ^c	5.3 $\pm$ 0.6 ^b	<0.0001	14.03
Crumbliness	1.4 $\pm$ 0.4 ^b	3.1 $\pm$ 0.7 ^d	0.7 $\pm$ 0.5 ^a	2.0 $\pm$ 0.5 ^c	<0.0001	28.20
Salty taste	2.7 $\pm$ 1.0	2.8 $\pm$ 1.1	2.7 $\pm$ 1.1	3.2 $\pm$ 1.0	0.33	0.33
Seed flavor	1.0 $\pm$ 0.6 ^a	5.3 $\pm$ 0.9 ^c	0.5 $\pm$ 0.4 ^a	4.3 $\pm$ 1.1 ^b	<0.0001	70.37
Pastiness	4.6 $\pm$ 0.9 ^b	4.4 $\pm$ 0.8 ^b	3.0 $\pm$ 0.9 ^a	3.6 $\pm$ 0.6 ^a	0.0012	6.98
Aftertaste	2.5 $\pm$ 1.0 ^a	3.4 $\pm$ 0.8 ^b	1.7 $\pm$ 0.7 ^a	3.6 $\pm$ 0.9 ^b	0.0006	7.74

Different letters in the same row indicate significant statistical differences ($p < 0.05$).

**Figure 5** Radar chart derived from the Quantitative Descriptive Analysis (QDA) of gluten-free sliced breads prepared without (Cb-C and Cb-D, control breads) or with W20-fermented sourdough (15% and 50% w/w for premix C and D, respectively) and ground seeds (10% w/w) (Eb-C and Eb-D).

Crumb color intensity was significantly higher in breads prepared with premix C than in those prepared with premix D. According to the ingredient declaration provided by the manufacturer, the premix C formulation contains buckwheat and sorghum flours, both of which naturally exhibit darker pigmentation than the major ingredients declared for premix D, namely corn starch, rice flour, and cassava starch. Since buckwheat and sorghum flours exhibit naturally darker pigmentation than refined starches and rice flour, their presence probably contributed to the greater crumb color intensity observed in these breads [21]. In addition, the incorporation of seeds further increased crumb color intensity, probably due to their natural pigments and enhanced Maillard reactions during baking.

Breads prepared with premix C exhibited higher crumb porosity than those prepared with premix D, whereas neither seed addition nor fermentation with strain W20 significantly affected this attribute. This finding may reflect differences in the composition of the commercial premixes. Previous studies have reported that incorporating buckwheat flour into GF bread formulations improves crumb porosity and gas-cell distribution compared with starch-based formulations [22]. The crumb structure of GF breads is strongly influenced by the ability of the formulation to stabilize gas cells during fermentation and baking, which depends on interactions among starches, proteins, hydrocolloids, and other flour components [23].

The experimental breads (Eb-C and Eb-D) exhibited a markedly different odor and flavor profile from their respective controls (Cb-C and Cb-D). As expected, seed odor and seed flavor were practically absent in the control samples but significantly increased in both experimental formulations, with Eb-C presenting the highest intensities. Similar findings have been reported in GF formulations enriched with buckwheat or flaxseed-derived ingredients, where the addition of seed-based materials intensified characteristic odor and flavor notes associated with these raw materials [21, 24]. In addition, the fermentation process may have further contributed to the development and perception of these sensory attributes. Sourdough fermentation is known to promote the formation and release of volatile compounds through microbial metabolism and enzymatic reactions, enhancing the complexity and intensity of bread aroma and flavor [25].

Regarding texture, Cb-D exhibited the highest crumb firmness, followed by Eb-D, whereas Cb-C and Eb-C showed the lowest crumb firmness values. Seed addition and fermentation with W20 reduced crumb elasticity after deformation. Accordingly, both control breads were more elastic than their corresponding experimental counterparts, with Eb-C showing the lowest elasticity among all samples. Crumbliness increased in the following order: Cb-D < Cb-C < Eb-D < Eb-C. Thus, seed addition combined with fermentation using strain W20 resulted in breads with a greater tendency to crumble. Nevertheless, the mean crumbliness scores remained low, with average intensity values below the midpoint of the 10-cm scale. Additionally, breads prepared with premix C exhibited slightly higher pastiness than those prepared with premix D. Texture is considered one of the most important quality attributes of GF bread because the absence of a continuous gluten network results in weaker structural cohesion, reduced elasticity, and a greater tendency of the crumb to fracture during mastication [23, 26]. The greater crumbliness may also be related to the incorporation of seeds, which can modify the structural continuity of the GF matrix and reduce crumb cohesiveness. Recent studies have shown that seed-derived ingredients may alter textural parameters such as cohesiveness and springiness depending on their type and concentration [27]. In GF breads, reduced crumb cohesion and elasticity are closely related to the absence of a continuous gluten network and are perceived as increased crumbliness [23]. Although sourdough

fermentation is also known to influence crumb texture through acidification, enzymatic activity, and the production of exopolysaccharides by lactic acid bacteria, the present experimental design does not allow discrimination between the individual effects of fermentation and seed incorporation, since both factors were simultaneously introduced in the experimental formulations. Therefore, the greater crumbliness observed in Eb-C and Eb-D is likely the result of the combined interaction between seed addition, sourdough fermentation, and the composition of each commercial premix.

Saltiness was perceived weakly and did not differ significantly among samples. Aftertaste flavor intensity was lower in the Cb than in the Eb samples, regardless of the commercial premix used. Seed addition and fermentation with strain W20 increased flavor persistence after swallowing. Seeds contain characteristic volatile and non-volatile compounds that contribute to flavor persistence. In contrast, sourdough fermentation promotes the formation of organic acids and other metabolites that increase the complexity and persistence of sensory perception. Consequently, these compounds may prolong flavor perception after swallowing, resulting in a more pronounced aftertaste [25].

When asked to describe the residual sensations perceived after swallowing, panelists most frequently reported bitter, seed-like, and fermented flavor notes.

3.4.2 Texture Profile Analysis (TPA)

The mean values and standard deviations of the texture parameters obtained by texture profile analysis (TPA) are presented in Table 4. Sample Cb-D did not fracture after being compressed to 50% of its original height. In contrast, the remaining samples exhibited fracture during compression, with the experimental breads (Eb-C and Eb-D) requiring significantly higher fracture forces.

Table 4 Texture characterization of breads elaborated with premixes C and D, with (experimental breads) or without (control breads) substitution with W20-sourdough (15% and 50% p/p for C and D, respectively). All breads were added of 10% p/p of seeds.

	Premix C		Premix D	
	Cb-C	Eb-C	Cb-D	Eb-D
Fracturability	6.4 ± 0.9 ^a	11.1 ± 0.2 ^b	nf	11.2 ± 0.9 ^b
Firmness	12.1 ± 3.2 ^a	14.0 ± 2.4 ^a	47.5 ± 4.6 ^c	20.6 ± 1.6 ^b
Cohesivity	0.224 ± 0.040 ^b	0.188 ± 0.016 ^a	0.403 ± 0.027 ^d	0.288 ± 0.021 ^c
Elasticity	0.489 ± 0.042 ^b	0.372 ± 0.031 ^a	0.858 ± 0.019 ^d	0.701 ± 0.027 ^c
Chewiness	1.5 ± 0.8 ^a	1.3 ± 0.3 ^a	15.2 ± 1.6 ^c	3.9 ± 0.6 ^b

Cb and Eb, control and experimental samples.

^{a-d} Values in the same row with different lowercase letters differ significantly ($p < 0.05$).

nf: not fractured.

Regarding firmness, breads prepared with premix D were significantly firmer than those prepared with premix C. Among the breads prepared with premix D, control breads (Cb-D) exhibited higher firmness than experimental ones (Eb-D). In contrast, no significant differences were observed between those elaborated with premix C (Cb-C and Eb-C).

Since cohesiveness ranges from 0 to 1, the values obtained indicate that all breads exhibited relatively low cohesiveness. Cohesiveness decreased in the following order: Cb-D > Eb-D > Cb-C > Eb-C.

Formulation also influenced bread elasticity. Breads made with premix D were significantly more elastic than those prepared with premix C, and both seed addition and fermentation with strain W20 further affected this parameter. Elasticity decreased in the following order: Cb-D > Eb-D > Cb-C > Eb-C.

Chewiness, as a secondary TPA parameter derived from hardness, cohesiveness, and springiness, followed a similar trend. Breads made with premix C (both Cb-C and Eb-C) exhibited the lowest chewiness values, whereas Cb-D showed significantly higher chewiness than Eb-D.

Instrumental texture results were consistent with those from the quantitative descriptive analysis (QDA). Both firmness and elasticity followed the same trend in both studies.

3.4.3 Crumb Structure Evaluation

Digital image analysis was used to quantitatively assess the crumb grain structure. Significant differences ($p < 0.05$) were observed between control (Cb) and experimental breads (Eb) prepared with both premixes for most crumb parameters (Figure 6 and Figure 7), except for alveolar density in breads prepared with premix D.

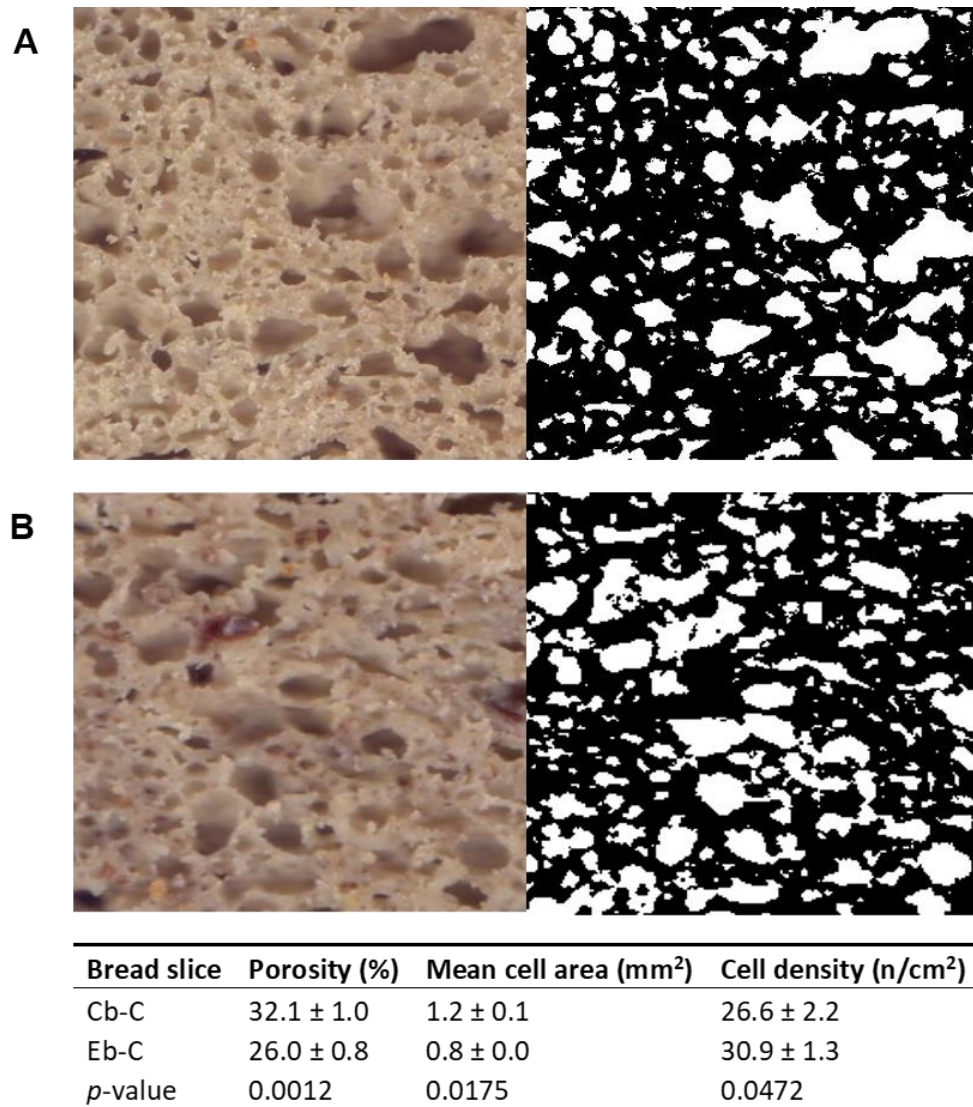


Figure 6 Original (left) and computed binary (right) images of crumb structure of GF breads corresponding to control (A) and experimental (B) breads made using premix C. The table below shows crumb parameters (mean ± standard deviation) and the corresponding statistical analysis. Cb-C and Eb-C correspond to control and experimental samples.

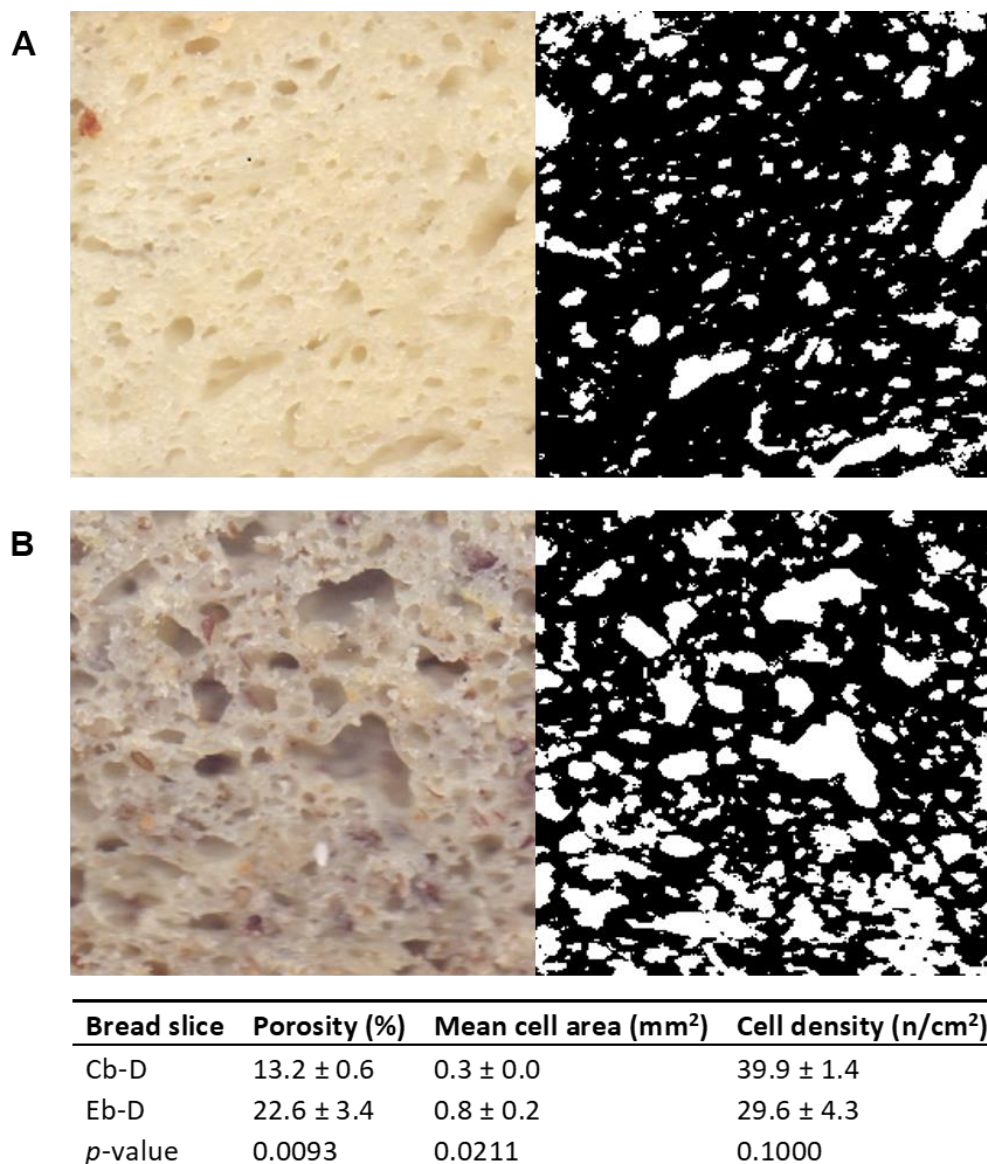


Figure 7 Original (left) and computed binary (right) images of crumb structure of GF breads corresponding to control (A) and experimental (B) breads made using premix D. The table below shows crumb parameters (media ± standard deviation) and the corresponding statistical analysis. Cb-D and Eb-D correspond to control and experimental samples.

The Eb bread prepared with premix C (Eb-C) exhibited a higher number of smaller alveoli and lower porosity than its control (Cb-C), together with a slightly more uniform alveolar distribution (Figure 6). These results were consistent with the preliminary sensory evaluation. The low and similar variability among slices indicated good structural uniformity. At the same time, the lower alveolar density of the experimental bread was also considered positive, as it reflected a more homogeneous crumb structure.

The breads prepared with premix D exhibited similar numbers of alveoli, but the Eb-D sample showed higher porosity and larger alveoli than Cb-D. Alveolar distribution agreed with the preliminary assays, whereas alveolar size did not; larger alveoli were perceived in Cb-D during the sensory evaluation, while image analysis indicated larger alveoli in Eb-D slices (Figure 7).

The Eb-D samples showed greater variability among slices in porosity and alveolar density, whereas the other parameters exhibited low and similar variability. Overall, the higher porosity and alveolar size observed in the experimental bread indicated a more aerated crumb.

According to the scheme proposed by Dallman [28], sliced bread is characterized by numerous small, uniformly distributed alveoli. Based on these criteria, the slices from Eb-C exhibited a more desirable crumb structure than its control. Although the opposite trend was observed for the breads made with premix D, Eb-D (50%) showed a crumb structure similar to that of Eb-C (15% W20), as both exhibited the same value (0.8). Although W20-fermented MM has been reported to reduce alveolar size [14], the presence of ground seeds may have modified this effect by physically disrupting the alveoli [29, 30] and altering their expansion [31]. Since Dallman's scheme was developed for breads without seeds, the use of scales specifically developed for seeded breads may provide a more appropriate basis for comparison, as their crumb structure may differ.

3.5 Bread Moisture and Bake Loss

Bake loss between Cb and Eb differed significantly ($p < 0.05$) in breads prepared with premix C, but remained unchanged in premix D formulations (Cb-D and Eb-D). In contrast, bread moisture showed no significant differences ($p > 0.05$) (Table 5). High hydrocolloid (xanthan gum) content in premix D could probably mask any additional water-retention effect from seeds and the EPS produced by W20. Conversely, in *Celidarina* breads, crumb moisture was similar between the control and experimental formulations, but bake loss was higher in the control. Since premix C also contains xanthan gum, its recipe requires a higher sucrose concentration-essential for EPS production- than premix D. Therefore, enhanced EPS production (capable of binding water) during the W20 fermentation might correlate with higher water retention. The synthesized EPS, along with the added seeds, may have effectively retained water during baking. The lack of significant differences in crumb moisture could be attributed to the water-binding mechanisms within the matrix and the analytical method used (oven-drying), which only measures free water. Consequently, the Eb-C likely possesses higher overall water content than the Cb-C, but held as bound water due to the EPS and seeds, thereby explaining the lower bake loss.

Table 5 Bread moisture (%) and baking loss (%) obtained from GF breads made with premix C and D. Values correspond to the mean $\pm$ SD, along with the statistical analysis.

	Bread moisture (%)	Baking loss (%)
Premix C		
Cb-C	42.5 $\pm$ 0.0	9.0 $\pm$ 0.1
Eb-C	41.5 $\pm$ 0.0	7.1 $\pm$ 0.5
<i>p</i> -value	0.3333	0.0041
Premix D		
Cb-D	41.6 $\pm$ 0.0	6.6 $\pm$ 0.4
Eb-D	41.6 $\pm$ 0.1	6.6 $\pm$ 0.6
<i>p</i> -value	>0.9999	>0.9999

Cb-C and Cb-D, control breads made with premix C and D, respectively (original recipe); Eb-C and Eb-D, experimental breads made with premix C and D with 15% and 50% of substitution with W20-SD, respectively, added of ground seeds (10% w/w).

p-values < 0.05 indicate significant statistical differences.

3.6 Nutritional Composition

Based on theoretical calculations obtained for both GF premixes, the partial replacement of the formulation with ground seeds in the experimental breads led to a significant increase in fiber and total fat content, without substantially affecting saturated fat levels or the remaining nutritional components. This outcome is favorable considering the nutritional benefits provided by seed-derived fiber and fats, which are largely health-promoting and include essential omega-3 and omega-6 fatty acids.

Notably, the experimental bread made with premix C (Table 6) qualified as a “source of fiber” (>2.5 g/serving) and a “source of protein” (>6 g/serving), according to the criteria established by the CAA [15]. In contrast, experimental bread prepared with premix D (Table 7) did not achieve these classifications; however, its fiber content was 5.7 times higher than that of its respective control.

Regarding energy value and the remaining nutrients, no significant differences were observed between experimental breads and their respective controls (Table 6 and Table 7). This outcome is favorable, since it indicates that both experimental breads provide energy and not excessive saturated fat levels. Nonetheless, all breads exhibited high sodium content (>300 mg/100 g) [32], attributable to the composition of the commercial premixes used.

Table 6 Nutritional label of experimental and control breads prepared with premix C. Experimental breads contain 10% w/w of seeds, and 15% w/w of substitution with W20-fermented sourdough; control breads were prepared following the original recipe.

Nutritional facts	Control		Experimental	
	Per serving ^a	DV ^b	Per serving ^a	DV ^b
Calories	136 kcal = 5692 kJ	7	144 kcal = 602 kJ	7
Carbohydrates	19.3 g	6	16.2 g	5
Proteins	9.2 g	12	8.9 g	12
Total fat	2.4 g	4	4.8 g	9
Saturated fat	0.3 g	1	0.5 g	3
Trans fat	0.0 g	-	0.0 g	-
Dietary fiber	1.5 g	6	2.6 g	10
Sodium	323 mg	13	293 mg	12

^a 60 g, one slice; ^b Percent daily value (%).

Table 7 Nutritional label of experimental and control breads prepared with premix D. Experimental breads contain 10% w/w of seeds, and 50% w/w of substitution with W20-fermented sourdough; control breads were prepared following the original recipe.

Nutritional facts	Control		Experimental	
	Per serving ^a	DV ^b	Per serving ^a	DV ^b
Calories	144 kcal = 602 kJ	7	155 kcal = 684 kJ	8
Carbohydrates	26.4 g	9	22.3 g	7
Proteins	2.7 g	4	3.8 g	5
Total fat	3.9 g	7	6.2 g	11
Saturated fat	0.5 g	2	0.8 g	4
Trans fat	0.0 g	-	0.0 g	-
Dietary fiber	0.3 g	1	1.7 g	7
Sodium	238 mg	10	235 mg	10

^a 60 g, one slice; ^b Percent daily value (%).

Although not quantified in this study, the incorporation of seeds also implies an additional contribution of vitamins and minerals, including B-complex vitamins, fat-soluble vitamins (particularly E and K), and minerals such as potassium, calcium, iron, magnesium, zinc, and phosphorus [33]. Additionally, there is scientific evidence that prolonged fermentation with SD can enhance the bioavailability of macro- and micronutrients [34, 35].

4. Conclusions

The application of W20 lactic starter was successfully extended to diverse commercial GF premixes, effectively enhancing the organoleptic profile of the developed breads.

Microbiological analyses confirmed the proper performance and predominance of W20 starter during fermentation, as well as its compatibility with baker's yeast, ensuring optimal W20 growth and appropriate dough leavening in both formulations.

The incorporation of W20-SD into GF bread making process resulted in products with improved sensory attributes compared with breads fermented solely with baker's yeast, demonstrating the versatility of strain W20 to adapt to new GF matrices. Fermentation with W20 type II SD significantly enhanced the odor, flavor, and texture of breads produced with the selected premixes (C and D) by replacing part of the dough with SD at different proportions (15% w/w for C and 50% w/w for D).

The addition of 10% w/w of seeds in flour form favored a more homogeneous integration into the dough, avoiding a marked reduction in loaf volume and contributing to a crumb structure comparable to that of traditional sliced bread. Furthermore, fortification with seeds provided a pleasant flavor and, based on theoretical estimations, significantly improved the nutritional value of the GF bread by elevating the dietary fiber content and enriching its composition with key nutritional compounds.

The results suggest that the incorporation of W20 as a lactic starter culture, fortified with optimal seed concentration (10% w/w), can enhance the sensory and nutritional characteristics of GF bread formulations. These optimized formulations could offer consumers a high-quality alternative with improved sensory and nutritional profiles.

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Author Contributions

Natalia G. Saez: Conceptualization, Investigation, Formal analysis, Visualization, Writing-original draft; María V. Lancelle Cedrolla: Investigation; Alicia E. Gómez: Investigation; Yanina Pavón: Methodology, Supervision; Daniela M. Guglielmotti: Conceptualization, Funding acquisition, Methodology, Supervision, Writing-review & editing; María L. Capra: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing-review & editing.

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Competing Interests

The authors have declared that no competing interests exist.

AI-Assisted Technologies Statement

The authors declare that ChatGPT AI tool was used exclusively to facilitate language editing and improve sentence clarity during manuscript writing. All scientific content, data synthesis, and conclusions are entirely the responsibility of the authors.

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