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Harnessing the Power of Natural Mineral Waters in Bread Formulations: Effects on Chemical, Physical, and Physicochemical Properties

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Abstract: This study aimed to evaluate the feasibility of incorporating natural mineral waters (NMW), including thermal water (TW) and bottled mineral water (BMW), into bread ('biju' type) to enhance its mineral content and explore their impacts on physicochemical, technological, biochemical, and chemical composition. NMW, rich in sodium, potassium, and magnesium, resulted in bread formulations with higher contents of these minerals and greater total mineral levels, thus potentially enriching food products. Proximate composition analysis showed no significant differences in moisture, proteins, carbohydrates, and energy, except for lipids and soluble sugars. Texture analysis revealed that water type influenced textural properties, with salt content affecting hardness, springiness, and cohesiveness. Viability analysis of *Saccharomyces cerevisiae* showed consistent results across formulations, suggesting water pH and mineral content did not significantly affect fermentation. In addition, bread formulations without added salt were developed to assess the potential of sodium-rich NMW as a natural source of salt. For these samples, and considering the parameters assessed, except for salt and sodium content, the differences observed were slight compared to salt-added formulations, highlighting NMW's potential to produce low-salt bread. These findings not only enhance the value of local resources but also offer an innovative and sustainable strategy for utilizing NMW springs across Europe.

Keywords: natural mineral waters; bread; chemical composition; physical properties; minerals

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1. Introduction

Due to continuous changes in the market and increased competition between industries, the food sector is progressively under pressure to provide healthy foods that meet consumers' daily nutritional needs. This has led the food industry to prioritize the development of new products and the search for innovative ingredients that may introduce novel dietary, functional, and organoleptic qualities to food products [1,2].

In this context, bread has been a staple food in the human diet for ages and is one of the world's most consumed foods, representing an important source of carbohydrates and energy for people worldwide. Different types of bread can also provide proteins, fibers, minerals, and vitamin B [3]. Therefore, due to its high consumption and accessibility, bread represents a good model for developing innovative, nutrient-enriched, value-added products.

Natural mineral waters (NMW) originate from underground reservoirs, exhibiting specific microbiological features and, occasionally, health-promoting effects [4]. They differ from drinking water due to their spring purity and consistent mineral content, encompassing a spectrum of elements such as calcium, magnesium, potassium, sodium, and trace minerals [5]. Among different types of NMW, thermal water (TW) is also distinguished due to its unique mineral and oligo-element profiles, attributed to myriad therapeutic effects [4,6]. TW receives this designation independently of the temperature at the source as long as it emerges inside a thermal resort and has therapeutic applications [6]. Portugal is one of the European countries richest in thermal sites, predominantly located in the northern region.

Despite the well-recognized richness of NMW as a source of mineral compounds, the potential of this resource remains underexplored as an ingredient in food products. Notably, the potential of TW in food manufacturing has been thoroughly neglected. Thus, this study proposed that NMW be utilized beyond its conventional purposes and incorporated into a bread formulation, aiming to prospect the potential of this natural resource to enrich bread with mineral compounds, creating a nutritive and innovative product with distinct sensory flavors and characteristics. Additionally, NMW is typically abundant at its source but has limited practical applications. By demonstrating its feasibility in bread production, we introduce a novel approach to its utilization, serving as a model for sustainable exploration of NMW springs across Europe, which is paramount to promoting their valorization and local development.

Importantly, the final quality of bread, including technological, biochemical, and nutritional aspects, may be strongly affected by the added components [7,8], making it essential to evaluate the impact of incorporating novel ingredients on these parameters. In light of this, the aims of this study were: (a) to produce 'biju' bread, a popular and traditional bread in Portugal, with NMW, including TW; (b) to evaluate the impact of NMW incorporation on the mineral content of the formulated bread; and (c) to determine the effect of NMW on the biochemical (yeast viability), texture, chemical, and physicochemical properties of bread.

2. Materials and Methods

2.1. Food Ingredients

All the ingredients for the bread production were acquired in local markets in the city of Chaves, Portugal. TW was manually collected in local public sources in sterile polyethylene bottles and used directly in the bread formulation. Drinking water was collected from the tap, while bottled mineral water was purchased in local markets of Chaves, Portugal. Quality control analyses (physicochemical measurements, microbiological assessment) were made on both water types to ensure their suitability for food production.

2.2. Bread Preparation

The bread production process goes through several stages: weighing and mixing the ingredients, molding, fermentation, and baking. All the ingredients, including the water, were weighed in this work. To make 2.5 kg of dough, 2.250 kg of Type 45 flour, 250 g of Type 70 flour, 125 g of Bavaria, 25 g of special Grampan, 2 kg of water, 45 g of salt (NaCl), 75 g of yeast (*S. cerevisiae*), and 75 g of olive oil were weighed. First, in a spiral mixer (model AEF012, Ferneto), the salt and flours were mixed for 3 min (min) on rotation I. After this time, 1.5 kg of drinking water (DW), bottled mineral water (BMW), or thermal water (TW) was added for another 3 min. At 6 min, the rotation was changed to position II. Two minutes after switching to rotation II, the olive oil was slowly added, followed by the remaining water, leaving it to beat until 6 min had passed. At 12 min, the yeast was added, and the dough was left to beat for another 6 min on rotation II. After this time, the dough rose for approximately 40 min. Once this step was completed, the dough was divided into two parts of roughly 2.750 kg, each of which resulted in 30 breads of approximately 90 g. After this step, the doughs were modulated and left to rest at a temperature of ±30 °C for 30 min. At the end of this time, the breads were placed in an oven for 10 min at 41 °C and 77% humidity. Finally, the bread was baked for 13 min at a top temperature of ±233 °C and a bottom temperature of ±212 °C. Therefore, three bread formulations were developed: (1) biju bread with drinking water (B_DW), (2) biju bread with thermal water (B_TW), and (3) biju bread with bottled mineral water (B_BMW).

Given the high sodium content observed in both BMW and TW (Table 1), which caused an increment in the levels of sodium in these samples, bread incorporated with these types of water was also prepared with no added salt, yielding two more samples, namely (4) B_TWns and (5) B_BMWns.

Table 1. Mineral composition (mg/L) of drinking water (DW), thermal water (TW), and bottled mineral water (BMW).

Chemical Composition	DW	TW	BMW
Calcium (Ca)	9.8	23	102
Magnesium (Mg)	<1.0	5.0	24
Silica (SiO_2)	-	70	62
Nitrate (NO_3)	<2.0	<0.30	<0.25
Sodium (Na)	2.8	511	577
Potassium (K)	<1-	56	28
Lithium (Li)	<0.1	2.3	1.9
Iron (Fe)	<0.05	0.23	<0.01
Manganese (Mn)	<0.015	0.05	0.20
Lead (Pb)	<0.003	<0.003	<0.003
Cadmium (Cd)	<0.0005	<0.0004	<0.0004
Aluminum (Al)	0.032	<0.003	0.021
Copper (Cu)	<0.020	<0.002	<0.002
Chromium (Cr)	<0.002	<0.001	0.0022
Nickel (Ni)	<0.005	<0.005	<0.005
Selenium (Se)	<0.0005	<0.0004	<0.0004
Arsenic (As)	<0.00059	0.119	0.0011
Zinc (Zn)	-	<0.05	0.0028
Total	12.63	667.70	795.13

BMW had higher levels of Ca (102 mg/L), Mg (24 mg/L), Na (577 mg/L), Mn (0.20 mg/L), and Cr (0.0022 mg/L) compared to DW and TW. On the other hand, TW had higher levels of silica (70 mg/L), potassium (56 mg/L), lithium (2.3 mg/L), iron (0.23 mg/L), and arsenic (0.119 mg/L) than DW and BMW.

2.3. Chemical Analysis

2.3.1. Nutritional Profile

The nutritional profile of the biju bread was analyzed following the official AOAC methodology [9] unless otherwise stated.

Moisture: The moisture content was analyzed following AOAC method 925.09 [9], in which 2 g of the sample was placed in a metal dish that was closed and weighed. The dish was placed in an oven (Scientific Series, Contherm, Lower Hutt, New Zealand) at 100 °C for five hours, and after cooling down, it was weighed again. The moisture was calculated by subtracting the final weight from the initial one.

Crude protein: Protein content was calculated using the Macro-Kjeldahl method, following the AOAC 920.87 method [9], using a conversion factor of 5.8. Briefly, 0.5 g of samples were digested in a $K_2SO_4/CuSO_4$ catalyst and sulphuric acid at 400 °C for three h. Then, an integrated alkaline stead distillation and titration took place in a Kjeldahl distiller (model Pro-Nitro-A, JP Selecta, Barcelona, Spain). The crude proteins were expressed as g/100 g of fresh weight (fw).

Crude fat: A Soxhlet apparatus was used to extract and quantify the crude fat, using 3 g of a sample and petroleum ether as an extracting medium. Crude fat was expressed as g/100 g of fresh weight.

Ash: Ash content was calculated following the AOAC 923.03 [9], in which 0.5 g of the sample was incinerated in a muffle (Lenton ECF 12/22, Hope Valley, UK) at 550 °C. The ash content was expressed as g/100 g of fresh weight.

Total carbohydrates were calculated by difference. The energy value was calculated using the European Parliament and Council Regulation No. 1169/2011 [10]. Energy (Kcal/100 g fw) = 4 × (g crude protein + g total carbohydrates) + 9 × (g crude fat).

2.3.2. Salt Determination (NaCl)

The concentration of NaCl was determined by multiplying the concentration of the Na^+ cation, obtained by flame atomic absorption spectrometry, by a factor of 2.5 [10].

2.3.3. Soluble Sugar Determination

The soluble sugars were determined through (HPLC) coupled to a refraction index (RI) detector. The procedure followed that previously reported by [11], using melezitose as the internal standard. The equipment consisted of a pump and degasser (Knauer, Smartline system 1000, Berlin, Germany) and an autosampler (AS-2057 Jasco, Easton, MD, USA) coupled to a refraction index detector (Knauer). Sugars were identified by comparing their peaks to the retention times of commercial standards, with the data being analyzed with the Clarity 2.4 software (DataApex, Prague, Czech Republic). Results were expressed as g/100 g of fresh weight.

2.3.4. pH

The pH was measured directly in three different points of the samples using a portable pH meter (Hanna Instruments, Woonsocket, RI, USA).

2.3.5. Water Activity

Measurements of the water activity (a_w) of bread crumbs were obtained according to [12] at 20 °C with the use of Aqualab equipment (4TE Decagon, Pullman, Washington, USA). Aw was measured on each of the three central slices of the bread, and results were averaged.

2.3.6. Individual Fatty Acids

Fatty acid measurements were performed by gas chromatography (GC) coupled to a flame ionization detector (FID) with a split/splitless injector (DANI 1000, DANI Instruments, Contone, Switzerland) [13]. Identification was carried out by comparing the relative retention times of the samples' fatty acid methyl esters (FAME) with the commercial standards. The fatty acids were determined after the transesterification procedure, as described by Barros and collaborators [13].

2.3.7. Mineral Composition: Macro and Microelements

Minerals and metals were determined by Atomic Absorption Spectrometry (AAS) using the Thermo Scientific™ iCE™ 3000 Series spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Potassium (K), sodium (Na), zinc (Zn), magnesium (Mg), and lithium (Li) were determined by AAS using flame atomization. At the same time, aluminum (Al), arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), iron (Fe), manganese (Mn), nickel (Ni), and selenium (Se) were detected by EAA using electrothermal atomization in a graphite furnace. To determine the minerals and metals, 500 mg of a sample were weighed and digested in a solution of nitric acid/hydrochloric acid for 4 h at 105 °C in a digester unit (VELP Scientifica DKL12, VELP Scientifica Srl, Usmate Velate, Italy). After cooling, the samples were filtered, and the volume was made up to 50 mL with ultrapure water. A calibration curve was made for the different elements, and the concentration of each component in the samples was tested in triplicate and determined using the respective calibration curve. The results were expressed in g/100 g of fresh weight.

2.3.8. Texture Profile

The texture profile was conducted using a texture analyzer (Stable Micro Systems TA.XT Plus (Vienna Court, Godalming, UK)) with a 30 kg load cell. The probe used was the P/36 aluminum cylinder, which performed a texture profile analysis (TPA), a typical test that simulates the chewing of the human mouth by performing two compressions of the matrix. The pre- and post-test speeds were set at three mm/s, the target mode was set to 25% strain, and the target mode was set to 25% strain, starting at 50 g of force. The results were combined and processed through a macro to determine the various texture dimensions, namely hardness, adhesiveness, springiness, cohesiveness, chewiness, and resilience. The results were analyzed through the Exponent program.

2.4. Evaluation of the Viability of S. cerevisiae Yeast in Bread Dough by Flow Cytometry

To assess the effect of TW and BMW on the viability of *S. cerevisiae* during the dough fermentation process using flow cytometry, 15 g of each type of dough was weighed after fermentation but before baking in a BagFilter bag (Interscience, Saint-Nom-la-Bretèche, France), and a 1/10 dilution was made with buffered peptone water. Each bag, with its diluted dough sample, was placed in the BagMixer (Interscience) and the maximum speed (level 4) was selected for 3 min to process the sample. Next, 1 mL of the processed sample was collected in a 1.5 mL Eppendorf tube and centrifuged at 3000× *g* for 1 min to sediment any debris collected. After centrifugation, 200 µL of the supernatant obtained was pipetted into a new 1.5 mL Eppendorf tube, centrifuged again at 3000× *g* for 1 min, and the pellet was resuspended in 500 µL of 0.01% (*v/v*) TWEEN 20 solution/PBS 1×. Finally, 5 µL of Thiazole Orange (TO) and 5 µL of Propidium Iodide (PI) were added to the contents of each tube and then incubated at room temperature for 5 min in the dark. After incubation, the contents of the tubes were analyzed by flow cytometry. To obtain uniform and easily comparable results, 10 µL of the total content of each tube, previously homogenized, was acquired.

2.5. Statistical Analysis

In this study, all the tests were carried out in triplicate, and all the data are expressed as mean ± standard deviation. All data were subjected to analysis of variance (ANOVA) and a multiple range test (Tukey's test) using IBM SPSS 27.0 software (SPSS Inc., Chicago, IL, USA). Differences between mean values were considered significantly different for $p < 0.05$.

3. Results and Discussion

3.1. Chemical Characterization of the Different Water Matrices Used

Table 1 shows the contents of minerals in DW, TW, and BMW used to develop the different bread formulations. Generally, the chemical composition varied according to the different water matrices, with TW and BMW having the highest contents for most minerals than DW. This fact was expected, as TW is enriched with minerals originating from deep circulation through geological structures [14].

Despite the observed differences, the levels of minerals in the DW lie within the applicable legislation by Decree-Law Nº 69/2023 of 21 August [15], which establishes the Parametric Value (PV) or Maximum Value Recommended (MVR) for the parameters mentioned. On the other hand, for NMW, the applicable legislation is Order Nº 14413/2016 [16], which only contains the quantification limits (QL) and does not reference the PV or MVR values. Nevertheless, according to the Codex Alimentarius, the mineral contents found in TW and BMW are in conformity; according to this legislation, the bottled natural mineral waters must not contain more than the following amounts: nitrate (50 mg/L), manganese (0.4 mg/L), lead (0.01 mg/L), cadmium (0.003 mg/L), copper (1 mg/L), chromium (0.05 mg/L), nickel (0.02 mg/L), selenium (0.01 mg/L), arsenic (0.01 mg/L) [17]. Since it is essential to ensure consumer health safety when new ingredients are introduced in food products, our data confirm that TW and BMW used are appropriately food grade from a chemical point of view.

3.2. Chemical and Physicochemical Composition of the Produced Bread

3.2.1. Formulations with Added Salt

pH, Water Activity, and Salt Concentration

The pH and water activity (a_w) values obtained for the different bread formulations are shown in Table 2.

Table 2. Chemical, physicochemical, and nutritional composition of different breads (g/100 g of fresh weight).

Parameter	B_DW	B_TW	B_TWns	B_BMW	B_BMWns
pH	5.69 ± 0.05 ^{a}	6.18 ± 0.02 ^{bc}	6.34 ± 0.02 ^{cd}	6.12 ± 0.14 ^{b}	6.45 ± 0.06 ^{d}
Water activity (a_w)	0.88 ± 0.01 ^{a}	0.87 ± 0.01 ^{a}	0.89 ± 0.00 ^{a}	0.90 ± 0.03 ^{a}	0.89 ± 0.01 ^{a}
Salt (g/100 g)	1.03 ± 0.00 ^{c}	1.12 ± 0.00 ^{d}	0.25 ± 0.00 ^{b}	1.16 ± 0.00 ^{e}	0.19 ± 0.01 ^{a}
Moisture (g/100 g)	26.51 ± 1.54 ^{a}	29.07 ± 2.66 ^{a}	31.54 ± 0.68 ^{a}	30.64 ± 2.26 ^{a}	30.34 ± 2.85 ^{a}
Proteins (g/100 g)	7.74 ± 0.14 ^{a}	7.60 ± 0.50 ^{a}	7.27 ± 0.26 ^{a}	6.96 ± 0.18 ^{a}	7.42 ± 0.37 ^{a}
Lipids (g/100 g)	1.51 ± 0.04 ^{a}	1.90 ± 0.08 ^{b}	1.49 ± 0.10 ^{a}	1.44 ± 0.12 ^{a}	1.47 ± 0.02 ^{a}
Ash (g/100 g)	1.97 ± 0.16 ^{cd}	2.10 ± 0.04 ^{d}	1.07 ± 0.12 ^{b}	1.80 ± 0.11 ^{c}	0.71 ± 0.02 ^{a}
Carbohydrates (g/100 g) *	62.27 ± 1.57 ^{a}	59.33 ± 2.23 ^{a}	58.63 ± 0.48 ^{a}	59.17 ± 2.00 ^{a}	60.08 ± 2.44 ^{a}
Sugars (maltose) (g/100 g)	5.08 ± 0.39 ^{b}	6.22 ± 0.13 ^{c}	5.17 ± 0.01 ^{b}	5.37 ± 0.27 ^{b}	2.25 ± 0.32 ^{a}
Energy (kcal/100 g)	293.64 ± 6.55 ^{a}	284.80 ± 10.38 ^{a}	277.00 ± 2.24 ^{a}	277.44 ± 9.12 ^{a}	283.15 ± 11.48 ^{a}

Values are represented by mean ± standard deviation in triplicate. Different lowercase letters in a row represent a significative difference among samples obtained from Tukey's test at a 95% confidence level. * Calculated by difference. B_DW = bread with drinking water; B_BMW = bread with bottled mineral water; B_BMWns = bread with bottled mineral water without salt; B_TW = bread with thermal water; B_TWns = bread with thermal water without salt.

B_TW and B_BMW showed higher pH values (6.18 ± 0.02 and 6.12 ± 0.14, respectively) than B_DW (5.69 ± 0.05). Previous studies reported bread with pH between 5.3 and 5.8 [18,19], enclosing the values found for the B_DW sample. The pH increases in B_TW and B_BMW compared to B_DW can be attributable to the higher concentration of salts in natural mineral waters, which may promote the increase in the pH of the product. Indeed, previous research has shown that the components needed to make bread dough impact the final product's pH [7,19].

With no statistical differences between the formulations, the a_w ranged between 0.87 ± 0.01 and 0.90 ± 0.03, which agrees with the range expected for this product category.

Concerning salt content (Table 2), significant differences were found between B_TW (1.12 g/100 g) and B_BMW (1.16 g/100 g), with a significant increase compared to B_DW (1.03 g/100 g). This increase is directly related to the greater amount of Na in TW and BMW (Table 1). Despite the observed increment, the NaCl values for B_TW and B_BMW are within the limits stipulated by Law no. 75/2009, which establishes the maximum permitted salt content in bread after it has been baked at 1.4 g of NaCl per 100 g of bread [20].

Nutritional Profile

Regarding energy, the values obtained from the different bread formulations ranged between 277.44 and 293.64 Kcal/100 g, corroborating data from the INSA food composition table (2006) [21], where wheat bread has an energy value of 289 Kcal/100 g. The energy values for various types of bread (multi cereal, Bavaria, wholemeal, rye, and oat) were assessed in a study by Carocho and collaborators [11], and they ranged from 242 to 265 Kcal/100 g, showing that the profiles of each formulation were different. In this study, it was also possible to observe that the various formulations varied between them, with the B_BMW formulation having the lowest energy (277.44 Kcal/100 g) despite no statistically significant differences being observed among them.

Concerning moisture, proteins, and carbohydrates, no statistically significant differences were observed, suggesting no effect of the incorporation of NMW on these properties compared to DW. The moisture contents found are, in general, lower than those found by Carocho and collaborators [11], who had values between 33.0 and 38.4 g/100 g for five types of bread (multi cereal, Bavaria, wholemeal, rye, and oat), and by [22], who investigated the moisture content in 19 types of bread commercially available, ranging from 31 to 45 g/100 g.

Regarding protein content, the observed concentration in this work was between 6.96 and 7.74 g/100 g, corroborating the range of previous studies [7,8,11,23].

The ash content in this study ranged from 1.80 to 2.10 g/100 g, showing higher values compared with the work developed by Carocho and colleagues, which obtained 0.97 g/100 g [11].

The results obtained from carbohydrates were between 59.17 and 62.27 g/100 g, while in the study carried out by Carocho and collaborators [11], the values ranged from 41 to 56.0 g/100 g FW. Altamirano-Fortoul and Rosell [23] studied the physicochemical changes in different baked breads after storing and freezing the dough, and the results obtained by the authors for protein and carbohydrates, 7.05 g/100 g and 62.5 g/100 g, respectively, were like the results obtained in this study. These differences could be attributed to the distinct characteristics between bread types, given that 'biju' bread is wheat-based with no additional ingredients such as whole wheat flour, rye, or seeds.

On the other hand, lipids were impacted, with B_TW (1.90 g/100 g) showing slightly but significantly higher content than B_BMW (1.44 g/100 g) and B_DW (1.51 g/100 g). These differences observed in lipid levels may be related to the hydrolysis reactions during the baking process or even the activity of lipases naturally present in the flour [24]. Likewise, soluble sugars (maltose) were greater in B_TW (6.22 g/100 g) than in B_BMW (5.37 g/100 g) and B_DW (5.08 g/100 g), which could be attributable to an increase in the activity of the amylase enzyme present in wheat flour in this sample. In water, these enzymes convert wheat starch into other fermentable sugars, such as maltose [25].

Individual Fatty Acids

Table 3 shows the individual fatty acids in the different bread formulations developed, expressed in g/100 g FW.

Regarding individual fatty acids, only the most abundant were considered. Although there was a higher number of saturated fatty acids (SFA) in all the bread formulations, it was interesting that polyunsaturated fatty acids (PUFA) had the highest amounts. Individually, linoleic acid (C18:2n6c) was the PUFA with the highest content in all the formulations developed, while the highest SFA content identified was palmitic acid (C16:0).

Overall, the formulation with the highest statistically significant differences compared to B_DW (SFA: 0.2925 g/100 g, MUFA: 0.4274 g/100 g, PUFA: 0.7915 g/100 g) was B_TW (SFA: 0.3901 g/100 g, MUFA: 0.5192 g/100 g, PUFA: 0.9912 g/100 g), which in turn was also the formulation with the highest individual fatty acid content. The investigation's findings were consistent with the research conducted by Carocho and collaborators in 2020 [11].

Mineral Composition

The concentrations of minerals presented in the different biju bread formulations developed in this work are shown in Table 4. The most abundant minerals were K, Na, and Mg. B_TW and B_BMW showed significantly higher contents of these elements than B_DW. This was also expected, since these elements are the most abundant in TW and BMW (Table 1).

Table 3. Individual fatty acids profile of different bread formulations (g/100 g fresh weight).

	B_DW	B_TW	B_TWns	B_BMW	B_BMWns
C14:0	0.0016 ± 0.0001 ^{ab}	0.0026 ± 0.0003 ^{c}	0.0015 ± 0.0000 ^{a}	0.0019± 0.0002 ^{ab}	0.0020 ± 0.0001 ^{b}
C16:0	0.2065 ± 0.0101 ^{a}	0.2905 ± 0.0225 ^{b}	0.1958 ± 0.0029 ^{a}	0.2105 ± 0.0024 ^{a}	0.2257 ± 0.0120 ^{a}
C16:1	0.0050 ± 0.0002 ^{a}	0.0068 ± 0.0004 ^{c}	0.0051 ± 0.0003 ^{a}	0.0057 ± 0.0005 ^{ab}	0.0062 ± 0.0000 ^{bc}
C18:0	0.0660 ± 0.0009 ^{ab}	0.0797 ± 0.0016 ^{c}	0.0673 ± 0.0012 ^{b}	0.0626 ± 0.0014 ^{a}	0.0623 ± 0.0023 ^{a}
C18:1n9c	0.4161 ± 0.0139 ^{b}	0.5055 ± 0.0024 ^{c}	0.4015 ± 0.0237 ^{ab}	0.3743 ± 0.0055 ^{a}	0.3821 ± 0.0062 ^{ab}
C18:2n6c	0.7159 ± 0.0055 ^{b}	0.8979 ± 0.0139 ^{b}	0.7172 ± 0.0177 ^{a}	0.6915 ± 0.0058 ^{a}	0.6974 ± 0.0069 ^{a}
C18:3n3	0.0756 ± 0.0008 ^{a}	0.0933 ± 0.0031 ^{b}	0.0755 ± 0.0023 ^{a}	0.0692 ± 0.0029 ^{a}	0.0707 ± 0.0024 ^{a}
C20:0	0.0067 ± 0.0008 ^{bc}	0.0077 ± 0.0001 ^{c}	0.0076 ± 0.0001 ^{c}	0.0059 ± 0.0001 ^{ab}	0.0055 ± 0.0006 ^{a}
C20:1	0.0062 ± 0.0007 ^{a}	0.0069 ± 0.0008 ^{a}	0.0067 ± 0.0003 ^{a}	0.0055 ± 0.0005 ^{a}	0.0055 ± 0.0004 ^{a}
C22:0	0.0116 ± 0.0007 ^{d}	0.0095 ± 0.0006 ^{bc}	0.0105 ± 0.0010 ^{cd}	0.0079 ± 0.0002 ^{ab}	0.0076 ± 0.0009 ^{a}
SFA	0.2925 ± 0.0091 ^{a}	0.3901 ± 0.0208 ^{b}	0.2827 ± 0.0048 ^{a}	0.2888 ± 0.0025 ^{a}	0.3031 ± 0.0096 ^{a}
MUFA	0.4274 ± 0.0145 ^{b}	0.5192 ± 0.0028 ^{c}	0.4133 ± 0.0238 ^{ab}	0.3856 ± 0.0057 ^{a}	0.3939 ± 0.0065 ^{ab}
PUFA	0.7915 ± 0.0062 ^{a}	0.9912 ± 0.0163 ^{b}	0.7927 ± 0.0199 ^{a}	0.7607 ± 0.0073 ^{a}	0.7680 ± 0.0077 ^{a}

Values are represented by mean ± standard deviation in triplicate. Different lowercase letters in a row represent a significative difference among samples obtained from Tukey's test at a 95% confidence level. B_DW = bread with drinking water; B_BMW = bread with bottled mineral water; B_BMWns = bread with bottled mineral water without salt; B_TW = bread with thermal water; B_TWns = bread with thermal water without salt. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 4. Mineral composition and content in bread formulations (mg/100 g fresh weight).

Element	B_DW	B_BMW	B_BMWns	B_TW	B_TWns
Potassium [K]	104.82 ± 0.08 ^{a}	128.11 ± 0.56 ^{d}	115.97 ± 0.98 ^{c}	117.23 ± 0.96 ^{c}	112.22 ± 0.46 ^{b}
Sodium [Na]	404.49 ± 1.70 ^{c}	454.47 ± 1.73 ^{e}	76.24 ± 5.33 ^{a}	441.62 ± 0.48 ^{d}	96.86 ± 0.21 ^{b}
Magnesium [Mg]	26.08 ± 0.17 ^{a}	30.82 ± 0.03 ^{c}	29.48 ± 0.04 ^{b}	31.24 ± 0.12 ^{d}	31.61 ± 0.07 ^{e}
Iron [Fe]	5.67 ± 0.05 ^{c}	5.80 ± 0.22 ^{cd}	3.54 ± 0.19 ^{a}	6.12 ± 0.04 ^{d}	4.13 ± 0.11 ^{b}
Zinc [Zn]	0.70 ± 0.006 ^{b}	0.67 ± 0.00 ^{a}	0.67 ± 0.02 ^{a}	0.78 ± 0.00 ^{c}	1.28 ± 0.02 ^{d}
Arsenic [As]	0.002 ± 0.000 ^{b}	0.003 ± 0.000 ^{c}	0.002 ± 0.000 ^{ab}	0.01 ± 0.00 ^{d}	0.001 ± 0.000 ^{a}
Chromium [Cr]	0.01 ± 0.00 ^{c}	0.01 ± 0.00 ^{b}	0.02 ± 0.00 ^{d}	0.03 ± 0.00 ^{e}	0.01 ± 0.00 ^{a}
Copper [Cu]	0.08 ± 0.00 ^{a}	0.16 ± 0.00 ^{e}	0.10 ± 0.00 ^{b}	0.15 ± 0.00 ^{d}	0.10 ± 0.00 ^{c}
Manganese [Mn]	0.15 ± 0.01 ^{a}	0.46 ± 0.03 ^{b}	0.55 ± 0.00 ^{c}	0.46 ± 0.01 ^{b}	0.54 ± 0.01 ^{c}
Nickel [Ni]	0.05 ± 0.00 ^{a}	0.11 ± 0.00 ^{b}	0.22 ± 0.02 ^{c}	0.20 ± 0.00 ^{c}	0.11 ± 0.00 ^{b}
Aluminum [Al]	0.26 ± 0.02 ^{a}	0.23 ± 0.00 ^{a}	0.25 ± 0.03 ^{a}	0.31 ± 0.00 ^{b}	0.31 ± 0.01 ^{b}
Lead [Pb]	nd	0.001 ± 0.000 ^{a}	0.026 ± 0.000 ^{d}	0.013 ± 0.00 ^{c}	0.005 ± 0.000 ^{b}
Cadmium [Cd]	nd	nd	nd	nd	nd
Selenium [Se]	nd	nd	nd	nd	nd
Total	543.16 ± 1.61 ^{c}	621.43 ± 1.21 ^{e}	227.95 ± 4.42 ^{a}	599.42 ± 0.64 ^{d}	248.14 ± 0.62 ^{b}

The values represent the mean ± standard deviation of triplicates. Different lowercase letters on the same row indicate samples with statistically significant differences at a confidence level of 95% ($p < 0.05$). B_DW = bread with drinking water; B_BMW = bread with bottled mineral water; B_BMWns = bread with bottled mineral water without salt; B_TW = bread with thermal water; B_TWns = bread with thermal water without salt. nd = not detected.

Considering that bread is a staple food for millions of humans, the daily intake of many nutrients and minerals depends on bread, making it important that the amount of each mineral be known. K is an essential mineral in the human diet. It plays an important role in many physiological processes in the human body, including the distribution of body fluids, nerve impulse transmission, and muscle contraction. It is an essential electrolyte, required for normal cellular function and has a recommended intake set at 3500 mg/day; intake below this level correlates with a higher risk of stroke [26–28].

The results obtained for K in different formulations showed a significant increase in this mineral for B_BMW (128.11 mg/100 g) and B_TW (117.23 mg/100 g) compared to B_DW (104.82 mg/100 g).

Sodium, a part of salt (NaCl), is essential in human health, though its excessive consumption is linked with high blood pressure, coronary issues, and other circulatory diseases. However, both elements are essential to body electrolytes. EFSA considers that a sodium intake of 2.0 g/day represents a level of sufficient confidence in reduced risk of cardiovascular diseases in the general adult population [29,30]. The Na values obtained in this study are within the range stipulated of Tolerable Upper Intake Level (UL) (404.49 and 454.47 mg/100 g).

Magnesium is a cofactor of more than 300 enzymatic reactions, acting either on the enzyme itself as a structural or catalytic component, or on the substrate, primarily for reactions involving ATP, which makes magnesium essential in the intermediary metabolism for the synthesis of carbohydrates, lipids, nucleic acids, and proteins, as well as for specific actions in various organs in the neuromuscular or cardiovascular system [31]. The EFSA has set adequate Mg intake at 350 mg/day for men and 300 mg/day for women [32,33].

The contents of micro minerals, namely Fe, Zn, Cu, Mn, and Se, were also determined. For these compounds, higher levels of Fe and Zn were detected in B_TW (6.12 mg/100 g and 0.78 mg/100 g, respectively) compared with B_BMW (5.80 mg/100 g and 0.67 mg/100 g) and B_DW (5.67 mg/100 g and 0.70 mg/100 g). Additionally, both B_TW and B_BMW presented greater concentrations of Cu (0.16 mg/100 g and 0.15 mg/100 g, respectively) and Mn (0.46 mg/100 g) than B_DW (0.08 mg/100 g and 0.15 mg/100 g, respectively). These increments could have been caused by the incorporation of BMW and TW in these samples, as they were richer in these compounds (Table 1) than DW and could have contributed to the increase in their concentration in the final product. Selenium has not been detected in any of the samples.

Iron is an essential mineral as it participates in the synthesis of hemoglobin, transports oxygen to the blood hemoglobin, transports oxygen to the blood, and is an enzyme cofactor [34]. The recommended daily intake of Fe is 8 to 10 mg/day, according to [35], but according to the Dietary Reference Intakes (DRI), there is a maximum intake value for iron of 45 mg [36].

Zinc is an essential trace element for humans, vital for many biological functions, and plays an important role in the normal functioning of more than 300 enzymes in the human body [37]. It is vital during pregnancy, in skin care, and immune resistance. Intake reference values of Zn are divided between genders: they range from 6.2 to 10.2 mg/day for women with a reference weight of 58.5 kg and from 7.5 to 12.7 mg/day for men with a reference weight of 68.1 kg [38].

Copper is an essential micronutrient required for electron transfer processes. It is a central component of many enzymes, including those involved in neurotransmitter synthesis, energy metabolism, and collagen and elastin cross-linking [39]. Cu also plays a role in hemoglobin synthesis and redox reactions [34,40]. It has anti-inflammatory properties, helps to reduce arthritis symptoms, and is an essential element for growth [41]. For adults, adequate intakes (AIs) of 1.6 mg/day for men and 1.3 mg/day for women are proposed. For children, AIs are 0.7 mg/day for children aged 1 to <3 years, 1 mg/day for children aged 3 to <10 years, and 1.3 and 1.1 mg/day for boys and girls aged 10 to <18 years [39]. The observed Cu concentrations are within the limits of the recommended AIs of Cu [36].

Manganese is an essential dietary mineral that is a component of several metalloenzymes involved in amino acid, lipid, and carbohydrate metabolism. However, excessive consumption causes toxicity, especially neurotoxicity [31]. As insufficient evidence is available to derive an average requirement or a population reference intake, an adequate intake (AI) is proposed. The mean intake of manganese in adults in the EU is around 3 mg/day. In addition, null or positive balances have consistently been observed with manganese intakes above 2.5 mg/day. With an AI of 3 mg/day [31], the concentration of Mn present in the formulations developed in this work is within the desirable limits.

In a recent study, Rybicka [22] analyzed the mineral contents in 73 commercial samples of bread in Poland, including conventional (wheat-based or other gluten-containing material such as rye and whole meal bread), dairy-free, egg-free, gluten-free, and low-protein bread types. According to the results, the levels of Fe, K, Mg, and Na ranged between 0.19 and 2.10 mg/100 g, 28.68 and 266.40 mg/100 g, 1.43 and 69.6 mg/100 g, and 79.92 and 846.22 mg/100 g, respectively. Therefore, from the data obtained in this study, it is possible to affirm that the developed bread formulations stand out from commercially available bread types in supplying Fe, but were in the same range regarding Na, K, and Mg.

Therefore, our findings suggest that the incorporation of NMW yields bread with mineral contents higher or equivalent to those in the market, contributing significantly or enhancing the intake of minerals essential to the body's health.

Additionally, macro- and microminerals are essential for health maintenance; when introducing new ingredients in food products, it is crucial to determine whether toxic or undesirable trace elements are incorporated. In the present study, the presence of nickel (Ni), aluminum (Al), lead (Pb), cadmium (Cd), and arsenic (As) was evaluated.

Nickel is an element that can be useful as an activator of some enzyme systems [42]. However, if Ni concentrations exceed permissible limits, they can create toxic effects in humans. The UL value for Ni is 1 mg/day [36]. The Ni levels obtained in this study were 0.05 mg/100 g in the B_DW, 0.12 mg/100 g in the B_BMW, and 0.20 mg/100 g in the B_TW, verifying that these values are within the recommended limits. Feyzi and colleagues [41] and Zioła-Frankowska and collaborators [43] determined in their study a range for Ni concentration between 0.000625 and 0.002125 mg/100 g and 0.001 and 0.041 mg/100 g, respectively, showing lower Ni concentration values than those obtained in this study.

Arsenic is highly toxic. Ingesting large amounts of As leads to gastrointestinal symptoms and serious cardiovascular and central nervous system disorders [41]. According to the FAO/WHO [44], the maximum permissible level of As in food is 100 µg/kg, corresponding to 0.010 mg/100 g. The permitted As content in bread is 0.02 mg/kg [43]. The results obtained showed that the concentration of As found in the bread was 0.0021 mg/100 g (B_DW), 0.0026 mg/100 g (B_BMW), and 0.0058 mg/100 g (B_TW), revealing that the levels of As detected in the different formulations are within the permissible levels. A study by Feyzi and colleagues [41] revealed low levels of As in the bread samples analyzed, with values of 0.00125 mg/100 g in all samples. On the other hand, Zioła-Frankowska and collaborators [43] determined As levels in their samples ranging from 0.00029 to 0.001622 mg/100 g, with the highest As value detected in a gluten-free loaf packed hermetically.

Given the persistence of aluminum in the body, the EFSA panel considered it appropriate to establish a tolerable weekly intake (TWI) rather than a tolerable daily intake, setting a value of 1 mg/kg body weight/per week [45]. The Al contents obtained were 0.26 mg/100 g (DW), 0.23 mg/100 g (B_BMW), and 0.31 mg/100 g (B_TW). Zioła-Frankowska and colleagues [43] quantified the Al levels present in their bread samples in the 0.206 and 0.656 mg/100 g range, with the lowest value quantified in fresh wheat bread. Higher Al contents compared to this study were determined by Woldetsadik and collaborators [46], with concentrations between 24.9 and 34.4 mg/100 g.

Lead occurs primarily in an inorganic form in the environment. Human exposure is mainly via food and water, with some via air, dust, and soil. In average adult consumers, lead dietary exposure ranges from 0.36 to 1.24, up to 2.43 µg/kg body weight per day in European high-consumers [47]. On the other hand, long-term exposure to this metal can cause kidney damage, reproductive and immune system issues, as well as adverse effects on the nervous system [48]. The lead content in food corresponds to the 0.02 to 0.25 mg/100 g [49,50]. In this study, only the B_BMW and B_TW formulations presented Pb levels, with 0.001 and 0.013 mg/100 g values, respectively. The results demonstrated lead levels lower than the lowest recommended Pb intake value, which do not pose any risk to consumer health. Feyzi, Zioła-Frankowska, and Magomya and their collaborators [41,43,51] reported Pb levels in the ranges of 2.951 to 9.862 mg/100 g, 0.034 to 0.313 mg/100 g, and 0.04719 to 0.1025 mg/100 g, respectively, with these authors obtaining higher Pb levels than those obtained in this study.

The main toxic effect of cadmium is its toxicity to the kidney, although it is associated with lung tumors and skeletal changes [52,53]. The permissible limit for Cd in food is 2.5 µg/kg body weight [53]. In this study, no Cd was detected in the formulations developed. Therefore, both TW and BMW can be safely introduced into bread and can potentially increase the levels of certain mineral compounds, contributing to their daily intakes through diet.

Texture Profile

The texture analysis results (hardness, springiness, cohesiveness, gumminess, chewiness, and resilience) obtained from the different bread formulations are shown in Table 5.

Table 5. Texture parameters of the developed bread formulations.

Parameter	**B_DW**	**B_BMW**	**B_BMWns**	**B_TW**	**B_TWns**
Hardness (g)	11,701.2 ± 171.4 ^{b}	8068.3 ± 822.3 ^{a}	11,847.9 ± 299.1 ^{b}	11,624.3 ± 1187.8 ^{b}	8269.1 ± 1023.4 ^{a}
Springiness (%)	0.592 ± 0.012 ^{ab}	0.712 ± 0.062 ^{cd}	0.549 ± 0.024 ^{a}	0.788 ± 0.044 ^{d}	0.676 ± 0.036 ^{bc}
Cohesiveness (%)	0.386 ± 0.016 ^{a}	0.518 ± 0.056 ^{b}	0.325 ± 0.011 ^{a}	0.654 ± 0.014 ^{c}	0.547 ± 0.029 ^{b}
Gumminess (%)	4522.9 ± 252.3 ^{b}	4273.0 ± 52.6 ^{b}	3957.4 ± 334.6 ^{b}	6475.3 ± 210.3 ^{c}	2480.6 ± 143.1 ^{a}
Chewing (%)	2706.1 ± 161.1 ^{a}	3084.4 ± 185.0 ^{ab}	2889.9 ± 218.4 ^{ab}	4386.6 ± 589.6 ^{c}	3577.5 ± 206.9 ^{bc}
Resilience (%)	0.138 ± 0.009 ^{a}	0.171 ± 0.004 ^{b}	0.165 ± 0.011 ^{b}	0.235 ± 0.006 ^{c}	0.163 ± 0.004 ^{b}

The values represent the mean ± standard deviation of triplicates. Different lowercase letters on the same line indicate samples with statistically significant differences at a confidence level of 95% ($p < 0.05$). B_DW = bread with drinking water; B_BMW = bread with bottled mineral water; B_BMWns = bread with bottled mineral water without salt; B_TW = bread with thermal water; B_TWns = bread with thermal water without salt.

Through the analysis of the results obtained, it was possible to verify that using different water matrices influenced the textural properties of the final product.

Hardness is the most important attribute for determining consumer acceptability of food products [54–57], defined as the force that teeth exert on the food, and is measured in grams [58]. The values of hardness obtained for B_TW (11,624.3 g) did not present statistically significant differences from B_DW (11,701.2 g). In contrast, the B_BMW formulation presented a value for the hardness parameter much lower than B_DW, with a value of 8068.3 g. The fact that B_BMW presents this difference in hardness may be related to the significant increase in air retention capacity [59], as it is carbonated water. Furthermore, according to Young [60], hardness may be related to the moisture content of the food, meaning that the higher its moisture content, the lower its hardness, which follows the results obtained in the B_BMW formulation, since out of the three formulations developed, it was the one that presented the highest moisture content (30.64 g/100 g, Table 2) [60].

Another important texture parameter, which revealed statistically significant differences between the B_BMW and B_TW formulations compared to the B_DW, was the elasticity. Elasticity is defined as the speed at which the deformed material returns to its initial state after the removal of the force [61]. In this study, the value obtained for this parameter was 0.712% for the B_BMW formulation and 0.788% for the B_TW formulation, with values higher than B_DW (0.592%).

Cohesiveness is the degree of resistance of food to undergoing a second deformation with regard to the resistance in the first deformation, being expressed as a percentage. In this parameter, all formulations presented statistically significant differences between them, with values of 0.386% (B_DW), 0.518% (B_BMW), and 0.654% (B_TW), with B_TW presenting the highest cohesion percentage. The results obtained by Bawa and colleagues [62] for this parameter corroborate the results obtained in this work, with a cohesion value for the control bread of 0.34%.

Gumminess is the force required to chew a semisolid food to the point of deglutition. On the other hand, chewiness is generally defined as the energy needed to chew solid foods until swallowing. Both gumminess and chewiness are secondary parameters resulting from hardness, cohesiveness, and elasticity [63]. With respect to gumminess and chewiness parameters, the B_TW showed significantly higher values than B_DW with percentages of 6475.3%, 4522.9%, and 4386.6% to 2706.1%, respectively.

Resilience is similar to elasticity, although it measures both the speed and the forces involved in the recovery of food when a deforming force is removed, also being measured in percentage [63]. For this parameter, all formulations presented statistically significant differences between them, with values between 0.138% (B_DW), 0.171% (B_BMW), and 0.235% (B_TW). Resilience, which simulates how a slice of bread maintains its initial height, was significantly higher in the B_TW formulation (0.235%) than other formulations.

3.2.2. Formulations without Added Salt

NMWs have been classified based on their pH, emergence temperature, total mineralization, and mineral content. Regarding the latter classification, "water with sodium" is that in which the sodium content is >200 mg/L [6]. As demonstrated in Table 1, both the TW and BMW used in this study are high in sodium concentration, promoting a significant increase in the concentration of this element in bread samples (Table 2).

The growing global concerns about the effects of excessive salt intake on consumer health have motivated the industry to develop food products with different nutritional claims ranging from "low sodium/salt" to "very low sodium/salt", "sodium-free or salt-free", or "no added sodium/salt". Therefore, the obtained results for the incorporation of NMW raised the question of the potential of NMW as a natural salting agent to produce bread, allowing for labeling with the claim of "no added sodium/salt". To investigate this, B_TW and B_BMW were prepared with no salt addition, and the results are shown in Table 2.

Comparing B_TW and B_BMW with their corresponding formulations without salt (B_TWns and B_BMWns, respectively), no statistically significant differences were observed regarding moisture content, protein content, carbohydrates, and energy value. Conversely, significant differences were found between the above formulations regarding ash, maltose, and salt contents, with the formulations with added salt exhibiting higher levels.

Similarly, for a_w (Table 2), there were no statistically significant differences between the formulations regarding water activity. However, both B_TWns and B_BMWns showed higher pH values than the B_TW and B_BMW, respectively, with the differences being significant only between the B_BMW and B_BMWns formulations.

In respect to individual fatty acids (Table 3), the contents in SFA, MUFA, and PUFA decreased significantly in the formulation B_TWns regarding B_TW, but in the case of B_BMW and B_BMWns, the SFA, MUFA, and PUFA were similar between formulations.

Regarding total minerals (Table 4), it was possible to observe that the B_BMW and B_TW formulations presented higher values when compared to B_BMWns and B_TWns, respectively. The value obtained for B_BMW was 621.43 mg/100 g, and for B_BMWns 227.95 mg/100 g, while B_TW showed a content of 599.42 mg/100 g and B_TWns a content of 248.14 mg/100 g. This discrepancy in total mineral concentrations between formulations with and without salt addition seems to be directly related to lower Na levels in formulations without salt addition, once the Na concentration ranged from 76.24 to 96.86 mg/100 g (B_BMWns and B_TWns, respectively). In comparison, formulations with added salt B_BMW (454.47 mg/100 g) and B_TW (441.62 mg/100 g) presented significantly higher Na values.

Therefore, the data indicate the possibility of using the nutritional claim "no added sodium/salt" in salt-free bread formulations since it is defined as: "*the product does not contain added sodium/salt or any other ingredient containing added sodium/salt, and the product does not contain more than 0.12 g of sodium, or the equivalent value of salt, per 100 g or 100 mL*", as described by Commission Regulation (EU) Nº 1047/2012 [64]. In this sense, considering that both formulations without salt addition had Na values lower than 0.12 g/100 g (B_BMWns: 0.076 g/100 g and B_TWns: 0.096 g/100 g), this premise is fulfilled.

Concerning texture parameters (Table 5), in the B_BMW and B_BMWns formulations, significant differences were observed in the parameters of hardness, springiness, and cohesiveness, with B_BMWns showing higher hardness (11,847.9 g), but lower values of springiness, cohesiveness and gumminess. Regarding the B_TW and B_TWns formulations, the latter presented significantly lower values for the parameters of hardness (8269.1 g), springiness (0.676%), cohesiveness (0.547%), gumminess (2480.6%), and resilience (0.163%). Salt in bread formulations is essential because it affects the dough, and its baking or bread properties and palatability. Salt may also exert substantial effects on texture parameters, such as by affecting water retention capacity, consequently impacting texture properties [65].

Rybica and colleagues [22] assessed the content of Na in 73 commercial bread types from Poland and found Na levels of up to 846 mg/100, higher than the values observed in both formulations (with and without added salt) (Table 4). According to the authors, the high content of NaCl (and hence Na) in bread results from the fact that salt is an essential ingredient crucial for breadmaking, such as for obtaining desirable dough structure and crumbs. In this sense, despite slightly affecting the bread's rheological properties, our results indicate that removing salt from formulations and replacing it with sodium naturally present in NMW can be a promising alternative for low-added-sodium bread products.

3.3. Evaluation of the Effect on the Viability of *S. cerevisiae*

The effect of TW and BMW on the viability of *S. cerevisiae* yeast was evaluated in bread formulations with and without added salt using the flow cytometry technique. Considering the distinct physicochemical characteristics of each water source, speculation was made about their influence on the fermentation capacity of *S. cerevisiae* during the leavening process of the studied bread doughs. Thus, after the fermentation process, a portion of each type of dough was collected for subsequent analysis in the flow cytometer.

After analyzing the graphs obtained by flow cytometry, it was found that the viability of *S. cerevisiae* cells, that is, the number of live cells, was similar in the five types of bread dough studied (Figures 1 and 2).

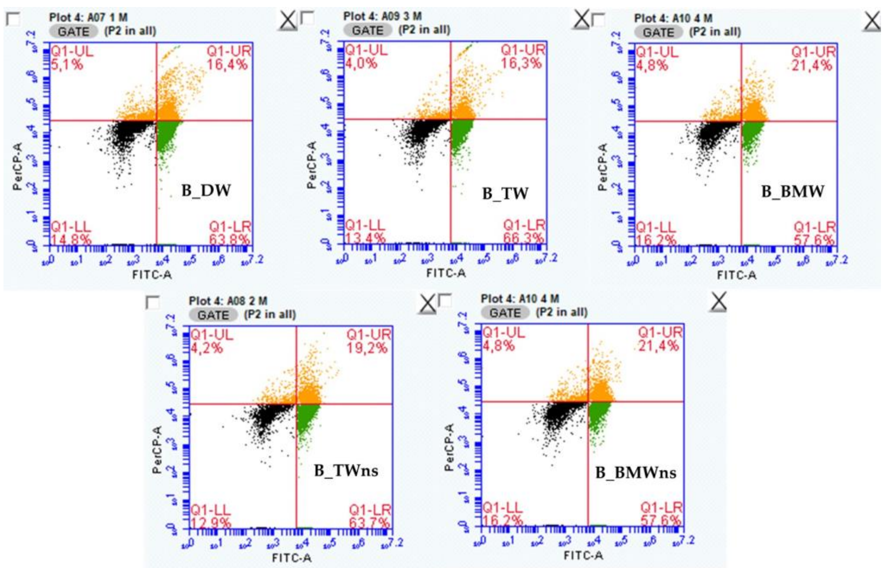

Figure 1. Results of flow cytometry were obtained for the analysis of *S. cerevisiae* viability after the fermentation process. B_DW—dough with drinking water; B_TW—dough with thermal water; B_BMW—dough with bottled mineral water; B_TWns—dough with thermal water without salt addition; B_BMWns—dough with bottled mineral water without salt addition. Dead or damaged *S. cerevisiae* cells are in orange, and viable *S. cerevisiae* cells are in green.

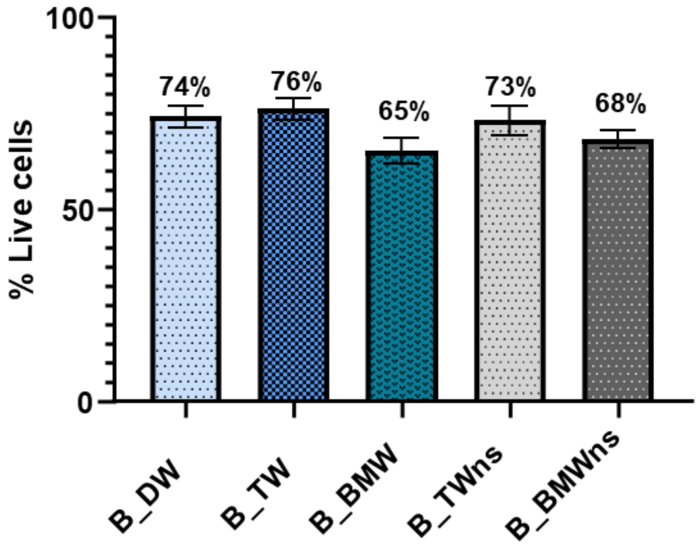

Figure 2. Effect of thermal and bottled mineral waters on the viability of *S. cerevisiae* during the fermentation process of different bread doughs. B_DW—dough with drinking water; B_TW—dough with thermal water; B_BMW—dough with bottled mineral water; B_TWns—dough with thermal water without salt addition; B_BMWns—dough with bottled mineral water without salt addition.

In the bread dough produced with DW, 74% of *S. cerevisiae* cells were viable (Figure 2); in the bread dough produced with TW with and without added salt, 76% and 73% of *S. cerevisiae* cells were alive, respectively (Figure 2). Finally, in the bread dough produced with BMW with and without added salt, it was observed that 65% and 68% of *S. cerevisiae* cells were alive, respectively (Figure 2). According to studies by Peña and colleagues [66], for *S. cerevisiae* to reach its optimal growth, the pH of the medium should be between 4 and 7. Thus, in this case, the pH of the different waters used (pH value of 6.80 for TW, 6.10 for BMW, and 6.92 for DW; values obtained at the Water Characterization Laboratory—LCA, AquaValor) did not influence the fermentation process, considering that there are no significant differences in the viability of this yeast in the different bread formulations developed.

Regarding the chemical compositions of the different waters used in this study (Table 1), the concentration of nitrates is relatively low in all of them, being less than 2.0 mg/L in the tap water, less than 0.30 mg/L in the TW, and less than 0.25 mg/L in BMW. Considering that the presence of nitrates as a nitrogen source is essential for the growth of *S. cerevisiae*, the authors [67] studied the effect of increasing concentrations of this compound (16.5 to 805 mg/L) on the multiplication of that yeast, concluding that higher nitrogen concentrations lead to higher biomass production. However, this was not observed in this study, probably due to the minimal differences observed between the nitrate concentrations present in the tested waters, as well as in the tested waters and their low levels. On the other hand, in a study by Casey and collaborators [68], it was found that the presence of K and Na in the culture medium increased the growth of *S. cerevisiae*. However, concerning the types of bread developed in this work, it seems that the observed differences in the chemical compositions of the waters used in terms of K and Na did not affect the viability of *S. cerevisiae* (Table 1, Figure 2).

4. Conclusions

Thermal and bottled mineral waters as food ingredients allowed the development of nutritionally balanced bread formulations, potentially contributing to the intake of essential mineral compounds. Furthermore, thermal and bottled mineral waters did not affect the viability of *S. cerevisiae* during the bread dough fermentation process, thus serving as an alternative to drinking water for bread production without affecting the essential fermentation step of the dough. However, since the viability study was only conducted at the end of the fermentation process, it will be pertinent for the baking sector in the future to evaluate the influence of thermal and bottled mineral waters on the various stages of *S. cerevisiae* development throughout the fermentation process period. Further studies can investigate the influence of mineral water addition on the rheological property characterization of bread proteins that can be evaluated, for instance, through protein structure analysis by Fourier transform infrared spectroscopy (FTIR).

In conclusion, the use of endogenous resources such as thermal and bottled mineral waters, which presently have great significance in the Portuguese and even global markets, can serve as the basis for developing innovative and value-added products like bread.

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