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## **Special Article: Food Characterization**

# Physical, Nutritional, and Chemical Profile of Innovative Bakery Products

Pascoalino LA<sup>1,2</sup>; Ayuso M<sup>1,2</sup>; Pereira E<sup>1,2\*</sup>; Ferreira E<sup>3</sup>; Ferreira ICFR<sup>1,2</sup>; Barros L<sup>1,2</sup>

<sup>1</sup>Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

<sup>2</sup>Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

<sup>3</sup>Pão de Gimonde<sup>®</sup>, M Ferreira & Filhas, LDA, EN218, n° 3798, 5300-553, Gimonde, Portugal

### \*Corresponding author: Pereira E

Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Portugal.

Email: eliana@ipb.pt

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## Introduction

Bread has been part of the human diet for millennia and is still widely consumed today, with approximately 70 kg of bread per year and per capita worldwide [1]. This food is daily consumed as bakery products by all social classes, being an important source of macro (carbohydrates, protein, and fat) and micronutrients (minerals and vitamins), which makes it an attractive food vehicle for growing the intake of bioactive compounds [2,3].

Its origins date back to ancient Egypt, where it was made mainly from barley. Nowadays, wheat is the most commonly used cereal for baking, although bread can be made with many types of grains or pseudocereals, either alone or mixed. In Portugal, in addition to wheat, other cereals, such as maize or rye, are traditionally used since they are essential crops in some regions of the country [4,5].

Refining wheat during bread baking reduces its nutritional quality as it loses fibre, vitamins, minerals, and phytochemicals [6]. Compared to other breads made with different whole grains, traditional bread is less satiating and increases the postprandial glycemic index [7]. So, the great challenge for the cereal industry today is to innovate and reinvent much of its product, especially by changing its traditional composition, as this seems to be an effective way to improve nutrition. Alterna-

## Abstract

Bakery products are the most eaten up foods worldwide. Among them, bread is regular consumed, due to their composition in macro and micronutrients. Nevertheless, the refined wheat used in traditional baking reduces its nutritional quality, causing some health problems related to obesity and diabetes. In this study, a comparative analysis among five non-conventional bread supplemented with different grains (rye (RB), Chickpea Flour (CFB), Multi-Seed (MSB), Biological Spelt (BSB), Chickpea and Wheat Sprouted Brains (CWSB)) was performed. Their physical profile (texture, colour and pH), centesimal composition, free sugars, and fatty acids was evaluated. Regarding the nutritional profile, MSB and BSB showed a high protein concentration. The highest concentration of PUFA and lowest SFA was registered for the BSB and MSB, respectively. CWSB revealed the highest concentration of soluble sugars. This study demonstrates that partially replacing wheat flour for bread baking is an alternative to improve their nutritional quality.

**Keywords:** Bread; Nutritional characterization; Chemical composition; Texture; Physicochemical properties; Flour

tives such as whole grains, the use of other grains, or the addition of protein-rich flours such as legumes can help improve the nutritional quality of bread and have a significant impact on consumer health [8,9].

The aim of this study was to conduct a comparative analysis of the physical, nutritional, and chemical parameters of five different types of bread, with different flours, made in a traditional Portuguese bakery (Pão de Gimonde®) aiming to develop novel products with better nutritional quality and health benefits. The analysis included the evaluation of colour, pH, texture, nutritional profile (proteins, moisture, ash, fat, carbohydrates, and energy), soluble sugars, and fatty acids. Subsequently, all samples were compared to determine which formulation showed the most promising nutritional, chemical and physical properties. In addition, this work provides a deeper understanding of the differences between the different cereals in the overall physicochemical profiles of the bread, which directly correlate with consumer preference.

#### **Materials and Methods**

## Sample Preparation

For breadmaking, all breads were made at the Pão de Gimonde<sup>®</sup> bakery facilities in Gimonde, Bragança, Portugal. Five

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Table 1: Bread flour ingredients	expressed in percentage.
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table 1. bread hour ingredients expressed in percentage.							
Bread type	Main flour	Added flour					
Rye (RB)	70% Rye	30% Wheat					
Chickpea flour (CFB)	90% Wheat	10% Chickpea flour					
Multi-seed (MSB)	90% Wheat	10% Protein-rich seeds flour*					
Biological spelt (BSB)	100% Whole spelt	-					
Chickpea and wheat sprouted grains (CWSB)	90%Wheat	10% Flour from sprouted chickpea and wheat					

\*Flour composed by linseed, sunflower, and sesame seed

types of bread, with different percentage of different flours, were analysed - namely Rye (RB), Chickpea Flour (CFB), Multi-Seed (MSB), Biological Spelt (BSB), and Chickpea and Wheat Sprouted Grains (CWSB) (Table 1). After baking, they were cooled, packed in plastic bags, and taken to the laboratory for analysis. Physical parameters (texture, colour and pH) were analysed with fresh bread; and nutritional and chemical evaluations were performed after lyophilised (Telstar LyoQuest Lyophilizer), cruched (model A327R1, Moulinex, Barcelona, Spain), and homogenised the samples.

## **Standards and Reagents**

The Fisher Scientific (Lisbon, Portugal) was the company were all chemicals and reagents were obtained. The water used in this research was Milli-Q (TGI Pure Water Systems, Greenville, SC, USA). The bread was acquired from Pão de Gimonde<sup>®</sup> bakery.

## **Physical Analysis of the Different Breads**

The physical analysis for the bread samples included a complete determination of the texture profile, encompassing the hardness, springiness, cohesiveness, and resilience parameter. Additionally, the colour of the bread and the pH were also analysed.

**Texture:** The bread's texture profile was determined using a procedure previously described by [1]. A Stable MicroSystems TA.XTPlus texture analyser (Vienna Court, Godalming, UK) with a 5 kg load cell was used and the probe used was the P/36 R aluminium radiused AACC, which performed a Texture Profile Analysis (TPA) which simulates the chewing of the human mouth by performing two compressions of the matrix. The pre and posttest speeds were set at two mm/s and three mm/s, respectively, and the target mode was set to 30% strain which started at 10 g of force. The results were combined and processed by a macro to obtain the different texture dimensions, namely hardness, springiness, cohesiveness, and resilience, which were then analysed by the Exponent programme.

**Colour:** The bread's colour was determined according to a methodology described by [1]. A portable colourimeter CR400 from Konica Minolta (Chiyoda, Toko, Japan) was used and the data obtained was expressed by the CIE  $L^*a^*b$  colour space.  $L^*$  represents lightness (100 for white and 0 for black),  $a^*$  represents redness when positive and greenness when negative (red-green), and  $b^*$  represents yellowness when positive and blueness when negative (yellow-blue). The colourimeter was calibrated with a standard white plate.

**pH:** The pH of the samples was measured directly in the samples with a Wireless pH Meter Foodcare HALO<sup>®</sup> - FC2022 calibrated before each measurement following a previously methodology made by [10].

Nutritional and Chemical Analysis of Different Breads

**Nutritional Profile:** The nutritional profile of the breads was analysed according to the official AOAC methods, 20<sup>th</sup> edition [11].

Moisture content was analysed by AOAC method 925.09; the crude protein was calculated using Macro-Kjedahl method (model Pro-Nitro-A, JP Selecta, Barcelona) and following the AOAC 920.87 procedure; the crude fat was carried out with a soxhlet apparatus, using AOAC 948.22 method; the ash was determined following the AOAC 923.03; and dietary fibre was calculated according to AOAC procedure 993.19. These results were expressed as g 100g<sup>-1</sup> of fw (fresh weight).

In addition, total available carbohydrates were calculated by difference (*Total available carbohydrates* ( $g \ 100 \ g^{-1}$ ) =  $100 - (g \ fat + g \ ash + g \ protein + g \ moisture$ )); and the energy was determined based on the European Parliament and Council Regulation No. 1169/2011 (*Energy* (kcal/(100g fw)) = 4 x (g \ protein + g \ total available \ carbohydrates) + 2 x (g \ dietary \ fibre) +  $9 \times (g \ crude \ fat$ )

Soluble sugar determination: Free sugars were determined by HPLC-RI system using a methodology described by (12). Freeze-dried samples were extracted with ethanol:water (80:20; v/v). The equipment of analysis consisted of an integrated system with a pump (Knauer, Smartline system 1000, Berlin, Germany), a degassing system (Smartline manager 5000), an auto-sampler (AS-2057 Jasco, Easton, MD, USA), and an RI detector (Knauer Smartline 2300, Berlin, Germany). Chromatographic separation was performed using a Eurospher 100-5 NH2 column (4.6×250 mm, 5 μm, Knauer) at 30°C. The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 mL min<sup>-1</sup>. Data were analysed using Clarity 2.4 Software (DataApex, Prague, Czech Republic). Compounds were identified by chromatographic comparisons with commercially available standards, and quantification was performed using the internal standard (melezitose IS, 25 mg mL-1). The sugars concentration was expressed in g 100 g<sup>-1</sup> of fw.

**Individual fatty acids:** Fatty acid composition was determined by gas-liquid chromatography with flame ionization detection (GC-FID)/capillary column after extraction and derivatization to Fatty Acid Methyl Esters (FAME) following a procedure previously described by [12].

The equipment consisted of a DANI GC (DANI 1000, Contone, Switzerland) with a split/splitless injector, and a Flame Ionization Detector (FID). The column used was a Zebron-Kame (30m×0.25 mm i.d., 0.20 μm). The oven temperature was set according to the following pattern: starting temperature 100°C, held for 2 min, then, a ramp from 10°C min<sup>-1</sup> to 140°C, followed by a ramp from 3 °C min<sup>-1</sup> to 190°C, 30°C min<sup>-1</sup> to 260°C held for 2 min. The carrier gas (hydrogen) was held at 1.1 mL min<sup>-1</sup> (0.61 bar), measured at 100°C. Split injection (1:50) was performed at 250°C, and identification of individual fatty acids was performed by comparing the relative retention times of the FAME peaks of the samples with commercial standards, namely FAME Mix C4-C24 (standard 4788-U, Sigma-Aldrich). Results were expressed as relative percentages (%) of each fatty acid and crude fat concentration was used to determine their concentration in g 100 g<sup>-1</sup>of fw.

**Statistical analysis:** The results were expressed as mean  $\pm$  Standard Deviation (SD). All extractions and analysis experiments were performed in triplicate. For statistical analysis a SPSS Statistics (IBM SPSS Statistics v. 25., IBMCorp, Armonk, NY,

USA) program was used. In addition an one-way analysis of variance (ANOVA one-way) followed by Tukey's HSD post hoc test (prior confirmation of their homoscedasticity) was applied.

#### **Results and Discussion**

#### **Physical Parameters**

The different parameters of the bread's texture (hardness, springiness, cohesiveness, and resilience) are shown in Table 2. The force required for the teeth to compress a food is defined as hardness, measured in grams [13]. Considering the statistical treatment, the CFB was the bread which presented higher hardness values (2177±60 g) followed by RB (1183±28 g); while CWSB, BSB and MSB had very similar hardness values (the values ranging between 585±29 and 402±8 g). Studies revealed that chickpea flour can lead to an increase in hardness. This can be explained due to the thickening of the crumb walls surrounding the air cells and the strengthening of the crumb structure by the protein particles [14].

Another parameter evaluated was springiness, which is described as the rate at which a deformed food reverts to its original length after removing the deforming force, measured in percentage [15]. The statistical treatment showed that MSB was the springiest bread ( $3.52\pm0.04\%$ ), followed by BSB ( $1.01\pm0.02\%$ ), and this is perhaps because of a spongier dough, aided by the seeds that could minimize dough density [1]. The other types of bread did not show statistical differences between them. Although, all the values of springiness were low, principally, due to bread not be a food with elasticity.

Cohesiveness is another texture parameter that evaluates a food's ability to withstand a second deformation in relation to its resistance to the first deformation, i.e. the force of the internal bonds compounds the food, and is measured in percentage [13]. Considering the relative compressive strength of the bread, the cohesion was relatively uniform for all breads, with values ranging between 0.91±0.01 to MSB and 0.81±0.01 for CFB and CWSB, also results in line with the bibliography [1].

Lastly, resilience measures both the speed and the forces used in the regeneration of food, also measured as a percentage. In general, the variation of the analysed breads was quite similar, in spite of the significative differences, only ranging from 0.59±0.01% (MSB) to 0.44±0.01% (CWSB). So, in general, the different flours used for breadmaking had no real influence on the resilience and cohesiveness, but caused some statistical differences in hardness and springiness parameters.

The colour are expressed in Table 2. L\* measured the lightness of the bread. A high number means a light colour bread, as is the case with the BSB sample (65±2), followed by the RB (63±2) and CWSB (62±3), without significative differences between them. L\* could decrease with the substitution of wheat flour for cereal brans [3]. So, it could explain the darker colouring of MSB (54±2) and CFB bread (53±2), also without statistical differences between them. The  $a^*$  parameter measures the range between red and green (from -100 to 100). The BSB bread presented a value very close to 0 (0.65±0.02), while the others showed the highest amounts of red (positive values). Finally, b\* express blueness, and in this case, the values did not show a large fluctuation and were between 17±1 for BSB and 13.3±0.5 for MSB. Figure 1 shows the global breads colour read by the colourimeter and compiled by joining each sample's different L\*, a\* and b\* coordinates and converted to RGB (<https:// www.e-paint.co.uk/convert-lab.asp>). As expected, and accord-



**Figure 1:** The bread colour, using the CIELab  $L^*$ ,  $a^*$ ,  $b^*$  colour space converted to RGB. Rye (RB), Chickpea Flour (CFB), Multi-Seed (MSB), Biological Spelt (BSB), and Chickpea and Wheat Sprouted Grains (CWSB).

Table 2: Physical	narameters	of the	different	studied breads	

Physical parameters	RB	CFB	MSB	BSB	CWSB	
Hardness (g)	1183±28 <sup>b</sup>	2177±60ª	402±8 <sup>d</sup>	567±4°	585±29°	
Springiness (%)	0.98±0.01 <sup>bc</sup>	0.92±0.02°	3.52±0.04ª	1.01±0.02 <sup>b</sup>	0.96±0.01 <sup>bc</sup>	
Cohesive- ness (%)	0.85±0.01°	0.81±0.01 <sup>d</sup> 0.91±0.01 <sup>d</sup>		0.88±0.01 <sup>b</sup>	0.81±0.01 <sup>d</sup>	
Resilience (%)	0.56±0.01⁵	0.45±0.01 <sup>d</sup>	0.59±0.01ª	0.54±0.01°	0.44±0.01 <sup>d</sup>	
		Col	our			
L*	63±2ª	53±2 <sup>b</sup>	54±2 <sup>b</sup>	65±2ª	62±3ª	
a*	2.5±0.1 <sup>b</sup>	3.6±0.1ª	2.2±0.1 <sup>c</sup>	0.65±0.02 <sup>d</sup>	2.5±0.1⁵	
b*	15.7±0.4 <sup>b</sup>	15±1°	13.3±0.5 <sup>d</sup>	17±1ª	15.7±0.5⁵	
Conversion to RGB	165 150 125	140 124 101	140 127 106	168 157 127	162 148 122	
	123	p		127	TZZ	
	5.32±0.04°	5.64±0.03 <sup>b</sup>	4.6±0.1 <sup>e</sup>	5.0±0.1 <sup>d</sup>	5.9±0.1ª	

Rye (RB), Chickpea Flour (CFB), Multi-Seed (MSB), Biological Spelt (BSB), and Chickpea and Wheat Sprouted Grains (CWSB). The results were expressed as average ± Standard Deviation. Diverse letters in the row mean to statistically significant variances (*p*<0.05 according to Tukey's HSD test).

ing to statistical treatment, it was possible to verify that MSB and CFB breads have a darker shade due to the dark colour of its grains and flour.

Regarding the pH parameter, this was also measured at three different points for each sample, and the results are presents in Table 2. Overall, the pH values for the bread ranging between 4.6  $\pm$  0.1 and 5.9  $\pm$  0.1 for MSB and CWSB, respectively. Since MSB has multicereal in your formulation, your pH could vary based on the cereals used. Channainah et al. (2019) studied the pH of wheat multigrain bread and evaluated the values in both fermentation, and period of baking until the cooling. During fermentation, the pH of the bread dough decreased significantly from 5.87 $\pm$ 0.07 to 5.46 $\pm$ 0.06, during the 35 min of baking pH ranged from 5.52  $\pm$  0.03 to 5.4  $\pm$  0.1, and after ambient cooling change to 5.23 $\pm$ 0.02 [16]. So, considering the final pH values, it can be seen that the results found in the present work are in line with literature.

#### **Nutritional Profile and Soluble Sugars Composition**

The results of the nutritional evaluation is in the Table 3. In the evaluation of the moisture content, it was verified that, on most breads, the values were very similar ranging between 40±1 and 42±1 g 100g<sup>-1</sup>, without significative statistical difference; however, the CWSB bread stood out for presenting a slightly higher value (49±2 g 100g<sup>-1</sup>). Likewise, the ash content was similar for some breads (concentrations between  $1.05\pm0.03 - 1.46\pm0.05$  g  $100g^{-1}$ ), without statistically significative differences for RB, CFB, and MSB samples.

In the protein evaluation, it was possible to verify a more visible statistic heterogeneity of values, having obtained the highest amount in BSB and MSB, followed by CFB bread. The

increase in protein content in the multicereal bread could be explained because the nutrients of sesame seeds, sunflower, and linseed, which are part of the protein-rich seeds flour, which is in accordance with another work [1]. Additionally, studies show that it is possible to produce high-protein bread by partially replacing wheat flour with chickpea flour, as well as the use of biological spelt [17-19]. Thus, the use of chickpea could be an significant source of vegetal proteins and fibres; so their ingesting has several benefits for the health of human [20-23].

Concerning fiber content, the highest values were registered for CWSB bread, followed by CFB. Considering the Regulation (EU) No. 432/2012 and Regulation (EC) No. 1924/2006, the CWSB bread demonstrated a "high dietary fibre", because has further than 6 g 100g<sup>-1</sup> of fw. Farther, bread enriched with chickpea flour and spelt are known to contain more dietary fibre compared to wheat bread [17-19]. In this way, high fibre content can be attributed to the presence of these flours in the bread's recipe. So, in terms of protein and fibre intake, CFB bread is the best rated and recommended bread, although it has a high fat content. Regarding the total energy of the bread, the statistical treatment of the data revealed significant differences between the different bread samples. The values ranged between 187±8 kcal 100g<sup>-1</sup> for CWSB and 243±5 kcal 100g<sup>-1</sup> for MSB, being the later the type of bred that presents the higher energy content.

Kraska et al. (2020) studied wholemeal spelt bread and found similar results in what concerns protein content, with values around 11% [18]. Carocho et. al (2020) compared different bread types and the multicereal bread also revealed the highest content in protein and the rye bread the smallest amount of protein, with values of 9.5 ± 0.1 g 100g<sup>-1</sup> and 5.68±0.06 g 100g<sup>-1</sup> respectively [1]. In general, the nutritional profile of the breads was similar, with moisture around 38%, and higher content in proteins and fibres, with means that the nutritional composition presented in this work are in line with the literature.

The results corresponding to the soluble sugars identified in all bread samples are represented in Table 3. The major sugar identified was maltose in all bread samples, with values ranging between 1.7±0.1 and 3.4±0.1 g 100g<sup>-1</sup> for RB and CWSB, respectively. Considering the statistical treatment, it was possible to observe that the content of each detected sugar molecule differs according to the different type of bread (fructose, glucose, maltose), with significant differences between them. Glucose was also detected in very small amounts in RB, CFB, and CWSB breads. This could be due to the low glucose content of most grains and is exacerbated by the leavening period, which consumes a significant portion of the available glucose [24]. As a result, the total sugar content also differs significantly between some samples, presenting highest values to CWSB bread (4.1±0.1 g 100g<sup>-1</sup> of fw). This high concentration could be explained by the catalyst of starch by amylases in sprouted grains [25]. Carocho et al. (2021) studied different breads made with different flours and the profile of soluble sugars was also maltose as the main sugar followed by fructose, and glucose [1].

## Individual Fatty Acids

The individual fatty acids profile for all tested samples is exposed in Table 4. Considering the results, it was possible observed that the linoleic acid (C18:2n6c) was the highest unsaturated fatty acid present in RB, BSB, and CWSB bread samples, and oleic acid (C18:1n9c) for CFB and MSB. On the other hand, the palmitic acid (C16:0) was the majority SFA (saturated fatty

Table 3: Nutritiona	l profile and	d sugar c	composition	of different b	preads.

Table 3: Nutritional profile and sugar composition of different breads.									
Centesimal composition	RB	CFB	MSB	BSB	CWSB				
Moisture (g 100g <sup>-1</sup> )	41.9±0.2 <sup>b</sup>	41±1 <sup>b</sup>	40±1 <sup>b</sup>	42±1 <sup>b</sup>	49±2ª				
Protein (g 100g⁻¹fw)	4.6±0.1°	7.5±0.1ª	7.8±0.1ª	7.8±0.1ª	5.8±0.3 <sup>b</sup>				
Ash (g 100g⁻¹ fw)	1.46±0.05ª	1.40±0.03ª	1.39±0.02ª	1.28±0.01 <sup>b</sup>	1.05±0.03°				
Crude fats (g 100g <sup>-1</sup> fw)	0.30±0.01°	4.3±0.1ª	3.64±0.04 <sup>b</sup>	0.30±0.01°	0.28±0.01°				
Dietary fibre (g 100g <sup>-1</sup> fw)	$4.1\pm0.2^{\circ}$ $5.2\pm0.3^{\circ}$ $4.3\pm0.1^{\circ}$ $3.8\pm0.1^{\circ}$		3.8±0.2°	7.2±0.4ª					
Carbohydrates (g 100g <sup>-1</sup> fw)	47.6±0.4ª	41±1°	43±1 <sup>b</sup>	45±1 <sup>b</sup>	37±2 <sup>d</sup>				
Total energy (kcal 100g <sup>-1</sup> fw)	220±1 <sup>b</sup>	240±4ª	243±5ª	221±3 <sup>b</sup>	187±8°				
Total energy (kJ g 100g <sup>-1</sup> fw)	919±5 <sup>b</sup>	1005±16ª	1016±19ª	923±11 <sup>b</sup>	781±31°				
		Soluble s	ugars						
Frutose (g 100g⁻¹fw)	0.52±0.02ª	0.49±0.02 <sup>b</sup>	0.33±0.01 <sup>d</sup>	0.15±0.01 <sup>e</sup>	0.40±0.01°				
Glucose (g 100g <sup>-1</sup> fw)	0.45±0.01°	0.44±0.01°	0.95±0.03ª	0.54±0.01 <sup>b</sup>	0.33±0.01 <sup>d</sup>				
Maltose (g 100g <sup>-1</sup> fw)	1.7±0.1 <sup>d</sup>	2.22±0.03°	1.8±0.1 <sup>d</sup>	2.5±0.1⁵	3.4±0.1ª				
Total sugars (g 100g <sup>-1</sup> fw)	2.6±0.1°	3.2±0.1⁵	3.1±0.1 <sup>b</sup>	3.2±0.1⁵	4.1±0.1ª				

Rye (RB), Chickpea Flour (CFB), Multi-Seed (MSB), Biological Spelt (BSB), and Chickpea and Wheat Sprouted Grains (CWSB). The results were expressed as average ± standard deviation and represent g 100g <sup>-1</sup> of fresh weight and in the case of total energy, kcal 100 g<sup>-1</sup> of fresh weight. Diverse letters in the row mean to statistically significant variances (p<0.05 according to Tukey's HSD test). acid) for all types of bread. Studies revealed that diets with high contents of linoleic and oleic acids improve blood sugar levels, decrease the risk of coronary disease, and also have antiinflammatory effects [26].

The Monounsaturated Fatty Acids (MUFA) were found in highest quantities in the MSB samples, with a significant proportion of unsaturated fats found in the seeds [1]. Otherwise, the highest concentration of SFA were found in RB and CWSB breads, showing statistically significant differences compared to the remaining. High consumption of SFA has been associated with heart disease, while consumption of MUFA and PUFA generally has health-promoting effects, namely acting specifically reducing the total plasma concentrations of LDL-cholesterol [27,28]. Interestingly, although higher levels of SFA were found in all bread sampling, the Polyunsaturated Fatty Acids (PUFA) had statistically the highest levels, what makes these breads a healthy option for the consumption of unsaturated fats. Overall, the highest PUFA content and the lowest SFA content were found in BSB and MSB, respectively. These results are according to Kraska et al. (2020), which also found a higher PUFA content, specifically linoleic acid (C18:2n6c), in bakery products made with spelt grain [18]. Carocho et al. (2020) also found linoleic acid (C18:2n6c) as the main PUFA and palmitic acid (C16:0) as the highest saturated one in breads made with different flours [1].

#### **Conclusions**

The study showed that it is possible to replace part of the wheat flour with alternative flours in the production of bread. Table 4: Fatty acid composition of the different bread in relative percentage (%) and in mg 100g<sup>-1</sup>.

	% of total fatty acids				mg 100g <sup>-1</sup> fw					
Fatty acids	RB	CFB	MSB	BSB	CWSB	RB	CFB	MSB	BSB	CWSB
C10:0	0.09	nd	nd	nd	nd	0.27±0.01	nd	nd	nd	nd
C11:0	0.10	0.03	0.02	0.27	nd	0.30±0.01°	1.3±0.1ª	0.75±0.02 <sup>b</sup>	0.80±0.03 <sup>b</sup>	nd
C12:0	0.29	0.02	0.02	0.19	nd	0.87±0.01ª	0.62±0.02 <sup>b</sup>	0.86±0.02ª	0.57±0.01 <sup>c</sup>	nd
C13:0	0.09	nd	nd	nd	nd	0.28±0.01	Nd	nd	nd	nd
C14:0	0.89	0.06	0.05	0.25	0.23	2.7±0.1ª	2.43±0.02 <sup>b</sup>	1.66±0.02°	0.75±0.03 <sup>d</sup>	0.65±0.02
C15:0	0.18	0.02	0.02	0.09	nd	0.54±0.02 <sup>c</sup>	0.88±0.02ª	0.71±0.02 <sup>b</sup>	0.27±0.01 <sup>d</sup>	nd
C16:0	16.41	7.93	7.78	13.6	15.31	49±2°	341±2ª	283±2 <sup>b</sup>	40.8±0.4 <sup>d</sup>	42.9±0.3 <sup>d</sup>
C16:1	0.22	0.12	0.07	0.16	nd	0.65±0.01°	5.0±0.1ª	2.7±0.1 <sup>b</sup>	0.46±0.01 <sup>d</sup>	nd
C17:0	0.24	0.06	0.06	0.14	nd	0.72±0.03°	2.69±0.02ª	2.1±0.1 <sup>b</sup>	0.41±0.01 <sup>d</sup>	nd
C17:1	0.07	0.04	0.03	nd	nd	0.22±0.01°	1.8±0.1ª	1.24±0.04 <sup>b</sup>	nd	nd
C18:0	5.54	4.45	nd	3.86	3.84	16.6±0.4 <sup>b</sup>	191.6±0.2ª	nd	11.6±0.3°	10.7±0.1d
C18:1n9c	26.34	47.74	50.43	29.67	32.06	79.0±0.5 <sup>d</sup>	2053±5ª	1836±2 <sup>b</sup>	89±3°	89.8±0.5°
C18:2n6t	0.15	0.03	nd	nd	nd	0.46±0.02°	1.35±0.02 <sup>b</sup>	3.26±0.02ª	nd	nd
C18:2n6c	39.40	20.92	22.88	43.52	45.43	118±1 <sup>d</sup>	900±2ª	833±1 <sup>b</sup>	131±4°	127±1°
C18:3n6	0.14	0.10	0.12	nd	nd	0.41±0.01 <sup>c</sup>	4.5±0.1ª	4.2±0.1 <sup>b</sup>	nd	nd
C18:3n3	8.37	17.18	16.99	6.61	1.99	25±1°	739±6ª	618±2 <sup>b</sup>	20±1°	5.6±0.2 <sup>d</sup>
C20:0	0.36	0.39	0.42	0.41	0.6	1.09±0.03 <sup>d</sup>	16.8±0.1ª	15.2±0.3 <sup>b</sup>	1.2±0.1 <sup>d</sup>	1.66±0.01
C20:1	0.38	0.19	0.23	0.38	nd	1.15±0.04 <sup>b</sup>	8.2±0.1ª	8.4±0.1ª	1.15±0.03 <sup>b</sup>	nd
C22:0	0.33	0.47	0.48	0.46	0.56	0.99±0.04 <sup>e</sup>	20.2±0.1ª	17.5±0.1 <sup>b</sup>	1.4±0.1 <sup>d</sup>	1.56±0.01
C22:1	0.15	0.03	0.03	0.07	nd	0.44±0.02 <sup>c</sup>	1.23±0.02ª	1.04±0.02 <sup>b</sup>	0.22±0.01 <sup>d</sup>	nd
C23:0	nd	nd	0.03	0.04	nd	nd	nd	1.18±0.02ª	0.13±0.01 <sup>b</sup>	nd
C24:0	0.25	0.22	0.27	0.29	nd	0.75±0.03 <sup>b</sup>	9.68±0.04ª	9.6±0.2ª	0.87±0.01 <sup>b</sup>	nd
Total SFA	24.8	13.7	9.1	19.6	20.5	74±1°	587±2ª	333±1 <sup>b</sup>	59±1 <sup>d</sup>	57.5±0.4 <sup>d</sup>
Total MUFA	27	48.1	50.8	30.2	32.1	81.0±0.5 <sup>d</sup>	2068±5ª	1848±2 <sup>b</sup>	91±3°	90±1°
Total PUFA	48.2	38.3	40.2	50.2	47.4	145±2°	1645±7ª	1459±3ª	151±3°	133±1 <sup>d</sup>

Rye (RB), Chickpea Flour (CFB), Multi-Seed (MSB), Biological Spelt (BSB), and Chickpea and Wheat Sprouted Grains (CWSB). The results were expressed as average for relative percentage and average  $\pm$  standard deviation for mg 100g<sup>-1</sup> of fresh weight (fw). C10:0, Capric acid; C11:0, Undecaenoic acid; C12:0, Lauric acid; C13:0, Tridecanoic acid; C14:0; Myristic acid; C15:0, Pentadecaenoic acid; C16:0, Palmitic acid; C16:1, Palmitoleic acid; C17:0 Heptadecaenoic acid; C17:1, Cis-10heptadecaenoic acid; C18:0, Stearic acid; C18:1n9c, Oleic acid; C18:2n6t, Elaidic acid; C18:2n6c, Linoleic acid; C18:3n6, y-Linolenic acid; C18:3n3,  $\alpha$ -Linolenic acid; C20:0, Arachidic acid; C20:1, Eicosenoic acid, C22:0, Behenic acid; C22:2, Erucic acid; C23:0, Tricosylic acid; C24:0, Lignoceric acid; SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids. Diverse letters in the row mean to statistically significant variances (p<0.05 according to Tukey's HSD test).

The development of new products using these alternative flours is stimulated by consumers who want to improve their nutritional status and by the industry that seeks to respond in an innovative way to these new demands. When evaluating the physical and chemical profile of the samples, the variances and peculiarities of each type of bread stand out, all of which seem to have their advantages and inconveniencies. Thus, considering the obtained results, consumers seeking bread with high protein and fibres would choose CFB bread because adding chickpea flour increase the concentration of these macronutrients. A consumer looking for bread high in fibre and low in calories would choose CWSB bread but need to be careful with its sugar content. In addition, MSB presents high levels of fats; nevertheless, its unsaturated fats and high protein content make is more suitable for consumers who choose to follow a healthier diet. This work demonstrates that innovation in the bakery industry could significantly improve the quality of bread, a staple food in most diets worldwide.

## **Author Statements**

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## **Conflict of Interest**

The authors declare they have no conflict of interest.

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