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Comparison of different bread types: Chemical and physical parameters

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ABSTRACT

In this work, the chemical and physical profile of 5 different bread types (Multicereal bread, Bavaria wheat bread, Wholemeal bread, Rye and Oat bread) were analysed in depth, namely the nutritional profile, individual fatty acids and soluble sugars through GC-FID and HPLC-RI, respectively, as well as the mineral profile, including micro and macroelements. Furthermore, a texture profile analysis was carried out in addition to the measurement of the crust colour. Each bread type showed a distinct profile, with Wholemeal and Bavaria having the lowest calories, and Oat the highest. Multicereal showed the highest amount of unsaturated fatty acids, while Wholemeal and Rye scored the least sodium amounts. The hardest bread was Rye and the easiest to chew were Oat and Bavaria breads. The latter was also the one with the darkest crumb of all the analysed breads. This work shows that bread can be baked to meet the needs and particularities of various kinds of diets.

1. Introduction

The importance of bread in the development of mankind is undeniable. Bread has been a staple food in Human diets for millennia, dating its consumption to Mesopotamia, with scriptures linking bread to the delays in the construction of the great pyramids of Egypt. Bread is consumed throughout the World in many shapes and forms, averaging a consumption of 70 kg per year per capita, although Europe consumes less bread, averaging only 59 kg (De Boni, Pasqualone, Roma, & Acciani, 2019; Edwards, 2007; Gębski, Jezewska-Zychowicz, Szlachciuk, & Sosicka- Gębski, 2019). Two of the most important details behind breads' success as a staple food are its simplicity in terms of ingredients and preparation, as well as the multiplicity of cereals that can be used to bake it. Flour, water, yeast and salt (sodium chloride) are the basic components, that when kneaded, fermented and baked produce bread, although there are thousands of recipes for breadmaking, almost as much as different bread types (Pico, Bernal, & Gómez, 2015). Many cereals can be used to produce bread, namely maize, probably the most used cereal, but also rice, wheat, barley, sorghum, millet, Oat, rye and others. J.P. Edwards (2017) listed some of the different bread types that are baked around the world, namely unleavened bread, sour dough bread, French breads, brown and wholemeal, wheat germ breads, high protein breads, high fibre and multigrain breads, soft grain breads, ethnic multigrain breads, slimming and health high fibre breads, added malt grain breads, bread with cereals other than wheat, crisp bread, bread for special dietary needs and finally war and famine breads. This multitude of bread types shows how much mankind has tried to vary in terms of baking bread, and how this staple food shaped generations, having deep roots in religion, conflicts and health.

The Western countries in the past decades have seen a considerable decrease in the consumption of bread, especially white bread varieties. This shift in consumer choice seems to be rooted in bread quality, consumer perception of bread, but also to trending consumer choices, namely the increase of gluten-free diets. These countries are also known for having low consumption of dietary fibre and high intake of salt, which has considerable health issues, and thus have increased the consumption of wholemeal bread, with higher amounts of fibre, being an overall healthier option (Sajdakowska, Gębski, Żakowska-Biemans, & Jezewska-Zychowicz, 2019).

In this study, a comparative analysis of chemical and physical parameters of 5 different types of bread, made with different flours, is carried out, encompassing the nutritional profile, soluble sugars, fatty acids, crust colour and texture, and subsequently comparing all the samples with each other, in order to evaluate which formulation has the most promising chemical and physical characteristics. This study allows a deeper understanding of the differences of various grain types in the overall physico-chemical profiles of breads, being the grains highly correlated to consumer preference. Furthermore, the importance of

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bread to mankind and the innovations that the food industry constantly pursues, further justifies a deeper knowledge on bread types. The choice of these breads relates to them being some of the most consumed in Portugal, and thus deserving an in-depth study.

2. Materials and methods

2.1. Chemicals, reagents and ingredients

All chemicals and reagents were acquired from scientific retailers, and were of, at least, analysis purity, unless when used for High Performance Liquid Chromatography (HPLC), which were of HPLC grade. Carob flour was acquired from Industrial Farense, an enterprise that trades dry fruits. All other flours were acquired from bakery enterprises, namely Cerealis (Maia, Portugal) and Prodipani (Mirandela, Portugal).

2.2. Bread preparation

Five types of breads were baked, each one of them with wheat as the base flour and varying flours for each individual sample, namely Oat bread (OB), Rye bread (RB), Light Wholemeal bread, made from wholemeal wheat flour (LB), Dark wholemeal bread made with dark Bavaria rye (DB) and finally a bread made with a mix of flours from different cereals, namely sesame, sunflower and linseed, and carob flour, named Multicereal bread (MB). All the bread samples were made with the same amount of water (600 mL), and sourdough (200 g) following the same baking procedures. Given the particularities of each bread type and to display a pleasant appearance, flavour and crust color, the added amounts of special flours could not be the same for all samples and thus their specific quantities are detailed in Table 1. For the breadmaking, all breads were made at the Pão de Gimonde bakery facilities in Gimonde, Bragança, Portugal. The flours were added to the water and sourdough, and thoroughly kneaded in an industrial spiral bread mixer (Mondial Forni, Verona, Italy) for 20 min. Each bread was left to leaven for specific intervals, as detailed in Table 1, which varied from 20 min for Rye bread and 24 h for all the other bread types. After this, all the bread samples were baked in the same industrial bakery oven (Ramalhos, Valongo do Vouga, Portugal) for 30 min at a constant temperature of 250 °C, after which they were left to cool down and immediately packed into microperforated plastic bags and taken to the laboratory for analysis. Each batch consisted of 20 breads, and three were picked randomly for analysis from each of the three batches that were baked under the same conditions.

2.3. Chemical analysis

The chemical analysis encompassed the full nutritional profile, as well as the determination of soluble sugars through HPLC, individual fatty acids through GC (Gas Chromatography) and mineral elements through atomic absorption spectroscopy.

2.3.1. Nutritional profile

The nutritional profile of the breads was analysed following the official AOAC methodology, 17th Ed. (AOAC, 2016), unless otherwise stated.

Moisture: The moisture content was analysed following AOAC method 925.09, in which 2 g of the sample were placed in a metal dish which was closed and weighed. The dish was placed in an oven (Scientific Series, Contherm, New Zealand) at 100 °C for 5 h, and after cooling down was weighed once again. The moisture was calculated by subtracting the final weight to the initial one.

Total available carbohydrates: Carbohydrates were calculated using the anthrone method, devised by Osborne and Voogt (1986), in which 0.3 g of the samples were added to perchloric acid and left overnight to digest. After filtration, anthrone (9,10-dihydro-9-oxoanthracene was added to the samples in a test tube, and detection was carried out at 630 nm with a spectrophotometer (EZ210 Perkin Elmer, Waltham, Massachusetts, USA) with prior boiling and cooling of the test tubes. The total available carbohydrates were expressed as g/100 g of fresh weight.

Dietary fibre: The dietary fibre was calculated using the AOAC procedure 993.19, through the enzymatic–gravimetric method, in which the sample is degraded with α-amylase, protease and amyloglucosidase prior to being filtered through crucibles with celite. The dietary fibre was expressed as g/100 g of fresh weight.

Crude protein: Protein content was calculated relying on the Macro-Kjedahl method, following the AOAC 920.87 method, using a conversion factor of 5.8. 0.5 g of the samples were digested in K_2SO_4/CuSO4 catalyst and sulphuric acid at 150 °C for 3 h. Then, an integrated alkaline stead distillation and titration took place in a Kjeldahl distiller (model Pro-Nitro-A, JP Selecta, Barcelona). The crude proteins were expressed as g/100 g of fresh weight.

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

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

Energy: The following formula, based on the European Parliament and Council Regulation No. 1169/2011 was used to calculate the energy:

$$Energy\left(\frac{kcal}{100gfw}\right) = 4\times(g\ protein + g\ total\ available\ carbohydrates) + 2 \times (g\ dietary\ fiber) + 9\times(g\ crude\ fat)$$

2.3.2. Soluble sugar determination

The soluble sugars were determined through (HPLC) coupled to a refraction index (RI) detector. The procedure followed the one previously reported by Carocho et al. (2015), using melezitose as the internal standard. The equipment consisted of a pump and degasser (Knauer, Smartline system 1000, Berlin, Germany), and an auto sampler (AS-2057 Jasco, Easton, MD, USA), coupled to a refraction index detector (Knauer). Sugars were identified comparing their peaks to the retention times of commercial standards, with the data being analysed with the Clarity 2.4 software (DataApex, Prague, Czech Republic).

Table 1
Ingredients and leavening times for the breads, expressed in grams.

Bread Type	Base Flour	Added Flour	Leavening Time
Multicereal (MB)	850	150	24 h at 4 °C
Dark Bavaria Wholemeal Rye (BB)	500	500	24 h at 4 °C
Light Wholemeal Wheat (LB)	500	500	24 h at 4 °C
Rye (RB)	300	700	60 min room temperature
Oat (OB)	800	200	60 min room temperature

The added flour is the different type of flour (multicereal, wholemeal, rye and Oat) added to each specific type of bread beyond the base flour.

Results were expressed as g/100 g of fresh weight.

2.3.3. Individual fatty acids

Fatty acids were determined by gas chromatography (GC) coupled to a flame ionization detector (FID). The equipment consisted of a DANI GC (DANI 1000, Contone, Switzerland) with a split/splitless injector. Briefly, the fat was extracted with petroleum ether in a Soxhlet apparatus, then methylated with 5 mL of a solution of methanol:sulphuric acid:toluene (2:1:1) overnight at 50 °C and 160 rpm. 3 mL of deionized water was added for phase separation and the fatty acid methyl esters were recovered to vials and injected in a GC-FID system. The column used was a Zebron-Kame (30 m × 0.25 mm i.d., 0.20 µm). The oven temperature followed the pattern: starting temperature of 100 °C, held for 2 min, then, a ramp of 10 °C/min to 140 °C, followed by a 3 °C/min to 190 °C, 30 °C/min ramp to 260 °C held for 2 min. The carrier gas (hydrogen) was maintained at 1.1 mL/min (0.61 bar), measured at 100 °C. Split injection (1:50) was performed at 250 °C, and the identification of the individual fatty acids was achieved by comparing the retention times of the fatty acid methyl esters to commercial standards, namely FAME Mix C4-C24 (standard 4788-U, Sigma-Aldrich). The same software used for the soluble sugars was employed for the fatty acids. The results were presented in relative percentage of each fatty acid.

2.3.4. Mineral composition: Macro and micronutrients

The mineral composition was determined using an atomic absorption spectrophotometer Perkin Elmer, Waltham, MA, USA), following the procedure described by Carocho et al. (2015). The samples were subject to a dry-ash mineralization at 550 °C with the resulting residue being extracted with HCL and HNO_3 and finally adjusting the volume with distilled water. The microelements Fe, Co, Zn and Mn were directly measured, while the macroelements Ca, Mg, Na and K were diluted in a 1/10 reason to avoid interferences. The determination of the mineral elements was achieved by comparing the absorbance responses to pure analytical solutions of $Fe(NO_3)_3$, $Zn(NO_3)_2$, NaCl, KCl, $CaCO_3$ and Mg. The results were expressed in mg/100 g of fresh weight.

2.4. Physical analysis

The physical analysis carried out for the bread samples encompassed a full determination of the texture profile, including the hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience. Furthermore, the colour of the bread crumb was also analysed with a portable colorimeter.

2.4.1. Texture profile

In terms of the texture profile, it was conducted using a Stable Micro Systems (Vienna Court, Godalming, UK) TA.XT Plus texture analyser with a 30 kg load cell. The probe used was the P/45 45 aluminium cylinder, which performed a texture profile analysis (TPA) which is 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 3 mm/s and the target mode was set to 25% strain which started at 50 g of force. The results were combined and processed through a macro to reach the various dimensions of texture, namely hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience. The results were analysed through the Exponent program.

2.4.2. Colour measurement

The crumb colour was analysed with a portable colorimeter CR400 from Konica Minolta (Chiyoda, Toko, Japan), using the D65 illuminant, a standard one from the International Commission of Illumination (CIE) representing the midday light in Europe. The CIE L^* a^* b^* colour space of 1976 was used, in which L^* represents the lightness, the a^* represents the redness (red-green), and b^* the yellowness (yellow-blue), with a 10° observer angle and 8 mm of aperture. The variation in total colour difference (ΔE*) was also calculated between the bread samples following the equation:

$$\Delta E^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

2.5. Statistical analysis

All results were expressed as mean ± standard deviation (SD), with the number of decimal places according to the magnitude of the standard deviation. To compare the different parameters between the different bread types, an analysis of variance (ANOVA) was carried out, after analysing the homoscedasticity and distribution of the means. When possible, the breads were classified using a Tukey's test relying on a significance level of 0.05. All bread samples were produced in triplicate and all extractions and analysis were also carried out in triplicate.

3. Results and discussion

The increase of consumer awareness and a pursue of a healthier lifestyle has pushed the market to develop new products with beneficial health effects. Thus, the bakery industry has tagged along this trend and developed breads with non-wheat cereals, which pose challenges in terms of the dough, texture behaviour, volume and sensory quality. Thus, the mixture of different non-wheat cereal flours with wheat has been tested in this work. Comparing the chemical and physical profile of different types of breads with non-wheat cereals like Rye, Oat and seeds with a portion of wheat flour has proven to have interesting and acceptable outcome for consumers. Table 1 shows the composition of the breads, with Multicereal showing the highest amount of wheat flour (850 g/kg), while the lowest amount is used to make the Rye bread. In terms of the leavening time, all breads except Rye and Oat are kept at 4 °C for 24 h. Rye and Oat bread require less temperature and less leavening, and thus, are allowed to leaven for only one hour at room temperature.

3.1. Nutritional profile

In terms of the chemical parameters analysed, the nutritional profile of all breads was analysed following AOAC procedures and is displayed on Table 2. The water content of the breads was generally similar, Oat and Multicereal showing the lowest content and statistically different from Rye and Wholemeal. Bavaria showed the highest amount, about 38%. The Multicereal bread showed the highest amount of proteins,

Table 2
Nutritional profile of each bread type, represented as g/100 g of fresh weight. Energy represented as kcal/100 g of fresh weight.

	Moisture	Proteins	Crude Fat	Ash	Total Available Carbohydrates	Insoluble Fibre	Soluble Fibre	Total Fibre	Energy
Multicereal	34.1 ± 0.5^{a}	9.5 ± 0.1^{e}	3.65 ± 0.02^{d}	1.05 ± 0.01^{d}	41 ± 4^{a}	4.9 ± 0.4^{d}	5.8 ± 0.4^{c}	10.7 ± 0.9^{d}	256 ± 8^{b}
Bavaria	38.4 ± 0.9^{c}	7.28 ± 0.05^{c}	0.95 ± 0.1^{c}	0.97 ± 0.03^{b}	50 ± 2^{b}	2.0 ± 0.1^{c}	0.33 ± 0.01^{a}	2.4 ± 0.1^{b}	242 ± 5^{a}
Wholemeal	37.1 ± 0.9^{b,c}	6.55 ± 0.04^{b}	0.18 ± 0.01^{a}	0.87 ± 0.05^{a}	52 ± 4^{b}	2.0 ± 0.2^{c}	1.3 ± 0.2^{b}	3.3 ± 0.3^{c}	242 ± 6^{a}
Rye	36 ± 19^{b}	5.68 ± 0.06^{a}	0.51 ± 0.02^{b}	0.821 ± 0.007^{a}	56 ± 6^{b}	0.67 ± 0.01^{a}	0.35 ± 0.02^{a}	1.02 ± 0.03^{a}	253 ± 5^{b}
Oat	33 ± 18^{a}	9.1 ± 0.1^{d}	0.94 ± 0.04^{c}	1.04 ± 0.03^{c}	54 ± 2^{b}	1.18 ± 0.05^{b}	1.06 ± 0.02^{b}	2.24 ± 0.08^{b}	265 ± 4^{c}

Each letter in each column represents a significative difference among samples, obtained from a Tukey's test with a significance interval of 0.05.

crude fat and fibres, although the one with the highest energy was Oat. The variation in carbohydrates showed an interesting result, namely similar amounts of carbohydrates in all bread types with only Multicereal showing lower statistically different values. Still, the high amount of proteins, fat and fibre in the Multicereal bread can be attributed to the nutrients present in the seeds of sesame, sunflower, linseed and carob flour that take part in the Multicereal mixture. Inversely, Rye bread showed the least amount of proteins and fibre, while Wholemeal had the least amount of fat. Regarding energy, Bavaria and Wholemeal showed the lowest amounts, with no statistical difference among them, but statistically different from Rye and Multicereal and with statistical difference among each other. Thus, considering protein intake, the highest scoring breads were Multicereal followed by Oat, while for consumers seeking high fibre breads, the recommended one is clearly Multicereal, although it has a high amount of fat. According to European Regulation (EC) No 1924/2006 the claim "source of dietary fibre" may be used for Wholemeal and Multicereal breads as their content in total dietary fibre (TDF) is higher than 3 g per 100 g. Moreover, Multicereal bread could be also considered as "high dietary fibre" because it provides more than 6 g/100 g fw, the following health claims "Wheat bran fibre contributes to an acceleration of intestinal transit" and "Wheat bran fibre contributes to an increase in fecal bulk" can be stated according to Regulation (EU) No 432/2012 and Regulation (EC) No 1924/2006.

3.2. Individual fatty acids and soluble sugars

Table 3 shows the individual fatty acids and soluble sugars present in the different bread samples. Fatty acids were expressed in relative percentage of themselves, while soluble sugars were quantified by comparison to commercial standards and retention times. In terms of the individual fatty acids, only the most abundant ones were considered, and thus, although there is a higher number of saturated fatty acids (SFA) in all bread samples, interestingly the polyunsaturated (PUFA) ones showed the highest amounts, making these breads a healthy option in terms of unsaturated fatty acid consumption. Globally, the highest amount of PUFA and lowest SFA was registered for the Rye bread, and thus making this bread the healthiest. Saturated fatty acids are related to metabolic syndromes, coronary hearts disease and changes in the gut microbiota, among other diseases, while MUFA and PUFA have generally health improving effects (Julibert, Bibiloni, & Tur, 2019; Li et al., 2015; Wolters et al., 2018). Monounsaturated fat was found in the statistically highest quantities in the Multicereal bread followed by Oat, with a clear contribution of unsaturated fats found in the seeds. The highest amounts of SFA were found in Bavaria and Oat breads, statistically higher than the rest of the breads, whilst the lowest statistical difference was found for Rye and Multicereal bread. Individually, the highest unsaturated fatty acid in all bread samples was linoleic acid, while the highest saturated one was palmitic acid. Regarding soluble sugars, the compounds were detected in all bread samples, namely fructose, glucose and maltose. Overall, the amount of total soluble sugars was very similar, with only the Oat bread showing a higher amount of these compounds. As expected, the highest soluble sugar was maltose, a common sugar found in cereal. Glucose was not detected in Wholemeal and Oat breads and detected in very low quantities in the other three bread types. This could be due to the low glucose present in most cereals (Žilić et al., 2017), and furthered by the long leavening time that most breads underwent, namely Multicereal, Wholemeal and Light Wholemeal bread, which leavened for 24 h, thus consuming a considerable amount of the few glucose available.

3.3. Mineral composition

On Table 4, the mineral fraction of the different bread types can be found, displaying four microelements (Fe, Cu, Zn and Mn) and four macroelements (Ca, Mg, Na, and K), all detected through atomic absorption spectroscopy. As expected, the most prevalent minerals were the macroelements, with magnesium and potassium being the most abundant. The least quantified minerals were copper and manganese. Bavaria was the bread with the least amount of all mineral content. Considering bread as a staple food for millions of human beings, the daily intake of many nutrients and minerals relies on bread, making the amount of each mineral important to be known. In terms of iron, an important player in the synthesis of haemoglobin and a co-factor for enzymes (Abbaspour, Hurrell, & Kelishadi, 2014), only the Bavaria bread was statistically significant from all the other breads, showing a slightly lower amount. The recommended daily intake of Fe is set at 8 to 10 mg/day, according to the EFSA (2006). Considering an intake of about 50 g of bread daily, these breads only represent about 5% of the daily intake, which is quite low. Copper is also known for having a role in the synthesis of haemoglobin, but also important in redox reactions and for cuproenzymes (Abbaspour et al., 2014; Askwith & Kaplan, 1998). For this mineral, the major quantity was found in the multicereal bread with 0.67 mg/100 g, and the least in the Bavaria bread. The upper level of Copper for the adult population of the EU is set at 5 mg/day, making these breads contribute between 5 and 10% of this limit. Zinc has a differentiated upper limit, split between genders; men have a limit of 9.5 mg/day, while woman should not consume above 7. This element is one of the most important minerals for human health, with more than 300 enzymes depending on it for normal function. Furthermore, 10% of the human genome encodes for proteins that can bind to zinc, being the deficiency of zinc related to a myriad to illnesses

Table 3
Representation of the major fatty acids, expressed in relative percentage, found in the different bread types, the monounsaturated (MUFA), polyunsaturated (PUFA) and saturated fatty acids (SFA) determined through GC-FID, the soluble sugars detected through HPLC-RI.

	Multicereal	Bavaria	Wholemeal	Rye	Oat
C12:0	0.068 ± 0.001[a]	0.95 ± 0.01[d]	1.732 ± 0.005[e]	0.886 ± 0.002[c]	0.66 ± 0.02[b]
C14:0	0.966 ± 0.002[a]	2.0 ± 0.8[c]	0.98 ± 0.05[a, b]	0.46 ± 0.07[a]	1.8 ± 0.6[b, c]
C16:0	9.64 ± 0.05[a]	25 ± 3[c]	19.5 ± 0.4[b]	11.2 ± 0.6[a]	23 ± 3[b, c]
C18:0	4.7 ± 0.2[a]	5.1 ± 0.5[a]	4.2 ± 0.2[a]	3 ± 1[a]	6 ± 3[a]
C18:1	15.6 ± 0.1[c]	13 ± 2[c]	7.55 ± 0.08[b]	3.2 ± 0.3[a]	14 ± 2[c]
C18:2	63 ± 0.3[c]	51 ± 2[b]	60.8 ± 0.6[c]	28.2 ± 0.9[a]	52 ± 4[b]
C18:3	7.03 ± 0.03[b]	3.14 ± 0.08[a]	4.96 ± 0.09[a, b]	54 ± 3[c]	3.14 ± 0.07[a]
MUFA	15.6 ± 0.1[d]	13 ± 2[c]	7.54 ± 0.08[b]	3.2 ± 0.3[a]	14 ± 2[c, d]
PUFA	69.8 ± 0.3[b]	55 ± 3[a]	66.0 ± 0.8[b]	81 ± 2[c]	56 ± 5[a]
SFA	14.4 ± 0.2[a]	33 ± 5[c]	26.3 ± 0.7[b]	16 ± 2[a]	30 ± 6[b, c]
Fructose	0.111 ± 0.001[c]	0.046 ± 0.009[a]	0.18 ± 0.01[d]	0.28 ± 0.03[e]	0.08 ± 0.01[b]
Glucose	0.09 ± 0.01[c]	0.031 ± 0.003[a]	n.d.	0.08 ± 0.01[b]	n.d.
Maltose	0.93 ± 0.03[a]	0.8 ± 0.2[a]	0.87 ± 0.08[a]	0.81 ± 0.05[a]	1.6 ± 0.1[b]
Total Soluble Sugars	1.13 ± 0.01[a]	0.9 ± 0.1[a]	1.05 ± 0.08[a]	1.170 ± 0.008[a]	1.7 ± 0.2[b]

Each letter in each column represents a significative difference among samples, obtained from a Tukey's test with a significance interval of 0.05.

Table 4
Detailed description of the mineral fraction of the different bread types, expressed as mg/100 g, including the microelements Fe, Cu, Zn and Mn, and the macroelements Ca, Mg, Na, K, detected through atomic absorption spectrometry.

	Iron (Fe)	Copper (Cu)	Zinc (Zn)	Manganese (Mn)	Calcium (Ca)	Magnesium (Mg)	Sodium (Na)	Potassium (K)
Multicereal	1.6 ± 0.1^{b}	0.67 ± 0.02^{c}	2.0 ± 0.1^{c}	0.54 ± 0.05^{b}	3.5 ± 0.6^{b}	56 ± 1^{c}	25 ± 1^{b}	54 ± 7^{b}
Bavaria	1.25 ± 0.02^{a}	0.38 ± 004^{a}	1.12 ± 0.06^{a}	0.49 ± 0.03$^{a, b}$	8.3 ± 0.7^{a}	31 ± 2$^{a, b}$	19 ± 1^{a}	25 ± 3^{a}
Wholemeal	1.6 ± 0.1^{b}	0.84 ± 0.03^{d}	1.08 ± 0.09^{a}	0.43 ± 0.04^{a}	10.8 ± 0.1^{c}	33 ± 2^{b}	18 ± 2^{a}	43 ± 1^{b}
Rye	1.69 ± 0.07^{b}	0.50 ± 0.05^{b}	1.50 ± 0.05^{b}	0.49 ± 0.04$^{a, b}$	9.7 ± 0.6^{b}	28 ± 2^{a}	26 ± 1$^{b, c}$	56 ± 18^{b}
Oat	1.7 ± 0.01^{b}	0.50 ± 0.02^{b}	1.8 ± 0.3^{c}	0.51 ± 0.04^{b}	7.9 ± 0.2^{a}	33 ± 1^{b}	28 ± 1^{c}	50 ± 4^{b}

Each letter in each column represents a significative difference among samples, obtained from a Tukey's test with a significance interval of 0.05.

(Nriagu, 2018). Multicereal and Oat bread stand out as the ones with the highest amount of zinc, respectively 2 and 1.8 mg/100 g. Rye is statistically lower, and Bavaria and Wholemeal have the least amount. All these quantities are well inside the upper limit consumption for this mineral. Manganese, a trace microelement that is essential for many metabolic functions, namely metalloenzymes, although its over-consumption is related to toxicity, especially neurotoxicity. The upper limit has not been defined by EFSA, although it has placed an adequate intake which is set at 3 mg/day for adults. Grain is known for having high quantities of manganese, reaching, in some types, a level of 10 mg/kg, thus, it is important to produce bread with levels that will not cause an excess in consumption of this mineral (EFSA, 2006; EFSA, 2013; Röllin & Nogueira, 2019). Interestingly, all the breads showed a very similar quantity of manganese, varying from 0.43 for Wholemeal bread and 0.54 for Multicereal, which, considering an intake of 50 g of any bread daily, does not constitute a risk of overconsumption of manganese. Considering the analysed macroelements, calcium, widely known for being an essential compound in the formation and maintenance of bones and skeletal integrity, has a tolerable upper intake of 2500 mg/day for adults set by the EFSA (EFSA, 2012; Wimalawansa, Razzaque, & Al-Daghri, 2018). Thus, the bread with the highest amount of calcium, Wholemeal, having 10.8 mg/100 g is still considered poor in calcium, while Multicereal and Rye bread has lower amounts, but undistinguishable between each other, and the breads with the least amount of calcium where Bavaria and Oat. Magnesium is another important mineral for the human body, being a co-factor of over 600 enzymes while also playing a role in the stabilization of nucleotides. Its adequate intake has been set by EFSA at 350 mg/day for men and 300 for woman (Curry & Yu, 2018; EFSA, 2015; Sun, Wang, Li, & Zhang, 2019). Regarding the breads, the highest amount was registered for Multicereal bread, with 56 mg/100 g, which makes this bread particularly rich in this mineral, followed by Bavaria, Wholemeal and Rye breads. Oat showed the least amount of magnesium, but alongside with Rye had the highest amount of Sodium, being the least registered in Bavaria and Wholemeal. Sodium, a part of salt (NaCl), has a very high importance in human health, linking its excessive consumption with high blood pressure, coronary problems and other circulatory diseases, although booth elements are essential body electrolytes. Considering the daily needs and the dangers of excess consumption, the EFSA established an adequate intake of 1.5 g/day of sodium, which corresponds to 3.8 g of salt per day (Alderman, 2000; EFSA, 2006). Considering the breads analysed in this work, none of them had over 0.04 g/100 g, and thus, all of them can be considered a "very low salt" food. Rye and Multicereal had the highest amount, and only reached 26 and 25 mg/100 g, respectively. The lowest amount was detected in Wholemeal bread. Finally, potassium, an essential mineral for the human body, an important electrolyte, required for normal cellular function has a recommended intake set at 3500 mg/day, which, below this level are correlates with a higher risk of stroke (EFSA, 2016). The breads all showed similar quantities of potassium, only Bavaria bread showing statistically less quantity. Overall, the breads did not show mineral amounts that could go beyond the daily adequate intake, making them indicated for all genders and ages, including pregnant and lactating females. Bavaria was the bread with the worst mineral profile due to having the lowest iron, copper, zinc, magnesium and potassium, but also displayed the least sodium which is desirable. Inversely, Oat bread showed high quantities of various minerals, including the infamous sodium, making it the worst in terms of this mineral among the lot, but still being considered a very low salt food. Overall, in terms of calcium, all breads could have higher amounts of this mineral.

3.4. Physical analysis

3.4.1. Texture

The top section of Table 5 shows the different dimensions of the bread's texture, as well as the crust colour. To maintain homogeneity among the different bread types, the analysis was carried out on the center slice of the breads, with a defined width of 2 cm. Three TPA's were carried out on each slice, which, after running a macro allowed to obtain the six dimensions displayed on the top section of Table 5 (hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience). The first dimension, hardness, is defined as the force the teeth

Table 5
The top section of the table details the various texture dimensions analysed for each bread type. The bottom section shows the total colour difference (ΔE) between each of the breads Detailed description of the texture dimensions.

	Hardness (g)	Adhesiveness (g.*sec*)	Springiness (%)	Cohesiveness (%)	Chewiness	Resilience (%)
Multicereal	1247 ± 18^{b}	−0.10 ± 0.06^{a,b}	2.5 ± 0.5^{b}	0.84 ± 0.06	1890 ± 44$^{c, d}$	0.45 ± 0.02^{b}
Bavaria	1952 ± 17^{c}	−0.23 ± 0.09$^{b, c}$	0.976 ± 0.001^{a}	0.84 ± 0.03	1640 ± 75$^{a, b}$	0.374 ± 0.003^{a}
Wholemeal	513 ± 7^{a}	−0.04 ± 0.01^{a}	3.82 ± 0.01^{c}	0.92 ± 0.04	1825 ± 73$^{b, c}$	0.54 ± 0.02^{d}
Rye	2594 ± 62^{d}	−0.3 ± 0.2^{c}	2.5 ± 0.9^{b}	0.88 ± 0.04	2114 ± 37^{d}	0.51 ± 0.02$^{c, d}$
Oat	633 ± 167^{a}	−0.14 ± 0.02$^{a, b}$	1.8 ± 0.7$^{a, b}$	0.86 ± 0.01	1563 ± 251^{a}	0.48 ± 0.02$^{b, c}$
		Multicereal	Bavaria	Wholemeal	Rye	Oat
Multicereal			6 ± 2^{b}	2 ± 1^{a}	4 ± 2$^{a, b}$	4 ± 1$^{a, b}$
Bavaria		6 ± 2^{b}		6 ± 2^{b}	2 ± 1^{a}	3 ± 1^{a}
Wholemeal		2 ± 1^{a}	6 ± 2^{b}		7 ± 3^{b}	5 ± 2^{b}
Rye		4 ± 2$^{a, b}$	2 ± 1^{a}	5 ± 3$^{a, b}$		2.5 ± 0.5^{a}
Oat		4 ± 1$^{a, b}$	3 ± 1^{a}	5 ± 2$^{a, b}$	2.5 ± 0.5^{a}	

Each letter in each column represents a significative difference among samples, obtained from a Tukey's test with a significance interval of 0.05.

have to apply on the food, and is measured in grams (Monaco, Cavella, & Masi, 2008). By analysing the top section of Table 5, it appears clear that Rye is the hardest bread, followed by Bavaria and Multicereal, while Oat and Wholemeal have the same hardness, which is quite low. This is in line with literature (Salehifar & Shahedi, 2007; Young, 2012), and partially explained by the elasticity of gluten, which is found in higher amounts in wheat flour, and thus, Rye and Bavaria by having the lowest amount of this flour, scored the hardest in this dimension. Oat is known for its soft doughs and breads and showed the lowest hardness of all the analysed samples, while also having a high amount of wheat flour, allowing it to be softer than Multicereal bread. The second analysed dimension is adhesiveness, which is defined as the capacity that food has to adhere to the teeth while chewing and is expressed in negative values due to the measuring force being applied from bottom to top in the texturometer (Paula, 2014). As expected, all the values were quite low, not even scoring 1 g.*sec*. Bread is not known for being very adhesive, although Rye and Bavaria scored −0.3 and −0.23 g per second, respectively, while Wholemeal was the least adhesive with only −0.04 g.*sec*. This dimension is somewhat linked to hardness, showing that the hardest breads are the most adhesive. Another dimension of texture is the springiness, defined by the rate at which a deformed food reverts to the undeformed state after removing the deforming force (Faber, Jaishankar, & McKinley, 2017), and is measured in percentage. Wholemeal was the springiest bread, followed by Multicereal and Rye *in exequo* with 2.5%, while Bavaria was the least springy. Bread is not a very springy food, and thus, all breads showed a very low value, with Wholemeal and Multicereal showing the highest values, probably due to a spongier dough, in the case of Multicereal aided by the seeds that reduced dough density, although, all values were very low, only ranging from 3.8 to 0.9. Cohesiveness is considered as the success of a food to withstand a second deformation relative to its resistance to the first deformation, and also expressed in percentage. Given the relative compressive capacity of the bread, cohesiveness was quite uniform for all breads, with no difference being recorded for any. Chewiness is the product of hardness, cohesiveness and springiness, and usually defined as the energy required to masticate food, although it is dimensionless (Chandra & Shamasundar, 2015). It would be expected for chewiness to be quite uniform provided it derives from other dimensions, namely hardness and springiness, which showed different values for the same bread. Rye was the chewiest bread of all samples, followed by Multicereal and Wholemeal with no statistical differences among the two, and finally Oat scoring the lowest, only 1563. Still, the hardest bread is the chewiest, meaning that it requires more energy to be eaten, which is quite expected due to the higher force needed to chew a hard substance. Resilience is similar to springiness, although it measures both the speed and forces involved in the recovery of a food when a deforming force is removed, being also measured in percentage. Some describe it as how a food “fights” to regain its original height (Chandra & Shamasundar, 2015). Overall, the variation of the analysed breads was quite small, only ranging from 0.374 (Bavaria) to 0.54% (Wholemeal), which means the different flours used for the bread formulation did not really affect resilience adhesiveness and cohesiveness, while they did induce some differences in the hardness and springiness.

3.4.2. Crust colour

Section a) of Fig. 1 reports the crust colour of the breads, analysed with a portable colorimeter that measured the L^*, a^* and b^*. L^* measured the lightness of the breads, where the higher the number the lighter the bread is, as represented on the L^* bars of Fig. 1. There was no statistical difference for L^* in the analysed breads, meaning the differences in the flour lightness do not affect the lightness of the breads. For the a^*, which measures the interval between the red and green (as seen in the a* bars), where the values from 0 to + 100 represent the red, and 0 to −100 represent the green colour. All breads showed values very close to 0, with Bavaria and Rye *in exequo* showing the highest amounts or red and Oat showing the greenest. Finally, b^* represent the blueness, where positive values show an increase in blue, and negative ones show an increase in yellow (as seen in the bars of b^* in Fig. 1). Once again, the values did not show a high variation, only ranging between 19.3 for Bavaria and 15 for Multicereal and Wholemeal, with the same amount.

Section b of Fig. 1 shows 6 circles that represent the overall colour read by the colorimeter and compiled by joining the different L^*, a^* and b^* coordinates of each bread. It is clear that Bavaria bread has, as expected, a darker tone, given the dark colour of its grains and flour, and confirmed by the lower L^* values in Fig. 1, while Wholemeal showed the lightest tone. On the bottom section of Table 5, the total colour difference between the breads can be assessed using the formula described in section 2.4.2., and complements the information provided in section b of Fig. 1. Here, it is clear that the highest statistical difference in colour can be found between Wholemeal and Bavaria, and Bavaria and Multicereal *in exequo*, followed by Rye and Wholemeal. Furthermore, the breads with the most similar crumb colour are Bavaria and Rye, displaying a higher amount of red, but also between Multicereal and Wholemeal with a* close to zero. Overall, the breads did not show very drastic colour changes due to having a relatively high amount of wheat flour. This wheat could not be reduced to maintain its acceptability from consumers. A table with the results of the crust colour can be found in Table S1 (Supplementary material).

4. Conclusion

The use of different flours for bread baking is becoming prevalent throughout the world, and efforts must be made to understand the effects of flours on the chemical and physical profiles of bread. The use of these flours is motivated not only by the consumers that seek healthier foods, but also by the industry itself, which seeks innovations to maintain profits and seduce customers to buy new products. By analysing the chemical and physical profile, the differences and specificities of each bread type stands out, and all of them seem to have their benefits and disadvantages. For instance, a consumer seeking a bread with low calories would choose Wholemeal for its low-fat content, or Rye, although Rye has the hardest crumb of all. A consumer seeking bread with low sodium would prefer Bavaria or Wholemeal, although Bavaria has the highest saturated fatty acids, making it less healthy. Oat scores the highest sodium, but also the least fat, and at par with Bavaria, are the easiest to chew. Multicereal, on the other hand, has the highest fat, but because its unsaturated fat makes it desirable for healthy diet consumers, and furthermore for its high fibre quantity. Wholemeal scores the highest in calcium amount and is also the softest bread of all. The variety of diets these breads can blend into proves that bread can, in fact, continue to be a staple food in many diets around the world, and still be a desirable food for future generations, and seduce the industry to continue investing in innovation for this sector, especially by introducing new flours beyond wheat.

CRediT authorship contribution statement

Márcio Carocho: Methodology, Investigation, Writing - original draft. **Patricia Morales:** Methodology, Writing - original draft. **María Ciudad-Mulero:** Methodology, Writing - original draft. **Virginia Fernández-Ruiz:** Investigation, Methodology. **Elisabete Ferreira:** Conceptualization, Methodology. **Sandrina Heleno:** Methodology, Writing - original draft. **Paula Rodrigues:** Methodology, Writing - original draft. **Lillian Barros:** Methodology, Writing - original draft, Writing - review & editing. **Isabel C.F.R. Ferreira:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

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

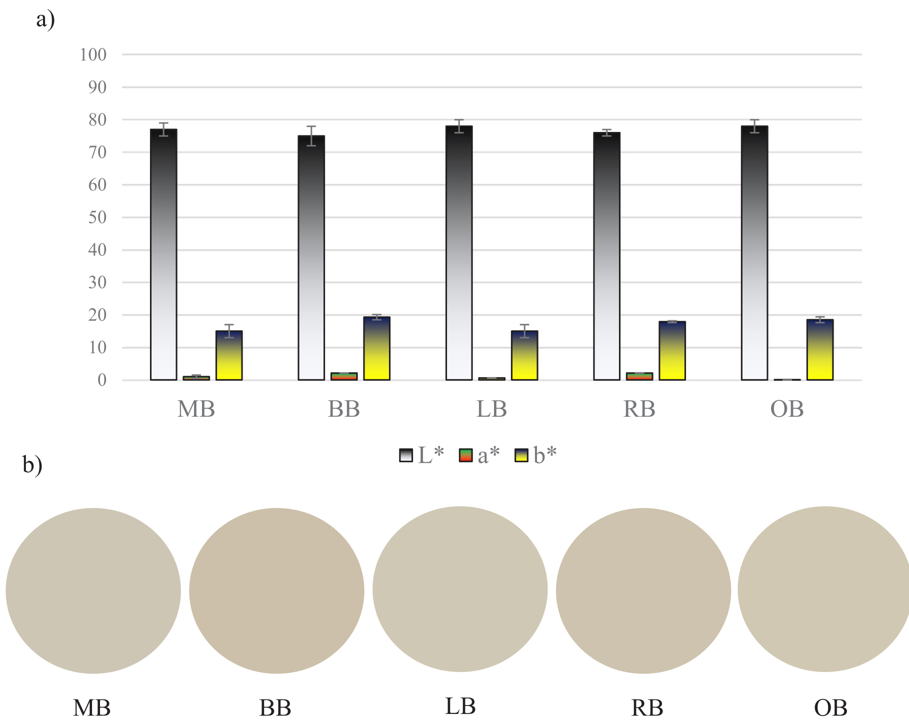

Fig. 1. a) Graphical representation of the crust colour, following the CIELab L^*, a^*, b^* representation of the different bread types. MB – Multicereal bread, BB – Bavaria bread, LB – Light Wholemeal bread, RB – Rye bread and OB – Oat bread. b) Representation of the colour of the bread crumb read by the colorimeter using the L^*, a^* and b^*.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125954.

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