

Techno-functional and nutritional performance of commercial breads available in Europe

Paola Conte^{1,2}, Costantino Fadda², Antonio Piga², and Concha Collar^{1*}

¹Cereals and Cereal-based Products. Food Science Department. Instituto de Agroquímica y Tecnología de Alimentos (CSIC). Avda. Catedrático Agustín Escardino, 7. 46980 Paterna. SPAIN.

²Dipartimento di Agraria. Sezione di Scienze e Tecnologie Ambientali e Alimentari. Università degli Studi di Sassari. Viale Italia, 39. 07100 Sassari. ITALY

*Corresponding author. Tel.: +34 963 90 00 22; Fax: +34 963 63 63 01

Email address: ccollar@iata.csic.es

Abstract

In recent years, the growing interest for well-being and healthy lifestyle together with an increasing awareness of the close relationship between food and health have boosted the production of an increasing number of novel goods to be placed in both gluten-containing and gluten-free products market. The objective of this study was to provide a realistic and detailed overview of the current bread-market supply, in order to evaluate the overall quality of the available offer in this prioritised food industry area. Twenty commercial breads consisting of gluten (n=10) and gluten-free (n=10) samples currently available in the European market, have been assessed by physical-chemical, technological, nutritional, and sensory determinations. The quality parameters obtained were related to each other by using Pearson correlations, while sample classification was achieved by applying factor analysis. Although the main distinction was between gluten and gluten-free samples as it was expected, classification of breads allowed differentiating samples with different formulations in terms of presence/absence of alternative, innovative and nutrient-dense raw materials.

Keywords

Bread, gluten-free, physico-chemical characteristics, nutritional features, sensory analysis

Introduction

Bread has been one of the staple foods most widely used and consumed around the world and one of the major constituents of the human diet since ancient times. Although the simplicity of the basic recipe (flour, water, salt and leavening agent) the long-term success of bread is ascribed to its typical flavour, taste, and its high nutritional value. Bread is a good source of energy mainly due to the high content of starch, besides protein, lipids rich in essential fatty acids, dietary fibre, antioxidants, and micronutrients (Rubel et al., 2015). Nowadays, despite some differences between countries depending on bread type or region, the level of bread consumption in the world has been declining (Cauvain, 2015). This trend does not apply to the gluten-free market that is experiencing a double-digit growth, as one of the most prosperous market niche in the immediate and near future (Miranda et al., 2014). Even in Europe, bread market has been showing contrasting patterns within countries (Collar, 2015). A study for European Commission in 2010 (made through 27 European Union states) reported that bread consumption patterns differ widely within the European Union but most countries have an average consumption of 50 kg of bread per capita per year (Federation of Bakers, 2013). This slight decline can be caused by several factors including the changes in consumers' food preferences (increasing consumption of alternative and energy-dense foods often rich in fat) and evolution of eating habits (growth of out-of-home meals), often associated with a lack of physical activity. Over the past decades these changes in people's lifestyles have also resulted in a dramatic increase of several non-communicable diseases including obesity, type 2 diabetes, cardiovascular diseases, and certain forms of cancer. However, consumer's demands in the field of food production have changed in depth (Betoret et al., 2011). In recent years, the increasing awareness of the relationship between food and health, the growing demand for healthy, natural and innovative foods as well as the increasing prevalence of food intolerances (in particular coeliac disease) led both scientific

research and bakery industry to make considerable efforts in order to meet the needs of consumers, and to improve the variety, quality and taste of bakery products available in the market. Thus, breads made from grains, grain flours, and bran alternative to wheat or containing other functional ingredients are acquiring a privileged position in the bakery market. The use of whole grains as partial substitutes of wheat flour in bread formulations is of nutritional interest because of their lower glycaemic index and health-related composition including dietary fibre, minerals, vitamins and antioxidants. The intake of whole-grain bread, which provides more health benefits than refined-grain bread, is generally associated with a reduced risk of coronary heart disease and type-2 diabetes (Blandino et al., 2013). Bread products enriched/fortified with functional components such as ω -3 fatty acids (Gökmen, 2011), prebiotic oligosaccharides (Angioloni and Collar 2011a), inulin (Rubel et al., 2015), and calcium (Salinas and Puppo, 2015) as well as multigrain breads obtained by the addition of minor cereals, pseudocereals, and grain legumes flours (Collar et al., 2014a), are also in good agreement with the current nutritional and nutraceutical dietary trends. Conversely, in case of allergies and food intolerances such as coeliac disease, the production of bread products made with gluten-free alternative raw materials becomes a necessity. Furthermore, in gluten-free breadmaking, a consumer-satisfying structure, an adequate nutritive value and a good taste of bread can only be achieved using a combination of different ingredients (Houben et al., 2012). Apart from most basic gluten-free ingredients such as rice and corn flour blended with structuring agents (hydrocolloids) and dairy proteins (Lazaridou et al., 2007), also different gluten-free flours (corn, teff, buckwheat, quinoa, sorghum) (Hager et al., 2012) and starches (corn, cassava, potato) (Collar et al., 2014b) as well as enzymes (transglutaminase, proteases) (Hamada et al., 2013), and other non-gluten proteins (from both animal and plant origin, e.g. milk protein, egg albumins and soy protein) (Ziobro et al.,

2013) are being used in order to mimic the viscoelastic properties of gluten and to improve the overall quality of gluten-free bakery products.

In this context, the objective of the present study was to provide a comprehensive, realistic and detailed overview of the current bread-market supply through the physical-chemical, technological, nutritional, and sensory characterization of 20 European commercial breads, in order to obtain an overall quality picture of the available offer in this prioritised food industry area.

[insert Figure 1.]

Materials and Methods

Materials

Twenty commercial breads (10 gluten-containing and 10 gluten-free samples) from major brands were selected and purchased from the European market (Figure 1). The chemical and nutrition facts of breads were retrieved from the labels provided by the manufacturers, with the only exception of both moisture and ash contents. Moisture determination was performed according to the AACC method 44-15.02 (AACC, 2005), while the ash content was estimated by difference. The ingredient composition of breads is compiled in Table 1. A four digit bread sample code was defined for commercial breads according to their crumb colour (1st digit), absence/presence of seeds (2nd digit), and sample number (3rd and 4th digits). The first digit of the code was set referring to white bread (1), mixed bread (2), and dark bread (3), the second digit to absence (1) or presence (2) of seeds, and the third digit to sample number (from 1 to 10 for gluten breads and from 11 to 20 for gluten-free breads), as it follows: 1101, 2202, 1103, 3104, 1105, 1206, 1107, 3208, 3209, 3210, 1211, 1112, 2213, 1114, 1115, 2116, 1117, 1118, 1119, 3220 (Figure 1).

[insert Table 1]

Methods

Bread measurements

Physical-chemical properties. The volume of bread samples was measured according to the AACC 10-05.01 method of rapeseed displacement (AACC, 2005). The specific volume was calculated as bread volume (ml) / bread weight (g). Aspect ratio was calculated as width/height ratio of central slices.

Colour measurements were determined on both crumb and crust using a Photoshop system in accordance with the method previously described by Angioloni and Collar (2009) and the results were expressed in accordance to the Hunter Lab colour space. The Photoshop (PS Adobe Photoshop CS5 extended) system (L, a, b colour coordinates) was calibrated using colour sheets from Pantone®Formula Guide (Pantone, Inc., USA). Pantone colour sheets and bread slices (three slices per sample) were used for calibration and for colour measurement, respectively. Images were acquired at 300 pixel resolution with a ScanJet II cx flatbed scanner (Hewlett-Packard, USA). Parameters determined were L (L = 0 [black] and L = 100[white]), a (-a = greenness and +a = redness), b (-b = blueness and +b = yellowness), WI - whiteness index (crumb), and BI - Browning Index (crust), as described earlier (Collar and Angioloni, 2014). Hunter Lab colour space parameters from Minolta colorimeter were calculated from the calibration linear equation Colorimeter vs Photoshop (Angioloni and Collar, 2009).

Crumb grain characteristics were assessed in bread slices using a digital image analysis system. Images were previously acquired with a ScanJet II cx flatbed scanner (Hewlett-Packard, USA). The analysis was performed on 40×40 mm or 60×60 mm squares (depending on the size of breads) taken from the centre of the images and data were processed using SigmaScan Pro 5 (Jandel Corporation, USA). The crumb grain parameters determined were: cell area, cell density (cell/cm²), cell/total area ratio, and wall to total area ratio (Collar et al., 2005). According to the

pre-selected cell size range (<0.4 mm², 0.4-1.0 mm², 1-10 mm², 10-80 mm², and >80 mm²), cell area distribution and cell number distribution were also determined.

Bread primary and secondary mechanical characteristics (Texture Profile Analysis, TPA, using a double compression cycle) of breads were recorded in a TA-XT2 texture analyser (Stable Micro System, Surrey, UK) using a 25 mm diameter probe, a 30 Kg load cell, 50% penetration depth and a 30 s gap between compressions on slices of 25 mm width (Collar et al., 2005).

For stress relaxation (SR) measurements, samples from the centre of the crumb slices were cut into cubes (2x2x2 cm) and compressed using a TA-XT plus texture analyser (Stable Micro System, Surrey, UK). Samples were compressed using a cylindrical upper die of 50 mm diameter at a cross speed 0.5 mm/sec. The strain used was 20% and the whole relaxation experiment lasted 10 min. The obtained stress relaxation curves were normalized and converted to linear form according to the Peleg and Pollak (1982) model, previously applied by Angioloni and Collar (2009) for bread:

$$\frac{F_0 t}{F_0 - F(t)} = k_1 + k_2 t \quad (1)$$

where F_0 is the initial force, $F(t)$ the momentary force at time (t) and k_1 (s), k_2 are constants related to the initial rate of relaxation (intercept) and to the extent of relaxation (slope), respectively. Relaxation time (RT) was calculated as the time required for the maximum force to drop to 60% of its value. All measurements were made in triplicate.

Enzymatic/Biochemical determinations. Bioaccessible polyphenols were determined in commercial breads using an *in vitro* digestive enzymatic mild extraction that mimics the conditions in the gastrointestinal tract according to the procedure of Glahn et al. (1998) and

adapted by Angioloni and Collar (2011b) for breads. The enzymes used to simulate the gastric and intestinal digestion were pepsin and bile/pancreatin solution, respectively. The obtained digestive extracts were used for the determination of bioavailable polyphenols after removing the proteins by addition of trichloroacetic acid (20% w/w), precipitation and centrifugation. The same extracts were used to determine the radical scavenging capacity of breads using the DPPH• (2,2-diphenyl-1-picrylhydrazyl) method (Brand-Williams et al., 1995), modified by Sánchez-Moreno et al. (1998) and adapted by Collar et al. (2014a). In brief, aliquots of 0.1 mL were taken, and 3.9 mL of a solution of DPPH 0.025 g/L (equivalent to 0.0634 μmol/mL) was added. Tubes were gently shaken, and 4 mL of each tube were added to 4 mL cuvettes, and A515 nm was read at 1 min and every 5–10 min until the plateau was reached. A cuvette containing 4 mL of DPPH 0.247 μmol in methanol was read at the same periods. A blank of methanol was used. Lectures were taken in duplicated samples. Plots of μmol DPPH vs time (min) were drawn, and calculations were made to know the antiradical activity (AR). $AR = \frac{100 \times ([DPPH]_{INITIAL} - [DPPH]_{PLATEAU})}{[DPPH]_{INITIAL}}$.

In vitro starch hydrolysis kinetics and relevant starch nutritional fractions were determined in accordance with the AACC (2005) method 32-40 with the modification reported by Angioloni and Collar (2011a). As stated by Englyst et al. (2003) different fractions of starch were determined: rapidly digestible starch (RDS) and slowly digestible starch (SDS) were measured after incubation for 20 and 120 min; total digestible starch (DS) was determined after 16 h of incubation while resistant starch (RS) was determined in the pellet as the starch remaining after 16 h incubation.

The starch hydrolysis kinetics and expected glycaemic index (eGI) of breads were calculated in accordance with the procedure followed by Chung et al. (2008) based on the method established by Goñi et al. (1997), and applied previously (Angioloni and Collar, 2011a). A first order kinetic

equation $[C = C_{\infty} (1 - e^{-kt})]$ was applied to describe the kinetics of starch hydrolysis, where C was the hydrolysis degree at each time, C_{∞} the equilibrium concentration or maximum hydrolysis extent, and k the kinetic constant. The hydrolysis index (HI) was calculated as the relation between the area under the hydrolysis curve (0–16 h) of breads and the area of a standard material (white bread) (Chung et al., 2008). The eGI was calculated using the equation proposed by Granfeldt et al. (1992): $eGI = 8.198 + 0.862HI$.

Sensory evaluation

Sensory analysis of fresh breads was performed with a panel of eight trained judges (four males and four females aged 24–57) using a semi-structured scale, scored 1–10 in which extremes (lowest: 1; highest: 10) were described for each sensory attribute according to Setser (1996). Evaluated attributes were grouped into visual, textural and organoleptic characteristics (Collar et al., 2005).

Statistical analysis

Statistical analysis of the results was performed using Statgraphics V.7.7 program (Bitstream, Cambridge, MN). Pearson correlation analysis for relationship between bread properties and factor analysis for breads classification were used.

Results and Discussion

Relationships between biochemical, physical and sensory parameters of breads

Associations between the evaluated bread quality parameters were analysed by using Pearson correlations. Biochemical vs physical properties (Table 2) and biochemical vs sensory ratings (Table 3) explicated major significant relationships. [\[insert Table 2 and 3\]](#)

Values of correlation coefficients (r) revealed significant relationships ($0.01 < p < 0.05$) between biochemical and physical properties of breads ($r = 0.46 - 0.77$), especially for starch hydrolysis parameters, protein and bio-accessible polyphenol contents with mechanical characteristics of breads, respectively (Table 2). C_{∞} and eGI as well as H_{90} and HI positively affected all bread primary and secondary mechanical characteristics ($0.50 < r < 0.76$), while only a few correlations were found for the starch nutritional fractions. DS negatively correlated with springiness ($r = -0.51$) and RS positively correlated with kinetic parameters for stress relaxation k_1 ($r = 0.73$), k_2 ($r = 0.65$), and RT ($r = 0.77$) (Table 2). Protein and bio-accessible polyphenol contents negatively correlated with hardness ($r = -0.53, -0.54, p < 0.05$) and positively correlated with springiness ($r = 0.55, p < 0.05$) and cohesiveness ($r = 0.61, 0.68, p < 0.01$), respectively.

Crumb texture is an important attribute of bread quality, and the protein fraction plays a key role in the formation of the structure, gas retention and volume of breads (Scanlon and Zghan, 2001). In this study, commercial breads analysed showed wide variation, with gluten-free breads exhibiting inferior crumb texture profile compared to wheat-based breads (softer and springier crumb with high cohesiveness). In fact, hardness, springiness, and cohesiveness values for gluten breads ranged from 4.5 to 9.7 (N), from 0.8 to 1 and from 0.59 to 0.68, respectively (except for sample 3210, which showed the highest hardness (96 N) and the lowest cohesiveness (0.18) values, respectively); while in gluten-free breads the following intervals were found: 8.5-47.1 for hardness, 0.7-0.9 for springiness, and 0.38-0.6 for cohesiveness. This is certainly due to the lack of a coherent and continuous protein matrix that, in gluten-free breadmaking, led to a low dough development important in determining the crumb structure and, consequently, the mechanical properties of bread. Also, changes in the structure can be linked to changes in starch digestibility. Bread can be considered as a composite material in which the protein network does not represent an isolate system but interacts with other constituents like starch granules (Guerrieri et

al., 1997). Depending on the kind of protein, starch and lipid interactions may block enzyme active sites with a consequent reduction of starch hydrolysis rate and expected glycaemic index.

Moreover, significant correlations were found between protein and polyphenols content and cell to total area ratio ($r = 0.76$, $p < 0.01$), as well as between eGI and cell to total area ratio ($r = -0.47$, $p < 0.005$) (Table 2).

Sensory attributes grouped into visual, textural (tactile and biting) and organoleptic characteristics were correlated with biochemical properties of breads, and, although r values were discreet, significant ($0.01 < p < 0.05$) correlations (from 0.46 to 0.77) were found (Table 3). Relationships between these properties evidenced that the digestible carbohydrates and dietary fibre content were the bread nutritional fractions that most influenced visual and taste and aroma properties.

It is a common agreement that sensory visual and tactile perception of breads play a key role in the consumers' acceptability (Angioloni and Collar, 2009); besides, several authors (Scanlon and Zghan, 2001; Angioloni and Collar, 2009; Hager and Arendt, 2013) pointed out how the crumb feels to the touch or in the mouth is greatly influenced by the grain or cell structure of the crumb (cell size, cell uniformity and thin-walled cells).

In this work, higher content of dietary fibre corresponded to low cell uniformity ($r = -0.54$) high cell size ($r = 0.50$), thickness ($r = 0.70$), aroma and taste intensity ($r = 0.46, 0.48$), and saltiness ($r = 0.69$); instead, the opposite was observed for digestible carbohydrate content ($0.50 < r < 0.63$) (Table 3). The effect of dietary fibre addition (using ingredients with high-fibre content or adding functional fibre) on crumb grain characteristics have been studied in several works with no conclusive results. Angioloni and Collar (2011a) reported heterogeneity in the values related to crumb grain structure for unsupplemented and fibre-supplemented breads; but, the authors, also pointed out that overall acceptability ratings seem to depend more on organoleptic and textural than on tactile and visual characteristics.

Protein and bio-accessible polyphenols content influenced the organoleptic properties but, unlike fibre and carbohydrates, these fractions were in good accordance with aroma (for both $r = 0.49$, $p < 0.05$) and taste ($r = 0.69, 0.79$, $p < 0.01$) quality and aftertaste ($r = -0.51, -0.72$) (Table 3).

In bread, sensory texture parameters are often connected and well predicted by instrumental measurement such as TPA (Bollaín et al., 2005). Consistent with this and in accordance with the correlations previously reported (biochemical vs texture properties) good correlations between starch hydrolysis parameters, protein and polyphenols content and sensory texture characteristics were also found. The higher C_{∞} , eGI, H_{90} and HI, the lower sensory cohesiveness ($-0.46 < r < -0.57$) and elasticity ($-0.48 < r < -0.55$); while, the higher protein and polyphenols content, the higher sensory cohesiveness ($r = 0.58, 0.66$ $p < 0.01$) and gumminess ($r = 0.64, 0.74$ $p < 0.01$) (Table 3).

Classification of breads

Classification of 20 European commercial breads (10 gluten containing and 10 gluten-free) on the basis of their distinctive and significant responses in terms of crumb and crust colour features, rheological behaviour, relevant nutritional fractions, bioactive components, and sensory ratings was achieved by means of multivariate data handling.

From more than 70 functional variables analysed in the different commercial breads, 17 independent variables were selected to perform sample classification using factor analysis (FA). FA grouped techno-functional and nutritional bread parameters into five different factors that explained 78.22% of the cumulative variance (VE), with the first three factors explaining 59.83% of the variability of the results (Table 4). [\[insert Table 4 and Figure 2\]](#)

Factor 1, which makes the highest contribution accounting for 31.87% of the total variation, grouped bioactive components and taste and aroma sensory features, factor 2 (16.53%) grouped

mechanical properties and starch hydrolysis parameters, while factor 3 (11.43%) included biting and tactile sensory attributes (Figure 2). Factor 1 correlated positively with protein content, bio-accessible polyphenols, aroma and taste quality. Factor 2 correlated positively with hardness, C_{∞} and eGI. Factor 3 showed negative relationships with softness and smoothness sensory characteristics. Plots of scores of Factor 1 vs Factor 2 and Factor 1 vs Factor 3 illustrating variable and sample location in the scatterplots are respectively depicted in Figure 3. In both plots, the separation between gluten and gluten-free breads was observed clearly according to the factor 1, located along the x-axis. Gluten breads were located in the positive zone (side) of the x-axis in both of plots; while, gluten-free breads were located in the negative zone (side).

The plot 1 (Figure 3) allowed identifying three different groups of samples as described below. In the positive side of the x-axis is located the group I that included all gluten breads with the exception of sample 3210, which general behaviour appeared closer to that of gluten-free breads. The samples of this group exhibited higher values for protein and bio-accessible polyphenols content, and aroma and taste quality. The group II (2213, 1114, 2116, 1117, 1118, 3220 samples) that showed intermediate and low (2116, 1117, 1118) values of the above-mentioned characteristics (especially in terms of protein and polyphenols content), and group III (1211, 1112, 1115, 1119 and 3210) in which the values for variables in factor 1 are always very low, were instead located in the left side of the x-axis. [insert Figure 3]

Most of gluten bread formulations were based primarily on common wheat flour except for samples 3104, 1105 and 1107 based on whole wheat flour, durum wheat remilled semolina and Khorosan kamut wheat flour, respectively (Table 1); but, it is noticeable that the protein content of breads of group I, which ranged from 8.5 to 12.5 (g/100 g bread, as is), was found to be highest in bread 1206 (12.5 g/100g) closely followed by bread 3209 (12g/100g). This highest level of protein content is probably due to the presence of soybean grain/seeds, which are an excellent

source of high-quality protein and isoflavones in bread formulation. These values are consistent with the significant increase in protein content for soy-supplemented wheat breads previously observed by Dhingra and Jood (2001). Moreover, the presence of flaxseeds and flaxseed oil, as ingredients, in bread 3208 closely followed by these same samples 1206 and 3209 could be responsible for the high content of both protein (Marpalle et al., 2014) and polyphenols (Meral et al., 2013). Among the samples grouped in the other two populations, it should be noticed the prominent level of both protein and polyphenols provided by the gluten-free sample 1112 (8.5 g/100g; 1474 mg of gallic acid/100g of fresh bread) (group III), but also the relevant polyphenol content observed in gluten-free bread 2213 (1444 mg of gallic acid/100 g) (group II). Sample 1112 includes eggs and soy protein isolate (ingredients normally used as source of protein in gluten-free bread), while sample 2213 includes flaxseeds. With regard to the sensory parameters, breads of group I were scored higher for taste and aroma quality than those of group II and group III. In the group I values ranged between 5.6-7.1 for aroma quality and between 5.1-6.6 for taste quality highlighting the clear preference given to these breads; in group II and III, instead, the only samples to be awarded a score higher than 5 for both of parameters were samples 2213 (5.7-5.5), 3220 (5.3-5.4) (group II), and 3210 (6.4-6.3) (group III). This result showed that, among the gluten-free breads, the judges gave high acceptability to those samples that, in their formulations, included either flours of minor cereals and pseudocereals or seeds in significant percentages (Table 1).

Furthermore, considering Factor 2, it is possible to clearly identify three groups of breads also in terms of mechanical properties and starch hydrolysis parameters (Figure 3). From the top along the y-axis, the groups were characterized by gradually decreasing values of eGI, C_{∞} , and hardness: once again, group I and group III exhibited the best and the poorest behaviour,

respectively. All samples of group I showed low and moderate eGI with values ranging from 52.2 (1105) to 72.52 (3209), with the only exception of sample 1101 (91.20) (Table 5). [\[insert Table 5\]](#)

The reason for this results is probably due to the poorer formulation of this bread that includes only refined wheat flour in its recipe and that, therefore, showed a glycaemic response very close to that of white bread (GI=100) generally used as a reference food. All breads belonging to the other two groups can be classified as high glycaemic index (GI) showing values of eGI higher than 70 (group II) and 86 (group III), with the only exception of sample 1114 (65.22) in group II (Table 5). This extreme variability shown by commercial breads in eGI values is directly related to the degree and rate of carbohydrate digestion. In fact, the starch digestion of cereal products is a complex process and the rate of digestion of the starch seems to be influenced by several factors such as characteristics of the starch, food processing, and the presence of fibre, protein, lipids and their interactions (Singh et al., 2010; Annor et al., 2013). As above, group I showed a low extent of starch hydrolysis with the lowest values for C_{∞} and eGI (Table 5). It seems that the high protein content of these breads may account for the reduced digestibility of the starch and for the low eGI. In several cereal products, starch-protein interactions lead to the formation of protein network that surrounds the starch granules reducing the availability to enzyme attack. Therefore, it should be noticed the rather low eGI (55.2) reported of sample 1105 made from durum wheat semolina, which is characterized by stronger starch-protein interactions. This fact, probably, may contribute to a further reduction in the degree of starch digestion.

Despite Factor 3 explained less than 12% of the cumulative variance, it is useful to further classify the samples in terms of sensory textural parameters such as smoothness (tactil parameter) and softness (biting parameter). Thus, considering the plot of Factor 1 vs Factor 3 (Figure 3) three groups can be defined: a first group (A) in which, along with most of the gluten-free bread (1211,1112,2213,1115,1117,1119,3220) were grouped some of the gluten breads (1103, 3104

and 3210); a second group (B) formed by the most of gluten breads (1101, 2202, 1105, 1206, 1107, 3208, 3209); and a third group (C) that showed the highest values (data not shown) for sensory smoothness and softness, composed of 1114, 2116 and 1118 gluten-free breads. It should be noticed the higher values showed by the three gluten-free breads when compared to all other samples, including gluten-breads.

Conclusions

In conclusion, characterization of different commercial breads evidenced that the main distinction, as it was expected, was between gluten and gluten-free breads, although the latter have shown a great variability in terms of overall quality. Classification of breads also allowed differentiating samples with different formulations. The highest values for the most significant variables were observed for breads characterized by rich formulations in terms of the presence of other and alternative flours (rye, buckwheat, quinoa, millet, durum wheat semolina, and whole wheat), grains (soybean) and seeds (flaxseed, sunflower, and sesame). Among these samples, the best overall behaviour was observed in gluten breads (1206, 3209, 2202 and 3208 particularly) but, intermediate values were also found in gluten-free samples 1112, 3220 and 2213. Conversely, the lowest values were found in gluten-free breads characterized by poorer formulations in terms of the absence or the presence of low percentages of the above-mentioned ingredients (1211, 1115, and 1119). Although the efforts of research and bakery industry are moving in the right direction to develop and produce high-quality gluten and gluten-free products, data obtained in this study confirm that, from a nutritional point of view, there is still substantial room for improvement on both of areas.

Acknowledgements

Authors acknowledge the financial support of the Spanish institutions Consejo Superior de Investigaciones Científicas (CSIC) and Ministerio de Economía y Competitividad (Project AGL2011-22669). Paola Conte gratefully acknowledges Sardinia Regional Government for the financial support of her PhD scholarship (P.O.R. Sardegna F.S.E. Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2007-2013 - Axis IV Human Resources, Objective I.3, Line of Activity I.3.1.).

Conflict of interest

The Authors declare that there is no conflict of interest.

References

- AACC (2005) *Approved methods of the American Association of Cereal Chemists*, 10th ed. AACC St. Paul, MN: The Association.
- Angioloni A and Collar C (2009) Bread crumb quality assessment: a plural physical approach. *European Food Research and Technology* 229: 21–30.
- Angioloni A and Collar C (2011a) Physicochemical and nutritional properties of reduced-caloric density high-fibre breads. *LWT - Food Science and Technology* 44(3): 747–758.
- Angioloni A and Collar C (2011b) Polyphenol composition and “in vitro” antiradical activity of single and multigrain breads. *Journal of Cereal Science* 53(1): 90–96.
- Annor GA, Marcone M, Bertoft E and Seetharaman K (2013) In vitro starch digestibility and expected glycemic index of Kodo Millet (*Paspalum scrobiculatum*) as affected by starch–protein–lipid interactions. *Cereal Chemistry* 90(3): 211–217.

- Bakers-Federation (2013) European bread market. Available at: <http://www.bakersfederation.org.uk/the-bread-industry/industry-facts/european-bread-market/> (accessed 10 August 2015).
- Betoret E, Betoret N, Vidal D and Fito, P. (2011) Functional foods development: trends and technologies. *Trends in Food Science and Technology* 22(9): 498–508.
- Blandino M, Sovrani V, Marinaccio F, Reyneri A, Rolle L, Giacosa S, Locatelli M, Bordiga M, Travaglia F, Coisson JD and Arlorio, M (2013) Nutritional and technological quality of bread enriched with an intermediated pearled wheat fraction. *Food Chemistry* 141(3): 2549–2557.
- Bollaín C, Angioloni A and Collar C (2005) Bread staling assessment of enzyme-supplemented pan breads by dynamic and static deformation measurements. *European Food Research and Technology* 220(1): 83–89.
- Brand-Williams W, Cuvelier ME and Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology* 28(1): 25–30.
- Cauvain S (2015) Bread: the product. In: Cauvain, SP (eds) *Technology of Breadmaking third edition*. New York: Springer Science and Business Media, pp. 1–21.
- Collar C (2015) Role of bread on nutrition and health worldwide. In: Bajerska J, Rosell CM and El Sheikh AF (eds) *Bread and its Fortification for Nutritional and Healthy Benefits*. CRC Press / Taylor and Francis Group, pp. 26–52.
- Collar C and Angioloni A (2014) Nutritional and functional performance of barley flours in breadmaking: mixed breads vs wheat breads. *European Food Research and Technology* 238: 459–469.
- Collar C, Bollaín C and Angioloni A (2005) Significance of microbial transglutaminase on the sensory, mechanical and crumb grain pattern of enzymes supplemented fresh pan breads. *Journal of Food Engineering* 70(4): 479–488.

- Collar C, Jiménez T, Conte P and Fadda C (2014a) Impact of ancient cereals, pseudocereals and legumes on starch hydrolysis and antiradical activity of technologically viable blended breads. *Carbohydrate Polymers* 113: 149–158.
- Collar C, Conte P, Fadda C and Piga A (2014b) Gluten-free dough-making of specialty breads: significance of blended starches, flours and additives on dough behaviour. *Food Science and Technology International* 21(7): 523–536.
- Chung HJ, Liu Q, Pauls PK, Fan MZ and Yada, R (2008) In vitro starch digestibility, expected glycaemic index and some physicochemical properties of starch and flour from common bean (*Phaseolus vulgaris* L.) varieties grown in Canada. *Food Research International* 41(9): 869–875.
- Dhingra S and Jood, S (2001) Organoleptic and nutritional evaluation of wheat breads supplemented with soybean and barley flour. *Food Chemistry* 77(4): 479–488.
- Englyst KN, Vinory S, Englyst HN and Lang V (2003) Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. *British Journal of Nutrition* 89(3): 329–339.
- Glahn RP, Lee OA, Yeung A, Goldman MI and Miller DD (1998) Caco-2 cell ferritin formation predicts nonradiolabeled food iron availability in an in vitro digestion/Caco-2 cell culture model. *The Journal of Nutrition* 128(9): 1555–1561.
- Goñi I, Garcia-Alonso A and Saura-Calixto F (1997) A starch hydrolysis procedure to estimate glycaemic index. *Nutrition Research* 17(3): 427–437.
- Gökmen V, Mogol BA, Barona Lumaga R, Fogliano V, Kaplun Z and Shimoni E (2011) Development of functional bread containing nanoencapsulated omega-3 fatty acids. *Journal of Food Engineering* 105(4): 585–591.
- Granfeldt Y, Björck I, Drews A and Tovar J (1992) An in vitro procedure based on chewing to

- predict metabolic responses to starch in cereal and legume products. *European Journal of Clinical Nutrition* 46: 649–660.
- Guerrieri N, Eynard L, Lavelli V and Cerletti, P (1997) Interactions of protein and starch studied through amyloglucosidase action. *Cereal Chemistry* 74(6): 846–850.
- Hager AS, Wolter A, Czernv M, Bez J, Zannini E, Arendt EK and Czernv M (2012) Investigation of product quality, sensory profile and ultrastructure of breads made from a range of commercial gluten-free flours compared to their wheat counterparts. *European Food Research and Technology* 235(2): 333–344.
- Hamada S, Suzuki K, Aoki N and Suzuki Y (2013) Improvements in the qualities of gluten-free bread after using a protease obtained from *Aspergillus oryzae*. *Journal of Cereal Science* 57(1): 91–97.
- Houben A, Hochstotter A and Becker T (2012) Possibilities to increase the quality in gluten-free bread production: an overview. *European Food Research and Technology* 235(2): 195–208.
- Lazaridou A, Duta D, Papageorgiou M, Belc N and Biliaderis CG (2007) Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. *Journal of Food Engineering* 79(3): 1033–1047.
- Marpalle P, Sonawane SK and Arya SS (2014) Effect of flaxseed flour addition on physicochemical and sensory properties of functional bread. *LWT - Food Science and Technology* 58(2): 614–619.
- Meral R and Sait Dogan I (2013) Quality and antioxidant activity of bread fortified with flaxseed. *Italian Journal of Food Science* 25(1): 51–56.
- Miranda A, Lasa A, Bustamante MA, Churruca I and Simon E (2014) Nutritional differences between a gluten-free diet and a diet containing equivalent products with gluten. *Plant Foods of Human Nutrition* 69(2): 182–187.

- Peleg M and Pollak NT (1982) The problem of equilibrium conditions in stress relaxation curves of solids foods. *Journal of Texture Studies* 13(1): 1–11.
- Rubel IA, Pérez EE, Manrique GD and Genovese DB (2015) Fibre enrichment of wheat bread with Jerusalem artichoke inulin: Effect on dough rheology and bread quality. *Food structure* 3: 21–29.
- Salinas MV and Puppo MC (2015) Optimization of the formulation of nutritional breads based on calcium carbonate and inulin. *LWT - Food Science and Technology* 60(1): 95–101.
- Sánchez-Moreno C, Larrauri JA and Saura-Calixto F (1998) A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture* 76(2): 270–276.
- Scanlon MG and Zghal MC (2001) Bread properties and crumb structure. *Food Research International* 34(10): 841–864.
- Setser CS (1996). Sensory methods. In: Hebeda RE and Zobel HF (eds) *Baked goods freshness*. New York: Marcel Decker Inc, pp. 171–187.
- Singh J, Dartois A and Kaur L (2010) Starch digestibility in food matrix: a review. *Trends in Food Science and Technology* 21(178): 168–180.
- Ziobro R, Witczak T, Juszczak L and Korus J (2013) Supplementation of gluten-free bread with non-gluten proteins. Effect on dough rheological properties and bread characteristic. *Food Hydrocolloids* 32(2): 213–220.

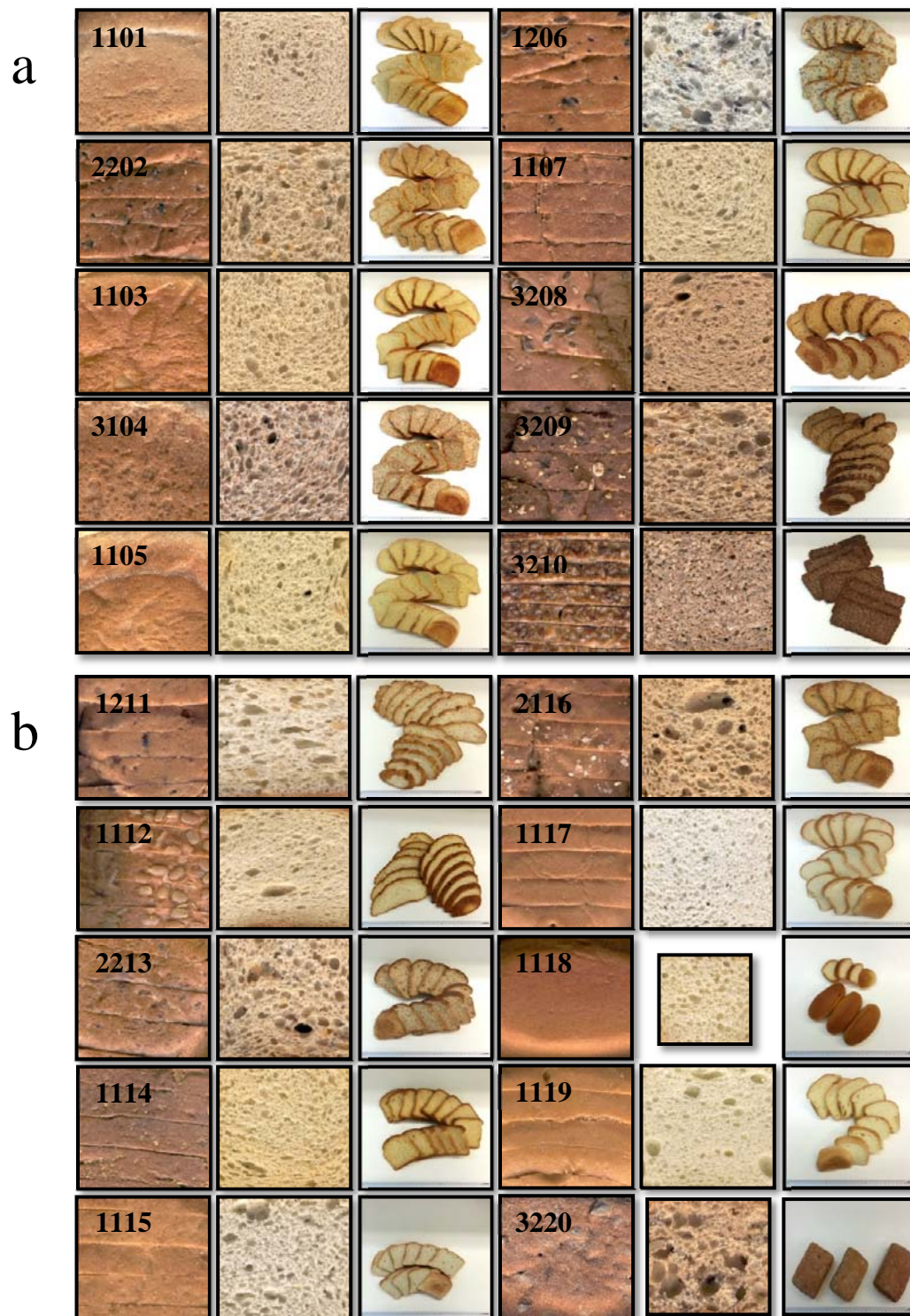


Figure 1. Crust and crumb slice digitalized images of gluten (a) and gluten-free (b) commercial breads. A four digit bread sample code refers to white (1), mixed (2), and dark (3) crumb colour (1st digit); absence (1) or presence (2) of seeds (2nd digit), and sample number (3rd and 4th digits) from 1 to 10 for gluten breads and from 11 to 20 for gluten-free breads.

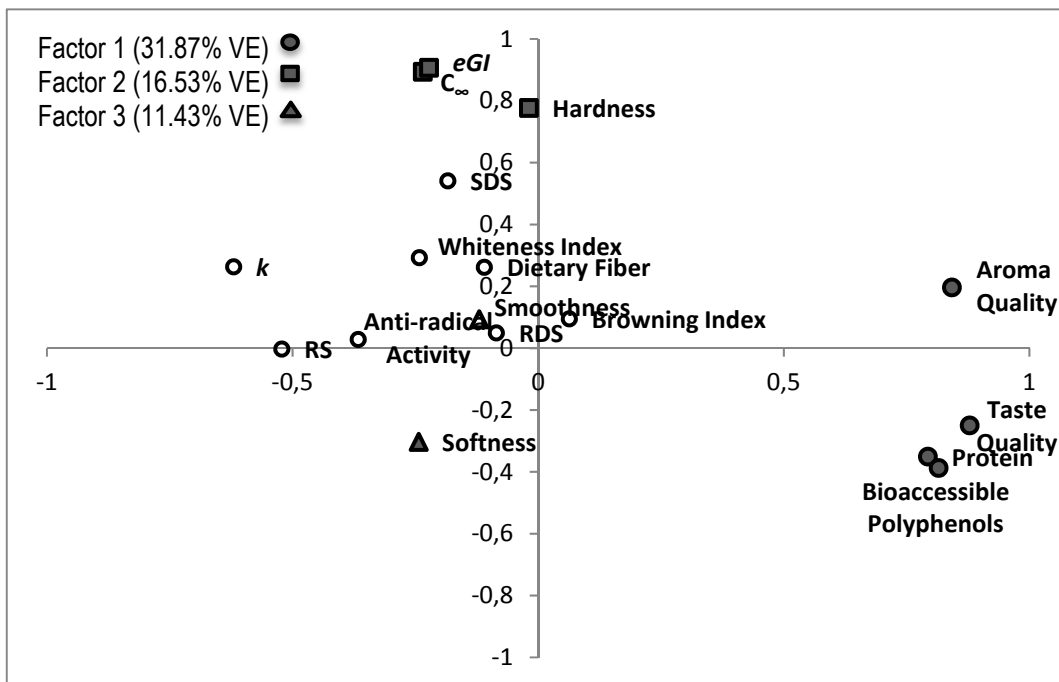


Figure 2. Scatterplots of technofunctional, nutritional and sensory parameters of commercial breads from factor analysis scores.

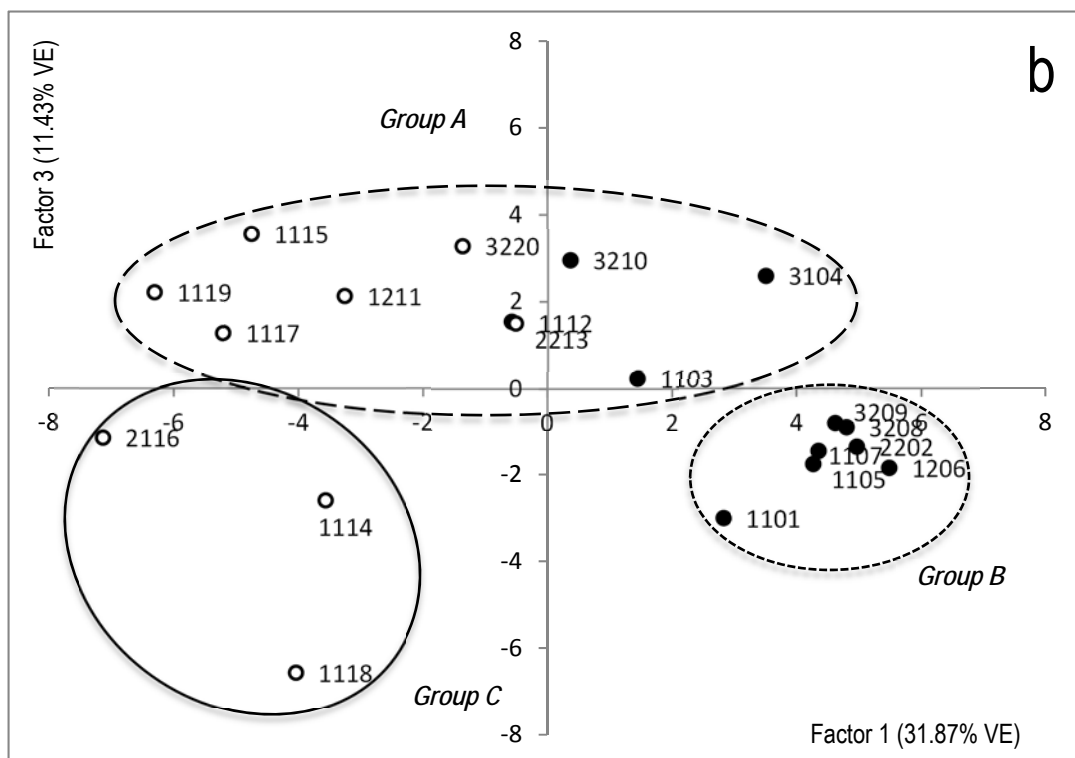
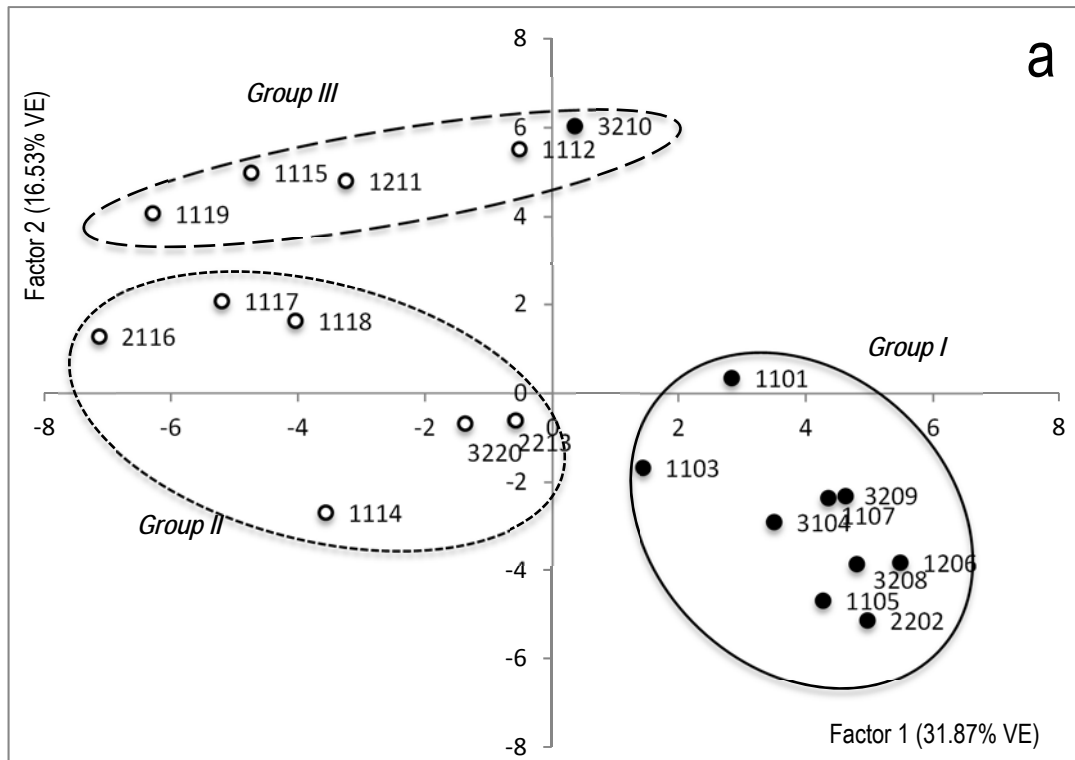


Figure 3. Scatterplots of scores of factor 1 vs factor 2 (a) and factor 1 vs factor 3 (b) of commercial breads.

Table 1. Ingredients of gluten and gluten-free breads. A four digit bread sample code refers to white (1), mixed (2), and dark (3) crumb color (1st digit); absence (1) or presence (2) of seeds (2nd digit), and sample number (3rd and 4th digits) from 1 to 10 for gluten breads and from 11 to 20 for gluten-free breads.

Breads	Ingredients
1101	Wheat Flour Tipe "I", Water, Extra-Virgin Olive Oil (3%), Rye Sourdough, Brewer's Yeast, Iodized Salt, Ethyl Alcohol
2202	Wheat Flour Tipe "0" (56.8%), Water, Cereal Flakes/Flours Mixture 5.7% (Oat, Barley, Durum Wheat, Toasted Corn, Rice, Rye, Millet), Soybean Grain (5.5%), Sunflower Oil (3.7%), Sourdough, Wheat Gluten, Salt, Malted Barley Flour, Malt Barley Extract, Ethyl Alcohol
1103	Wheat Flour Tipe "0", Water, Selected Wheat Bran (9.3%), Malted Barley Flour, Extra-Virgin Olive Oil (3.9%), Sourdough, Wheat Gluten, Salt, Glucose, Malted Barley Flour, Ethyl Alcohol
3104	Whole Wheat Flour (64.2%), Water, Sunflower Oil (4.3%), Wheat Gluten, Sourdough, Dextrose (1.5%), Salt, Malted Barley Flour, Ethyl Alcohol
1105	Durum Wheat Remilled Semolina (68.3%), Water, Extra-Virgin Olive Oil (2.9%), Sourdough, Salt, Glucose, Wheat Gluten, Malted Barley Flour, Wheat Flour Tipe "0", Ethyl Alcohol
1206	Wheat Flour Tipe "0" (55%), Water, Flax-seeds (4.9%), Soybean Grain (4.3%), Malted Barley Flour, Sunflower seeds (3.3%), Wheat Gluten, Olive Oil (1.2%), Sourdough, Dextrose, Flax Seed Oil (0.9%), Salt, Wheat Fiber (0.6%), Malted Barley Flour, Vitamin E (0.03%), Ethyl Alcohol.
1107	Khorasan Kamut Wheat Flour, Water, Rice Oil (2.8%), Salt, Yeast, Extra-Virgin Olive Oil (1.2%), Rice Flour, Malted Wheat Flour, Acacia Fiber, Ethyl Alcohol
3208	Wheat Flour Tipe "0", Water, Rye Flour (18.7%), Sunflower seeds (4%), Vegetable Oil (3.4%), Wheat Gluten, Sesame Seeds (2%), Sourdough, Flax-seeds (1.8%), Dextrose, Salt, Malt Barley Extract, Malted Barley Flour, Ethyl Alcohol
3209	Water, Wheat Flour Tipe "0" (34.5%), Whole Wheat Flour (16.5%), Seeds 9.5 (Sunflower seeds 3.7%, Soybeans 3.5, Flax-seeds 2.1%, Sesame Seeds 0.2%), Cereal Flours 6.3% (Oat Flour 1.6%, Barley Flour 1.6%, Rice Flour 1.6%, Rye Flour 0.9%, Millet Flour 0.4%, Corn Flour 0.2%), Vegetable Oil (2.8%), Sourdough, Dextrose (1.3%), Wheat Gluten, Salt, Oat Flakes 0.2%, Barley Flakes 0.2%), Rice Flakes (0.2%), Malted Barley Flour, Malt Barley Extract, Ethyl Alcohol
3210	Whole Rye Groats, Water, Sourdough, Flaxseeds (3%), Barley Flakes (3%), Oat Flakes (3%), Salt, Yeast
1211	Water, Corn Starch, Rice Flour, Sunflower seeds (5%), No-Hydrogenated Vegetable Margarine (refined palm oil, water), sugar, Brewer's Yeast, Thickening Agents: Guar Seed Flour-HPMC, Iodized Salt, Lupine Protein, Psyllium Fiber, Tartaric Acid, Flavouring
1112	Sourdough (Rice Flour, Corn Starch, Buckwheat Flour, Salt, Lactobacillus sanfranciscensis, Lactobacillus plantarum), Water, Sunflower seeds (5.4%), Potato Starch, Corn Starch, Glycerol, Inulin, Sunflower Oil, Olive Oil (3.2%), Egg White, Soy Protein Isolate, Sugar, Brewer's Yeast, Xanthan Gum, Glucose/Fructose Syrup, Salt, Guar Gum, Soy Lecithin, Mono and Diacetyltartaric Acid Esters of Mono and Diglycerides of Fatty Acids
2213	Corn Starch, Rice Starch, Sourdough 22.5% (Rice Flour), Water, Rice Flour, Millet Flour (2.3%), Quinoa Flour (1.6%), Apple Fiber, Rice Syrup, Beet Sugar Syrup, Sunflower seeds (1.8%), Soy Flakes (1.8%), Soy Bran (1.7%), Millet Flakes (1.2%),
1114	Water, Corn Starch, Rice Flour, Sugar, Eggs, No-Hydrogenated Vegetable Margarine (Palm Oil, Coconut Oil, Colza Oil), Glucose Syrup, Milk Powder, Apple Fiber, HPMC, Guar Gum, Mono and Diglycerides of Fatty Acids, Yeast, Tartaric Acid, Salt
1115	Potato Starch, Water, Rice Flour, Corn Flour, Vegetable Fibers, Non-Hydrogenated Vegetable Oils, HPMC, Xanthan Gum, Eggs, Yeast, Teff Flour (2.5%), Sugar, Modified Rice Starch, Maltodextrins, Salt, Invert Sugar, Potassium Sorbate, Quinoa Flour
2116	Water, Potato Starch, Corn Starch, Refined Sunflower Oil, Tapioca Starch, Egg White (Powder), Rice Bran, Yeast, Cellulose, Salt, Beet Pulp, Millet Flakes, Wine Vinegar, Xanthan Gum, HPMC, CMC, Calcium Propionate, Sorbic Acid, Potassium sorbate
1117	Water, Wheat Starch (No Gluten), Rice Starch, Cellulose Fiber, Guar Gum, HPMC, Soy Protein, Apple Fiber, Rice Flour, Millet Flour, Yeast, Sunflower Oil, Quinoa Flour, Sugar, Rice Syrup, Salt, Palm Oil, Honey, Folic Acid, Calcium Citrate
1118	Rice Flour, Corn Starch, Sugar, Eggs, Water, Vegetable Margarine (Palm fat, Coco fat, Canola oil, Salt, Mono and Diglycerides of Fatty Acids, Natural Flavour), Rice Starch, Glucose Syrup, Mono and Diglycerides of Fatty Acids, Guar Gum Seeds Flour, HPMC, Yeast, Salt, Flavouring, Citric Acid
1119	Corn Starch, Water, Rice Flour, Sunflower Oil, Sugar, Guar Gum Seeds Flour, HPMC, Lupine Protein, Yeast, Salt, Apple Fiber, Flavour, Mono and Diacetyltartaric Acid Esters of Mono and Diglycerides of Fatty Acids
3220	Corn Starch, Water, Rice Flour, Sunflower seeds (7.5%), Buckwheat Flour (7%), Flaxseeds (5.5%), Sugar beet Syrup, Rice Starch, Yeast, Apple Extract, HPMC, Soy Protein, Salt, Sunflower Oil, Tartaric Acid

Table 2. Significant Pearson correlations (* p<0.05, ** p <0.01) between biochemical and physical properties of commercial breads.

	Moisture	Protein	Dietary C _∞ Fibre	k	H ₉₀	HI	eGI	DS	RS	TS	Bioaccessible Soluble Polyphenols	Insoluble Polyphenols	Antiradical activity
Whiteness Index								0.6046**		0.5603*	-0.4649*		-0.4773*
Cell to total area ratio	-0.5241*	0.7566**				-0.4699*	-0.4699*				0.7647**		0.5898**
Hardness	0.5027*	-0.5296*	0.6345**	0.5206*	0.5552*	0.6446**	0.6446**				-0.5372*		
Springiness	-0.6687**	0.5511*	-0.6908**		-0.6824**	-0.7607**	-0.7606**	-0.5149*		-0.5536*	0.553*		0.6395**
Cohesiveness	-0.7385**	0.6105**	-0.6706**		-0.5409*	-0.7127**	-0.7127**				0.6771**	0.5062*	0.4721*
Chewiness			0.4718*	0.5479*	0.4907*	0.4983*	0.5201*	0.5201*					
Resilience	-0.6625**		-0.6219**		-0.5486*	-0.6782**	-0.6782**				0.5076*	0.4668*	
F ₀	0.6285**		0.5183*		0.5135*	0.5572*	0.5571*						
k ₁									0.7303*				0.5512*
k ₂		-0.6013**							0.6516**		-0.6605**		
RT		-0.5911**							0.7726**		-0.6425**		-0.4567*

C_∞: equilibrium concentration; k: kinetic constant; H₉₀: total starch hydrolysis at 90 min; HI: hydrolysis index; eGI: expected glycemic index; DS: digestible starch; RS: resistant starch; TS: total starch; F₀: initial force; k₁: stress decay rate; k₂: residual stress; RT: relaxation time.

Table 3. Significant Pearson correlations (* p<0.05, ** p <0.01) between biochemical properties and sensory parameters of breads.

	Moisture	Protein	Digestible carbohydrates	Dietary Fibre	Ash	C_{∞}	k	H_{90}	HI	eGI	RS	TS	Bioaccessible Polyphenols
Cell Uniformity			0.5771**	-0.5433*									
Cell Size			-0.5048*	0.5022*									
Thickness	0.4678*		-0.5576*	0.6986**		0.4934*	0.6657**				0.4838*		-0.494*
Moistness	0.47*				-0.4628*								
Elasticity	-0.4752*					-0.487*		-0.5169*	-0.5461*	-0.546*			
Softness					-0.6159**								
Coarseness				0.5251*			0.533*						
Cohesiveness	-0.5713*	0.5825**				-0.5664*	-0.4763*	-0.4628*	-0.5507*	-0.5506*			0.6636**
Gumminess		0.6428**										-0.4703*	0.7445**
Mouth Dryness				0.4769*									
Aroma Intensity	0.506*		-0.5087*	0.4659*							0.4741*		-0.4934*
Aroma Quality		0.4929*											0.4936*
Taste Intensity			-0.5074*	0.482*									
Taste Quality		0.6924**					-0.4994*					-0.4807*	0.7931**
Saltiness			-0.6274**	0.6943**									
Aftertaste	0.68**	-0.5102*					0.6155**						-0.7242*

C_{∞} : equilibrium concentration; k : kinetic constant; H_{90} : total starch hydrolysis at 90 min; HI : hydrolysis index; eGI : expected glycemic index; RS: resistant starch; TS: total starch.

1 Table 4. Loading Matrix After Varimax Rotation in Factor Analysis.

	Factor 1 (31,87%VE)	Factor 2 (16,53%VE)	Factor 3 (11,43%VE)	Factor 4 (10,42%VE)	Factor 5 (7,96%VE)
Protein	0,7936	-0,3507	-0,0866	0,2366	-0,0771
Dietary Fibre	-0,1094	0,2613	0,6121	0,4758	0,1232
Whiteness Index	-0,2417	0,2927	-0,2245	-0,7495	-0,2201
Browning Index	0,0636	0,0950	-0,2123	0,7734	-0,0792
Hardness	-0,0188	0,7767	0,1689	0,3922	-0,0873
C_{∞}	-0,2348	0,8939	0,0937	-0,0934	0,0967
k	-0,6198	0,2631	0,6117	0,0558	0,0031
eGI	-0,2222	0,9066	0,0765	-0,1256	0,0782
RDS	-0,0849	0,0492	0,0531	-0,2954	-0,8372
SDS	-0,1837	0,5407	-0,0024	-0,2606	0,6930
RS	-0,5215	-0,0032	0,5213	-0,2754	0,3216
Bioaccessible Polyphenols	0,8154	-0,3875	0,0010	0,2058	-0,1695
Antiradical activity	-0,3663	0,0279	-0,1678	-0,0997	0,4971
Aroma Quality	0,8424	0,1960	0,1465	-0,2081	-0,0291
Smoothness	-0,1202	0,0937	-0,8387	0,1749	0,1280
Softness	-0,2433	-0,3026	-0,7280	-0,0723	0,2525
Taste Quality	0,8786	-0,2498	0,1073	0,1387	-0,0208

2 VE: variance explained.

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17 Table 5. Starch hydrolysis kinetics parameters and expected glycemic index values of gluten and gluten-free
 18 commercial breads.

<i>Bread samples</i> ^{a,b}	Characteristics				
	C_{∞}	k	H_{90}	HI	eGI
<i>Gluten Breads</i>					
1101	80±0.9p	0.010±0.003a	71±1n	96±1l	91±2i
2202	49±0.7b	0.010±0.010a	47±0b	58±2b	58±1b
1103	60±1.1f	0.100±0.010b	53±1e	67±2d	66±1d
3104	55±0.5d	0.100±0.006b	50±1cd	65±2cd	65±1cd
1105	42±0.6a	0.010±0.009a	36±1a	51±0a	52±0a
1206	53±0.6c	0.010±0.004a	51±1d	63±3c	63±3c
1107	64±1h	0.010±0.003a	59±1i	73±4fg	71±1ef
3208	59±0.5e	0.010±0.010a	55±1g	68±1de	66±2d
3209	66±1.1i	0.010±0.006a	71±1n	75±1g	73±2g
3210	80±0.7p	0.100±0.006b	73±1o	95±4l	90±1i
<i>Gluten-free Breads</i>					
1211	82±0.5q	0.100±0.003b	69±0m	97±1lm	92±3il
1112	90±0.4s	0.100±0.005b	79±0p	100m	94±0l
2213	62±1.3g	0.100±0.008b	57±1h	74±0fg	72±0f
1114	56±0.5d	0.100±0.005b	53±1ef	66±4cd	65±1cd
1115	86±1.2r	0.100±0.007b	80±1p	100±0m	94±0l
2116	73±0.5n	0.100±0.005b	58±0h	80±1h	78±1g
1117	68±0.1l	0.100±0.009b	62±1l	80±0h	77±0g
1118	71±0.5m	0.010±0.001a	49±1c	82±3h	79±3h
1119	78±1.2o	0.100±0.002b	71±0n	90±1i	86±2i
3220	64±0.5h	0.100±0.002b	50±1d	71±3ef	70±3e

19 ^a Mean values ± standard deviation. Within rows, values (mean of three replicates) with the same following letter do
 20 not differ significantly from each other ($p > 0.05$).

21 ^b A four digit bread sample code refers to white (1), mixed (2), and dark (3) crumb color (1st digit); absence (1) or
 22 presence (2) of seeds (2nd digit), and sample number (3rd and 4th digits) from 1 to 10 for gluten breads and from 11 to
 23 20 for gluten-free breads.

24 C_{∞} : equilibrium concentration; k : kinetic constant; H_{90} : total starch hydrolysis at 90 min; HI : hydrolysis index; eGI :
 25 expected glycemic index.

26

27

28