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## **Technological, nutritional and sensorial characteristics of wheat bread fortified with calcium salts**

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## Abstract

The aim of this paper was to carry out a comprehensive study of wheat flour breads fortified with different sources of calcium, which includes: technological, nutritional and sensorial characteristics. Calcium salts (lactate: LA, carbonate: CA, and citrate: CI) at two fortification levels (20 and 50%) were analysed. Only the LA fortified breads were harder with lower specific volume and the LA 50% showed the higher chewiness value. The crust colour of the CI 50% breads presented the lower browning index. *In vitro* nutritional studies showed that calcium content on digest and dialysate was significantly higher in all fortified breads. The CA 20% and all the 50% fortifications showed a better contribution of bioaccessible calcium. Sensorial general quality was not significantly different between fortified and Control breads.

## 1 Introduction

Calcium is an essential nutrient that is necessary for many functions in human health and is the most abundant mineral in the body, being 99% found in teeth and bone and only 1% in serum (Beto, 2015). Normal growth and development of skeleton and teeth are associated with an appropriate calcium intake. Risk for osteoporosis and bone fractures in adulthood has been detected when calcium intake is low (Bailey et al., 2010).

Recommended dietary allowance (RDA) for calcium is different according to age, gender and local regulations. The USDA (US Department of Agriculture) and the Argentinean Alimentarius Codex (AAC, 2013) recommend a RDA of 1300 mg/day and 1000 mg/day, respectively. Data from Europe, the USA and Argentina allowed concluding that a large percentage of the population does not meet the requirements currently recommended for optimal calcium intake due to cultural or economic factors (NN&HS, 2007; Kaganov et al., 2015). Bailey et al. (2010) evaluated calcium intake in the USA finding that in the older age groups the prevalence of meeting the RDA increased because high percentage of the population in the USA takes calcium supplements. However, it is recommended to achieve the optimal intake of calcium through dietary sources and not through supplements. Therefore, it is necessary to search for alternatives in order to correct the deficiency in calcium intake, being food fortification an interesting option. The UK, for instance, decided to fortify wheat flour with calcium carbonate contributing in this way to approximately 14 % of total calcium intake (cited by Krupa-Kozak et al., 2012).

When fortifying food it is necessary to estimate the efficiency of the process by determining the bioavailability of the added nutrient (Rebellato et al., 2017). Dialysability is an *in vitro* test that simulates gastrointestinal digestion (Wolfgor et al., 2002). This method can be used as an alternative to human or animal studies in regards to bioavailability estimation, since significant correlation with *in vivo* methods has been reported (Miller et al., 1981). Furthermore, using dialysability and total calcium content of the food, potential calcium contribution can be determined (Zuleta et al., 2012).

Bread is one of the most consumed staple foods; consequently, it has been selected by some authors as a product to be fortified with calcium. Ranhotra et al. (1997) studied the relative bioavailability *in vivo* of calcium in breads fortified with different calcium sources, including inorganic (carbonate and sulfate), organic (citrate and lactate) and a brandname calcium supplement. They concluded that calcium was equally well available

from all sources. Salinas and Puppo (2015) analysed the impact of calcium carbonate combined with the prebiotic inulin on wheat bread properties, showing that the prebiotic negatively affected breadmaking quality. Moreover different researches evaluated the effect of several sources of calcium on dough rheological properties (Salinas and Puppo, 2014; Sehn et al. 2015; Tuhumury et al. 2016; Codinăa et al. 2018). Although the incorporation of calcium could affect the taste and the quality of bread, only a few authors carried out sensory analyses (Ziadeh et al., 2005, Krupa-Kozak et al., 2011).

The aim of the present work was to prepare breads with different calcium salts (inorganic and organic calcium sources) at two levels of calcium and carry out a complete study including technological, sensory and nutritional characteristics of bread.

## **2 Materials and methods**

### **2.1 Ingredients and chemicals**

Wheat flour type 000 (classified according to the AAC) was obtained from Favorita (Ciudad Autónoma de Buenos Aires, Argentina). Baker's yeast and salt were purchased from a local supermarket. Calcium carbonate (CA), calcium citrate (CI), and calcium lactate (LA) were purchased from Novalquim SRL (Rosario, Argentina). Enzymes:  $\alpha$ -amylase (A-3176, from porcine pancreas), pepsin (P-7000, from porcine gastric mucosa), pancreatin (P-1750, from porcine pancreas), and bile (B-8631, porcine extract) were obtained from Sigma Chemical Co. (MO, USA). Chlorhydric acid, nitric acid, and sodium bicarbonate were analytical grade.

### **2.2 Flour chemical analysis**

Chemical characterization of wheat flour was performed in order to determine moisture, protein, and ash contents, which were determined according to the AOAC methods 925.10; 979.09 and 923.03 respectively (AOAC, 1998).

### **2.3 Breadmaking**

The average bread intake in Argentina is 200 g and the AAC defines mineral fortified foods as those in which the mineral content is between 20% and 50% of the RDA. Consequently, flour fortification was made in two theoretical levels corresponding to 20% and 50% of the calcium RDA. Control and fortified breads were obtained using the



ingredients listed on **Table 1**. Ingredients were placed into an automatic bread maker (ATMA HP4060E, China), mixed, kneaded for 20 min and fermented for 40 min at 35°C (first proof). After that, dough was divided into 200 g pieces, hand-rounded, and let to rest for 15 min. Then, rounded pieces were sheeted and curled to form a “swiss roll” effect. A second proof, controlled with a push-meter used to measure the proofing (Aguirre et al., 2011), was carried out at 27°C and 80% relative humidity until dough pieces doubled their volume, after which they were baked at 180°C for 26 min in a pre-heated oven (Brafh HC 4.70, Rosario, Argentina). Finally, loaves were cooled at room temperature. Three loaves were obtained for each formula, which were considered as replications for the following determinations.

#### **2.4 Moisture content, weight, volume and specific volume**

Moisture content was determined according to the air-oven method AACC 44-15A (AACC, 2000). Loaf weight was determined with a Boeco BAS 31PLUS balance (Germany). Loaf volume was measured according to the rapeseed displacement method AACC 10-05 (AACC, 1983). Specific volume (mL/g) was calculated by dividing the bread volume by its weight.

#### **2.5 Crumb texture analysis**

Textural profile analysis (TPA) of the bread crumb was performed using a motorized test frame Mecmesin Multitest 2.5d (Sterling, VA, USA) equipped with a 100 N digital force gauge. Cylindrical shape crumb slices (36 mm diameter and 25 mm height) were cut from the middle part of the loaf and tested. Samples were compressed twice with no delay between them, to 40% of its original height with the 100 mm diameter cylindrical probe at a crosshead speed of 300 mm/min (Licciardello et al., 2014). Parameters obtained were: hardness (force determined on the first peak), cohesiveness (ratio between second and first compression peaks), springiness (distance between the start of the second cycle and its peak) and chewiness (hardness × cohesiveness × springiness).

#### **2.6 Digital image analysis**

A wooden box according to the design described by Soazo et al. (2015) was used. Samples were photographed employing a Canon Eos Rebel T3 digital camera (China) on

a matte black background. Camera was set in manual mode with lens aperture at  $f = 8$ , time of exposition 1/200, no flash, ISO sensibility 400, maximum resolution. Images were stored in RAW format. A Wolf Faust IT8 calibration card (Frankfurt, Germany) was photographed with setting described and used to obtain the International Color Consortium (ICC) profile employing the LProf software (Marti, 2005).

### 2.6.1 Crust colour analysis

Entire loaves were used to obtain the digital images. Colour was determined in the CIE-Lab colour space. In order to obtain  $L^*$  (lightness component),  $a^*$  (green to red component), and  $b^*$  (blue to yellow component) parameters, images were processed using Photoshop® (Adobe Systems Inc., CA, USA) acquiring average values. Browning Index (BI) was calculated using the following equation (Correa et al., 2017):

$$BI = \frac{100 \times (X - 0,31)}{0,172}$$

Where:

$$X = \frac{(a^* + 1,75 \times L^*)}{(5,645 \times L^* + a^* - 3,012 \times b^*)}$$

### 2.6.2 Crumb grain diameter

Crumb slices cut from the middle part of the each loaf were used to obtain the digital images. Images were processed using ImageJ together with BoneJ, a bone measurement ImageJ plugin that allowed us to measure the local thickness of the grains in the 2D images (Rasband, 2015; Doube et al., 2010). First, images were subjected to a binarization with ImageJ where the foreground, the crumb matrix, was converted into black, and the background, the grains, into white. The thickness at a point can be defined as the diameter of the greatest sphere that fits within a structure and contains the point. Diameters and their absolute frequencies were obtained and relative frequencies were calculated. Finally, the diameters were grouped in three intervals ranging from 0-0.10, 0.11-0.20 and 0.21-0.30 cm (small, medium and large holes, respectively).

## 2.7 Dialysability assay

Calcium dialysability assay was performed as described by Drago et al. (2005) with some modifications. Samples were grounded and 11.5 g of this powder was homogenized with 38.5 mL of distilled water. A first digestion was carried out by adding 5.0 mL of 4.0 g of  $\alpha$ -amylase in 60 mL of distilled water. The mixture was incubated in a shaking water bath at 37°C for 2 h. Once the incubation was finished, pH was adjusted to 2.0 using 6M HCl to perform the pepsin digestion by the addition of 1.6 mL of: 16.0 g of pepsin in 100 mL of 0.1M HCl, shaking incubation at 37°C was resumed for another 2 h and the solution constituted **the digest**. Afterwards, a 3 - 5 g aliquot of the digest was wet ashed by heating with 10 mL of HNO<sub>3</sub> to determine calcium by atomic absorption spectroscopy. A UNICAM SOLAAR 969 (Unicam Ltd., Cambridge, United Kingdom) spectrophotometer was used. A third stage was conducted adding a 15 - 16 g aliquot of the digest on a 100 mL beaker, in which a dialysis bag was incorporated. Spectra/Por dialysis tubing, diameter 14.6 mm, length 20.0 mm, 6000 - 8000 molecular cut off (Spectrum, USA) containing 18.75 mL of PIPES buffer (5.2 g of PIPES disodium salt in 100 mL of distilled water, pH adjusted to 6.6 - 6.9 with 6M HCl) were used. The beakers, with the pepsin digest aliquot and the dialysis bag, were taken to the shaking water bath (37°C, until pH was approximately 5.0). Afterwards, 3.75 mL of pancreatin-bile suspension (0.4 g of pancreatin and 2.5 g of bile in 100 mL of 0.1 M NaHCO<sub>3</sub>) were added on the beaker and digestion continued for 2 h. Finally, the dialysis bag was removed from the beaker, rinsed with distilled water and its content was transferred to a flask and weighed, constituting **the dialysate**. The dialysate was also wet ashed to determine calcium content. Also total calcium content on whole bread was determined by atomic absorption spectrophotometry according to the AACC 40-70 method (AACC, 2000).

Potential calcium contribution (PCaC) was calculated with the following equation:

$$\text{PCaC} = \frac{\text{total calcium content (mg of Ca/portion of 200g)} \times \text{DCa\%}}{100}$$

Where:

$$\text{DCa\%} = \frac{\text{calcium content on dialysate (mg of Ca/g of dialysate)} \times 100}{\text{calcium content on digest (mg of Ca/g of digest)}}$$

## 2.8 Sensory analysis

Sensory evaluation of loaves was carried out for various attributes namely foreign aroma, crust colour, crumb colour, foreign taste, hardness, chewiness, alveoli and general quality, according to the quantitative descriptive analysis methodology (QDA) proposed by Stone et al. (2012). A trained panel of eight members evaluated the samples for all the previously mentioned attributes, using scale of 10 cm nonstructured scale anchored at the extremes 1 by 'none/weak' and 9 'very strong'. Panelists were seated in a room free of noise and odours and suitably illuminated. Three slices of bread including crumb and crust were presented in white disposable plates with three-digit numbers randomly coded. Water was provided for palate cleansing.

## 2.9 Statistical analysis

All the analyses were carried out in triplicate. Statistical analysis was performed using Statgraphics Plus 5.1 (Statpoint Technologies, Inc., VA, USA). Analysis of variance was used and when the effect of the factors was significant ( $p < 0.05$ ), the test of multiple ranks honestly significant difference (HSD) of Tukey was applied (95% of confidence level).

# 3 Results and discussion

## 3.1 Flour composition

Average values obtained in our laboratory:  $12.9 \pm 0.3\%$  moisture,  $12.1 \pm 0.2\%$  protein, and  $0.65 \pm 0.01\%$  ash content. These results are in accordance with the AAC in regards to the wheat flour type 000 classification.

## 3.2. Moisture content

Moisture content of breads is shown in **Table 2**. No significant difference was observed when comparing Control and fortified breads. The amount of water used during bread making was constant for all the formulations, explaining the obtained moisture values.

### 3.3. Weight, volume and specific volume

Results of weight, volume and specific volume tests are shown in **Table 2**. Bread weight was not affected by the addition of the salts when comparing with Control breads. However, a significant decrease was observed in bread volume for the LA 50% fortification and in specific volume for both levels when fortification was made with LA. Salinas and Puppo (2014) reported a destabilization of the gluten network generated by the addition of LA into dough. Thus, this effect could be the cause of the decrease in bread specific volume observed for LA fortifications. However, Krupa-Kozak et al. (2012) studied the effect of calcium salts on gluten-free breads and observed that, when comparing LA, CA and CI with control breads, only LA reduced specific volume. Consequently, LA not only destabilized the gluten network but an additional effect may be involved. Pattison and von Holy (2001) studied the effect of selected compounds on baker's yeast (*Saccharomyces cerevisiae*) activity observing that LA reduced this activity up to 37.4% compared with a control. Furthermore, these authors detected that an increase in LA concentration conducted to a yeast activity reduction. One of the most important functions of baker's yeast in bread making is the production of CO<sub>2</sub> during proof due to the alcoholic fermentation of sugars. Gas produced during this stage will cause an increase in dough and bread volume. As a consequence of the addition of LA to dough, yeast activity could have been reduced and CO<sub>2</sub> production decreased leading to loaves with significantly lower specific volume as observed in LA fortifications compared with Control.

### 3.4. Crumb texture analysis

**Table 3** shows the parameters obtained from the TPA of breads. Cohesiveness and springiness were not affected by the addition of calcium salts. However, hardness significantly increased compared to Control when LA was used. Hardness is related with the sensory of lack of freshness in bakery products (Young, 2012), so the increase in this parameter might cause the consumer to reject the product due to the perception of stale bread. A negative correlation between loaf specific volume and crumb hardness was reported (Salinas and Puppo, 2014). This effect can be explained by the fact that high-volume bread will contain a larger volume of air cells which do not contribute to hardness (van Eijk and Hille, 1996). Considering that breads fortified with LA showed lower specific

volume than Controls, then it was expected to observe higher values for hardness. In addition, chewiness, parameter directly related to hardness, was significantly higher for LA 50%.

### **3.5. Digital image analysis**

#### **3.5.1. Crust colour analysis**

Digital images of breads are shown in **Figure 1**. Results of colour analysis are shown in **Table 4**. Fortification with CA at both levels has not affected any of the crust colour parameters when comparing to Control breads. However, fortification with LA and CI affected at least one of the colour parameters when calcium level was 50%. Furthermore, breads fortified with CI 50% showed a significantly lower BI than Control breads which means that CI 50% yielded paler breads. In agreement, Bell et al. (1998), when analyzing the effect of salt (phosphate and citrate) type in low moisture systems, observed that browning rate constants were lower in the presence of citrate than those for phosphate at the same buffer concentration. However, as salt content in the solid increased, the authors observed that the browning rate constant increased for phosphate but decreased for citrate. The authors related their observations with the fact that phosphate buffer has been reported to catalyze nonenzymatic browning in solutions and citrate is a much weaker catalyst, especially for the Maillard reaction, where no catalytic effect was reported.

#### **3.5.2. Crumb grain diameter**

The relative frequencies of the diameters of the holes grouped in the three intervals are shown in **Table 4**. The distribution of the holes showed a high frequency of small holes (average 85%), followed medium holes (average 13%), and big holes (average 2%). Most of the baked products have a characteristic crumb formed of holes of different shapes, sizes and distributions (Cauvain, 2007). No significant differences were observed between Control and fortified breads.

### **3.6. Calcium dialysability and potential calcium contribution**

Calcium content of digest and dialysate and PCaC are shown in **Table 5**. Calcium content of the digest of samples was nearly theoretical values (200 and 500 mg of

Ca/portion of 200 g of bread). The presence of calcium on Control breads can be attributed to the occurrence of the mineral in some ingredients i.e.: wheat flour, tap water, etc. In regards to calcium content on the dialysate, it was significantly higher for all of the fortifications in comparison to Control breads. Besides, 50% fortifications showed, as expected, significantly higher calcium contents than 20% fortifications. The PCaC, that represents the bioaccessible calcium in relation to the added calcium, is the best parameter to analyse different salts and fortification levels tested. The PCaC was significantly higher for CI 20%, when compared to Controls, while only an increasing trend was observed for the remaining 20% fortifications. All fortifications made at 50% showed a PCaC significantly higher than Controls, being both LA and CI higher than CA. This may be related to the lower solubility of CA. Some authors elaborated breads using mixtures of wheat flour and non-traditional flours rich in calcium like amaranth flour (Dyner et al., 2007) or American carob flour (Zuleta et al., 2012). The PCaC determined for our fortifications (average PCaC for 20% fortifications: 77.7 mg/100 g of bread, and average PCaC for 50% fortifications: 169.3 mg/100 g of bread) were higher than those reported for amaranth flour (PCaC: 8.07 mg/100 g of bread) and American carob flour (PCaC: 20.8 mg/100 g of bread). Thus, salts and fortification levels tested resulted to provide a good contribution of bioaccessible calcium thanks to high PCaC values.

### 3.7. Sensory analysis

The spider web for QDA sensory analysis of breads is shown in **Figure 2**. The incorporation of calcium salts did not significantly affect the foreign aroma, crumb colour, hardness, chewiness and alveoli of breads. In agreement, previous research of Romanchik-Cerpovicz and McKemie (2007) had shown that CA, CI and LA did not affect sensory characteristics of wheat-flour tortillas fortified with calcium at level of 10% of the adequate intake for adults. A foreign taste described as bitter was detected in the LA 50% breads. Krupa-Kozak et al. (2012), investigated fortification of gluten-free bread containing inulin with different calcium sources (CA, CI, LA and calcium chloride). The authors reported that breads with LA showed the lowest sensory scores as a consequence of their poor taste, which was described as bitter. The aforementioned results confirm the importance of determining the salt thresholds below which their incorporation is not detectable. Ziadeh et al. (2005) prepared pita bread loaves from

flours fortified with, CA and CI, and reported detection thresholds of 1994 and 2132 (mg/100 g bread) for CA and CI, respectively. Breads formulated in the present investigation with CA and CI had a fortification level below those thresholds. It is important to remark that the results obtained for LA breads could constitute an estimation of the threshold value perceived for panellist for this calcium salt. In reference to colour parameters, all salts studied in the present investigation are either white or colourless, however the colour of the CI 50% crust received a lower score in agreement with a lower  $L^*$ ,  $a^*$ ,  $b^*$  and BI parameters measured in the CI 50% breads. In spite of the minor differences previously mentioned, the general quality parameter was not significantly different between fortified and Control breads.

#### **4. Conclusion**

This study demonstrated that fortification of wheat flour bread with calcium salts is an effective approach to provide population with an important source of bioaccessible calcium. However, the impact of some calcium salts on the properties of breads must be taken in consideration. The effect of LA fortifications on hardness and chewiness of the breads could be a consequence of the effect of LA on the reduction of yeast activity and destabilization of gluten network. On the other hand, no major changes were observed for CI and CA in regards to bread physicochemical properties respect to the Control breads, though the CI 50% ones resulted to be paler. However, paler colour could be solved by a slight increase on baking time. Since all breads elaborated resulted to show high values of PCaC the fortification with the studied calcium salts can be recommended. Moreover, higher fortification levels can be tested to improve PCaC and also the addition of an enhancer of calcium absorption can be investigated. For a successful fortification, fortified foods should have similar sensory characteristics as their nonfortified counterparts. This requirement is particularly important for calcium fortification in which, compared with other micronutrients, this mineral will have to be added in larger amounts. Sensorial general quality was not significantly different between fortified and Control breads. This may be a promising result considering perspective consumer acceptability of breads.



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## 6. Ethics approval

Ethics approval was not required for this research.

## 7. Ethics approval

The paper has no conflict of interest.

## 8. Data Availability Statement

Research data are not shared.

## 9. References

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#### ANNOTATED REFERENCES

1. Krupa-Kozak, U., Altamirano-Fortoul, R., Wronkowska, M., & Rosell, C. M. (2012). Breadmaking performance and technological characteristic of gluten-free bread with inulin supplemented with calcium salts. *European Food Research and Technology*, **235**, 545-554.

**Annotations:** This paper was cited because the authors studied the effect of calcium lactate, citrate and carbonate in gluten-free breads. The paper was very useful to compare and discuss our results. Particularly, in physicochemical analysis and sensory analysis.

2. Salinas, M. V., & Puppo, M. C. (2014). Rheological Properties of Bread Dough Formulated with Wheat Flour–Organic Calcium Salts–FOS-Enriched Inulin Systems. *Food and Bioprocess Technology*, **7**, 1618-1628.

**Annotations:** This paper was cited because the authors studied the effect of calcium lactate and citrate and carbonate in wheat flour breads. The paper was very useful to compare and discuss our results. Particularly, to discuss the correlation between specific volume and crumb texture.

3. Ziadeh, G., Shadarevian, S., Malek, A., Khalil, J., Haddad, T., Haddad, J., & Toufeili, I. (2005). Determination of sensory thresholds of selected calcium salts and formulation of calcium-fortified pocket-type flat bread. *Journal of Food Science*, **70**(8), 548-552.

**Annotations:** This paper was cited because the authors studied bread loaves from flours fortified with calcium carbonate and citrate. They analysed and reported detection thresholds for those salts and were useful for the discussion of sensory analysis results.

4. Zuleta, Á., Binaghi, M. J., Greco, C. B., Aguirre, C., De la Casa, L., Tadini, C., & Ronayne de Ferrer, P. A. (2012). Diseño de panes funcionales a base de harinas no tradicionales. *Revista chilena de nutrición*, **39**, 58-64.

**Annotations:** This paper was cited because the authors discussed the potential calcium contribution. This parameter represents the bioaccessible calcium in relation to the added calcium and is the best parameter to analyse different salts and fortification levels tested.

**Table 1.** Ingredients employed for breadmaking.

<b>Fortification</b>	<b>Flour (g)</b>	<b>Salt (g)</b>	<b>Yeast (g)</b>	<b>Water (mL)</b>	<b>Calcium salt (g)</b>
Control	450.0	9.0	9.0	240	0.0
LA 20%	446.1	9.0	9.0	240	3.9
LA 50%	440.3	9.0	9.0	240	9.7
CA 20%	448.7	9.0	9.0	240	1.3
CA 50%	446.8	9.0	9.0	240	3.2
CI 20%	447.6	9.0	9.0	240	2.4
CI 50%	444.0	9.0	9.0	240	6.0

**Table 2.** Moisture content, weight, volume and specific volume of breads.

Fortification	Moisture content (%)	Weight (g)	Volume (mL)	Specific volume (mL/g)
Control	29±1 <sup>a</sup>	171±1 <sup>ab</sup>	497±39 <sup>b</sup>	2.9±0.2 <sup>b</sup>
LA 20%	28.8±0.5 <sup>a</sup>	172±1 <sup>b</sup>	468±12 <sup>ab</sup>	2.73±0.08 <sup>a</sup>
LA 50%	29.4±0.5 <sup>a</sup>	171±1 <sup>ab</sup>	420±9 <sup>a</sup>	2.46±0.06 <sup>a</sup>
CA 20%	29.2±0.7 <sup>a</sup>	171±1 <sup>ab</sup>	518±20 <sup>b</sup>	3.0±0.1 <sup>b</sup>
CA 50%	30±1 <sup>a</sup>	168±2 <sup>a</sup>	518±32 <sup>b</sup>	3.1±0.2 <sup>b</sup>
CI 20%	29±1 <sup>a</sup>	172.3±0.8 <sup>b</sup>	477±25 <sup>ab</sup>	2.8±0.1 <sup>ab</sup>
CI 50%	29±1 <sup>a</sup>	169.9±0.1 <sup>ab</sup>	487±20 <sup>ab</sup>	2.9±0.1 <sup>ab</sup>

Data corresponds to mean values and standard deviations of three samples.

Values with different letters in each column are significantly different ( $p < 0.05$ ).



**Table 3.** Parameters obtained from texture profile analysis of breads.

Fortification	Texture Profile Analysis			
	Hardness (N)	Cohesiveness	Springiness (cm)	Chewiness (N*cm)
Control	10±2 <sup>a</sup>	0.708±0.009 <sup>a</sup>	1.17±0.06 <sup>a</sup>	8±2 <sup>ab</sup>
LA 20%	17±2 <sup>b</sup>	0.65±0.02 <sup>a</sup>	1.16±0.05 <sup>a</sup>	12±2 <sup>abc</sup>
LA 50%	18±1 <sup>b</sup>	0.67±0.01 <sup>a</sup>	1.097±0.002 <sup>a</sup>	13.0±0.7 <sup>c</sup>
CA 20%	10±3 <sup>a</sup>	0.70±0.03 <sup>a</sup>	1.24±0.04 <sup>a</sup>	9±2 <sup>ab</sup>
CA 50%	8.9±0.2 <sup>a</sup>	0.72±0.01 <sup>a</sup>	1.20±0.01 <sup>a</sup>	7.7±0.3 <sup>a</sup>
CI 20%	14±2 <sup>ab</sup>	0.67±0.02 <sup>a</sup>	1.20±0.02 <sup>a</sup>	12±1 <sup>abc</sup>
CI 50%	13±3 <sup>ab</sup>	0.690±0.08 <sup>a</sup>	1.2±0.1 <sup>a</sup>	11±3 <sup>abc</sup>

Data corresponds to mean values and standard deviations of three samples.

Values with different letters in each column are significantly different ( $p < 0.05$ ).

**Table 4.** Colour parameters of the crust and relative frequencies of hole diameters of the crumb.

Fortification	Crust				Hole diameter (relative frequencies of intervals)		
	<i>L</i> <sup>*</sup>	<i>a</i> <sup>*</sup>	<i>b</i> <sup>*</sup>	BI	0.0 - 0.1 (cm)	0.1 - 0.2 (cm)	0.2 - 0.3 (cm)
Control	53.8±0.6 <sup>bc</sup>	12.7±0.4 <sup>bc</sup>	37±2 <sup>b</sup>	121±9 <sup>b</sup>	84±3 <sup>a</sup>	14±2 <sup>a</sup>	2±1 <sup>a</sup>
LA 20%	52.4±0.7 <sup>ab</sup>	13±1 <sup>c</sup>	37±2 <sup>b</sup>	127±14 <sup>b</sup>	83±1 <sup>a</sup>	14±1 <sup>a</sup>	3±1 <sup>a</sup>
LA 50%	50±1 <sup>a</sup>	13±1 <sup>bc</sup>	35±3 <sup>b</sup>	133±23 <sup>b</sup>	88±2 <sup>a</sup>	11±1 <sup>a</sup>	1.0±0.8 <sup>a</sup>
CA 20%	54.9±0.6 <sup>bcd</sup>	11±1 <sup>ab</sup>	33±4 <sup>ab</sup>	103±16 <sup>ab</sup>	84±2 <sup>a</sup>	15±3 <sup>a</sup>	1.0±0.3 <sup>a</sup>
CA 50%	54±2 <sup>bc</sup>	13.1±0.4 <sup>bc</sup>	39±2 <sup>b</sup>	133±5 <sup>b</sup>	85±3 <sup>a</sup>	13±1 <sup>a</sup>	2±2 <sup>a</sup>
CI 20%	56.4±0.3 <sup>cd</sup>	11.1±0.5 <sup>b</sup>	33±3 <sup>ab</sup>	98±10 <sup>ab</sup>	87±2 <sup>a</sup>	11±2 <sup>a</sup>	2.0±0.4 <sup>a</sup>
CI 50%	57±1 <sup>d</sup>	8.8±0.8 <sup>a</sup>	25±5 <sup>a</sup>	69±15 <sup>a</sup>	82.5±0.8 <sup>a</sup>	15.5±0.9 <sup>a</sup>	2.0±0.3 <sup>a</sup>

Data corresponds to mean values and standard deviations of three samples.

Values with different letters in each column are significantly different ( $p < 0.05$ ).

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**Table 5.** Calcium content on digest and dialysate, and potential calcium contribution (PCaC) of breads.

<b>Fortification</b>	<b>Digest (mg of Ca/portion of 200 g)</b>	<b>Dialysate (mg of Ca/portion of 200 g)</b>	<b>PCaC (mg of Ca/portion of 200 g)</b>
Control	65±3 <sup>a</sup>	34±6 <sup>a</sup>	33±8 <sup>a</sup>
LA 20%	269±20 <sup>b</sup>	92±6 <sup>b</sup>	68±9 <sup>ab</sup>
LA 50%	519±68 <sup>c</sup>	195±19 <sup>c</sup>	196±28 <sup>d</sup>
CA 20%	256±6 <sup>b</sup>	81±11 <sup>b</sup>	74±8 <sup>ab</sup>
CA 50%	551±29 <sup>c</sup>	140±17 <sup>c</sup>	116±5 <sup>c</sup>
CI 20%	222±31 <sup>b</sup>	91±3 <sup>b</sup>	91±16 <sup>b</sup>
CI 50%	498±64 <sup>c</sup>	194±16 <sup>c</sup>	196±34 <sup>d</sup>

Data corresponds to mean values and standard deviations of three samples.

Values with different letters in each column are significantly different ( $p < 0.05$ ).

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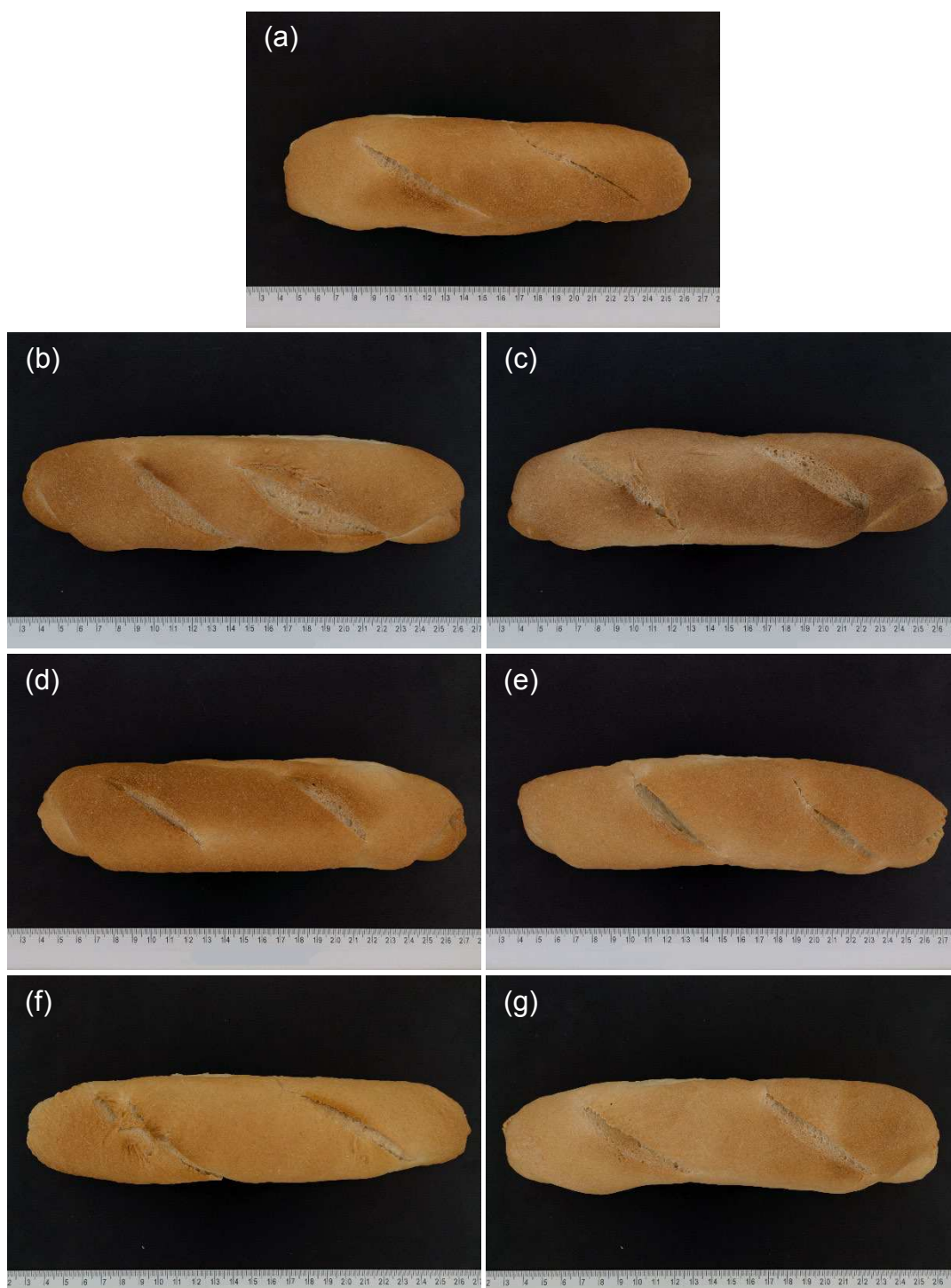
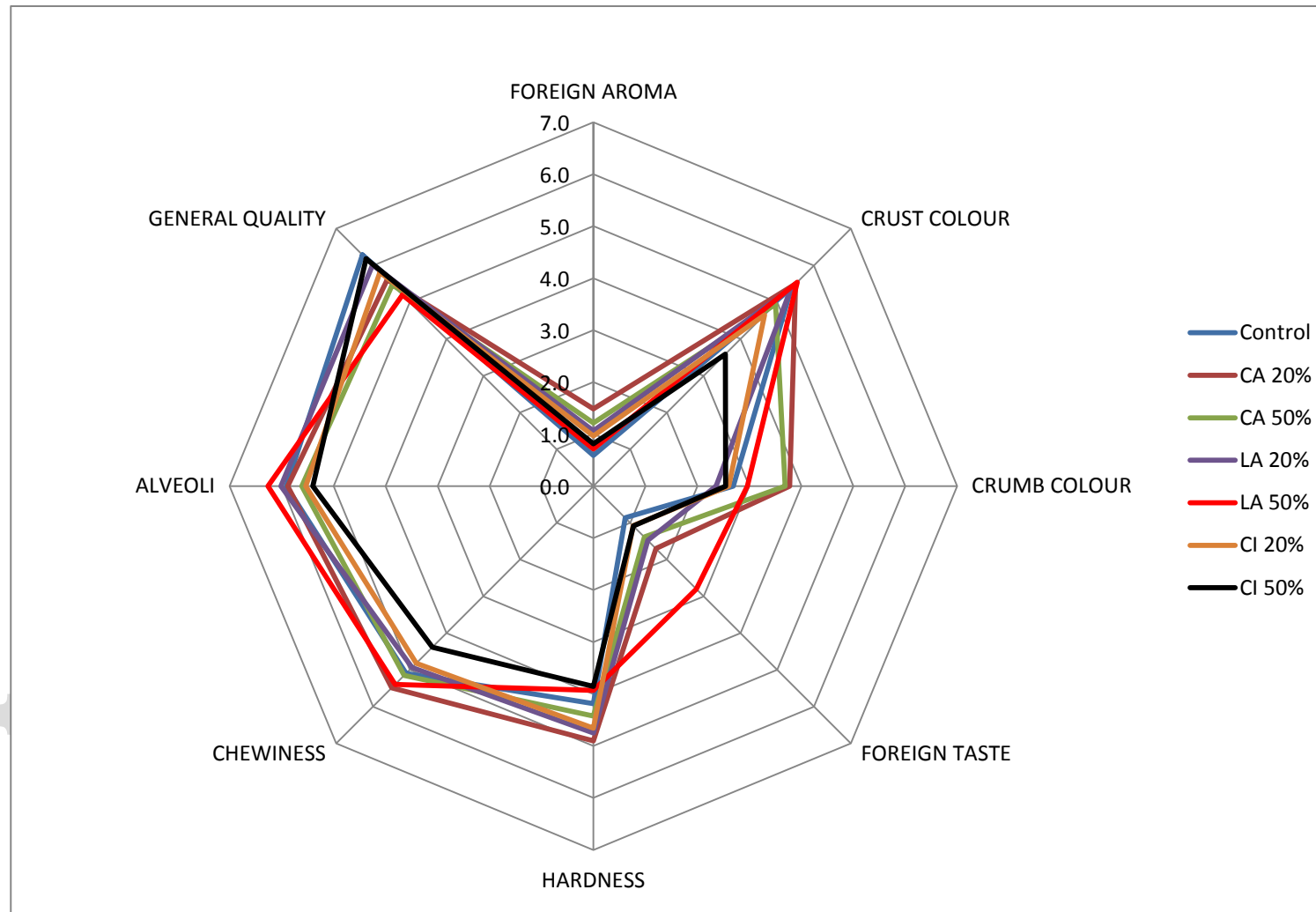
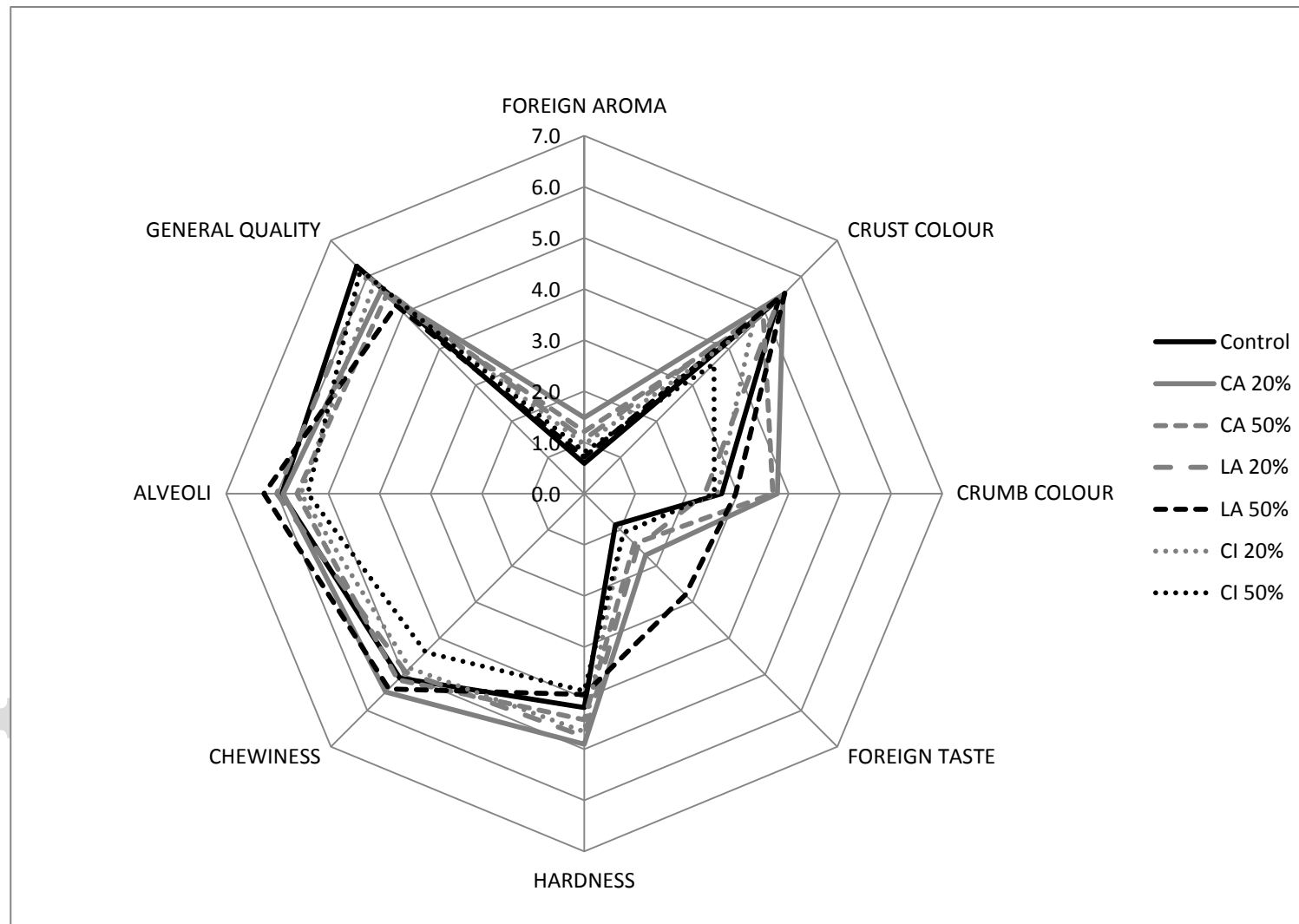


Figure 2



Colour for online version



Black and white for printed version

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