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**Effect of cocoa bean shells granulometries  
on qualitative properties of gluten-free bread during storage**

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1 **Abstract**

2 Cocoa bean shells (CBS) are by-products of cocoa processing. Their rich chemical composition makes them interesting  
3 ingredients to improve the overall quality of gluten free bread. The addition of different dimensional fractions (F1, 1.00-  
4 1.99 mm; F2, 0.50-0.99 mm; F3, 0.355-0.49 mm) of CBS to gluten-free bread was investigated in terms of volume,  
5 moisture content, crumb grain, texture and colour during 3 days of storage. No significant differences in moisture content  
6 were observed among fractions, but protein, fat and fibre content resulted influenced by the dimensional range. With  
7 reference to water absorption indexes, F2 showed the highest water binding capacity (WBC) and water holding capacity  
8 (WHC), having instead the lowest water solubility index (WSI), in comparison to the other fractions, and confirming a good  
9 retention of the fibres' structure. Results on bread showed that CBS addition modified crumb grain leading to a lower  
10 specific volume, especially for F2. The presence of CBS accelerated significantly the crumb staling process in comparison  
11 to the control, resulting however F1 softer than control at the end of the storage period. Finally, colour was improved by the  
12 presence of CBS becoming darker, with F3 showing the highest impact on  $L^*$  and  $a^*$ .

13

14 **Keywords:** cocoa by-products, celiac disease, shelf-life, water absorption, structure, colour

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## 18 **Introduction**

19 World cocoa beans (*Theobroma cacao* L.) production results of 5.2 Mtons in 2018 [1] with Côte d'Ivoire (2.0 Mtons),  
20 Ghana (0.9 Mtons), Indonesia (0.7 Mtons), Nigeria (0.3 MTons), Ecuador (0.3 Mtons), Cameroon (0.3 Mtons) and Brazil  
21 (0.2 Mtons) as the main producers. Cocoa bean shells (CBS) are the outer portions of beans that encase the nibs and, since  
22 they represent 12-20% of the cocoa seed, they result as a by-product produced in great quantity. Chemical composition of  
23 CBS is very interesting as protein content ranges from 11 to 18 g/100g dm, fat content from 2 to 21 g/100g dm, fibre  
24 content from 43 to 60.5 g/100g dm and polyphenols from 1 to 5.8 g/100g dm [2-4]. Several efforts were spent in valorising  
25 this waste material, starting from the use in animal feed with the limit of theobromine content (2–3%) [5] to the use for the  
26 production of extracts rich in fibres, polyphenols, antioxidants and pectins [6-9]. In addition, in the last years several studies  
27 on application of CBS fibre as a functional ingredient in food products were published. Martínez-Cervera et al. [10] used  
28 soluble cocoa dietary fibre as a fat replacer in chocolate muffins with some advantages such as higher moisture, more tender  
29 and crumbly structure and a reduction of hardening during storage. Similarly, Collar et al. [11] investigated the effects of  
30 soluble dietary fibre from cocoa shell wheat bread and suggested that dietary fibres from cocoa shell added up to 6% could  
31 be used to design technologically feasible, sensory acceptable, and long-term stored fibre-enriched wheat bread. In the same  
32 way, Grillo et al. [12] obtained dietary fibre powder from CBS and substituted oat and whole wheat with no significant  
33 differences between CBS powder and other fibres during organoleptic test. Recently, Jozinović et al. [13] successfully  
34 enriched extruded snacks with cocoa husks and reported a proportional increase in resistant starch, polyphenol content and  
35 antioxidant activity with the addition of cocoa husks, while starch damage slightly decreased. A similar approach was  
36 followed by Rojo-Poveda et al. [14] who successfully obtained new functional beverages with potential health benefits.  
37 Kreibich et al. [15] described CBS structure from a microscopical point of view and observed thick-walled protective cells  
38 providing somewhat rigid and flexible support properties. Interestingly, authors established that the shell has different layers  
39 and vascular bundles distributed among the tissues: the first tissue (epidermis) layer is comprised of long cells (smaller  
40 sizes) with thick walls and the second of long flattened parenchyma cells, closely surrounding the vascular bundles. It is  
41 possible to visualize different formats (spiral – tracheid), sizes and numbers of vascular tissues (protoxylem and phloem) for  
42 water and nutrient transportation. Starting from this morphological characteristic, a technological approach, similar to what  
43 reported by de Sousa et al. [16], could be applied for separating CBS into different granulometric fractions that potentially  
44 had different profiles in chemical composition and technological properties, as consequence. Rojo-Poveda et al. [14]

45 demonstrated that the extraction of polyphenols for obtaining a functional beverage from CBS was directly influenced by  
46 the grinding degrees when using percolation techniques, whereas the maceration technique showed no dependence on the  
47 particle size. The present paper represents the first attempt to use sieving of CBS as a preliminary method for modifying  
48 functional properties of this kind of industrial waste. Thus, the aim of this work was to evaluate the effects of the addition of  
49 different CBS granulometries on physico-chemical properties of a commercial gluten-free bread formulation during 3 days  
50 of shelf-life.

## 51 **Materials and Methods**

### 52 **Samples, breadmaking and storage**

53 A commercial (NT FOOD S.p.A., Altopascio, Lucca, Italy) gluten-free bread mixture with the following ingredients was  
54 used: corn, rice cream soup, tapioca starch, sugar, vegetable fibres, salt, thickening agents: guar flour and  
55 hydroxypropylmethylcellulose, flavourings. The proximate composition of the mixture was: moisture 9.7g/100g,  
56 carbohydrate 85.7g/100g, fibres 1.9g/100g, protein 1.8g/100g, fat 0.9g/100g, salt 1.9g/100g. Ground CBS were kindly  
57 donated by the company Ferri dal 1905, Castel Goffredo (MN), Italy.

58 To obtain the different particle sizes of CBS, it was classified in sieves with opening sizes 2.00 mm, 1.00 mm, 0.50 mm and  
59 0.355 mm, respectively with an automatic sieve (Giuliani Tecnologie, Torino, Italy). The obtained fractions were named F1  
60 (1.00 to 1.99 mm), F2 (0.50 to 0.99 mm) and F3 (0.355 to 0.49 mm).

61 Bread samples were prepared with the following recipe: gluten free mixture (250.0 g), water (225.0 g), sunflower oil (12.5  
62 g), compressed yeast (6.25 g) (control, C). CBS from F1, F2 and F3 fractions were then added by substituting the mixture at  
63 4 g/100g in order to achieve a total final fibre content equal to 3 g/100g for the claim “source of fibre” [17]. The amount of  
64 water was determined on the basis of preliminary tests until obtaining a workable dough for all the formulations. The same  
65 amount of water was added to all the recipes in order to evaluate the unique effect of the different CBS added fractions.

66 A domestic bread machine (Moulinex, Groupe Seb Italia S.p.A., Milano, Italy) was used for breadmaking, with the rapid  
67 program: stirring + kneading + rising, 80 min; baking, 55min at 210 °C, obtaining sandwich bread loaves with mean weight  
68 of about 400 g. Cooking losses after baking were measured and ranged from  $22.1 \pm 1.2$  g/100g for C and F1 samples to  
69 about  $24.5 \pm 1.3$  g/100g for F2 and F3 breads. The cooked breads were cooled at room temperature, packaged in alcohol-  
70 sprayed sealed air-tight plastic bags and stored in a 25 °C temperature-controlled chamber in the dark (ISCO 9000, Milan,  
71 Italy). Samples were analysed at 0, 1 and 3 days of shelf life. Three loaves were used for the characterization of the breads  
72 at each storage time analysing 9 loaves for each bread type.

73 **Proximate composition and water absorption tests of flour and CBS**

74 Moisture, protein, lipid and ash of cocoa bean shell fractions were determined using standard procedures [18]. Moisture was  
75 determined in oven at 105 °C for 24 h. Crude fat content was determined using an automatized Soxhlet extractor (SER  
76 148/3 VELP SCIENTIFICA, Usmate Velate, Italy) using diethylether. Total ash was determined after mineralization at 550  
77 °C for 5 h + 5 h. Total nitrogen was determined with a Kjeldahl system (DKL heating digester and UDK 139 semiautomatic  
78 distillation unit, VELP SCIENTIFICA).

79 Water absorption tests on CBS fractions were determined as described by Sarangapani et al. [19], as average of three  
80 replicates. Water binding capacity (WBC) and water holding capacity (WHC) were expressed as grams of water retained  
81 per gram of solid. Water absorption index (WAI) and water solubility index (WSI) were expressed as grams of gel per  
82 grams of sample (db) and grams of dried supernatant per grams of sample (db), respectively.

83

84 **Physical analyses**

85 Specific volume of breads was determined in triplicate according to the AACC Approved Method 10-05.01 [20] and  
86 expressed as the volume/weight ratio of cooked bread (mL/g).

87 Crumb grain was evaluated by means of a digital image analysis system, as reported previously [21]. Images of three central  
88 slices (20 mm thickness) of each loaf were acquired with a scanner (Hewlett Packard, Palo Alto, CA, USA) at 600 dots per  
89 inch (dpi) taking squares (40x40 mm) from the centre of the images after calibration, standardization and optimization by  
90 means of Image-Pro Plus 4.5 (Media Cybernetics Inc., USA) software. The number of pores (expressed as percentage of the  
91 total number) was obtained according to pre-selected dimensional classes based on their area. Selected classes were: class-1:  
92 0.01-0.099 mm<sup>2</sup>; class-2: 0.1-0.199 mm<sup>2</sup>; class.3: 0.2-0.99 mm<sup>2</sup>; class-4: > 1 mm<sup>2</sup>.

93 The crumb moisture content (g/100g) within the bread loaves was evaluated in triplicate at each shelf-life time for each  
94 bread type following the AACC standard method, 44-15.02 [20].

95 Texture analysis was performed on crust and crumb using a TA.XT2 Texture Analyzer equipped with a 25 kg load cell  
96 (Stable Micro Systems, Godalming, UK) and Texture Expert for Windows software (version1.22) for data analysis on each  
97 loaf. Crust hardness was measured by means of a puncture test using a 3 mm diameter stainless steel probe and a test speed  
98 of 2 mm/s. Maximum peak force (N) was measured from the penetration curve and taken as crust hardness. Measurements  
99 were taken on five preselected points of the crust.

100 Crumb evaluation was carried out on ten cubes of 20×20×20 mm extracted from two central slices of the samples. A TPA

101 test was performed with a 35 mm diameter cylindrical aluminium probe by means of a double compression with a speed of  
102 2mm/s up to the 40% of the original sample height. The textural parameters considered were hardness (maximum peak  
103 force of the first compression cycle, N), cohesiveness (ratio of positive force area during the second compression to that  
104 during the first compression area, dimensionless), resilience (area during the withdrawal of the penetration, divided by the  
105 area of the first penetration, dimensionless), and chewiness (product of hardness x cohesiveness x springiness, N) [22].

106 Colour was determined on ten pre-selected locations of the crust and crumb of each bread loaf. The analyses were  
107 performed using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D65  
108 and a 10° position of the standard observer. The instrument was calibrated before each analysis with white and black  
109 standard tiles.  $L^*$  (lightness),  $a^*$  (redness) and  $b^*$  (yellowness) were quantified on each sample using the Spectramagic  
110 software (Ver. 3.6).

### 111 **Statistical analysis**

112 Means and standard deviations calculated with SPSS (Version 25.0, SPSS Inc., Chicago, USA) statistical software were  
113 used to perform one way (ANOVA) to evaluate the effect of CBS addition at a significance level of 0.05 ( $p < 0.05$ ). A  
114 Tukey-Kramer post-hoc test at a 95% confidence level was also applied using the same software to verify the differences  
115 among groups.

## 116 **Results and Discussion**

### 117 **Chemical and technological analyses of CBS fractions**

118 Moisture content of CBS (Table 1) was in the range 4.79-5.01 g/100 g with no significant differences among the considered  
119 fractions. Observed moisture content was quite lower compared to those reported by Martínez et al. [2] but the composition  
120 of CBS is reported to be quite variable and it depends, among other factors, on its origin and mainly on the process to which  
121 it has been subjected. The highest ash content (Table 1) was observed for F3 while the lowest for F2 with F1 as intermediate  
122 value: in general, obtained data are in the range previously reported for this cocoa co-product [2]. Regarding protein  
123 content, no differences were observed between F2 and F3 while F1 presented a significantly lower value. Obtained data are  
124 in the range already published in the scientific literature [3]. Moreover, the fat content resulted significantly different among  
125 the three CBS size classes, being the lowest for F1 and the highest for F3, as also reported by other authors [3].

126 Finally, fibre content of F1 resulted the highest one with no differences between F2 and F3; the observed results confirm the  
127 feasibility of fibre enrichment by means of grinding and sieving [23]. Starting with the work of Knuckles et al. [24]  
128 separation of different fractions with different chemical composition and technological properties, as consequence, was

129 successfully achieved for cocoa bean shell.

130 Hydration properties of the fractions are reported in Table 1. WBC and WHC data presented a similar trend with the highest  
131 values for F2 probably due to the higher fibre structure retention as WHC and WBC are strictly related to them [25]. The  
132 same authors, working on coffee by-products, reported also an inverse relation with the granulometry of the material but in  
133 the present work, this relationship was not observed. Technological functionality was in the range published by Lecumberri  
134 et al. [26] for cocoa shell and according to their observations, it could be confirmed that all fractions presented only few  
135 amounts of soluble fibres.

136 WAI and WSI can be used to indicate the behaviour of the starches contained in the three fractions [19]: F1 gave the highest  
137 values while on the contrary F3 the lowest ones. Low WAI values are generally linked to high level of starch damage and  
138 interactions with non-starch components and cell wall matrices [19, 27]. Actually, F3 samples represent the littlest fraction  
139 with probably the highest impact of grinding on structure but with the highest content of starch that was subjected to  
140 damages during the whole process of cocoa beans. This hypothesis is confirmed by the high WSI value of F3 fraction  
141 probably due to the depolymerization of starch, which leads to the production of low molecular weight fragments of  
142 amylose and amylopectin [19]. Thus, the decrease in the values of water interaction indexes could be explained by the fact  
143 that some of the molecules of the solubilized material, in particular amylose molecules, may interact each other generating a  
144 modified structure that impedes water absorption [28].

#### 145 **Crumb grain characteristics and specific bulk volume of bread**

146 Specific volumes of tested breads are reported in Figure 1: at time 0 significant differences were observed among the cocoa  
147 bean shell fractions. Particularly, F2 presented a significantly lower value compared to F1 and F3 probably due to its high  
148 WHC and WBC values (Table 1) that are generally related to low volume in bread [29]. Moreover, F2 samples presented  
149 both a higher cooking loss ( $24.8 \pm 0.6$  %) and a higher percentage of holes belonging to the greatest class (Figure 2)  
150 compared to the other samples. Results are in agreement with Coda et al. [30] that reported a volume variation depending on  
151 wheat bran particle size. Similarly, Gómez et al. [31], studying the effect of fibre size on the quality of fibre-enriched cakes,  
152 reported a relation between specific volume, regardless the origin of fibres, and dimensions of them. During storage,  
153 specific volume of C samples remained constant with no significant variations. Otherwise, all CBS-enriched breads  
154 presented a significant volume reduction (Figure 1), also showing a significant increase in the percentage of holes belonging  
155 to the greatest class and a decrease in the lowest one, as consequence, with a significant variation in the total number of  
156 holes only for F1 samples (data not shown). During storage F1, F2 and F3 breads showed a little weight loss (data not



157 shown) ranging from 1 to 2 % that, together with water migration from crumb to crust, could have caused the reduction of  
158 wall thickness and the increase of holes' dimensions, as consequence. On the contrary, C samples did not show any weight  
159 loss during storage.

160 The presence of fibres contained in cocoa bean shells caused a significant reduction of total number of holes (about 350  
161 holes/16cm<sup>2</sup>) compared to C samples (about 600 holes/16cm<sup>2</sup>) with a higher presence of holes with large dimensions  
162 (Figure 2). Bigger holes are more sensible to collapse and this fact could explain the volume reduction observed for F1, F2  
163 and F3 samples (Figure 1). Probably, cocoa shell fibres interfered with thickening agents (guar flour and  
164 hydroxypropylmethylcellulose) of the gluten free mixture used for the preparation of breads as a competitor for water when  
165 water is limited [32].

166 Crumb moisture content (Figure 3) of enriched samples were significantly lower than C ones at all storage times confirming  
167 the higher evaporation caused by a coarser crumb grain characteristic observed for F1, F2 and F3 samples (Figure 2). As  
168 stated above, weight loss after cooking was significantly higher for F2 and F3 samples while no statistical differences were  
169 observed for others. During storage, no samples showed significant difference in crumb water content thanks to packaging  
170 and to the short-term shelf life.

171 Crust colorimetric data (Table 2) showed significant differences due to the addition of cocoa bean shell. Particularly, crust  
172 L\* values increased and a\* decreased thanks to the cocoa bean fibre probably due to the lower moisture content (Figure 3)  
173 that generally leads to lighter bread crust [33]. On the contrary, the impact of cocoa bean shell on bread crumb colour was  
174 evident: addition of CBS caused a significant reduction of L\* and simultaneously a significant increase of a\* and b\* (Table  
175 2) with darker crumb in accordance with Kārklīņa et al. [34], due to the natural colour of cocoa bean shells. F3 showed the  
176 greatest impact on L\* and a\* values probably due to the very low dimensions of particles. In general, colorimetric changes  
177 caused by cocoa bean shells' addition can be positively considered as gluten free breads are generally reported to present  
178 pale colour that is not attractive for consumers [35].

179 Bread crumb and crust hardness (Table 3) at time 0 days were not significantly affected by cocoa bean shell addition.  
180 During storage, F2 samples showed the highest hardness increase at time 3 with a value two folds higher compared to the  
181 control. This could be due to the presence of largest holes that generally tend to collapse (Figure 3) and to the high WHC  
182 value (Table 1) of this fraction with a probably strong competition for water with starches and thickening agents [36]. On  
183 the contrary, F1 gave the lowest hardness value at time 3 with significant lower values also compared to C, demonstrating  
184 an antistaling effect probably due to the high WAI value (Table 1) of this fraction related to a high water retention capacity

185 which could help to prevent the retrogradation of amylopectin [37]. Cohesiveness (Table 3) decreased for all samples with  
186 increasing storage time, as expected [38]. All samples added with cocoa bean shell fractions exhibited lower values at day 1  
187 compared to C in accordance with Rocha Parra et al. [39] probably in relation with crumb moisture content that resulted  
188 lower in F samples.

189 Regarding resilience data at time 0 (Table 3), F2 bread showed a significantly lowest value compared to the others probably  
190 due to the lowest volume and to the presence of a highest percentage of holes belonging to the greatest class. Characteristics  
191 of F2 breads could be probably related to water hydration properties of this CBS fraction. In general, CBS enriched breads  
192 presented significantly lowest resilience values compared to C at all times probably due to the lowest total number of holes  
193 per area and to the highest presence of holes with great dimensions. Collar et al. [11] already reported significant reduction  
194 of resilience in breads with soluble dietary fiber preparations obtained from cocoa shell.

195 Finally, chewiness values of C samples were in almost all cases significantly higher compared to the enriched ones:  
196 chewiness is a secondary texture parameter that is associated with difficulty in chewing the sample and forming a bolus  
197 before swallowing. These results could be related to the greater moisture retained by C samples (Figure 3), making them  
198 denser to chew [10].

## 199 **Conclusions**

200 This study attempted to evaluate the effect of the addition of three different dimensional fractions from cocoa bean shells on  
201 gluten free bread during 3 days of storage. Different proximate composition and water absorption properties observed for  
202 the different fractions of cocoa bean shell were reflected also in bread characteristics. The fibre deriving from CBS  
203 fractions, probably, negatively influenced the crumb grain, with coarser structure, as well as specific volume and moisture  
204 content, with significantly lower values. On the other hand, the CBS supplementation led to a pleasant darker colour, with  
205 F3 showing the more consistent improvement. Moreover, textural attributes (hardness) confirmed the possibility to add CBS  
206 F1 fraction with limited impact on product quality also during storage.

207 Overall, it was evidenced that cocoa bean shell fractions represent a promising functional ingredient for the development of  
208 improved gluten free bread formulations. Nevertheless, further studies would be necessary to improve the characteristics of  
209 final product also evaluating sensorial attributes as well as water dynamic during shelf life.

## 210 **Compliance with ethical standards**

211 **Conflict of interest** The authors declare that they have no conflict of interest.

212 **Compliance with ethics requirements** This article does not contain any studies with human or animal subjects.

213

## 214 **References**

- 215 1. Faostat, 2018. Food and Agriculture Organization of the United Nations. (accessed 12/05/2020)  
216 <http://www.fao.org/faostat/en/#data/QC>
- 217 2. Martínez R, Torres P, Meneses MA, Figueroa JG, Pérez-Álvarez JA, Viuda-Martos M (2012)  
218 Chemical, technological and in vitro antioxidant properties of cocoa (*Theobroma cacao* L.) co-  
219 products. *Food Res Int* 49:39-45
- 220 3. Okiyama DC, Navarro SL, Rodrigues CE (2017) Cocoa shell and its compounds: Applications  
221 in the food industry. *Trends Food Sci Technol* 63:103-112
- 222 4. Okiyama DC, Soares ID, Cuevas MS, Crevelin EJ, Moraes LA, Melo MP, Rodrigues CE  
223 (2018) Pressurized liquid extraction of flavanols and alkaloids from cocoa bean shell using  
224 ethanol as solvent. *Food Res Int* 114:20-29
- 225 5. Panak Balentić J, Ačkar Đ, Jokić S, Jozinović A, Babić J, Miličević B, Šubarí D, Pavlović N  
226 (2018) Cocoa Shell: A By-Product with Great Potential for Wide Application. *Molecules* 23:  
227 1404
- 228 6. Arlorio M, Coisson JD, Restani P, Martelli A (2001) Characterization of pectins and some  
229 secondary compounds from theobroma cacao hulls. *J Food Sci* 66:653-656
- 230 7. Barbosa-Pereira L, Guglielmetti A, Zeppa G (2018) Pulsed electric field assisted extraction of  
231 bioactive compounds from cocoa bean shell and coffee silverskin. *Food Bioproc Tech* 11:818-  
232 835

- 233 8. Hernández-Hernández C, Viera-Alcaide I, Sillero AMM, Fernández-Bolaños J, Rodríguez-  
234 Gutiérrez G (2018) Bioactive compounds in Mexican genotypes of cocoa cotyledon and husk.  
235 Food Chem 240:831-839
- 236 9. Mazzutti S, Goncalves Rodrigues LG, Mezzomo N, Venturi V, Ferreira SRS (2018) Integrated  
237 green-based processes using supercritical CO<sub>2</sub> and pressurized ethanol applied to recover  
238 antioxidant compounds from cocoa (*Theobroma cacao*) bean hulls. J Supercrit Fluids 135:52-59
- 239 10. Martínez-Cervera S, Salvador A, Muguerza B, Moulay L, Fiszman SM (2011) Cocoa fibre and  
240 its application as a fat replacer in chocolate muffins. LWT-Food Sci Technol, 44:729-736
- 241 11. Collar C, Rosell CM, Muguerza B, Moulay L (2009) Breadmaking performance and keeping  
242 behavior of cocoa-soluble fiber-enriched wheat breads. Food Sci Technol Int 15:79-87
- 243 12. Grillo G, Boffa L, Binello A, Mantegna S, Cravotto G, Chemat F, Dizhbite T, Lauberte L,  
244 Telysheva G (2019). Cocoa bean shell waste valorisation; extraction from lab to pilot-scale  
245 cavitation reactors. Food Res Int 115:200-208
- 246 13. Jozinović A, Panak Balentić J, Ačkar Đ, Babić J, Pajin B, Miličević B, Guberac S, Šubarić D  
247 (2019) Cocoa husk application in the enrichment of extruded snack products. J Food Process  
248 Pres, e13866
- 249 14. Rojo-Poveda O, Barbosa-Pereira L, Mateus-Reguengo L, Bertolino M, Stévigny C (2019)  
250 Effects of Particle Size and Extraction Methods on Cocoa Bean Shell Functional Beverage.  
251 Nutrients 11:867.
- 252 15. Kreibich HH, Moecke EOH, Scussel VM (2017) In: Méndez-Vilas A. (ed) Microscopy and  
253 imaging science: practical approaches to applied research and education. Formatex Research  
254 Center, Badajoz, Spain

- 255 16. de Sousa MF, Guimarães RM, de Oliveira Araújo M, Barcelos KR, Carneiro NS, Lima DS,  
256 Egea MB (2019) Characterization of corn (*Zea mays* L.) bran as a new food ingredient for  
257 snack bars. *LWT-Food Sci Technol* 101: 812-818
- 258 17. EC Regulation No 1924/2006 of the European Parliament and of the Council of 20 December  
259 2006 on nutrition and health claims made on foods. *Official Journal of the European Union*,  
260 L404 (2006): 9-25.
- 261 18. AOAC, Association of Official Analytical Chemists (2002). *Official Method of Analysis* (16th  
262 Edition), Association of Official Analytical, Washington DC.
- 263 19. Sarangapani C, Thirumdas R, Devi Y, Trimukhe A, Deshmukh RR, Annapure U.S. (2016)  
264 Effect of low-pressure plasma on physico-chemical and functional properties of parboiled rice  
265 flour. *LWT-Food Sci Technol*, 69:482-489.
- 266 20. AACC, American Association of Cereal Chemists (2000) *Approved Methods of the AACC*  
267 (10th ed.) St. Paul, USA: Am Assoc Cereal Chem
- 268 21. Rinaldi M, Paciulli M, Caligiani A, Scazzina F, Chiavaro E (2017) Sourdough fermentation and  
269 chestnut flour in gluten-free bread: A shelf-life evaluation. *Food Chem* 224: 144-152
- 270 22. Bourne MC (1978) Texture profile analysis. *Food Technol* 32: 62-66
- 271 23. Wu YV, Doehlert DC (2002) Enrichment of  $\beta$ -glucan in oat bran by fine grinding and air  
272 classification. *LWT-Food Sci Technol* 35:30-33
- 273 24. Knuckles BE, Chiu MM, Betschart AA (1992) Beta-glucan-enriched fractions from laboratory-  
274 scale dry milling and sieving of barley and oats. *Cereal Chem* 69:198-202
- 275 25. Raghavendra SN, Rastogi NK, Raghavarao KSMS, Tharanathan RN (2004). Dietary fiber from  
276 coconut residue: effects of different treatments and particle size on the hydration properties. *Eur*  
277 *Food Res Technol* 218: 563-567

- 278 26. Lecumberri E, Mateos R, Izquierdo-Pulido M, Rupérez P, Goya L, Bravo L (2007) Dietary  
279 fibre composition, antioxidant capacity and physico-chemical properties of a fibre-rich product  
280 from cocoa (*Theobroma cacao* L.). *Food Chem* 104:948-954
- 281 27. De Angelis D, Madodé YE, Briffaz A, Hounhouigan DJ, Pasqualone A, Summo C (2020)  
282 Comparing the quality of two traditional fried street foods from the raw material to the end  
283 product: The Beninese cowpea-based ata and the Italian wheat-based popizza. *Legum Sci*, e35.
- 284 28. Pérez Sira E, Méndez A, León M, Hernández G, Sívoli L (2015) In: Pérez Sira E (ed) *Cocoa*  
285 *byproducts technology, rheology, styling, and nutrition*. Nova Science Publishers, New York.
- 286 29. Martínez MM, Díaz Á, Gómez M (2014) Effect of different microstructural features of soluble  
287 and insoluble fibres on gluten-free dough rheology and bread-making. *J Food Eng* 142:49-56
- 288 30. Coda R, Kärki I, Nordlund E, Heiniö RL, Poutanen K, Katina K (2014) Influence of particle  
289 size on bioprocess induced changes on technological functionality of wheat bran. *Food*  
290 *Microbiol* 37:69-77
- 291 31. Gómez M, Moraleja A, Oliete B, Ruiz E, Caballero PA (2010) Effect of fibre size on the quality  
292 of fibre-enriched layer cakes. *LWT-Food Sci Technol* 43:33-38
- 293 32. Cappa C, Lucisano M, Mariotti M (2013) Influence of Psyllium, sugar beet fibre and water on  
294 gluten-free dough properties and bread quality. *Carbohydr Polym* 98:657-1666
- 295 33. Almeida EL, Chang YK, Steel CJ (2013) Dietary fibre sources in bread: Influence on  
296 technological quality. *LWT-Food Scie Technol* 50:545-553
- 297 34. Kārklīņa D, Gedrovica I, Reča M, Kronberga M (2012) Production of biscuits with higher  
298 nutritional value. *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact,*  
299 *and Applied Sciences* 66:113-116
- 300 35. Masure HG, Fierens E, Delcour JA (2016) Current and forward looking experimental  
301 approaches in gluten-free bread making research. *J Cereal Sci* 67:92-111

- 302 36. Guimarães RM, Pimentel TC, de Rezende TAM, de Santana Silva J, Falcão HG, Ida EI, Egea  
303 MB (2019) Gluten-free bread: effect of soy and corn co-products on the quality parameters. Eur  
304 Food Res Techn 245:1365-1376
- 305 37. Sharma P, Gujral HS (2014) Anti-staling effects of  $\beta$ -glucan and barley flour in wheat flour  
306 chapatti. Food Chem 145:102-108
- 307 38. Cai L, Choi I, Hyun JN, Jeong YK, Baik BK (2014). Influence of bran particle size on  
308 bread-baking quality of whole grain wheat flour and starch retrogradation. Cereal Chem 91:65-  
309 71
- 310 39. Rocha Parra AF, Ribotta PD, Ferrero C (2015) Apple pomace in gluten-free formulations:  
311 effect on rheology and product quality. Int J Food Sci Tech 50:682-690  
312  
313  
314

315 **Captions for figures**

316 **Figure 1:** Specific volume of tested breads. Error bars represent +/- 1 standard deviation, (n = 3,  
317 sample size = 3 for each bread type). Different capital letters indicate significant differences ( $p < 0.05$ )  
318 among different times for the same bread while different lowercase letters indicate significant  
319 differences ( $p < 0.05$ ) among the four types of bread at the same storage time.

320 **Figure 2:** Number of holes, as percentage of the total number, for the selected dimensional classes at  
321 time 0 day. Error bars represent +/- 1 standard deviation, (n = 9, sample size = 3 for each bread type).  
322 Bars of histograms with the same letters for each class are not significantly different ( $p < 0.05$ ).

323 **Figure 3:** Crumb moisture content for tested breads during storage. Error bars represent +/- 1 standard  
324 deviation, (n = 9, sample size = 3 for each bread type). Different capital letters indicate significant  
325 differences ( $p < 0.05$ ) among different times for the same bread while different lowercase letters  
326 indicate significant differences ( $p < 0.05$ ) among the four types of bread at the same storage time.



327

328 **Table 1.** Water binding capacity (WBC), water absorption index (WAI), water holding capacity (WHC), water solubility index (WSI) and proximate  
329 composition of cocoa bean shell fractions<sup>a</sup>

	<b>moisture</b>	<b>ash</b>	<b>protein</b>	<b>fat</b>	<b>fibre</b>	<b>WBC</b>	<b>WAI</b>	<b>WHC</b>	<b>WSI</b>
<b>F1</b>	4.9±0.3a	6.0±0.1b	15.2±0.4b	6.9±0.6c	63.7±0.6a	2.89±0.16b	5.52±0.16a	3.22±0.18ab	0.124±0.062a
<b>F2</b>	4.7±0.2a	5.7±0.1c	17.3±0.3a	14.6±1.6b	59.4±0.6b	3.75±0.13a	4.38±0.23b	3.50±0.03a	0.023±0.004b
<b>F3</b>	5.1±0.2a	6.7±0.1a	18.2±0.6a	21.8±1.9a	57.2±0.7b	2.95±0.20b	3.98±0.18c	3.08±0.11b	0.150±0.073a

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331 <sup>a</sup> n=3. Means in columns followed by different letter differed significantly ( $p < 0.05$ ).  
332 Hydration properties are expressed as g/g while proximate data are expressed as g/100g

333

334 **Table 2.** Crumb and crust colorimetric parameters for analysed breads. <sup>a</sup>

	<i>day</i>	<i>L*</i>	<i>a*</i>	<i>b*</i>	<i>L*</i>	<i>a*</i>	<i>b*</i>
		<i>Crust</i>			<i>Crumb</i>		
<i>C</i>	0	78.77±0.01 <b>B</b>	1.84±0.01 <b>aA</b>	13.01±0.03 <b>aB</b>	86.99±2.42 <b>aA</b>	0.128±0.035 <b>cB</b>	6.59±0.13 <b>aA</b>
	1	83.83±1.27 <b>bA</b>	1.98±0.31 <b>aA</b>	15.97±0.99 <b>aA</b>	81.12±2.18 <b>aB</b>	0.440±0.121 <b>dA</b>	6.97±0.43 <b>cA</b>
	3	83.26±0.38 <b>bA</b>	0.42±0.10 <b>bB</b>	7.92±0.36 <b>cC</b>	83.26±0.38 <b>aAB</b>	0.307±0.135 <b>dA</b>	6.70±0.78 <b>cA</b>
<i>F3</i>	0	84.04±0.88 <b>aA</b>	2.03±0.16 <b>aA</b>	12.67±0.71 <b>aA</b>	71.96±1.56 <b>cA</b>	2.207±0.251 <b>aA</b>	9.27±0.46 <b>bA</b>
	1	84.75±2.11 <b>bA</b>	1.61±0.28 <b>aB</b>	11.46±0.89 <b>bA</b>	73.82±2.18 <b>bA</b>	2.038±0.301 <b>aAB</b>	8.80±0.66 <b>bB</b>
	3	83.12±1.49 <b>bA</b>	1.84±0.22 <b>aB</b>	11.76±1.08 <b>bB</b>	75.42±2.18 <b>bA</b>	1.866±0.316 <b>aB</b>	10.21±0.99 <b>aA</b>
<i>F2</i>	0	84.23±2.10 <b>aA</b>	1.37±0.38 <b>bA</b>	14.09±1.30 <b>aA</b>	81.08±1.92 <b>bA</b>	1.367±0.38 <b>bA</b>	14.33±1.28 <b>aA</b>
	1	86.76±0.69 <b>abA</b>	0.84±0.16 <b>aA</b>	10.89±0.54 <b>bB</b>	76.42±2.19 <b>abB</b>	1.050±0.286 <b>cB</b>	8.46±0.48 <b>bB</b>
	3	84.36±1.72 <b>bA</b>	1.63±0.37 <b>bA</b>	15.17±1.16 <b>aA</b>	81.84±2.97 <b>aA</b>	1.321±0.2001 <b>bA</b>	11.08±0.94 <b>aAB</b>
<i>F1</i>	0	86.26±2.33 <b>aA</b>	1.19±0.13 <b>bA</b>	10.54±0.88 <b>bA</b>	73.09±1.85 <b>cA</b>	1.398±0.155 <b>bAB</b>	8.88±1.25 <b>bB</b>
	1	88.02±1.37 <b>aA</b>	0.80±0.19 <b>bB</b>	11.58±1.05 <b>bA</b>	74.75±2.11 <b>bA</b>	1.609±0.284 <b>bA</b>	11.45±0.89 <b>aA</b>
	3	87.85±1.37 <b>aA</b>	0.82±0.19 <b>bAB</b>	10.88±1.05 <b>aA</b>	73.73±5.01 <b>bA</b>	0.907±0.270 <b>cB</b>	8.03±0.88 <b>bB</b>

335 <sup>a</sup> n=10. Means in column followed by different capital letters significantly differ ( $p < 0.05$ ) among different times for the same bread. Means followed by different  
336 lowercase letters significantly differ ( $p < 0.05$ ) among the four types of bread at the same storage time.

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**Table 3.** Crumb textural profile analysis (TPA) and crust penetration parameters for the four analysed breads during storage.<sup>a</sup>

<i>crumb</i>						<i>crust</i>
	<i>day</i>	<i>Hardness (N)</i>	<i>Cohesiveness</i>	<i>Resilience</i>	<i>Chewiness (N)</i>	<i>Hardness (N)</i>
<i>C</i>	0	0.53±0.06 <b>aB</b>	0.77±0.10 <b>aA</b>	100.10±0.32 <b>aA</b>	0.43±0.09 <b>aB</b>	1.92±0.11 <b>aA</b>
	1	0.76±0.12 <b>bB</b>	0.84±0.09 <b>aA</b>	102.67±3.02 <b>aB</b>	0.90±0.22 <b>aA</b>	1.16±0.15 <b>bB</b>
	3	1.57±0.48 <b>bA</b>	0.64±0.08 <b>aB</b>	103.16±3.87 <b>aC</b>	1.10±0.42 <b>abA</b>	2.22±0.46 <b>bA</b>
<i>F3</i>	0	0.52±0.10 <b>aB</b>	0.78±1.07 <b>aA</b>	98.68±0.02 <b>aA</b>	0.40±0.19 <b>aB</b>	1.83±0.49 <b>aC</b>
	1	1.21±0.24 <b>aAB</b>	0.58±0.02 <b>aB</b>	97.05±0.71 <b>bA</b>	0.63±0.15 <b>bA</b>	2.39±0.51 <b>aB</b>
	3	1.43±0.34 <b>bA</b>	0.55±0.06 <b>bB</b>	91.53±3.99 <b>bA</b>	0.72±0.16 <b>bA</b>	3.30±0.31 <b>aA</b>
<i>F2</i>	0	0.61±0.21 <b>aC</b>	0.74±0.06 <b>aA</b>	81.55±15.48 <b>bB</b>	0.35±0.10 <b>aB</b>	1.84±0.43 <b>aA</b>
	1	1.31±0.29 <b>aB</b>	0.60±0.04 <b>bB</b>	98.00±0.53 <b>bA</b>	0.71±0.16 <b>bB</b>	1.52±0.31 <b>bB</b>
	3	2.93±0.47 <b>aA</b>	0.58±0.09 <b>aB</b>	97.06±1.14 <b>bA</b>	1.64±0.25 <b>aA</b>	1.81±0.19 <b>bA</b>
<i>F1</i>	0	0.57±0.16 <b>aB</b>	0.72±0.02 <b>aA</b>	98.77±0.42 <b>aA</b>	0.43±0.11 <b>aA</b>	2.18±0.60 <b>aAB</b>
	1	0.84±0.07 <b>bA</b>	0.60±0.04 <b>bB</b>	97.55±0.51 <b>bAB</b>	0.51±0.06 <b>bA</b>	2.61±0.27 <b>aA</b>
	3	0.95±0.24 <b>cA</b>	0.57±0.08 <b>aB</b>	92.62±4.46 <b>bB</b>	0.49±0.13 <b>bA</b>	1.91±0.26 <b>bB</b>

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341 <sup>a</sup> n=5 for crust and n=10 for crumb. Means in column followed by different capital letters significantly differ ( $p < 0.05$ )  
342 among different times for the same bread. Means followed by different lowercase letters significantly differ ( $p < 0.05$ )  
343 among the four types of bread at the same storage time

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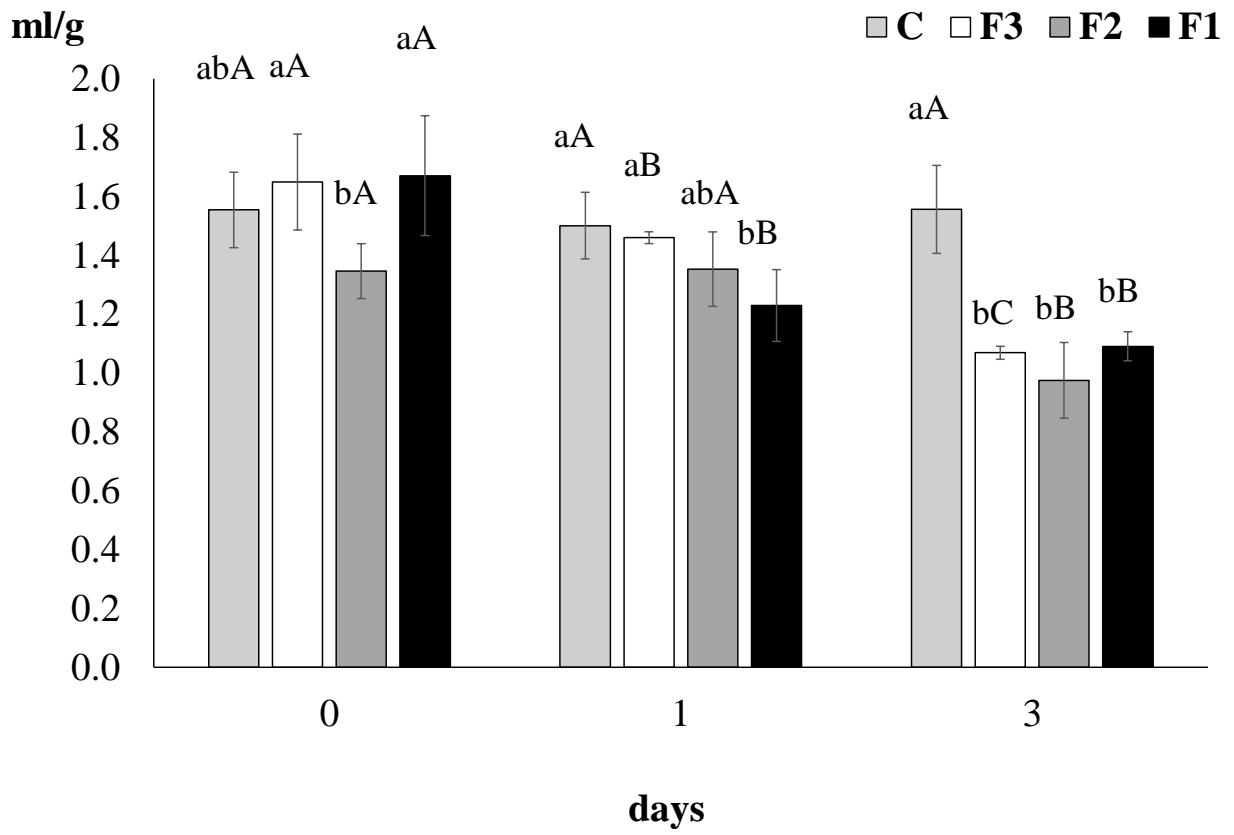
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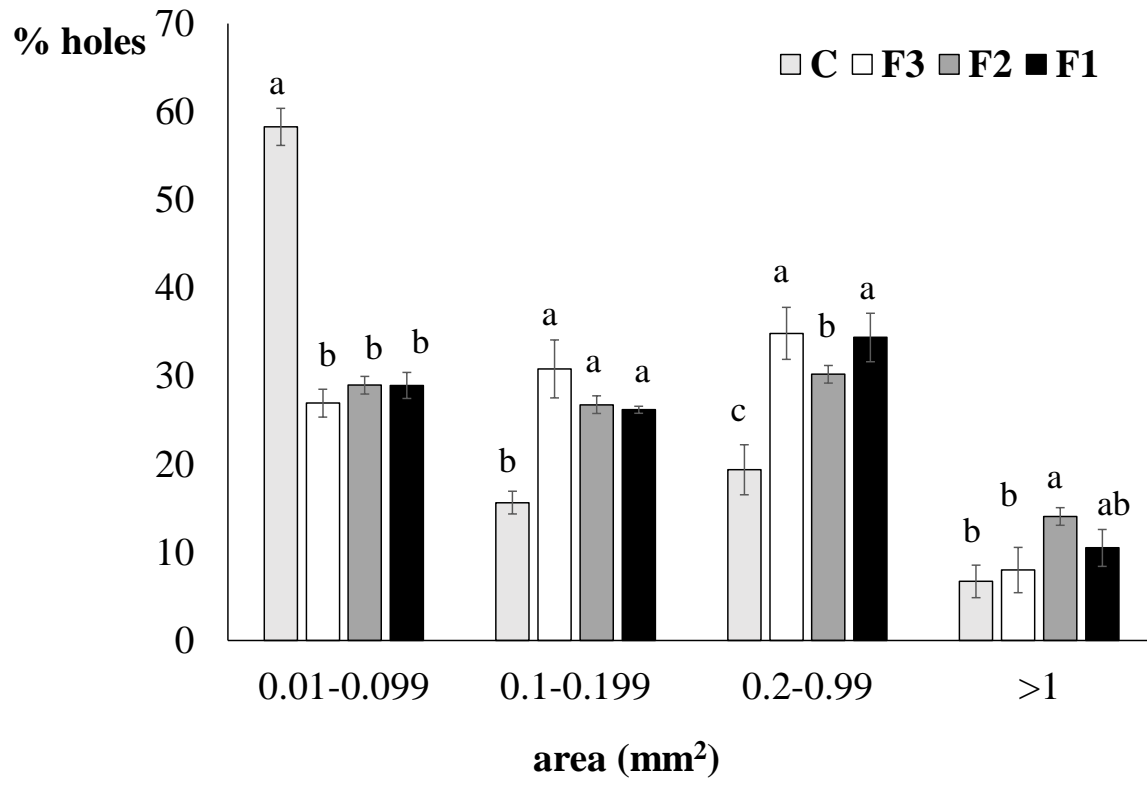
349 **Figure 1.**



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352 **Figure. 2.**



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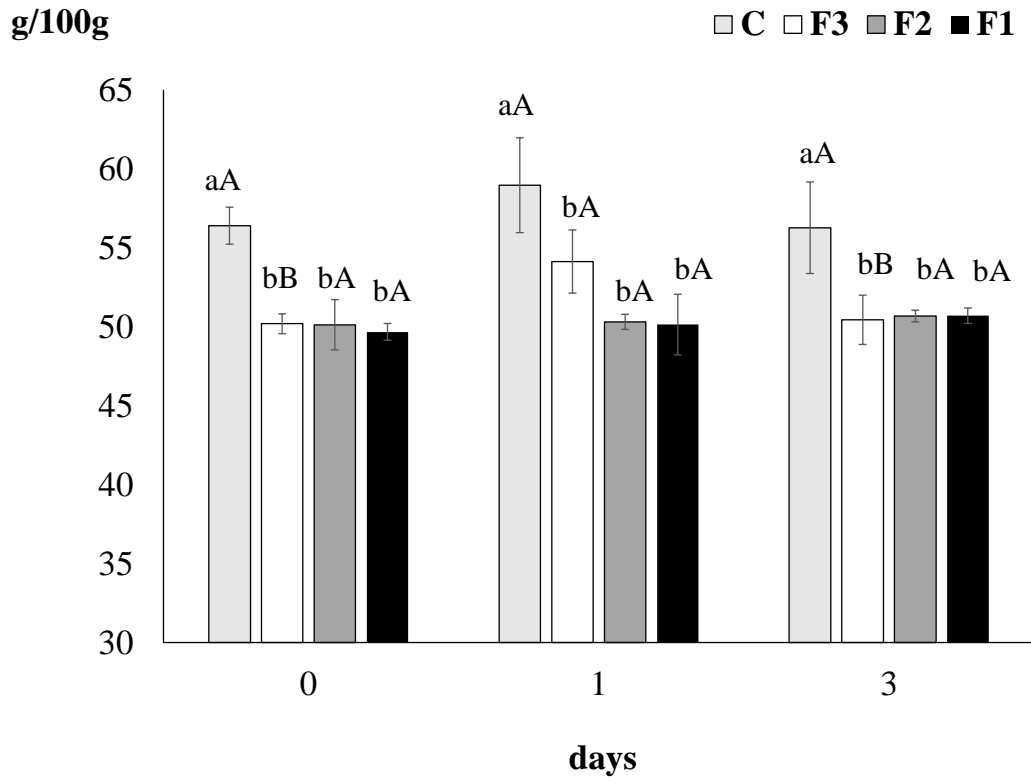
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357 **Figure. 3.**

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