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Original

Effect of chestnut flour supplementation on physico-chemical properties and oxidative stability of gluten-free biscuits during storage / Paciulli, Maria; Rinaldi, Massimiliano; Cavazza, Antonella; Ganino, Tommaso; Rodolfi, Margherita; Chiancone, Benedetta; Chiavaro, Emma. - In: LEBENSMITTEL-WISSENSCHAFT + TECHNOLOGIE. - ISSN 0023-6438. - 98:(2018), pp. 451-457. [10.1016/j.lwt.2018.09.002]

Availability:

This version is available at: 11381/2849364 since: 2021-11-03T09:22:37Z

Publisher:

Academic Press

Published

DOI:10.1016/j.lwt.2018.09.002

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(Article begins on next page)

Effect of chestnut flour supplementation on physico-chemical properties and oxidative stability of gluten-free biscuits during storage

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1 **ABSTRACT**

2 In the present paper, the effect of different levels of chestnut flour (0, 500, 800, 1000 g/kg) in
3 gluten-free biscuits' formulations was evaluated, during a storage of 60 days. Chestnut flour
4 increased water binding capacity and decreased water absorption index of the gluten-free mix used
5 as the basis of biscuits' recipe, leading also to a decrease of both viscous and elastic moduli as well
6 as hardness of the formulated doughs up to 70 % in comparison to control samples. At time 0 day,
7 the highest chestnut flour contents (1000 and 800 g/kg) gave the highest hardness values of the
8 biscuits; during storage all the formulations showed water content increase from about 26 to 35
9 g/kg. Correspondingly, also hardness values increased during storage while only the ones produced
10 with 1000 g/kg chestnut flour remained constant in time. In addition, gluten-free biscuits produced
11 with chestnut flour resulted darker (lower L^* and higher a^* and b^* values) and with a larger
12 oxidative stability values, in relation to the antioxidants present in chestnut. **Chestnut flour**
13 **improved technological and organoleptic quality of the gluten-free biscuits. 500 g/kg chestnut**
14 **flour replacement may be considered the best compromise between quality and storage**
15 **stability.**

16

17 **Keywords:** chestnut, **biscuits**, celiac disease, **colour**, storage, **texture**

18

19

20 1. INTRODUCTION

21 Recently, the market of gluten-free products is expanding, thanks to an increase in consumers
22 avoiding gluten for lifestyle reasons. The market value of this sector, in terms of volume, was
23 estimated to be 393.43 **Ktons** in 2015, projected to grow at a Compound Annual Growth Rate
24 (CAGR) of 10.4% in 2020 (Gluten-Free Products Market by Type, Source, Region – 2020).
25 Nevertheless, the nutritional and sensory properties of gluten-free products do not meet the demand
26 of celiac patients (Rosell & Matos, 2015).

27 Recently, it has been reported that in Italy celiac patients rely on biscuits and crackers as sources of
28 carbohydrates (Valiutti et al., 2017). The most important consequences of a gluten-free diet are: a
29 lack of micronutrients such as folates, iron, and dietary fibre (Moroni, Dal Bello & Arendt, 2009).
30 The long shelf-life of biscuits makes possible large-scale production and distribution, as well as the
31 good eating quality makes biscuits attractive for nutritional improvements (Gadallah & Ashoush,
32 2016).

33 The addition of **different classes of ingredients (pulses, herbs, pseudocereals, seeds)** in the
34 formulation of gluten-free biscuits has been tested to improve their nutritional value: Benkadri et al.
35 (2018) successfully produced gluten-free biscuits with a ratio 78/22 g/kg rice/chickpea flour by
36 using xanthan gum to achieve the proper technological properties of dough; Simons and Hall III
37 (2018) sensorially evaluated cookies prepared by means of raw, germinated and pre-treated pinto
38 beans flour up to 400 g/kg; okara was added to improve protein and fibre content (Ostermann-
39 Porcel, Quiroga-Panelo, Rinaldoni, Campderrós, 2017), raw and germinated amaranth for total
40 dietary fibre and antioxidant activity (Chauhan, Saxena, Singh, 2015), raw and germinated
41 *Chenopodium* (*Chenopodium album*) flour for antioxidant activity, total phenolic and dietary fibre
42 content (Jan, Saxena, Singh, 2016), **pumpkin seeds micro- and macronutrients,**
43 **monounsaturated fatty acids and polyunsaturated fatty acids content (Radocaj , Dimic,**
44 **Diosady, Vujasinovic, 2017).**

45 In this regard chestnut flour being naturally gluten-free, has been often proposed to improve the
46 quality of the bakery products for celiac patients, with bread as the most studied product (Dall'Asta
47 et al., 2013; Demirkesen, Mert, Sumnu & Sahin, 2010; Paciulli, Rinaldi, Cirlini, Scazzina &
48 Chiavaro, 2016). Chestnut flour presents high quality proteins with essential amino acids (4–7%), a
49 relatively high amount of sugars (20–30%), starch (50–60%), dietary fibre (4–10%); and low
50 amount of fat (2–4%), mostly unsaturated (Dall'Asta et al., 2013). In addition, chestnut is a good
51 source of phenolic compounds, oligominerals and vitamins, mainly group B (De Vasconcelos,
52 Bennett, Rosa & Ferreira-Cardoso, 2010). Furthermore, due to the peculiar composition, chestnut
53 flour may contribute to enhance colour and aroma in gluten-free bakery products (Rinaldi, Paciulli,
54 Caligiani, Scazzina & Chiavaro, 2017).

55 The effect of chestnut flour on biscuits has been investigated in gluten (Dokić, Nikolić, Šoronja–
56 Simović, Pajin & Juul, 2014; Hegazy, Kamil, Hussein & Bareh, 2015) or low fat (Inkaya, Gocmen ,
57 Ozturk & Koksel, 2009) products. Until now, only Demirkesen (2016) added chestnut flour at
58 different levels to gluten-free rice based cookies, by analysing the rheological properties of the
59 doughs and the quality of the final products, and finding in the 400 g/kg chestnut flour replacement
60 the best formulation. Storage evaluation on chestnut biscuits was not conducted.

61 **To extend the knowledge in this field and evaluate the main changes occurring on biscuits**
62 **during the first part of their shelf-life, different percentages of chestnut flour were**
63 **supplemented to a gluten-free commercial mix to measure the physico-chemical properties of**
64 **flours, doughs and biscuits during 60 days of storage.**

65

66 **2. MATERIALS AND METHODS**

67 **2.1 Samples preparation and storage conditions**

68 A commercial (NT FOOD S.p.A., Altopascio, Lucca, Italy) gluten-free multipurpose mixture was
69 purchased. The list of ingredients, as reported on label, was: **maize flour, pre-gelatinized rice**

70 **flour**, tapioca starch, **sucrose**, vegetable fibres, salt, thickening agents (guar flour and
71 hydroxypropylmethylcellulose-HPMC) and flavourings. The proximate composition of the mixture,
72 **on fresh basis**, was the following (g/kg): **moisture 97, carbohydrates 857 (sugar 36), fibres 19,**
73 **proteins 18, fats 9.**

74 Similarly, chestnut flour (**Molino Bassini, Anzola dell'Emilia, Bologna, Italy**) obtained from Italian
75 chestnuts, was purchased on the market and **had** the following proximate composition **on fresh**
76 **basis (g/kg): moisture 74, carbohydrates (sugar) 715 (275), proteins 67, fibres 108, fats 36.**

77 **Four biscuit samples were prepared:**

- 78 - **M1000: 1000 g/kg gluten-free mixture**
- 79 - **M200C800: 200 g/kg gluten-free mixture, 800 g/kg chestnut flour**
- 80 - **M500C500: 500 g/kg gluten-free mixture, 500 g/kg chestnut flour**
- 81 - **C1000: 1000 g/kg chestnut flour**

82 For each formulation, every 100g of **gluten free mix, chestnut flour, or mix of the two**, were
83 added 50g of **powdered sucrose**, 30g of water, 30g of butter (containing **150 g/kg** of water), 1g of
84 baking powder and 1g of salt.

85 The ratios chestnut flour/gluten-free mixture were selected based on preliminary experiments; the
86 optimal amount of water was obtained by means of preliminary baking tests **combined with a**
87 **consumer test with 20 untrained panellists for evaluating consumers' acceptance of the**
88 **obtained biscuits.**

89 All the ingredients (water at **20°C**) were mixed by using a Kitchen-Aid Professional mixer (KPM5,
90 KitchenAid, St. Joseph, Michigan, USA) with a dough hook (K45DH) at speed 2 for 90 seconds.
91 Subsequently, the dough was allowed to rest at 4 °C for 5 min and then rectangular biscuits of 2x5
92 cm and 0.8 mm thickness were obtained. Finally, the biscuits were **baked** at 180°C for 15 min in a
93 ventilated electrical oven (Electrolux mod. EVY9841AX, Stockholm, Sweden). Duplicates were
94 performed for each formulation.

95 The cooked biscuits were cooled at room temperature ($25 \pm 1^\circ\text{C}$), packaged in sealed air-tight
96 plastic bags and stored in a $25 \pm 1^\circ\text{C}$ and 40% RH temperature/relative humidity-controlled
97 chamber in the dark (ISCO 9000, Milan, Italy). Samples were analysed after 0, 7, 15, 30 and 60
98 days.

99 **2.2 Water absorption indexes of flours**

100 Water binding capacity (WBC) and water absorption index (WAI) were determined as described by
101 Sarangapani (2016) on gluten-free mix, chestnut flour and their mixes. Briefly, for WBC $1.000 \pm$
102 0.005 g of flour were mixed with distilled water (10 ml) and centrifuged at $2000 \times g$ for 10 min
103 (Eppendorf 5810 R, Hamburg, Germany). WBC was expressed as grams of water retained
104 per gram of solid. For WAI, flour ($50.0 \text{ mg} \pm 0.1 \text{ mg}$) sample was dispersed in 1.0 ml of
105 distilled water and cooked at 90°C for 10 min. The cooked paste was cooled and then
106 centrifuged at $3000 \times g$ at 4°C for 10 min. The supernatant was decanted into an evaporating
107 dish and the weight of dry solids was recovered by evaporating the supernatant at 105°C till
108 constant weight. WAI was expressed as the ratio between residue and initial weight of the
109 sample. The results were expressed as average of three replicates.

110 **2.3 Rheological characterization of doughs**

111 Rheological measurements were carried out using a controlled stress ARES rheometer (Ta
112 Instruments, New Castle, DE, USA). The plate-plate system was used with a diameter of 50 mm
113 and a gap between plates of 2 mm. The temperature of the system was $30.0 \pm 0.01^\circ\text{C}$ (Peltier
114 system). A test time of 15 min was applied to all samples before measuring. Five replicates with
115 different dough sample at each experimental condition were carried out. In addition, Texture profile
116 analysis (TPA) were performed, with slight modification from the study of Armero & Collar, 1997,
117 using a TA-XT2i texture analyser (Stable Microsystems, Surrey, UK) with a 3.5-cm-diameter probe
118 on a preformed cylinder of dough with diameter and height of 2.2 and 1.2 cm, respectively. The test

119 speed was 3 mm/s with a compression of the sample till 50% of its height. Values given are the
120 means of five determinations.

121 **2.4 Thermal properties of flours and biscuits**

122 The DSC-curves were obtained using a Q-100 thermal analysis systems (TA Instruments, New
123 Castle, DE, USA). Distilled water was added to flour and biscuits in a ratio 3:1 (w/w), sealed in
124 hermetic pans, and left to equilibrate for 24 h at room temperature. Samples were heated at a rate of
125 $5\text{ }^{\circ}\text{C min}^{-1}$ **in a range of temperature from 25 to 125 °C** (Walker S, Seetharaman K, Goldstein,
126 2012) with a void pan as reference. The enthalpy (ΔH) and the onset (T_{on}), peak (T_{p}), and offset
127 (T_{off}) temperatures of the observed transitions were computed from the thermal curves by the
128 Universal Analysis Program 2000 (TA Instruments). Three replicates were analyzed for each
129 sample.

130 **2.5 Analysis on biscuits**

131 *- Moisture content and water activity*

132 The moisture content was evaluated following the AACC standard method, 44-15.02 (AACC,
133 2000), while a_w was measured using a water activity meter (4TE, AquaLab, Decagon Devices, Inc.,
134 WA, USA) after equilibration at room temperature ($20 \pm 2^{\circ}\text{C}$). Three replicates were analyzed for
135 each sample.

136 *- Texture analysis*

137 Texture analysis was performed using a TA.XT2i Texture Analyzer equipped with a 25 kg load cell
138 (Stable Micro Systems, Godalming, UK). Biscuit hardness was measured by means of a cutting-
139 shear test using a stainless steel knife blade (3 mm thick) which run perpendicular to the major
140 dimension of the sample, placed on a slot surface (4 mm wide), at a constant speed of 2 mm/s. The
141 maximum force (N) required to shear the sample was taken as a measure of hardness (Becker,

142 Damiani, de Melo, Borges, de Barros Vilas Boas, 2014); the distance at the maximum force was
143 also recorded. Ten replicates were analyzed for each sample.

144 - *Colour*

145 **Colour was determined on five pre-selected locations on two different biscuits for each**
146 **production.** For this measurement a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan)
147 equipped with a standard illuminant D65 and a 10° position of the standard observer, **to simulate**
148 **the human perception**, was used. L^* (lightness), a^* (redness) and b^* (yellowness) were quantified
149 on each sample using the Spectramagic software (Ver. 3.6) (CIE (Commission Internationale de
150 l'eclairage), 1978). The results are reported as the average of **twenty** replications.

151 - *Oxidative stability*

152 The oxidative stability of samples was measured with OXITEST (VELP, Usmate, MB, Italy),
153 according to Cavazza et al. (2015), on ground biscuits (30 g of grounded product). Each accelerated
154 oxidation test was repeated using two different reactors, for a total of four analytical replies (each
155 reactor is fitted with two separate oxidation chambers), under over-pressure of pure oxygen (0.6
156 MPa, degree 5.0) and constant high temperature (100 °C). The OXITEST response is the induction
157 period (IP), expressed as “stability time” before fat oxidation and corresponding to a drop of O₂
158 pressure due to the consumption of oxygen by the sample. The IP value is automatically calculated
159 from oxidation curve by graphical method (two tangent methods) by using the software OXISoft™.
160 The results are reported as the average of three replications.

161 **2.6 Statistical analysis**

162 Means and standard deviations were calculated with SPSS (Version 24.0, SPSS Inc., Chicago,
163 USA) statistical software. SPSS was used also to perform one way ANOVA, coupled with a Tukey-
164 Kramer post-hoc test, to evaluate the effect of chestnut flour addition and differences during
165 storage, at a significance level of $p \leq 0.05$. **Pearson correlation coefficients ($p \leq 0.01$; 0.05) between**

166 variables were also calculated.

167

168 3. RESULTS AND DISCUSSION

169 3.1 Water absorption indexes of flours

170 The values of WBC and WAI, obtained on flours and mixtures, are reported in **Table 1**. The gluten-
171 free mixture showed significant highest WBC values followed by M500C500 and finally by both
172 C1000 and M800C200. The large water binding capacity of the hydrocolloids contained in the
173 gluten-free mixture such as tapioca starch, vegetable fibres, guar flour and
174 hydroxypropylmethylcellulose is well known and contributes to the high WBC values (Guarda,
175 Rosell, Benedit & Galotto, 2004). However, chestnut flour exhibited a higher WBC value if
176 compared to other natural gluten-free flours (Martínez & Gómez, 2017) thanks to its high fibre
177 content. The addition of 200 g/kg gluten-free mixture to chestnut flour was not sufficient to cause a
178 significant increase in WBC value **while, on the contrary**, sample M500C500 presented a
179 significantly higher value.

180 Regarding WAI values, the gluten-free mixture showed the lowest value when compared to the
181 samples containing chestnut flour (Table 1). At the same time, the gluten-free mixture presented
182 values higher than other natural gluten-free sources, such as corn, rice flour and tapioca starch
183 thanks to the added thickening agents (Cappa, Lucisano & Mariotti, 2013). Chestnut flour (C1000)
184 was able to absorb more water when heated, probably because of the reactions of the high total fibre
185 and protein content. Moreover, since chestnut flour needs a long drying procedure (40 days at
186 40°C), it contained a high amount of damaged starch which is reported to increase the water
187 absorption (Ghodke, Ananthanarayan & Rodrigues, 2009). The mix **C50M50 gave the highest**
188 **values among all**, due to synergistic interaction and water absorption competition between different
189 starches, fibres and other hydrophilic components, such as low molecular weight polysaccharides
190 and sugars (Maninder, Sandhu & Singh, 2007).

191 **Despite the different absorption properties of the used flours, the same amount of water was**
192 **added to all the biscuits formulations, to evaluate the only effect of chestnut flour**
193 **supplementation.**

194

195 **3.2 Rheological characterization of doughs**

196 The G' elastic (storage) and G'' viscous (loss) moduli at 0.05, 5 and 10 s^{-1} for biscuit doughs are
197 reported in Table 2. **Both** moduli (G' and G'') for all samples increased with increasing frequencies
198 and G' was larger than G'' throughout the frequency range. All samples had higher storage moduli
199 than loss moduli, suggesting that they had more elastic properties than viscous properties (Lee &
200 Inglett, 2006). In general, M1000 showed slightly but significantly higher values of both G' and G''
201 at all the frequencies, when compared to the mixtures containing chestnut flour. These results
202 confirmed the work of Sozer (2009) for a rice dough supplemented with proteins and gums, in
203 which the increasing amount of polymers in the system resulted in increased elasticity of the dough.
204 Comparing the doughs containing chestnut, a subsequent but not significant decrease of G' and G''
205 was observed increasing the amount of substitution. Moreira, Chenlo, Torres & Rama, 2013
206 reported that both G' and G'' moduli strongly decreased in chestnut enriched dough when chestnuts
207 were dried at temperature above the onset gelatinisation temperature. The chestnuts used in this
208 study were subjected to 40 days of drying at constant temperature (40 °C) in a traditional drying
209 kiln called “metato” and for this reason the differences compared to gluten-free mixture were only
210 limited. When chestnut flour was used in formulation for biscuits, its effect on the dough rheology
211 resulted different accordingly to the nature of the other flour. Demirkesen (2016) found an increase
212 of G' and G'' in rice based doughs with the increase of chestnut percentage, on the contrary, Hegazy
213 et al. (2015) found a decrease of the peak viscosity when chestnut was added to wheat based
214 doughs. The studied doughs possessed all $\tan\delta$ values lower than the unit (Table 2), indicating that

215 they were more solid-like material than liquid-like. The chestnut flour in doughs caused an increase
216 in $\tan\delta$ increasing viscous-like response due to the effect of sugar and fibres contained in chestnut
217 flour (Skendi, Papageorgiou & Biliaderis, 2009).

218 TPA results on doughs are reported in Table 3. M100 presented the highest hardness value followed
219 by C100 with a value significantly lower. Both combinations between gluten-free mixture and
220 chestnut flour (C800M200 and C500M500) showed significantly lower values of hardness
221 compared to M1000 and C1000. On the contrary, samples with predominant chestnut flour (C1000
222 and C800M200) gave the lowest adhesiveness values caused by the high fibre content and damaged
223 starch with a well-known water absorption tendency and a coarser appearance (Mancebo, Picón &
224 Gómez, 2015). This is however not related with the obtained WBC and WAI (Table 1). In
225 accordance with Moreira et al. (2011), the combination of chestnut flour and gluten-free mixture
226 containing hydrocolloids allowed an doughs elasticity increasing with increase in springiness (Table
227 3). This fact was also confirmed by the high values of resilience obtained for C1000 samples.
228 Finally, cohesiveness **of the doughs containing chestnut** resulted higher if compared to M1000
229 due to the higher sugar content, in accordance with Manohar & Rao (1997).

230 **3.3 Thermal properties of flours and biscuits**

231 DSC parameters of the analysed samples are reported in Table 4. Similar phenomena for flours and
232 biscuits were observed. Regarding flours, significant differences were observed between M1000
233 and the samples containing chestnut. This phenomena is due to the different composition of the
234 gluten-free mixture and the chestnut flour in terms of starch and sugars, as reported in paragraph
235 2.1. The onset temperature as well as offset and peak temperature of gelatinisation (T_{on} , T_{off} and T_p)
236 were significantly lower for the samples with the highest percentages of chestnut flour (C1000 and
237 M200C800) in comparison to the others. These results, visible in a range already reported for
238 chestnut flour (Torres, Moreira, Chenlo & Morel, 2013), are due to the different nature of **maize**

239 and chestnut starch and their interactions with other hydrophilic components (Moreira, Chenlo
240 &Arufe, 2015). Moreover, values of gelatinization enthalpy were significantly lower in chestnut
241 flour compared to gluten-free mixtures (Table 4), because of the high sugar content of the chestnut
242 flours, which absorb water, reported to influence negatively the starch gelatinization (Demirkensen,
243 2016). The obtained results are in accordance with Hegazy et al. (2015) who reported a significantly
244 lower peak and onset temperature for chestnut flour compared to wheat flour.

245 Biscuits containing chestnut flour presented the lowest T_{on} , T_{off} and enthalpy values of starch
246 transition, confirming that the presence of other ingredients and the cooking procedure did not
247 change thermal properties of cookie dough, in accordance with Walker et al. (2012).

248 **3.4 Analysis on biscuits**

249 Moisture contents of the biscuits during 60 days of storage are reported in Figure 1. At time 0, the
250 sample M200C800 presented the highest value followed by C1000 and by both M1000 and
251 M500C500. During **storage**, all biscuits absorbed water from the **surrounding environment** and
252 showed an increase in moisture content, as expected. In particular, M1000 and M500C500 showed
253 the highest moisture increase during the first 7 days of storage. At the end of the **considered**
254 **storage period**, M1000 and M200C800 presented the highest moisture content, as they continued
255 to absorb humidity after 30 days. **C1000 and M500C500, on the other hand**, did not show
256 significant differences in moisture content from 30 to 60 days.

257 The values of water activity (a_w) showed a slight but not significant increase passing from 0 to 60
258 days, with values around 0.40 for all samples (data not shown), in accordance to the values reported
259 for similar products (Romani, Rocculi, Tappi & Dalla Rosa, 2016), with the increase due to the
260 water absorption. Among the tested **formulations**, M500C500 presented the highest values at all
261 storage times (0.42 ± 0.010) due to the competition for water absorption between sugars and fibres in
262 comparison to other samples. M1000 resulted the sample with the highest increasing of a_w during

263 **storage**, passing from values of 0.39 to 0.44.

264 Hardness values of the biscuits are **showed** in Figure 2. At time 0 days hardness values resulted
265 significantly different among biscuits, resulting highest for M200C800 and C1000 followed by
266 M1000 and M500C500. **The observed differences might depend on total fibre content and, as
267 consequence, water content and/or on the contribute of proteins or broken starch. In this regards a
268 high statistical correlation ($p<0.01$) was found between hardness and water content ($r =$
269 **0.416**).** This trend is in accordance with other studies reported in the scientific literature (Becker et
270 al. 2014) that observed a direct link between fibre content and hardness of wheat and gluten-free
271 biscuits. In addition, Moreira, Chenlo, Torres & Prieto 2012, observed that larger fibre size gives
272 **higher force required to break the biscuit confirming the large dimension of particles in chestnut
273 flour used in the present study that was obtained by sieving with 300 μm screen.** Demirkesen (2016)
274 justified the hardness increasing of rice biscuits enriched with increasing percentage of chestnut
275 flour, with the reduction of starch gelatinization, due to the high sugar content of chestnut flour that,
276 binding water, prevent the phenomena, in accordance with our results (Table 4). **Moreover, the
277 high sugar content of chestnut may contribute to the hardness increasing, with the
278 phenomena of recrystallization, as already reported by other authors on biscuits (Pittia, Di
279 Teodoro & Sacchetti, 2015).**

280 On the other hand, M200C800 and C1000 showed the lowest distance at rupture at time 0 days,
281 demonstrating that biscuits higher in fibre had a lower ability **to sustain the deformation**, resulting in
282 a more fragile product. In this regard, flours with low WBC are reported to affect the formation of
283 structural matrix and make products more fragile (Shrestha & Noomhorm, 2002). **To confirm of
284 this hypothesis, a significant correlation ($p<0.005$) was found between WBC and distance at
285 rupture of the biscuits at time 0 days ($r=0.645$).**

286 **All samples showed significant increases in hardness during storage (Figure 2), particularly
287 starting from day 15, in relation to the water increase (Figure 1). Guillard, Broyart, Bonazzi,**

288 **Guilbert & Gontard (2003) reported that water sorption could lead to food polymers swelling**
289 **and decrease in material porosity by generating a more compact structure with increase of the**
290 **instrumental hardness. C1000 was the only sample that did not change in hardness during**
291 **storage.**

292 Distance at rupture showed two different trends during **storage**: C1000 and M200C800 showed a
293 significant increase while M500C500 and M1000 showed a decrease. M200C800 and C1000 are the
294 samples richest in fibre and during **storage**, the increase in moisture content (Figure 2) may have
295 caused the hydration of fibres with a more flexible structure.

296 Colorimetric parameters of the analysed biscuits resulted deeply influenced by the colour of the
297 flours (Table 5), being chestnut flour darker than gluten-free mixture (Paciulli et al., 2016). M1000
298 biscuits, nevertheless, shows non-enzymatic browning during cooking, presented a pale appearance
299 with high L^* and low a^* and b^* . Chestnut flour caused a significant decrease of L^* as well as higher
300 a^* and b^* values, proportionally to its content. During **storage** the colour of biscuits has not
301 changed.

302 Finally, regarding the oxidative stability of the samples, measured with OXITEST instrument, IP
303 (min) values obtained for analysed samples were 590 ± 29 for M100, 1528 ± 69 for M500C500,
304 1.855 ± 35 for M800C200 and 1173 ± 92 for C1000. IP values of biscuits made only with chestnut
305 flour resulted significantly higher compared to those obtained from gluten-free mixture thanks to
306 the antioxidant content of chestnut flour as previously reported (Paciulli et al., 2016). Mixing the
307 two flours the IP values resulted even higher than C1000. This may be related to the gluten-free
308 mixture contribution to the reduction of the unsaturated fatty acids' content and to the addition of
309 antioxidant compounds from chestnut flour (Dall'Asta et al., 2013).

310 **4. CONCLUSIONS**

311 **The results of this investigation show how chestnut flour affects the physico-chemical**

312 **properties of gluten free biscuits. Chestnut flour, regardless the ratio to the gluten free mix,**
313 led to WBC reduction with a consequent improvement of the **doughs machinability: particularly,**
314 M200C800 and M500C500 doughs presented **lower hardness and higher springiness values** in
315 comparison to M1000 and C1000. Moreover, M500C500 dough showed also the lowest
316 adhesiveness values among all. The increase of chestnut flour in the doughs led to lower starch
317 gelatinization and to an increase of the hardness values of biscuits after baking. During storage
318 hardness of biscuits increased proportionally to the water content, with M2000C8000 resulting the
319 hardest sample. An improvement of the biscuits colour with the addition of chestnut flour was
320 observed up to 800 g/kg, resulting darker, browner and stable biscuits compared to the
321 corresponding, pale, controls. In the same way, also the oxidative stability, increased with the
322 chestnut flour content. Unfortunately, the C1000 sample, resulted too dark due to an excessive
323 sugars' caramelization as well as less stable against oxidation. The present study demonstrates that
324 addition of chestnut flour could be a feasible way to improve the technological, **organoleptic** and
325 **overall** quality of gluten-free biscuits, resulting 500 g/kg the best addition level. Further studies are
326 however necessary to confirm the obtained results for a **longer storage** and testing other
327 formulations in the range 500 g/kg and 800 g/kg chestnut flour on gluten-free mix.

328 **ACKNOWLEDGEMENTS**

329 This work was conducted within the project BIOCAST – La biofunzionalità della filiera del
330 castagno dalla farina agli scarti, funded by the Italian Ministry of Agriculture, Food and Forestry
331 Policies.

332 The authors declare no conflict of interest.

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461

462

463 **Table 1.** WBC and WAI of the used flours and mixtures of flours.

	WBC (g/g)	WAI (g/g)
M1000	2.50±0.13a	5.14±0.19d
M500C500	2.20±0.07b	8.31±0.25a
M200C800	2.09±0.03c	7.70±0.43bc
C1000	2.05±0.04c	6.83±0.24c

464 ^a Means in columns followed by different letter differed significantly ($p < 0.05$). Abbreviations: WBC: water binding
465 capacity; WAI: water absorption index; M1000: 1000 g/kg gluten-free mixture; M500C500: 500 g/kg gluten-free
466 mixture, 500 g/kg chestnut flour; M200C800: 200 g/kg gluten-free mixture, 800 g/kg chestnut flour; C1000: 1000 g/kg
467 chestnut flour.

468

469

470 **Table 2.** Rheological parameters (G' , G'' , $TAN\delta$) of the doughs at different oscillation frequencies^a

	0.05 s ⁻¹		
	G' (10 ⁵ Pa)	G'' (10 ⁴ Pa)	TAN δ
M1000	1.14±0.18 a	9.18±2.25 a	0.80±0.12 a
M500C500	0.34±0.04 bc	2.24±0.31 b	0.66±0.07 b
M200C800	0.28±0.03 c	2.01±0.18 b	0.74±0.06 ab
C1000	0.40±0.05 b	2.61±0.26 b	0.66±0.09 b
	5 s ⁻¹		
	G' (10 ⁵ Pa)	G'' (10 ⁴ Pa)	TAN δ
M1000	4.51±0.65 a	15.2±2.21 a	0.34±0.02 b
M500C500	1.21±0.15 b	4.16±0.52 c	0.34±0.02 b
M200C800	1.00±0.11b	3.93±0.43 c	0.39±0.01 a
C1000	1.38±0.16 b	5.52±0.65 b	0.40±0.01 a
	10 s ⁻¹		
	G' (10 ⁵ Pa)	G'' (10 ⁴ Pa)	TAN δ
M1000	5.33±0.73a	18.0±0.24 a	0.34±0.02 c
M500C500	1.37±0.17 b	5.27±0.74 bc	0.38±0.02 b
M200C800	1.22±0.14 b	5.06±0.61 c	0.38±0.02 a
C1000	1.60±0.16 b	6.69±0.65 b	0.42±0.01 a

471

472 ^a Means in column, within the same oscillation frequency group, followed by different letters significantly
 473 differ ($p < 0.05$) among the four types of dough. Abbreviations: G': elastic modulus; G'': viscous modulus;
 474 TAN δ : G''/ G'; M1000: 1000 g/kg gluten-free mixture; M500C500: 500 g/kg gluten-free mixture, 500 g/kg
 475 chestnut flour; M200C800: 200 g/kg gluten-free mixture, 800 g/kg chestnut flour; C1000: 1000 g/kg chestnut
 476 flour.

477

478 **Table 3.** Texture parameters of analysed doughs^a

	Hardness (N)	Springiness	Cohesiveness	Gumminess (N)	Resilience	Adhesivness (Nmm ⁻¹)
M1000	10.58±1.22a	0.11±0.01d	0.17±0.03c	1.79±0.25a	0.04±0.002b	-0.96±0.23bc
M500C500	4.46±0.47c	0.41±0.08a	0.28±0.05a	1.22±0.15b	0.04±0.002b	-1.07±0.23c
M200C800	5.33±0.45d	0.31±0.04b	0.23±0.02b	1.23±0.11b	0.04±0.002b	-0.78±0.15ab
C1000	9.16±0.86b	0.23±0.02c	0.20±0.02b	1.85±0.14a	0.05±0.002a	-0.67±0.13a

479 ^a Means followed by different letters significantly differ ($p < 0.05$) among the four types of biscuit. Abbreviations:
 480 M1000: 1000 g/kg gluten-free mixture; M500C500: 500 g/kg gluten-free mixture, 500 g/kg chestnut flour; M200C800:
 481 200 g/kg gluten-free mixture, 800 g/kg chestnut flour; C1000: 1000 g/kg chestnut flour.

482

483 **Table 4.** Starch gelatinization peak thermal (DSC) parameters of the of flours and biscuits, with
 484 excess of water, at time 0 days.^a

	Ton (°C)	Tp (°C)	Toff (°C)	Enthalpy (Jg ⁻¹)
Flours				
M1000	73.0±3.0b	81.0±1.4a	90.0±1.4a	1.35±0.11a
M500C500	75.4±0.7a	80.8±0.3b	86.5±1.0b	0.93±0.01b
M200C800	65.0±1.1c	70.2±0.7c	77.0±0.1c	0.54±0.06c
C1000	65.2±0.3c	71.3±0.6c	77.5±1.6c	0.55±0.08c
Biscuits				
M1000	72.4±0.1a	78.9±0.1ab	87.4±0.4ab	1.44±0.08a
M500C500	67.6±0.1b	80.7±0.6a	91.1±4.7a	1.30±0.31ab
M200C800	68.0±0.4ab	75.6±0.9b	86.2±0.2b	1.16±0.04b
C1000	67.3±0.9c	74.0±0.8c	81.6±0.8c	0.87±0.051c

485 ^a Means in columns, within the same group (flours or biscuits), followed by different letter differed significantly ($p <$
 486 0.05). Abbreviations: DSC: differential scanning calorimetry; Ton: onset temperature; Toff: offset temperature; Tp:
 487 peak temperature; M1000: 1000 g/kg gluten-free mixture; M500C500: 500 g/kg gluten-free mixture, 500 g/kg chestnut
 488 flour; M200C800: 200 g/kg gluten-free mixture, 800 g/kg chestnut flour; C1000: 1000 g/kg chestnut flour.

489 **Table 5.** Colorimetric parameters of analysed biscuits during storage^a.

	Time (days)	<i>L</i> *	<i>a</i> *	<i>b</i> *
M1000	0	87.67±0.96 aA	0.32± 0.08 cA	12.01±0.68 cA
	7	86.75±1.39 aA	0.23±0.17 c A	12.13±0.28 bA
	15	87.66±0.57 aA	0.33±0.12 d A	12.10±0.58 cA
	30	86.50±3.83 aA	0.28±0.13 cA	11.64±1.00 cA
	60	87.53±1.07 aA	0.42±0.13 cA	11.80±0.56 bA
M500C500	0	66.40±0.60 bAB	6.68±0.39 bA	19.25±0.43 aA
	7	66.72±0.39 bAB	6.27±0.23 bAB	19.04±0.73 aA
	15	64.94±1.11 bB	6.56±0.41 cAB	19.16±0.53 aA
	30	67.40±1.29 bA	6.19±0.39 bAB	19.10±0.79 aA
	60	67.05±2.44 bAB	6.13±0.22 bB	18.70±1.00 aA
M200C800	0	61.92±0.84 cA	7.81±0.20 aA	19.26±0.15 aA
	7	61.57±1.70 cA	8.06±0.48 aA	19.54±0.95 aA
	15	63.12±1.47 bA	7.43±0.37 bA	19.10±1.10 aA
	30	60.43±2.40 cA	8.01±0.50 aA	19.10±0.64 aA
	60	60.02±2.34 bcA	7.25±0.84 aA	18.24±2.84 aA
C1000	0	58.40±0.63 dA	8.04±0.06 aA	17.53±0.44 bA
	7	57.10±2.50 dA	8.10±0.38 aA	18.06±1.73 aA
	15	58.70±1.69 cA	8.10±0.62 aA	17.74±1.01 bA
	30	57.45±2.13 cA	8.03±0.53 aA	16.62±1.95 bA
	60	59.70±4.30 cA	8.00±1.15 aA	18.00±3.00 aA

490 ^a Means in column followed by different capital letters indicate significant differences ($p < 0.05$) measured on a single
491 type of biscuit during different storage days. Means in column followed by different lowercase letters indicate
492 significant differences ($p < 0.05$) between the four biscuit formulations at the same day of storage. Abbreviations: *L**:
493 lightness; *a**: greenness; *b** yellowness. M1000: 1000 g/kg gluten-free mixture; M500C500: 500 g/kg gluten-free
494 mixture, 500 g/kg chestnut flour; M200C800: 200 g/kg gluten-free mixture, 800 g/kg chestnut flour; C1000: 1000 g/kg
495 chestnut flour.

496

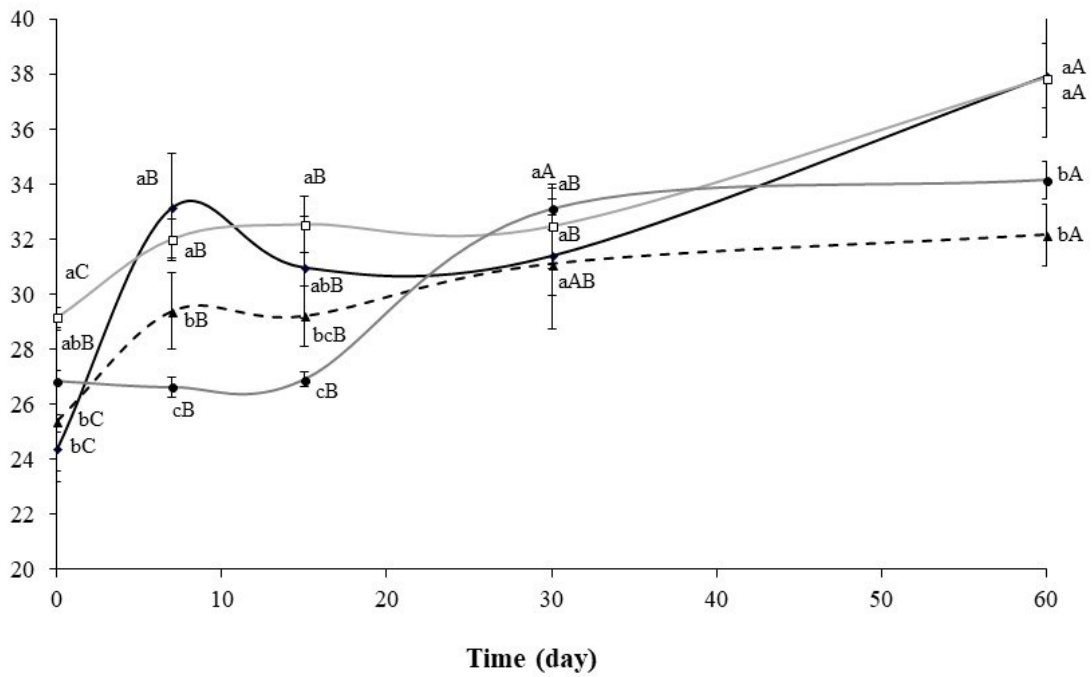
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499 **Captions for figures**

500 **Figure 1.** Moisture content of analysed biscuits^a.

Water content (g/kg)

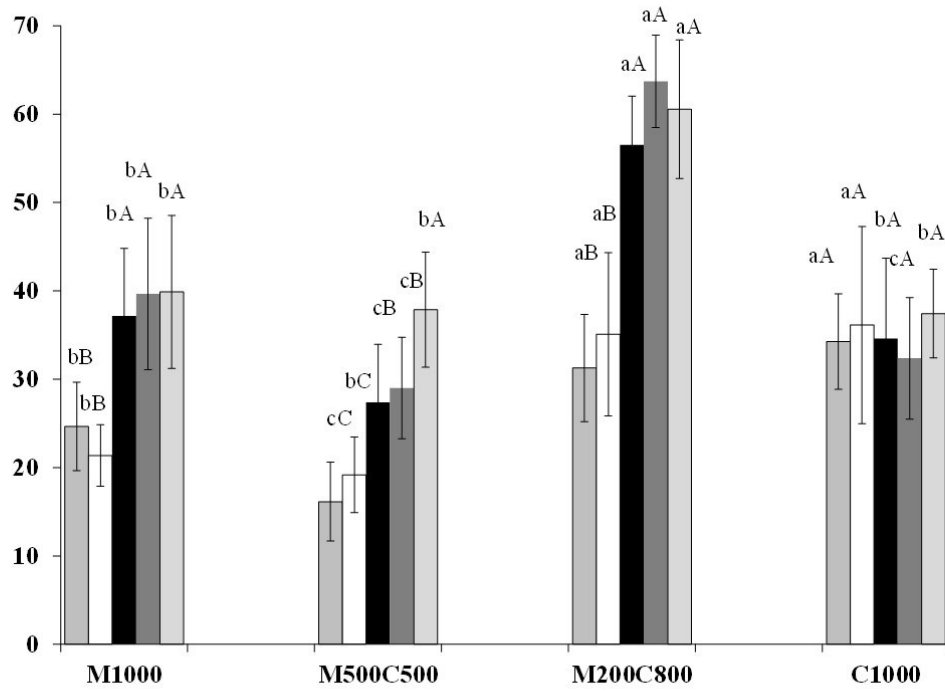


501

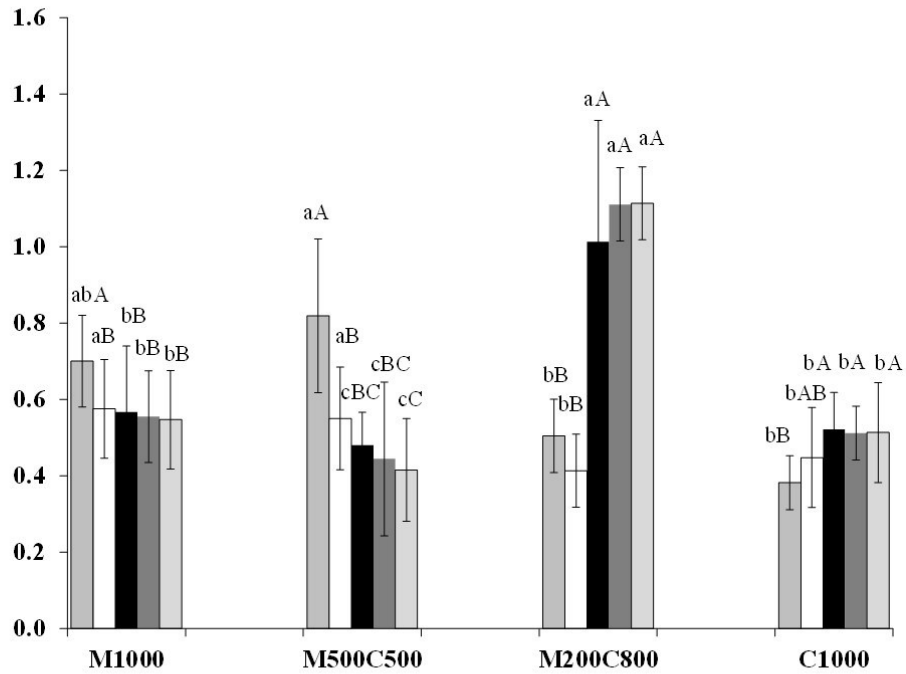
502 ^aMeans followed by different capital letters significantly differ ($p < 0.05$) among different times for the same biscuit.
 503 Means followed by different lowercase letters significantly differ ($p < 0.05$) among the four types of biscuit at the same
 504 storage time. ♦ M1000; □ M200C800; ▲ M500C500; ● C1000 Abbreviations: M1000: 1000 g/kg gluten-free
 505 mixture; M500C500: 500 g/kg gluten-free mixture, 500 g/kg chestnut flour; M200C800: 200 g/kg gluten-free
 506 mixture, 800 g/kg chestnut flour; C1000: 1000 g/kg chestnut flour.

507 **Figure 2.** Hardness (N) (panel a) and distance at rupture point (mm) (panel b) of the analysed
 508 biscuits, obtained by cutting test ^a.

Hardness (N)



Distance at rupture (mm)



509

510 ^aMeans followed by different capital letters significantly differ ($p < 0.05$) among different times for the same biscuit.

511 Means followed by different lowercase letters significantly differ ($p < 0.05$) among the four types of biscuit at the same

512 storage time. 0; 7; 15; 30; 60 days. **Abbreviations: M1000: 1000 g/kg gluten-free mixture; M500C500:**
513 **500 g/kg gluten-free mixture, 500 g/kg chestnut flour; M200C800: 200 g/kg gluten-free mixture, 800 g/kg**
514 **chestnut flour; C1000: 1000 g/kg chestnut flour.**