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

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ABSTRACT

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

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

1. INTRODUCTION

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

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

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

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

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

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

2. MATERIALS AND METHODS

2.1 Samples preparation and storage conditions

A commercial (NT FOOD S.p.A., Altopascio, Lucca, Italy) gluten-free multipurpose mixture was purchased. The list of ingredients, as reported on label, was: **maize flour, pre-gelatinized rice** flour, tapioca starch, sucrose, vegetable fibres, salt, thickening agents (guar flour and hydroxypropylmethylcellulose-HPMC) and flavourings. The proximate composition of the mixture, on fresh basis, was the following (g/kg): moisture 97, carbohydrates 857 (sugar 36), fibres 19, proteins 18, fats 9.

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

Four biscuit samples were prepared:

- M1000: 1000 g/kg gluten-free mixture
- M200C800: 200 g/kg gluten-free mixture, 800 g/kg chestnut flour
- M500C500: 500 g/kg gluten-free mixture, 500 g/kg chestnut flour
- C1000: 1000 g/kg chestnut flour

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

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

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

The cooked biscuits were cooled at room temperature **(25 ± 1°C)**, packaged in sealed air-tight plastic bags and stored in a 25 **± 1** °C and 40% RH temperature/relative humidity-controlled chamber in the dark (ISCO 9000, Milan, Italy). Samples were analysed after 0, 7, 15, 30 and 60 days.

2.2 Water absorption indexes of flours

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

2.3 Rheological characterization of doughs

Rheological measurements were carried out using a controlled stress ARES rheometer (Ta Instruments, New Castle, DE, USA). The plate-plate system was used with a diameter of 50 mm and a gap between plates of 2 mm. The temperature of the system was 30.0 ± 0.01 °C (Peltier system). A test time of 15 min was applied to all samples before measuring. Five replicates with different dough sample at each experimental condition were carried out. In addition, Texture profile analysis (TPA) were performed, with slight modification from the study of Armero & Collar, 1997, using a TA-XT2i texture analyser (Stable Microsystems, Surrey, UK) **with** a 3.5-cm-diameter probe on a preformed cylinder of dough with diameter and height of 2.2 and 1.2 cm, respectively. The test speed was 3 mm/s with a compression of the sample till 50% of its height. Values given are the means of five determinations.

2.4 Thermal properties of flours and biscuits

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

2.5 Analysis on biscuits

- *Moisture content and water activity*

The moisture content was evaluated following the AACC standard method, 44-15.02 (AACC, 2000), while a_w was measured using a water activity meter (4TE, AquaLab, Decagon Devices, Inc., WA, USA) after equilibration at room temperature (20 ± 2°C). Three replicates were analyzed for each sample.

- *Texture analysis*

Texture analysis was performed using a TA.XT2i Texture Analyzer equipped with a 25 kg load cell (Stable Micro Systems, Godalming, UK). Biscuit hardness was measured by means of a cutting-shear test using a stainless steel knife blade (3 mm thick) which run perpendicular to the major dimension of the sample, placed on a slot surface (4 mm wide), at a constant speed of 2 mm/s. The maximum force (N) required to shear the sample was taken as a measure of hardness (Becker, Damiani, de Melo, Borges, de Barros Vilas Boas, 2014); the distance at the maximum force was also recorded. Ten replicates were analyzed for each sample.

- *Colour*

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

- *Oxidative stability*

The oxidative stability of samples was measured with OXITEST (VELP, Usmate, MB, Italy), according to Cavazza et al. (2015), on ground biscuits (30 g of grounded product). Each accelerated oxidation test was repeated using two different reactors, for a total of four analytical replies (each reactor is fitted with two separate oxidation chambers), under over-pressure of pure oxygen (0.6 MPa, degree 5.0) and constant high temperature (100 °C). The OXITEST response is the induction period (IP), expressed as "stability time" before fat oxidation and corresponding to a drop of O_2 pressure due to the consumption of oxygen by the sample. The IP value is automatically calculated from oxidation curve by graphical method (two tangent methods) by using the software OXISoft™. The results are reported as the average of three replications.

2.6 Statistical analysis

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

3. RESULTS AND DISCUSSION

3.1 Water absorption indexes of flours

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

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

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

3.2 Rheological characterization of doughs

The G′ elastic (storage) and G″ viscous (loss) moduli at 0.05, 5 and 10 s^{-1} for biscuit doughs are reported in Table 2. **Both** moduli (G′ and G″) for all samples increased with increasing frequencies and G′ was larger than G″ throughout the frequency range. All samples had higher storage moduli than loss moduli, suggesting that they had more elastic properties than viscous properties (Lee & Inglett, 2006). In general, M1000 showed slightly but significantly higher values of both G′ and G″ at all the frequencies, when compared to the mixtures containing chestnut flour. These results confirmed the work of Sozer (2009) for a rice dough supplemented with proteins and gums, in which the increasing amount of polymers in the system resulted in increased elasticity of the dough. Comparing the doughs containing chestnut, a subsequent but not significant decrease of G′ and G″ was observed increasing the amount of substitution. Moreira, Chenlo, Torres & Rama, 2013 reported that both G′ and G″ moduli strongly decreased in chestnut enriched dough when chestnuts were dried at temperature above the onset gelatinisation temperature. The chestnuts used in this study were subjected to 40 days of drying at constant temperature (40 °C) in a traditional drying kiln called "metato" and for this reason the differences compared to gluten-free mixture were only limited. When chestnut flour was used in formulation for biscuits, its effect on the dough rheology resulted different accordingly to the nature of the other flour. Demirkesen (2016) found an increase of G′ and G″ in rice based doughs with the increase of chestnut percentage, on the contrary, Hegazy et al. (2015) found a decrease of the peak viscosity when chestnut was added to wheat based doughs. The studied doughs possessed all tanδ values lower than the unit (Table 2), indicating that they were more solid-like material than liquid-like. The chestnut flour in doughs caused an increase in tanδ increasing viscous-like response due to the effect of sugar and fibres contained in chestnut flour (Skendi, Papageorgiou & Biliaderis, 2009).

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

3.3 Thermal properties of flours and biscuits

DSC parameters of the analysed samples are reported in Table 4. Similar phenomena for flours and biscuits were observed. Regarding flours, significant differences were observed between M1000 and the samples containing chestnut. This phenomena is due to the different composition of the gluten-free mixture and the chestnut flour in terms of starch and sugars, as reported in paragraph 2.1. The onset temperature as well as offset and peak temperature of gelatinisation (T_{on}, T_{off} and T_p) were significantly lower for the samples with the highest percentages of chestnut flour (C1000 and M200C800) in comparison to the others. These results, visible in a range already reported for chestnut flour (Torres, Moreira, Chenlo & Morel, 2013), are due to the different nature of **maize** and chestnut starch and their interactions with other hydrophilic components (Moreira, Chenlo &Arufe, 2015). Moreover, values of gelatinization enthalpy were significantly lower in chestnut flour compared to gluten-free mixtures (Table 4), because of the high sugar content of the chestnut flours, which absorb water, reported to influence negatively the starch gelatinization (Demirkensen, 2016). The obtained results are in accordance with Hegazy et al. (2015) who reported a significantly lower peak and onset temperature for chestnut flour compared to wheat flour.

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

3.4 Analysis on biscuits

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

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

storage, passing from values of 0.39 to 0.44.

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

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

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

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

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

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

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

4. CONCLUSIONS

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

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

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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

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

Table 2. Rheological parameters (**G', G", TANδ)** of the doughs at different oscillation frequencies[a]

	0.05 s^{-1}		
	G' (10^5 Pa)	**G" (10^4 Pa)**	**TANδ**
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)**	**TANδ**
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)**	**TANδ**
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

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

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

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

Table 4. Starch gelatinization peak thermal (DSC) parameters of the of flours and biscuits, with 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

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

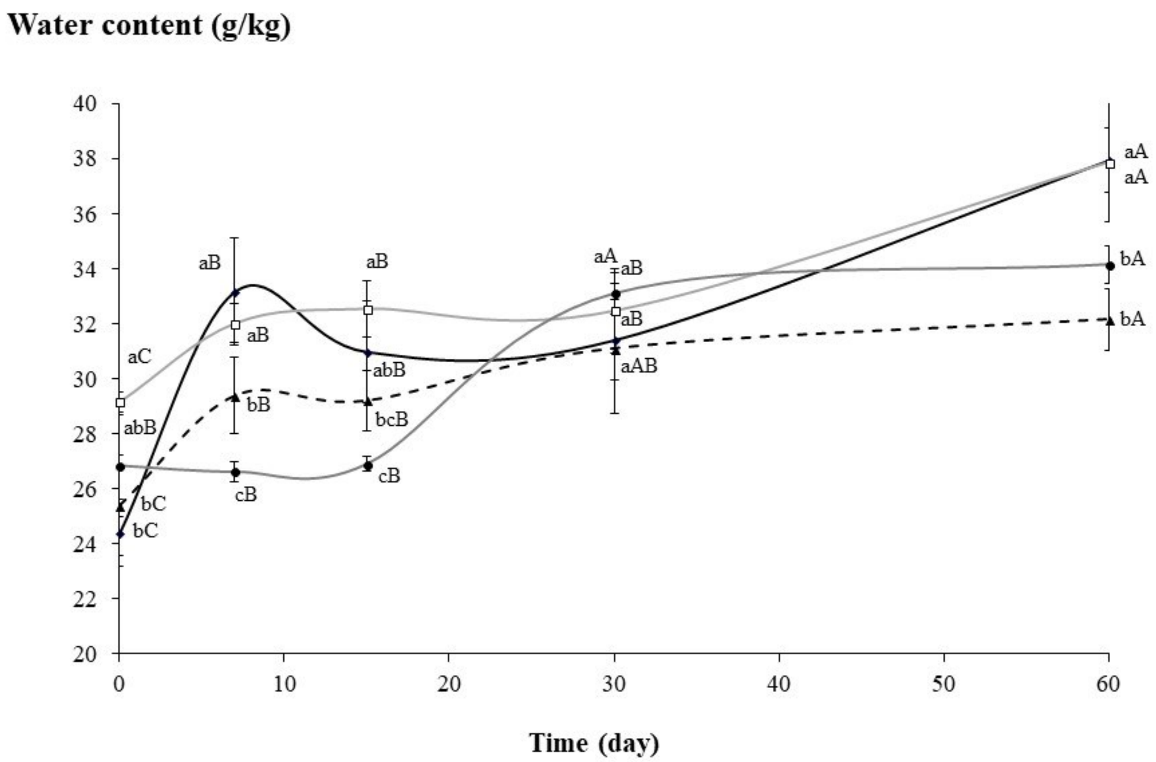
Table 5. Colorimetric parameters of analysed biscuits during storage[a].

	Time (days)	L^*	a^*	b^*
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

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

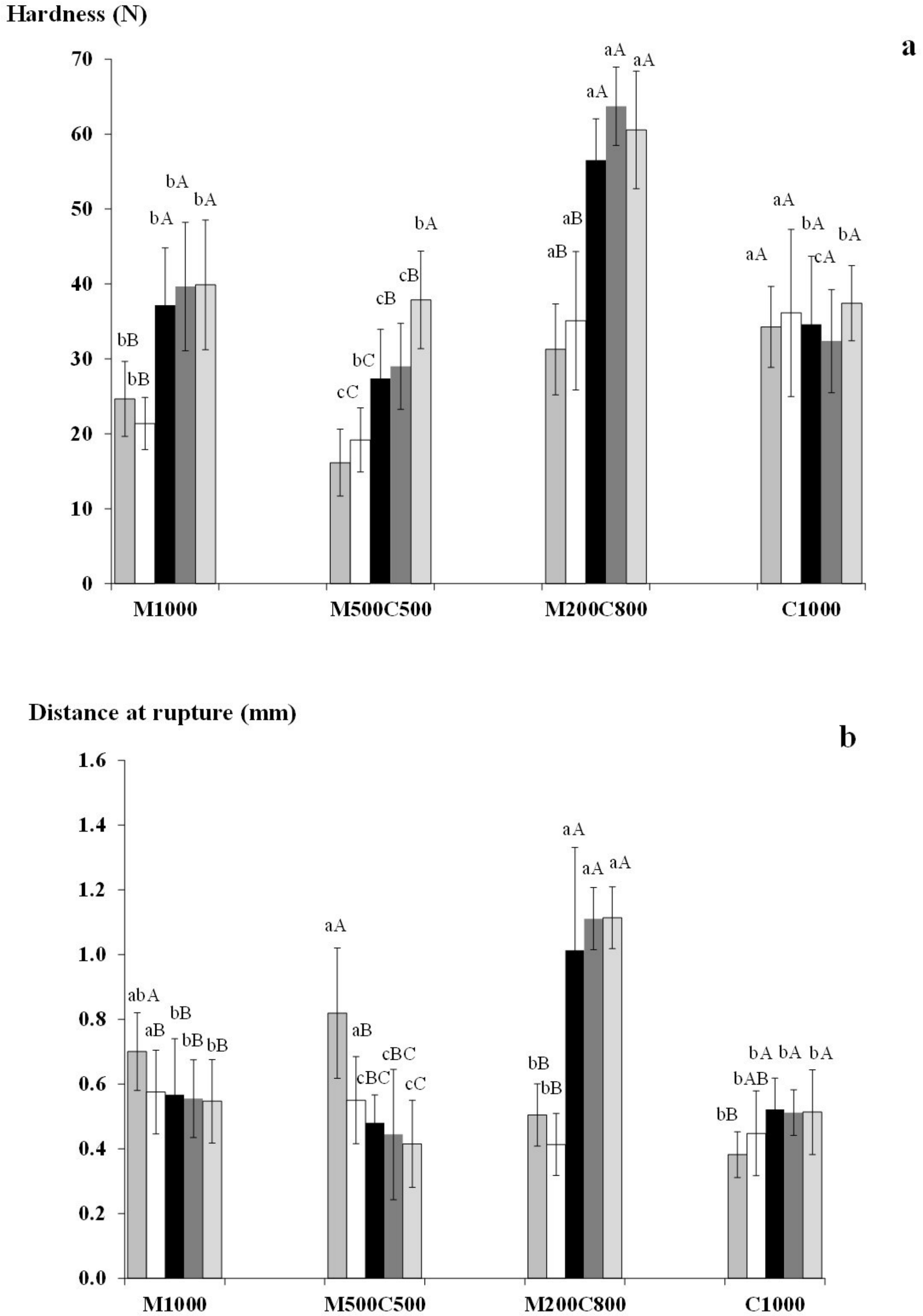
Captions for figures

Figure 1. Moisture content of analysed **biscuits**[a].



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

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



[a]Means followed by different capital letters significantly differ ($p < 0.05$) among different times for the same biscuit. Means followed by different lowercase letters significantly differ ($p < 0.05$) among the four types of biscuit at the same

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