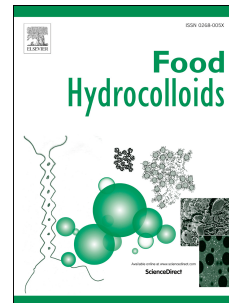# Accepted Manuscript

Hydration and rheological properties of amaranth-wheat flour dough: Influence of germination of amaranth seeds

Luciano M. Guardianelli, María V. Salinas, María C. Puppo

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Seeds

Amaranth seeds

Germinated amaranth seeds
(18h, 30°C)

Flours

Amaranth flour (A)

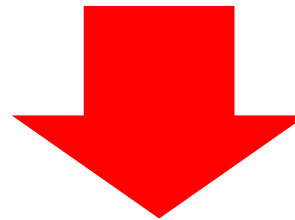Germinated amaranth flour (GA)

+ Wheat flour

Dough with A or GA

Dough

Control

Amaranth-wheat
(A5, A15, A25)

Germinated amaranth-wheat
(GA5, GA15, GA25)

Water-insoluble proteins
Water absorption
Hardness
Springiness

Molecular mobility

1 **Full Title: Hydration and rheological properties of amaranth-wheat flour dough:**

2 **influence of germination of amaranth seeds**

3

4 **Short Title: Wheat dough with germinated amaranth seeds**

5

6 **Authors:** Luciano M. Guardianelli[1], María V. Salinas[1], María C. Puppo[1,2,*]

7

8 [1] CIDCA (CIC-CONICET - Facultad de Ciencias Exactas - Universidad Nacional de La

9 Plata). 47 y 116, 1900 La Plata, Argentina.

10 [2] Facultad de Ciencias Agrarias y Forestales-Universidad Nacional de La Plata. 60 y

11 119, 1900 La Plata, Argentina.

12

13 Corresponding Author: (*)

14 María Cecilia Puppo

15 TE/FAX: 54+221+4254853

16 mcpuppo@quimica.unlp.edu.ar

17

20

21 **ABSTRACT**

22 The objective of this work was to analyze wheat dough combined with amaranth flour

23 to predict dough behavior during breadmaking. Blends with wheat and amaranth flours

24 from germinated (GA) and non-germinated (A) seeds at 5%, 15% and 25% were

25 formulated. The dry gluten content, as measurement of the amount of insoluble protein

26 of blends, was determined. Besides, the hydration (moisture-M_{cont}, water absorption-

27 W_{abs}, molecular mobility-λ, water activity-a_w) and rheological (texture and

28 viscoelasticity) properties of dough were also determined. Dough with 25% of amaranth

29 flour (A25, GA25) showed higher moisture but had lowed less λ than the compared to

30 wheat dough. Moreover, A25 was a bit harder compared to wheat dough though it

31 presented less relaxation of the matrix polymers but a viscous behavior higher than the

32 elastic one (> tan δ). The major difference was detected for GA25 dough, which

33 exhibited a structure with the lowest consistency and with the highest G"/G' ratio due to

34 the modification of proteins during germination, since these proteins contribute to

35 dough elasticity through the stabilization of polymeric gluten proteins.

36

37

1. Introduction

39

Amaranth seeds from different species (*Amaranthus cruentus, Amaranthus caudatus and Amaranthus hypochondriacus*) are usually consumed by humans either as seeds or as flours, a functional ingredient in foods (Gamel et al., 2006). Amaranth was cultivated on a large scale in Mexico and Central America until the early sixteenth century, and its seed was once a staple food of the Aztecs (Arendt & Zannini, 2013). Its consumption has gradually declined over time, but currently there is a growing demand to incorporate it into the diet. Amaranth seeds can be toasted, extruded, burst, germinated or they can be ground into flour and then consumed as such or be included in other cereal products such as bread, cakes, muffins, pancakes, cookies, crepes, noodles and snacks. Amaranth contains high levels of protein, fat, and dietary fiber compared to conventional cereals. Moreover, the amaranth seed proteins are rich in lysine, an amino acid that is generally deficient in cereal grains (Bressani, 2018; Singh et al., 2019), turning it into a seed of high nutritional quality.

Germination is one of seeds treatments usually used to may improve the functional and nutritional properties of cereals and legumes. Generally, this treatment of seeds causes the decomposition of the main seed reserves, such as carbohydrates, proteins and lipids, as a results of an increase in enzymatic activity. This process leads to the increase of free amino acids, simple sugars, and to the improvement of the fatty acid profile (Guardianelli, Salinas, & Puppo, 2019). In addition, Cornejo et al. (2019) studied the physicochemical and nutritional changes in two amaranth species (*Amaranthus quitensis* and *Amaranthus caudatus*) after germination. These authors reported a similar glycemic index and increased protein digestibility in sprouts. Furthermore, antinutritional compounds such as tannins were not modified while phytic acid and oxalate contents were reduced (Najdi Hejazi et al., 2016). This is the reason why the germination of seeds is considered a worthwhile process from the point of view of nutritional value. On the other hand, bread made with refined wheat flour, despite being a good source of energy, is considered nutritionally poor due to its low fiber and mineral content (Slavin, 2003). Therefore, the addition of ingredients with a high nutritional quality, such as some legumes, cereals or pseudocereals, or the sprouts thereof, is a good alternative to improve the nutritive value of wheat bread.

Flour prepared from germinated seeds may also have some positive effects on the structure of wheat dough and consequently bread quality. Therefore, the final structure of the bread crumb may be strongly related to the rheological behavior of the dough

73 before cooking (Armero & Collar, 1997; Dobraszczyk & Schofield, 2000; Dobraszczyk

74 & Morgenstern, 2003; Khatkar & Schofield, 2002; Angioloni & Dalla Rosa, 2007). After

75 kneading and fermentation, the air bubbles produced by the yeast must be kept in the

76 dough. During baking, the starch, proteins and water of dough form the matrix and the

77 air bubbles shape the alveoli in the bread. Dough must be viscoelastic to give space to

78 the bubbles and keep them confined (Houben et al., 2010). In general, not only the

79 type of flour determines the rheology of the dough, but also other factors such as the

80 system, the amount of water added and the time duration of kneading (Zheng et al.,

81 2000; Angioloni & Dalla Rosa, 2007). Furthermore, other ingredients or additional

82 treatment to the dough can influence the rheological properties (Mirsaeedghazi et al.,

83 2008; Salinas et al., 2012). According to Ayo (2001), reported up to 15% amaranth

84 wheat flour (85:15 wheat flour: amaranth flour) can be used in may be substituted with

85 amaranth flour for the production of wheat bread without significantly affecting the

86 physical and sensory quality, as well as the acceptance of the product by consumers.

87 On the other hand, it is possible to use higher levels up to 30% of amaranth flour

88 substitution (25%-30%) in amaranth-wheat cookies (Sindhuja, Sudha, & Rahim, 2005).

89 Ranhotra, Loewe, & Lehmann (1977) reported that by replacing wheat flour with 20%

90 sprouted wheat flour, bread obtained were completely acceptable with a good specific

91 volume and crumb texture. Several authors have studied the relationship between

92 germination and the technological quality of bread made with wheat flour and sprouted

93 peas (Sadowska et al., 2003) or with soy bean sprouts (Rosales-Juárez et al., 2008).

94 However, so far there is no evidence of the use of flour from germinated amaranth

95 seeds in wheat flour breads. Thus, the objective of this work was to evaluate the

96 hydration and rheological properties of wheat flour dough with the addition of

97 germinated and non-germinated amaranth seed flours, in order to be able to predict the

98 breadmaking behavior of these composite formulations.

99

100 **2. Materials and methods**

101 **2.1. Materials**

102 Commercial wheat flour for breadmaking (Molino Campodónico Ltda., Argentina) with

103 11.2% of proteins, 2.30% of lipids, 4.78% of total dietary fiber, 0.60% of ash and

104 12.19% of moisture was used. Alveographic parameters were 86 mm, 106 mm and 325

105 for tenacity (P), extensibility (L) and deformation work (W), respectively. Farinographic

106 parameters of this flour were 56.6%, 8 min, 8.5 min and 100 UB for water absorption,

107 development time, stability and softening degree, respectively.

108 Flour of non-germinated amaranth seeds (A) had 12.8% of protein content, 57.3% of
109 starch, 0.1% of fructose, 1.0% of glucose, 1.8% of sucrose, 6.3% of lipids, 9.3% of total
110 dietary fiber, 2.41% of ash, and 11.1% of moisture.

111 According to Guardianelli et al. (2019), flour of amaranth seeds germinated (GA) for 18
112 h at 30ºC presented 50.4% of starch, 1.2% of fructose, 4.7% of glucose, 2.0% of
113 sucrose, 14.6% of protein, 5.4% of lipids, 10.4% of total dietary fiber, 2.76% of ash, and
114 8.8% of moisture.

115

116 **2.2. Methods**

117 Blends with wheat flour complemented with amaranth flours were prepared.
118 Germinated amaranth flour (GA) or Amaranth flour (A) was added to wheat flour (100
119 g) at different levels: 0% (C), 5% (GA5 or A5), 15% (GA15 or A15), or 25% (GA25 or
120 A25). All mixes also contained 1.5% NaCl (wheat flour basis). The amount of water and
121 mixing time were established by farinographic assays. Water absorption was 55.9%,
122 55.2%, 56.0%, and 58.0% for C, A5%, A15%, and A25%, respectively. While
123 development time for different blends was 11.7 min (C), 9.0 min (A5), 6.5 min (A15),
124 and 7.0 min (A25). Farinogram parameters were similar for flour blends obtained with
125 sprouted or non-sprouted amaranth seeds.

126

127 **2.2.1. Dough preparation**

128 Dough was prepared in a small-scale kneader with planetary mixing action (Kenwood
129 Major, Italy). Dry ingredients (wheat flour, amaranth–GA or A; NaCl) were mixed for 1
130 min, and then the amount of distilled water corresponding to farinographic water
131 absorption was added to the solids. Dough was first kneaded for 1 min at 50 rpm first
132 and then at 90 rpm until it reached the development time reported by the farinogram.
133 Dough was laminated four times (rotating the dough 90° before each pass). Then, it
134 was left to rest for 15 min at 25 °C covered with a plastic film to avoid water loss. All
135 doughs were made in duplicate.

136

137 **2.2.2. Dough physicochemical properties**

138

139 **2.2.2.1. Moisture content.** The moisture of the dough was determined indirectly by air
140 drying in an oven (San Jor, Buenos Aires, Argentina) at 105 ºC until constant weight
141 (AACC, 2000). Determinations were carried out in triplicate.

142

143 **2.2.2.2. Water activity.** Measurements (n=4) were performed at 25 ⁰C with Aqualab

144 4TEV meter (Decagon Devices Inc., Washington, USA). Determinations were carried

145 out in duplicate.

146

147 **2.2.2.3. Molecular mobility**. The molecular mobility (λ) of the dough was analyzed by

148 relaxation assays using NMR Bruker Minispec (Bruker, USA) according to Salinas et al.

149 (2015). A portion of dough was placed in glass tubes (10-mm diameter) up to 3-cm

150 height, and the tubes were closed to avoid dehydration. ^{1}H spin-spin relaxation times

151 (λ) were measured using the Carr-Purcell-Meiboom-Gill pulse sequence. Nuclei are

152 excited for a few milliseconds, and when the pulse stops, they return to ground state

153 emitting a signal. Relaxation curves of the proton (^{1}H) signal intensity versus time have

154 exponential decays and can be fitted according to Eq. 1:

155

156 $$I(t) = A \exp(-t/\lambda) \tag{1}$$

157

158 Where I(t) represents the ^{1}H signal intensity (proportional to the mobile water fraction in

159 the dough), t is the time, λ is the relaxation time (a constant parameter), and A is the

160 signal intensity of protons at t=0. Assays (n=4) were performed in duplicate.

161

162 **2.2.2.4. Gluten determination.** The dry gluten (DG) content of the different

163 formulations was determined in accordance to AACC method 38-12 (2000) modified by

164 Salinas & Puppo (2014). Determinations were carried out in duplicate.

165

166

167 **2.2.3. Dough rheological properties**

168 For rheological measurements, dough was laminated (thickness =1 cm) and cylindrical

169 pieces (diameter = 3 cm) were cut using metallic cutters.

170

171 **2.2.3.1. Texture profile analysis (TPA)**

172 A dough cylinder (n = 15) was subjected to two cycles of compression up to 40% of the

173 original height with a cylindrical probe (diameter = 7.5 cm) using a TA.XT2i Texture

174 Analyzer (Stable Micro Systems, Surrey, U.K.) with a load cell of 25 kg and Texture

175 Expert for Windows version 1.2 Software was used. Force-time curves were obtained

176 at a crosshead speed of 0.5 mm/s. Dough hardness (Hard), consistency (Cons),

177 adhesiveness (Adh), springiness (Spring), and cohesiveness (Cohes) were

178 determined. Hardness is defined as the maximum force during the first compression.

179 Consistency is the sum of the areas under the force vs. time curve corresponding to

180   the first and second compression cycles. Adhesiveness is the negative area in the first

181   cycle. Springiness is calculated as the distance ratio between the beginning and the

182   maximum force of the second and first peaks. Cohesiveness is determined as the ratio

183   between the positive areas of the second and the first cycles (Bourne, 2002). Assays

184   were performed in duplicate.

185

186   **2.2.3.2. Relaxation test**

187   The relaxation test consists of deforming the material by applying a compression to

188   constant deformation and recording, as a function of time, the force that opposes the

189   material to maintain the deformation selected. For this, discs of dough (n=3) were

190   subjected to a compression of 40% at 0.5 mm/s for 20 min using a TA.XT2i Texture

191   Analyzer (Stable Micro Systems, Surrey, UK) with a load cell of 25 kg. Assays were

192   carried out at 25 °C. To prevent drying of the dough, the cylinders were covered with

193   semisolid Vaseline. A regression of second order of the exponential decay was

194   performed on stress-relaxation curves using Origin Pro 8 software (OriginLab

195   Corporation, MA, USA). A generalized Maxwell model (Steffe, 1996; Rodríguez-

196   Sandoval et al., 2009; Salinas et al., 2012) was applied (Eq. 3):

197

198    $$\sigma(t) = \sigma_1 * \exp(-t / T_1) + \sigma_2 * \exp(-t / T_2) + \sigma_3 \tag{3}$$

199

200   Where $\sigma(t)$ represents the stress measured at any time during the test, t is the time.

201   The relaxation time T_i is defined as the ratio between the viscosity and the elastic

202   modulus (Eq. 4) and the elastic relaxation modulus E_i is defined as the ratio between

203   the stress and constant strain (Eq. 5).

204

205    $$T_i = \eta_i / E_i \tag{4}$$

206

207    $$E_i = \sigma_i / \varepsilon_0 \tag{5}$$

208

209   Where ε_0 is a constant strain calculated as the ratio of deformation to the initial height

210   of the dough.

211   By applying this model, elastic relaxation moduli (*E*) and relaxation times (*T*) were

212   obtained for the first and second exponential terms. Modulus E_3 corresponds to the

213   equilibrium modulus at infinite time. The assay was performed in duplicate.

214

215   **2.2.3.3. Dynamic rheological assay**

216 For the rheometric tests, cylindrical pieces (diameter = 3 cm, height = 2 mm) were
217 obtained. Dynamic oscillatory tests were performed in a Haake RS600 controlled stress
218 oscillatory rheometer (Haake, Germany) at 25.0 ± 0.1 ºC, using a plate–plate sensor
219 system with a 1.0 mm gap between plates. Serrated plates were used and semisolid
220 Vaseline was applied to prevent sample drying during testing. All samples were left to
221 rest for 15 min between plates before measurements to allow dough relaxation. Two
222 types of rheological tests were carried out in the following way: (a) constant frequency
223 strain sweeps (1 Hz) to determine the linear viscoelastic range and (b) frequency
224 sweeps (from 0.005 to 100 Hz) at constant tension (5 Pa) within the linear viscoelastic
225 range. The mechanical spectra were obtained by recording the dynamic moduli G', G"
226 and tan δ (G" / G') as a function of frequency. Modulus G' corresponds to the elastic or
227 storage dynamic modulus, related to the response of the material as a solid, while G" is
228 the viscous dynamic or loss modulus, related to the response of the material as a fluid,
229 and tan δ is related to the general viscoelastic response. Assays were carried out in
230 triplicate.

231

232 **2.2.4. Statistical analysis**
233 The experiment was designed according to a factorial design, the factors being the
234 treatment and the percentage of addition of flour A and GA. The data were analyzed
235 with bidirectional ANOVA using the InfoStat software (Di Rienzo et al., 2012) and the
236 means were compared using the Duncan multiple range test at a significance level of
237 $p < 0.05$.

238

239 **3. Results and discussion**

240

241 **3.1. Gluten content of wheat-amaranth dough**
242 The content of dry gluten (DG), as an indicator of water-insoluble proteins of dough,
243 was analyzed. Values of DG increased with the incorporation of amaranth flour, being
244 more pronounced in gluten samples with non-germinated seed flour (Figure 1). Dough
245 with germinated amaranth (GA) presented a lower amount of gluten water-insoluble
246 proteins than non-germinated amaranth (A) dough, probably because the germination
247 process would hydrolyze proteins that may act in a synergic form with wheat proteins in
248 stabilizing the gluten matrix. This behavior could be due to the content of amaranth
249 proteins incorporated to wheat flour and also to the new structure that those proteins
250 acquired after germination (Aphalo, Martínez, & Añón, 2009).

251

252 **3.2. Hydration properties of dough**

253 Control dough had 43% of moisture. This parameter increased with the level of both

254 types of amaranth flours, reaching the highest values with the maximum content of

255 these flours (45%). The highest value of moisture agrees with the highest value of

256 farinograph water absorption obtained (from 55.9 to 58.0 for C and A25, respectively).

257 The increase in farinograph water absorption with the replacement of wheat flour with

258 amaranth flour was previously reported by Bojnanská & Smitalová (2014).

259 On the other hand, although the amount of water varied, the availability of water

260 represented by water activity (a_w) was statistically the same in all formulations ($a_w \approx$

261 0.97) (data not shown).

262 Molecular mobility of water in dough is represented by the ^{1}H spin-spin relaxation time

263 (λ) parameter. Systems with shorter relaxation times are less mobile (solid-like state)

264 than those with longer relaxation times (liquid-like state). High values of λ denote high

265 molecular mobility; it means that water in dough is linked to the other components in a

266 weak form and therefore is in a high-energy mobile state, leading to a more labile

267 gluten structure (Salinas et al., 2012). This phenomenon depends on the molecular

268 structure of all components present in dough. Values of λ of dough are shown in Figure

269 2. Control dough (C) and dough with 5% of amaranth flour (A5 and GA5) presented the

270 same high molecular mobility. Higher amounts of amaranth flour decreased λ values,

271 associated with less mobility of water due to the presence of the different components

272 of amaranth seeds, mainly proteins and starch, which are able to bind water. The

273 tendency observed for λ was opposite to that obtained for DG water-insoluble proteins

274 reported as dry gluten (Figure 1).

275

276 **3.3. Texture profile of dough**

277 Different texture parameters obtained from the analysis of the texture profile of dough

278 are listed in Table 1. The addition of amaranth flour, mainly the sample obtained from

279 non-germinated seeds, produced an increase in hardness (Hard) with respect to C;

280 being the highest value observed for A5 dough. This behavior could be due to the

281 incorporation of a certain proportion of globular proteins of 11S and P-globulin type

282 (Avanza & Añón, 2007; Quiroga et al., 2009). These proteins, which are able to bind a

283 higher amount of water than gluten proteins, contribute to the formation of a more

284 structured network because of the gelation process. The presence of these globular

285 proteins also contributes to promoting gluten development (Figure 1). As more amount

286 of amaranth flour is added (25%), there is a dilution effect of the gluten proteins that

287 amaranth proteins cannot compensate, therefore a bit softer dough is obtained at this

288 higher level (Table 1). Nevertheless, with the exception of GA25, higher hardness was

289 obtained for wheat-amaranth dough in comparison with C. This behavior suggests that

290 proteins and also fibers present in amaranth flour reinforce the gluten network. Bigne et
291 al. (2016) obtained similar results with mesquite-wheat dough. Consistency (Cons) also
292 increased in A5 and GA5 dough and a subsequent decrease with the increase in
293 amaranth flour level was observed (Table 1). This decrease can be attributed to
294 changes in amaranth protein structure because of germination, leading to a distinct
295 interaction with wheat proteins and water during matrix formation. No significant
296 differences were observed in adhesiveness (Adh), except for GA15 that presented the
297 highest value. Wheat dough had the lowest value for springiness (Spring), and this
298 parameter increased with the addition of amaranth flour to dough. On the other hand,
299 no significant differences in cohesiveness (Cohes) were observed between the control
300 and dough with 5% amaranth (A5 and GA5); in contrast, dough with 15% and 25%
301 amaranth flour (A15, A25, GA15 and GA25) showed a significant increase in
302 cohesiveness.

303 An increase in cohesiveness together with a decrease in adhesiveness and molecular
304 mobility with high levels of amaranth flour (≥ 15%) suggests a strong interaction of the
305 components of this flour (proteins, starch, fiber) with water, contributing to maintain or
306 slightly decrease the hardness and consistency of the dough.

307

308 **3.4. Viscoelastic behavior of dough**

309 In viscoelastic solids such as dough, the stress decays towards an equilibrium value.
310 Relaxation curves are decreasing stress curves as a function of time and exhibit three
311 zones (Yadav, Roopa, & Bhattacharya, 2006): a first zone of great decay, an
312 intermediate zone of decay, and a third zone with an insignificant slope that reaches an
313 equilibrium value of stress. Relaxation is a phenomenon related to the molecular and
314 structural reorientation of the system that is studied through the elastic and relaxation
315 moduli obtained from the generalized Maxwell model. The relaxation time (T) has an
316 inverse behavior to the elastic modulus (E) and proportional to the viscosity (η) and is
317 related to the degree of relaxation, that is, the higher the value of T, the greater the
318 viscous component with respect to the elastic one, and therefore the dough is more
319 relaxed. The dough relaxation parameters E and T of dough for the different zones of
320 the curve are shown in Figure 3.

321 Figure 3 a and b show elastic (E1) and relaxation time (T1), respectively (first zone).
322 Both parameters govern the relaxation at the beginning of deformation, attributed to the
323 reorientation of small molecules. Dough C and A5 presented the highest value of E1,
324 while dough with 15% and 25% of amaranth flour (A and GA) showed lower values of
325 E1 (Figure 3 a), the decrease being more pronounced in GA dough. Results suggest
326 that the germinated amaranth protein in GA25 dough formed small molecules of lower

327 elasticity. On the other hand, this significant variation in E was not reflected in T1,
328 which showed no differences with respect to the C dough with the exception of GA25
329 (Figure 3 b). This behavior suggests that the GA25 dough had the lowest degree of
330 relaxation (<T1) in zone 1 with a lower E1, indicating a greater contribution of viscosity.
331 All doughs presented one order higher values of E2, compared to E1 and E3 moduli
332 (Figure 3 a, c, e), due to the presence of polymeric gluten proteins that are greater in
333 size and undergo less relaxation and therefore greatly contribute to dough elasticity. No
334 significant differences in E2 values with respect to C were observed for dough with
335 non-germinated amaranth seeds (A), while for GA, E2 increased with the increment of
336 GA flour (Figure 3 c). This increase in E2 suggests the formation of a structure
337 stabilized by polymers of higher elasticity. This could be due to the contribution of
338 amaranth globular proteins that after germination changed their conformation, acquiring
339 a structure that improved the interaction with water and consequently the structure of
340 dough, which was also evidenced by a lower molecular mobility.
341 The behavior for relaxation time T2 (Figure 3 d) was similar to that observed for T1; a
342 decrease for GA dough with the increase in the amount of amaranth flour was
343 observed. The very low values of T2 for GA25 suggest a low relaxation degree of
344 gluten polymers in the presence of amaranth proteins, in concordance with the highest
345 value of E2.
346 Finally, Figure 3 e shows values of E3 that represent the energy storage in dough in a
347 zone (zone 3) where stress does not change with the deformation applied, reaching the
348 equilibrium state. The doughs with the higher values of E3 were those formulated with
349 non-germinated amaranth seeds (A doughs) and GA5, with values higher than C and
350 without significant differences between different levels of A flour. Dough GA15 and
351 GA25 presented lower values of E3, associated with a low elastic behavior at
352 equilibrium, after the deformation process.
353 In spite of the lower relaxation time T2 and the highest elastic modulus E2 of the
354 polymeric fraction of GA25 dough, this sample presented a higher contribution of the
355 low molecular mass molecules to viscosity (lower E1), accompanied by a very low
356 equilibrium elastic modulus (E3). The relaxation behavior of this dough is in
357 concordance with the low value of consistency observed in TPA (Table 1). Salinas &
358 Puppo (2014) found the same behavior for dough formulated with calcium citrate and
359 13% of inulin, i.e., low values of hardness together with low values of E3.
360 Another way to study the viscoelasticity of dough is through dynamic rheology at low
361 deformation. Dough was left to rest for few minutes before the measurement to favor
362 the molecular arrangement of gluten polymers. The viscoelastic parameters obtained
363 from mechanical spectra were storage (G') and loss (G") moduli and the ratio G"/G' =

364   tan δ (Table 1). Values of G' and G" for amaranth dough increased with respect to

365   sample C. Nevertheless, no significant differences were observed between dough A

366   and GA when the amount of amaranth flour was increased. On the other hand, values

367   of tan δ were the typical ones observed for wheat dough (Letang, Piau, & Verdier,

368   1999) with values around 0.3. In the case of amaranth-wheat dough, values were in the

369   range 0.341-0.425. Dough A5 and C presented the lowest values of tan δ associated

370   with a major elastic behavior, similar to that observed in the relaxation assay at high

371   deformation (high E1 and E3). At equal amount of amaranth flour, GA samples

372   exhibited higher values of tan δ, suggesting a net increase in the viscous behavior. The

373   increase of tan δ associated with a more viscous matrix agreed with the lower value of

374   consistency obtained by the texture assay, the effect being more pronounced for GA25

375   (Table 1).

376   Another alternative for analyzing mechanical spectra is by evaluating the dependence

377   between G' and G" in all the frequency range studied (Figure 4). The equality of the G'

378   and G" moduli (tan δ = 1) is evidenced by a red line at 45⁰. The relationship between

379   these two moduli was evidenced by a curve. The proximity of this curve to the red line

380   suggests a more viscous behavior of the sample. In turn, the slope of curve G' versus

381   G" has been used as an indicator of changes in the morphology of the different

382   polymers (Ahmed et al., 2013). A superposition of the curves indicates that there are

383   no differences in the morphology of the polymers, while no superposition suggests the

384   formation of a heterogeneous matrix. Dough with curves with a high slope refers to a

385   more elastic network (Salinas et al., 2015). At low frequencies (<0.05 Hz), at which

386   practically there is no deformation of dough and the changes observed are attributable

387   to the nature of the dough structure, the curve of dough C was the highest one, while

388   curves of A5, A15 and A25 were placed below, A5 being the lowest one (Figure 4 a). At

389   low level of amaranth flour (5%) a softening of the gluten matrix would be produced,

390   while at high levels this weakening effect would be compensated by a reinforcement of

391   the gluten structure probably due to the contribution of the globular amaranth proteins.

392   For this reason, at the highest level (25%) the amaranth-wheat dough (A25) presented

393   a G' vs. G" behavior similar to that obtained for the control dough. In contrast, dough

394   with GA (Figure 4 b) showed a similar tendency to that observed for the dough with

395   non-germinated amaranth seed flour at low frequencies (<0.03 Hz). The main

396   differences for the GA dough are that the curve furthest from that of C was GA25. This

397   behavior suggests that with a high amount of GA, the content of low molecular mass

398   molecules present in dough is higher, due to de-polymerization during germination that

399   leads to a less structured matrix with a more viscous rheological performance. For all

400   the doughs assayed, at high values of G' and G" a total superimposition of the curves

401 was observed, suggesting an equal behavior of both moduli at high deformation

402 frequencies.

403

404 **4. Conclusions**

405 The incorporation of amaranth flour (up to 25%) produced relatively minor changes in

406 the physicochemical and rheological properties of wheat dough, including higher water

407 absorption and presence of water-insoluble proteins. In addition, the flour obtained

408 from germinated seeds had a different behavior in the parameters studied with respect

409 to the flour obtained from the non-germinated seeds. The GA25 dough had the highest

410 water content and also a lower molecular mobility associated with a certain degree of

411 structure of dough. The GA25 dough presented the same hardness although a greater

412 elasticity (TPA) than the control dough, due to the modification of the globular

413 amaranth proteins as a consequence of seed germination. However, the dough was

414 more viscous (greater tan δ and smaller E3), possibly due to morphological changes in

415 the gluten structure with respect to wheat dough. Overall, wheat flour supplemented

416 with up to 25% amaranth flour obtained from germinated or non-germinated seeds

417 produced changes in water absorption, it was possible to obtain dough of acceptable

418 rheological properties for breadmaking.

419

423

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523

524 FIGURE CAPTIONS

525 **Fig. 1** Dry gluten content of germinated and non-germinated amaranth-wheat flour
526 dough. Levels of amaranth flours: non-germinated samples: 0% (C), 5% (A5), 15%
527 (A15), 25% (A25); germinated samples: 5% (GA5), 15% (GA15), 25% (GA25).
528 Different letters indicate significant differences (p < 0.05).

529

530 **Fig. 2** ^{1}H spin–spin relaxation time (λ). Non-germinated amaranth flour levels: 0% (C),
531 5% (A5), 15% (A15), and 25% (A25). Germinated amaranth flour levels: 5% (GA5),
532 15% (GA15), 25% (GA25). Different letters indicate significant differences (p < 0.05).

533

534 **Fig. 3** Relaxation parameters of germinated and non-germinated amaranth-wheat flour
535 dough. Relaxation parameters: Elastic moduli: E1 (**a**), E2 (**c**) and E3 (**e**). Relaxation
536 times: T1 (**b**) and T2 (**d**). Levels of amaranth flours: non-germinated samples: 0% (C),
537 5% (A5), 15% (A15), 25% (A25); germinated samples: 5% (GA5), 15% (GA15), 25%
538 (GA25). Different letters indicate significant differences (p < 0.05).

539

540 **Fig. 4** Elastic modulus (G') as a function of viscous modulus (G'') of wheat flour dough

541 with: non-germinated (**a**) or germinated (**b**) amaranth flours.

542

TABLES

Table 1. Rheological properties of amaranth-wheat flour dough.

Dough	Textural parameters					Viscoelastic parameters (1 Hz)		
	Hard (N)	Cons (N.s)	Adh (N.s)	Spring (-)	Cohes (-)	G' (kPa)	G'' (kPa)	tan δ (-)
C	1.5 ± 0.3 **a**	11.7 ± 2.0 **b**	5.0 ± 0.5 **ab**	0.87 ± 0.02 **a**	0.73 ± 0.04 **ab**	10.8 ± 0.3 **a**	3.7 ± 0.15 **a**	0.341 ± 0.002 **a**
A5	2.0 ± 0.2 **d**	15.9 ± 1.4 **d**	5.0 ± 1.3 **ab**	0.90 ± 0.02 **bc**	0.72 ± 0.04 **a**	21.7 ± 4.3 **b**	7.9 ± 1.9 **b**	0.362 ± 0.029 **ab**
A15	1.8 ± 0.3 **c**	13.0 ± 1.4 **c**	5.3 ± 1.1 **b**	0.90 ± 0.01 **bcd**	0.75 ± 0.02 **cd**	22.3 ± 1.9 **b**	8.4 ± 1.0 **b**	0.374 ± 0.019 **b**
A25	1.6 ± 0.2 **b**	12.2 ± 1.9 **b**	5.1 ± 0.6 **ab**	0.90 ± 0.02 **bcd**	0.75 ± 0.02 **bc**	23.5 ± 3.9 **b**	9.1 ± 1.7 **b**	0.388 ± 0.023 **bc**
GA5	1.7 ± 0.2 **bc**	13.5 ± 1.3 **c**	5.3 ± 0.8 **ab**	0.89 ± 0.02 **b**	0.73 ± 0.02 **ab**	21.2 ± 3.1 **b**	8.8 ± 1.4 **b**	0.415 ± 0.008 **cd**
GA15	1.7 ± 0.3 **bc**	12.1 ± 1.2 **b**	5.7 ± 0.5 **c**	0.90 ± 0.02 **cd**	0.76 ± 0.03 **cd**	21.0 ± 6.9 **b**	8.8 ± 3.3 **b**	0.412 ± 0.023 **cd**
GA25	1.5 ± 0.2 **a**	9.5 ± 1.3 **a**	4.9 ± 0.7 **a**	0.91 ± 0.01 **d**	0.77 ± 0.03 **d**	21.3 ± 0.9 **b**	9.0 ± 0.4 **b**	0.425 ± 0.007 **d**

Textural parameters: Hardness (Hard), Consistency (Cons), Adhesiveness (Adh), Springiness (Spring), Cohesiveness (Cohes). Viscoelastic parameters: Storage modulus (G'), loss modulus (G''), loss tangent (G''/G'). Non-germinated amaranth flour levels: 0% (C), 5% (A5), 15% (A15), 25% (A25). Germinated amaranth flour levels: 5% (GA5), 15% (GA15), 25% (GA25). Different letters in the same column indicate significant differences (p < 0.05).
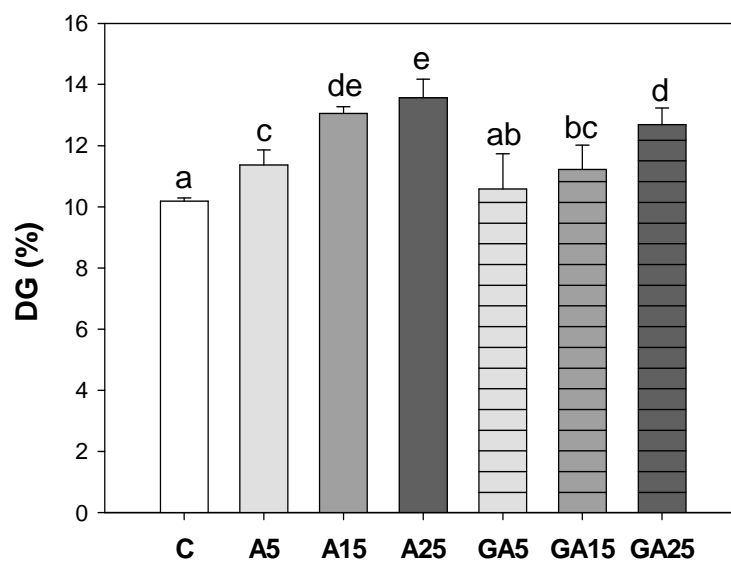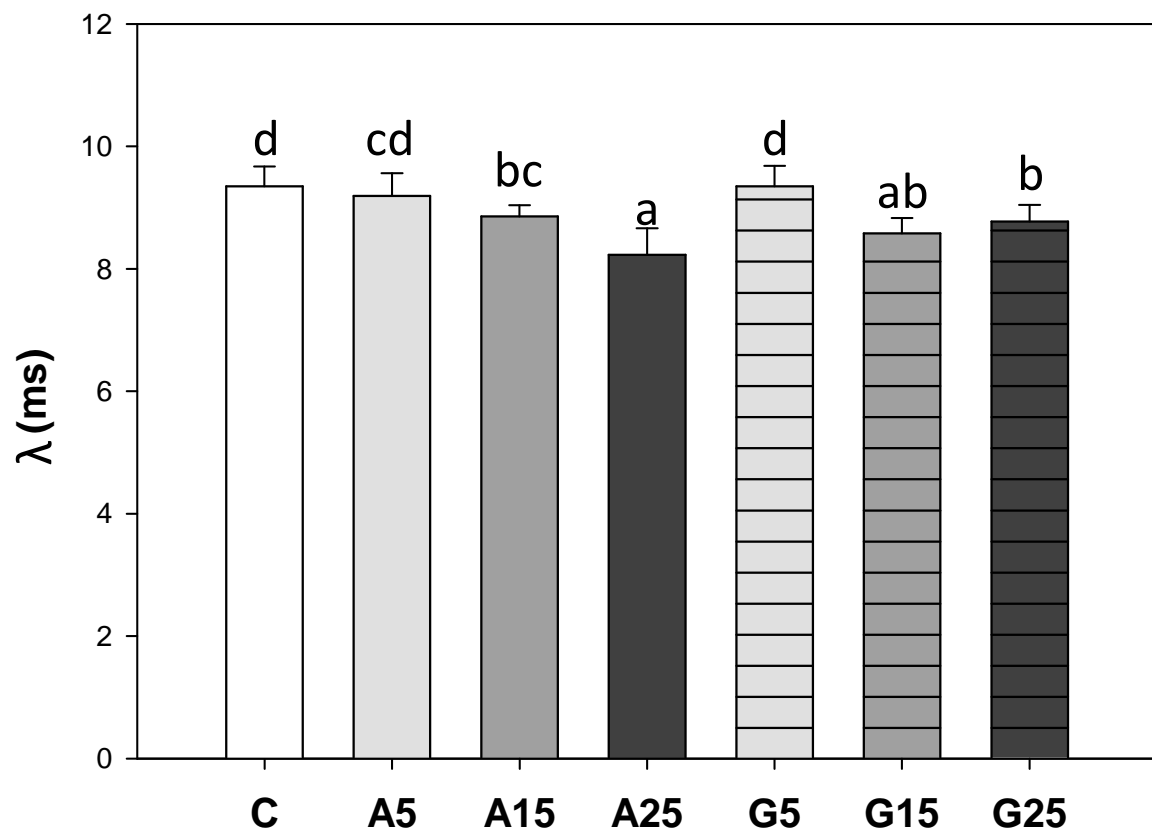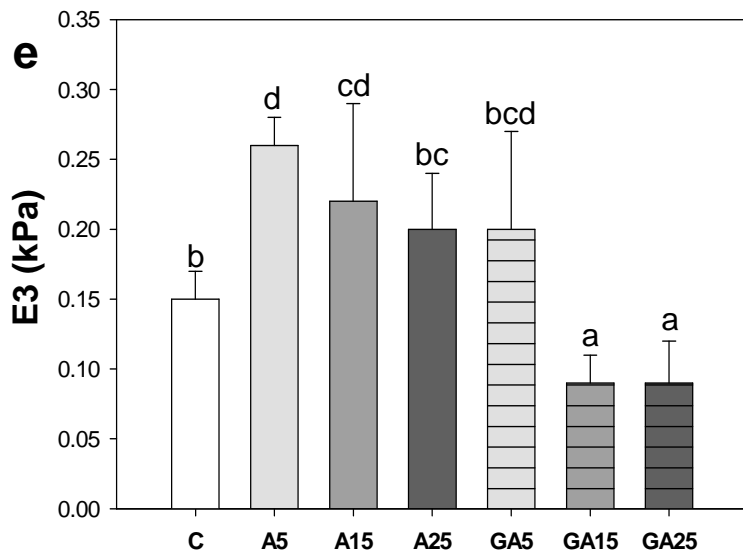
Figure 1

Figure 2

Figure 3

a

G' (Pa)

0.05 Hz

- ● C
- ○ A5
- ▲ A15
- △ A25

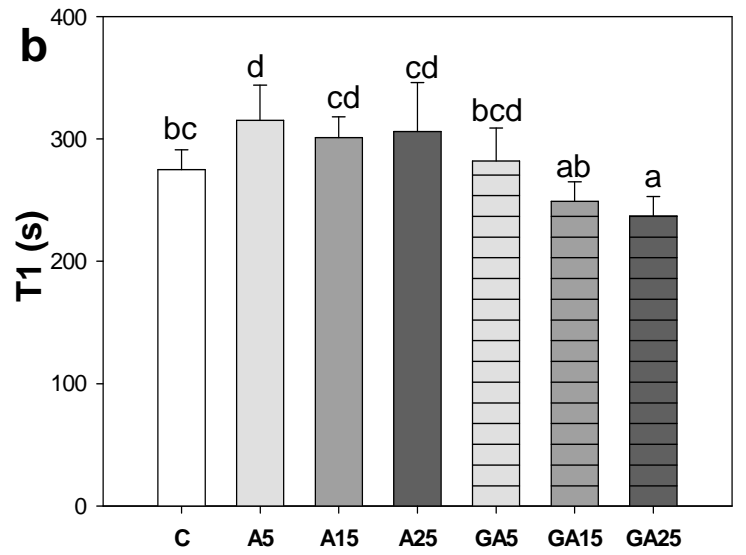G'' (Pa)

b

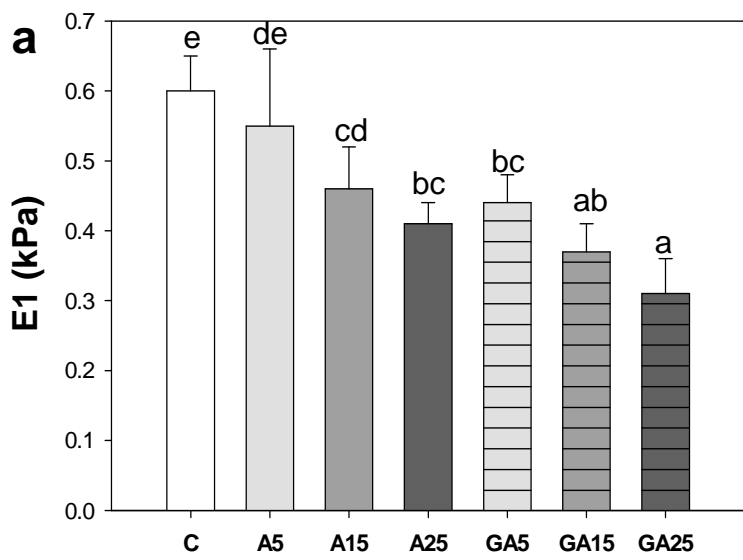G' (Pa)

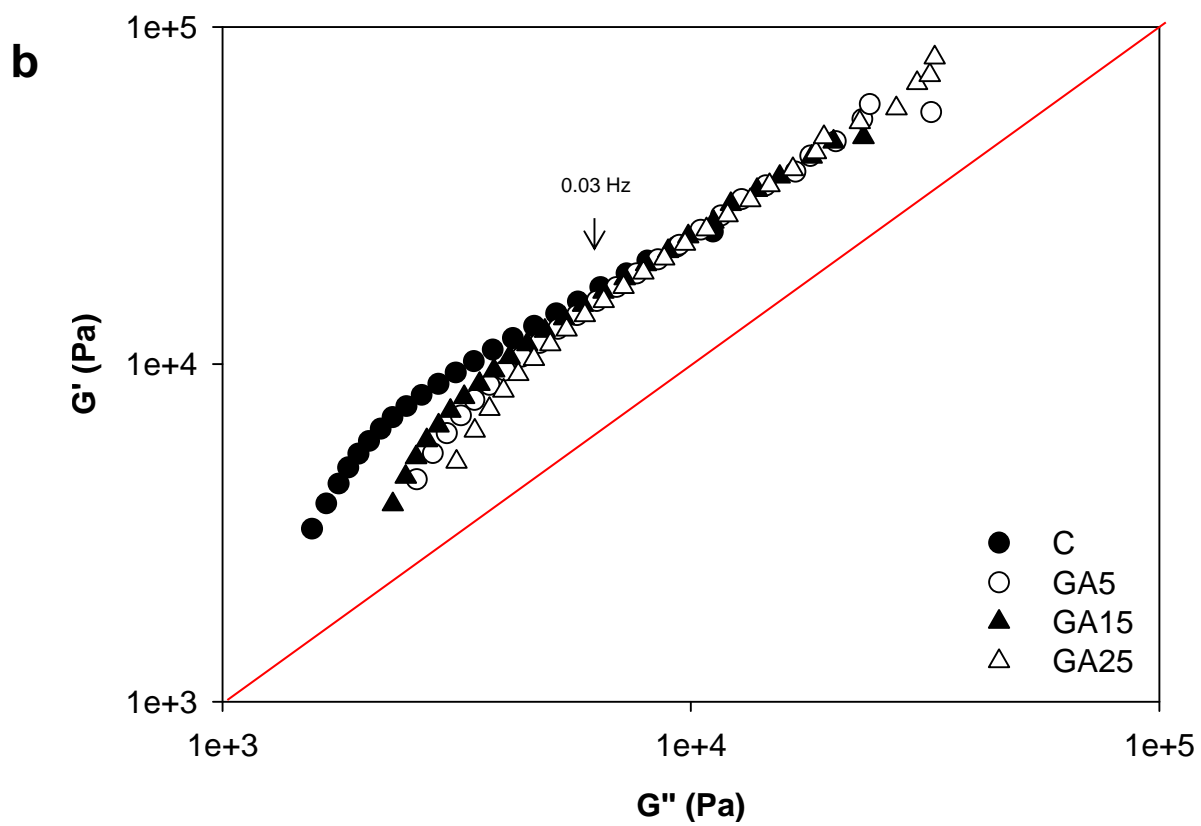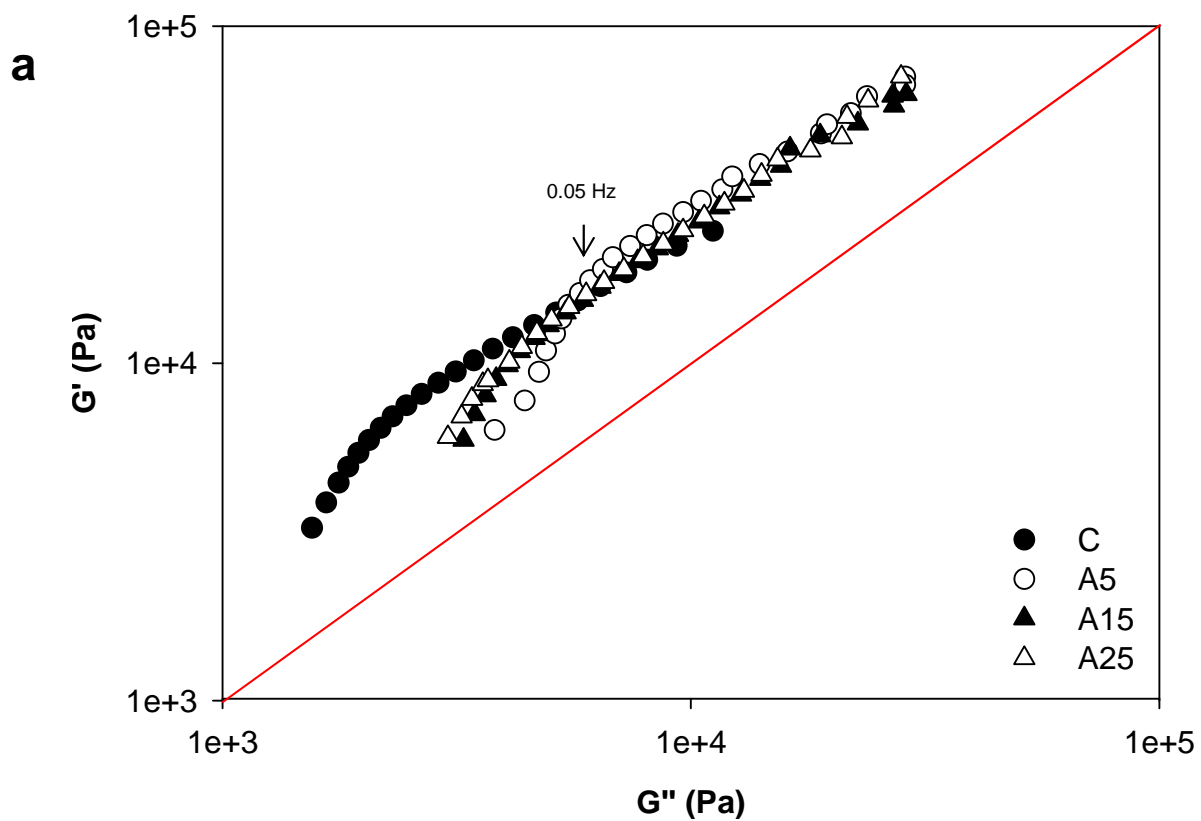0.03 Hz

- ● C
- ○ GA5
- ▲ GA15
- △ GA25

G'' (Pa)
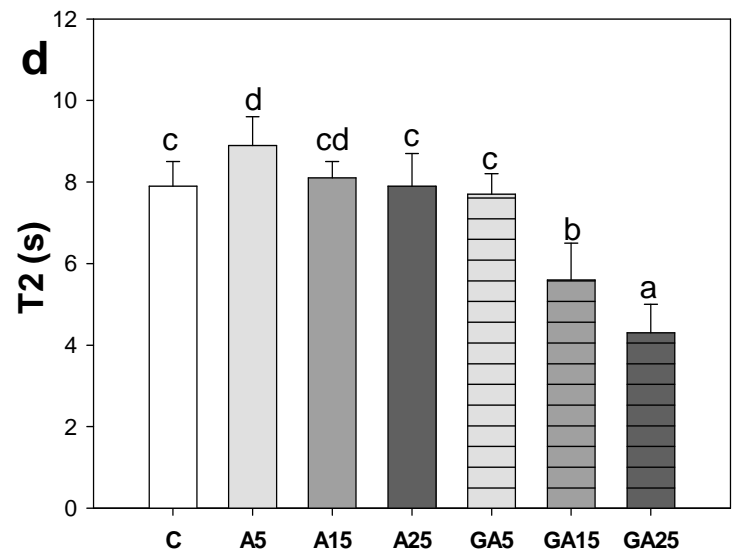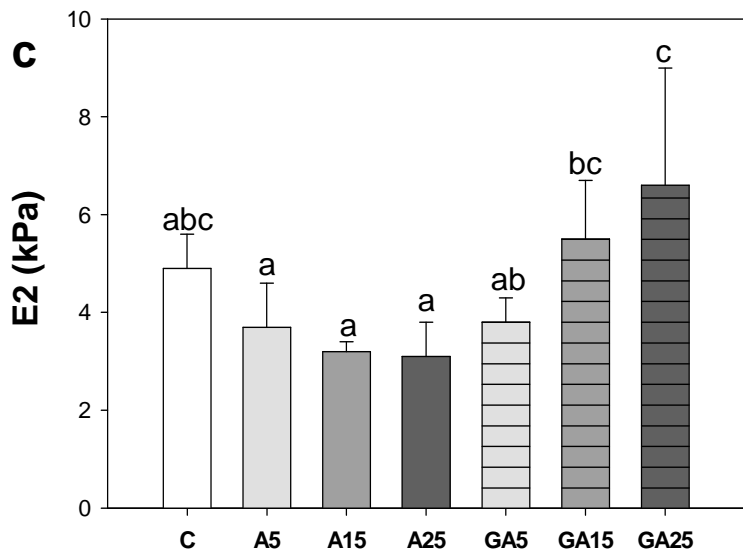
Figure 4

Highlights

- Amaranth flour, up to 15%, slightly strengthened wheat-based dough

- Amaranth flour from pre-germinated seeds had minor effects on rheological properties of dough

- Pre-germinated seeds-25% formed dough with slightly lower viscoelasticity but higher consistency

- Regardless of the treatment of the seed, acceptable dough for bread was obtained

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

Guardianelli L.
Salinas M.V.
Puppo M.C.