Full Title: Hydration and rheological properties of amaranth-wheat flour dough: influence of germination of amaranth seeds

Short Title: Wheat dough with germinated amaranth seeds

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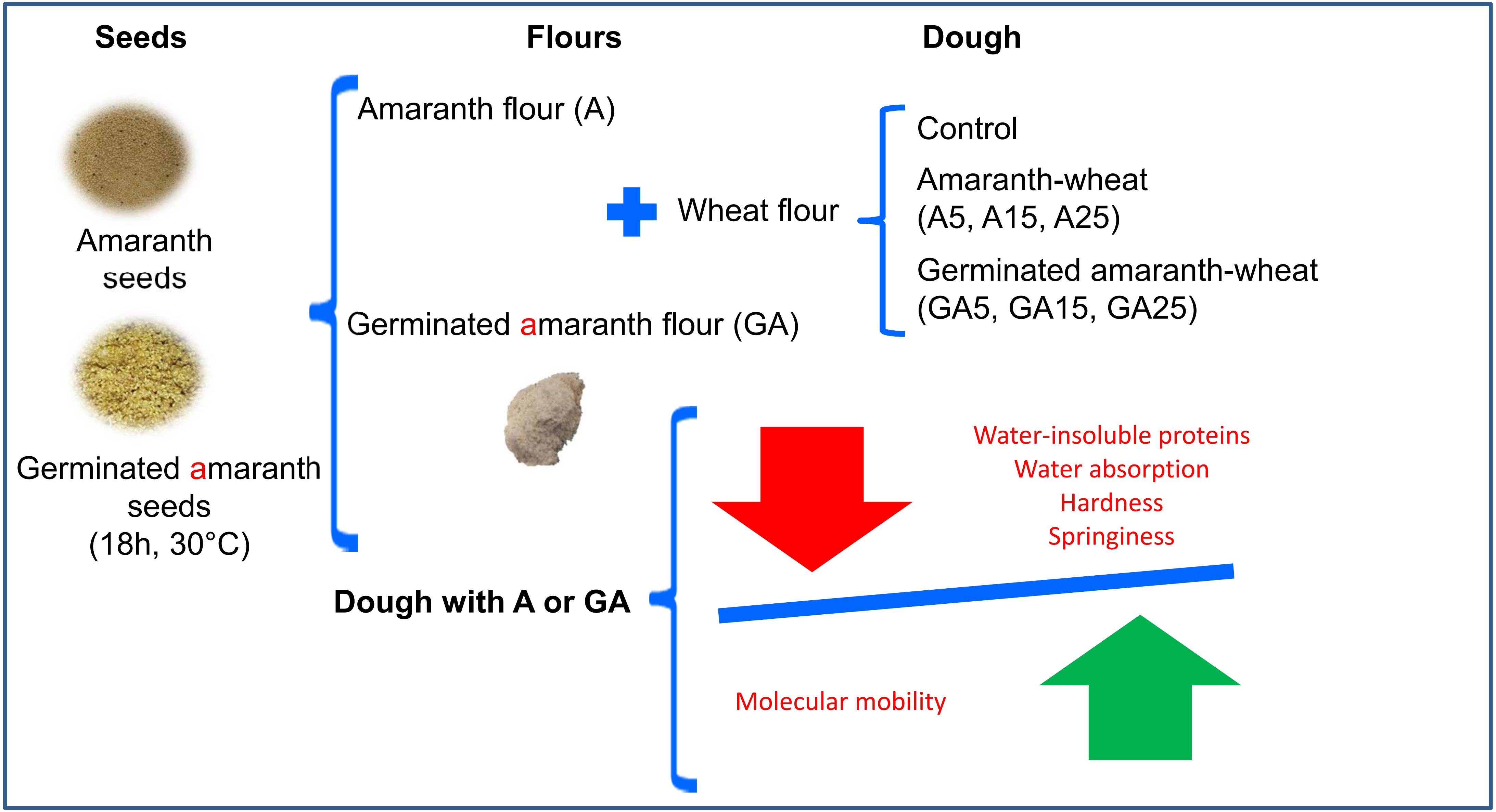
ABSTRACT

The objective of this work was to analyze wheat dough combined with amaranth flour to predict dough behavior during breadmaking. Blends with wheat and amaranth flours from germinated (GA) and non-germinated (A) seeds at 5%, 15% and 25% were formulated. The dry gluten content, as measurement of the amount of insoluble protein of blends, was determined. Besides, the hydration (moisture-M_{cont}, water absorption-W_{abs}, molecular mobility-λ, water activity-a_w) and rheological (texture and viscoelasticity) properties of dough were also determined. Dough with 25% of amaranth flour (A25, GA25) showed higher moisture but had lowed less λ than the compared to wheat dough. Moreover, A25 was a bit harder compared to wheat dough though it presented less relaxation of the matrix polymers but a viscous behavior higher than the elastic one (> tan δ). The major difference was detected for GA25 dough, which exhibited a structure with the lowest consistency and with the highest G''/G' ratio due to the modification of proteins during germination, since these proteins contribute to dough elasticity through the stabilization of polymeric gluten proteins.



Seeds

Flours

Dough

Amaranth seeds

Germinated amaranth seeds
(18h, 30°C)

Amaranth flour (A)

Germinated amaranth flour (GA)

Wheat flour

Control

Amaranth-wheat
(A5, A15, A25)

Germinated amaranth-wheat
(GA5, GA15, GA25)

Dough with A or GA

Water-insoluble proteins
Water absorption
Hardness
Springiness

Molecular mobility

1. Introduction

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 before cooking (Armero & Collar, 1997; Dobraszczyk & Schofield, 2000; Dobraszczyk & Morgenstern, 2003; Khatkar & Schofield, 2002; Angioloni & Dalla Rosa, 2007). After kneading and fermentation, the air bubbles produced by the yeast must be kept in the dough. During baking, the starch, proteins and water of dough form the matrix and the air bubbles shape the alveoli in the bread. Dough must be viscoelastic to give space to the bubbles and keep them confined (Houben et al., 2010). In general, not only the type of flour determines the rheology of the dough, but also other factors such as the system, the amount of water added and the time duration of kneading (Zheng et al., 2000; Angioloni & Dalla Rosa, 2007). Furthermore, other ingredients or additional treatment to the dough can influence the rheological properties (Mirsaeedghazi et al., 2008; Salinas et al., 2012). According to Ayo (2001), reported up to 15% amaranth wheat flour (85:15 wheat flour: amaranth flour) can be used in may be substituted with amaranth flour for the production of wheat bread without significantly affecting the physical and sensory quality, as well as the acceptance of the product by consumers. On the other hand, it is possible to use higher levels up to 30% of amaranth flour substitution (25%-30%) in amaranth-wheat cookies (Sindhuja, Sudha, & Rahim, 2005). Ranhotra, Loewe, & Lehmann (1977) reported that by replacing wheat flour with 20% sprouted wheat flour, bread obtained were completely acceptable with a good specific volume and crumb texture. Several authors have studied the relationship between germination and the technological quality of bread made with wheat flour and sprouted peas (Sadowska et al., 2003) or with soy bean sprouts (Rosales-Juárez et al., 2008). However, so far there is no evidence of the use of flour from germinated amaranth seeds in wheat flour breads. Thus, the objective of this work was to evaluate the hydration and rheological properties of wheat flour dough with the addition of germinated and non-germinated amaranth seed flours, in order to be able to predict the breadmaking behavior of these composite formulations.

2. Materials and methods

2.1. Materials

Commercial wheat flour for breadmaking (Molino Campodónico Ltda., Argentina) with 11.2% of proteins, 2.30% of lipids, 4.78% of total dietary fiber, 0.60% of ash and 12.19% of moisture was used. Alveographic parameters were 86 mm, 106 mm and 325 for tenacity (P), extensibility (L) and deformation work (W), respectively. Farinographic parameters of this flour were 56.6%, 8 min, 8.5 min and 100 UB for water absorption, development time, stability and softening degree, respectively.

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

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

2.2. Methods

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

2.2.1. Dough preparation

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

2.2.2. Dough physicochemical properties

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

2.2.2.2. Water activity. Measurements (n=4) were performed at 25 ºC with Aqualab 4TEV meter (Decagon Devices Inc., Washington, USA). Determinations were carried out in duplicate.

2.2.2.3. Molecular mobility. The molecular mobility (λ) of the dough was analyzed by relaxation assays using NMR Bruker Minispec (Bruker, USA) according to Salinas et al. (2015). A portion of dough was placed in glass tubes (10-mm diameter) up to 3-cm height, and the tubes were closed to avoid dehydration. ^{1}H spin-spin relaxation times (λ) were measured using the Carr-Purcell-Meiboom-Gill pulse sequence. Nuclei are excited for a few milliseconds, and when the pulse stops, they return to ground state emitting a signal. Relaxation curves of the proton (^{1}H) signal intensity versus time have exponential decays and can be fitted according to Eq. 1:

$$I(t) = A \exp(-t/\lambda) \qquad (1)$$

Where I(t) represents the ^{1}H signal intensity (proportional to the mobile water fraction in the dough), t is the time, λ is the relaxation time (a constant parameter), and A is the signal intensity of protons at t=0. Assays (n=4) were performed in duplicate.

2.2.2.4. Gluten determination. The dry gluten (DG) content of the different formulations was determined in accordance to AACC method 38-12 (2000) modified by Salinas & Puppo (2014). Determinations were carried out in duplicate.

2.2.3. Dough rheological properties

For rheological measurements, dough was laminated (thickness =1 cm) and cylindrical pieces (diameter = 3 cm) were cut using metallic cutters.

2.2.3.1. Texture profile analysis (TPA)

A dough cylinder (n = 15) was subjected to two cycles of compression up to 40% of the original height with a cylindrical probe (diameter = 7.5 cm) using a TA.XT2i Texture Analyzer (Stable Micro Systems, Surrey, U.K.) with a load cell of 25 kg and Texture Expert for Windows version 1.2 Software was used. Force-time curves were obtained at a crosshead speed of 0.5 mm/s. Dough hardness (Hard), consistency (Cons), adhesiveness (Adh), springiness (Spring), and cohesiveness (Cohes) were determined. Hardness is defined as the maximum force during the first compression. Consistency is the sum of the areas under the force vs. time curve corresponding to the first and second compression cycles. Adhesiveness is the negative area in the first cycle. Springiness is calculated as the distance ratio between the beginning and the maximum force of the second and first peaks. Cohesiveness is determined as the ratio between the positive areas of the second and the first cycles (Bourne, 2002). Assays were performed in duplicate.

2.2.3.2. Relaxation test

The relaxation test consists of deforming the material by applying a compression to constant deformation and recording, as a function of time, the force that opposes the material to maintain the deformation selected. For this, discs of dough (n=3) were subjected to a compression of 40% at 0.5 mm/s for 20 min using a TA.XT2i Texture Analyzer (Stable Micro Systems, Surrey, UK) with a load cell of 25 kg. Assays were carried out at 25 °C. To prevent drying of the dough, the cylinders were covered with semisolid Vaseline. A regression of second order of the exponential decay was performed on stress-relaxation curves using Origin Pro 8 software (OriginLab Corporation, MA, USA). A generalized Maxwell model (Steffe, 1996; Rodríguez-Sandoval et al., 2009; Salinas et al., 2012) was applied (Eq. 3):

$$\sigma (t) = \sigma_1 * \exp(-t / T_1) + \sigma_2 * \exp(-t / T_2) + \sigma_3 \qquad (3)$$

Where $\sigma (t)$ represents the stress measured at any time during the test, t is the time. The relaxation time T_i is defined as the ratio between the viscosity and the elastic modulus (Eq. 4) and the elastic relaxation modulus E_i is defined as the ratio between the stress and constant strain (Eq. 5).

$$T_i = \eta_i / E_i \qquad (4)$$

$$E_i = \sigma_i / \varepsilon_0 \qquad (5)$$

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

By applying this model, elastic relaxation moduli (*E*) and relaxation times (*T*) were obtained for the first and second exponential terms. Modulus E_3 corresponds to the equilibrium modulus at infinite time. The assay was performed in duplicate.

2.2.3.3. Dynamic rheological assay

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

2.2.4. Statistical analysis

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

3. Results and discussion

3.1. Gluten content of wheat-amaranth dough

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

3.2. Hydration properties of dough

Control dough had 43% of moisture. This parameter increased with the level of both types of amaranth flours, reaching the highest values with the maximum content of these flours (45%). The highest value of moisture agrees with the highest value of farinograph water absorption obtained (from 55.9 to 58.0 for C and A25, respectively). The increase in farinograph water absorption with the replacement of wheat flour with amaranth flour was previously reported by Bojnanská & Smitalová (2014).

On the other hand, although the amount of water varied, the availability of water represented by water activity (a_w) was statistically the same in all formulations ($a_w \approx 0.97$) (data not shown).

Molecular mobility of water in dough is represented by the ^{1}H spin-spin relaxation time (λ) parameter. Systems with shorter relaxation times are less mobile (solid-like state) than those with longer relaxation times (liquid-like state). High values of λ denote high molecular mobility; it means that water in dough is linked to the other components in a weak form and therefore is in a high-energy mobile state, leading to a more labile gluten structure (Salinas et al., 2012). This phenomenon depends on the molecular structure of all components present in dough. Values of λ of dough are shown in Figure 2. Control dough (C) and dough with 5% of amaranth flour (A5 and GA5) presented the same high molecular mobility. Higher amounts of amaranth flour decreased λ values, associated with less mobility of water due to the presence of the different components of amaranth seeds, mainly proteins and starch, which are able to bind water. The tendency observed for λ was opposite to that obtained for DG water-insoluble proteins reported as dry gluten (Figure 1).

3.3. Texture profile of dough

Different texture parameters obtained from the analysis of the texture profile of dough are listed in Table 1. The addition of amaranth flour, mainly the sample obtained from non-germinated seeds, produced an increase in hardness (Hard) with respect to C; being the highest value observed for A5 dough. This behavior could be due to the incorporation of a certain proportion of globular proteins of 11S and P-globulin type (Avanza & Añón, 2007; Quiroga et al., 2009). These proteins, which are able to bind a higher amount of water than gluten proteins, contribute to the formation of a more structured network because of the gelation process. The presence of these globular proteins also contributes to promoting gluten development (Figure 1). As more amount of amaranth flour is added (25%), there is a dilution effect of the gluten proteins that amaranth proteins cannot compensate, therefore a bit softer dough is obtained at this higher level (Table 1). Nevertheless, with the exception of GA25, higher hardness was obtained for wheat-amaranth dough in comparison with C. This behavior suggests that proteins and also fibers present in amaranth flour reinforce the gluten network. Bigne et al. (2016) obtained similar results with mesquite-wheat dough. Consistency (Cons) also increased in A5 and GA5 dough and a subsequent decrease with the increase in amaranth flour level was observed (Table 1). This decrease can be attributed to changes in amaranth protein structure because of germination, leading to a distinct interaction with wheat proteins and water during matrix formation. No significant differences were observed in adhesiveness (Adh), except for GA15 that presented the highest value. Wheat dough had the lowest value for springiness (Spring), and this parameter increased with the addition of amaranth flour to dough. On the other hand, no significant differences in cohesiveness (Cohes) were observed between the control and dough with 5% amaranth (A5 and GA5); in contrast, dough with 15% and 25% amaranth flour (A15, A25, GA15 and GA25) showed a significant increase in cohesiveness.

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

3.4. Viscoelastic behavior of dough

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

Figure 3 a and b show elastic (E1) and relaxation time (T1), respectively (first zone). Both parameters govern the relaxation at the beginning of deformation, attributed to the reorientation of small molecules. Dough C and A5 presented the highest value of E1, while dough with 15% and 25% of amaranth flour (A and GA) showed lower values of E1 (Figure 3 a), the decrease being more pronounced in GA dough. Results suggest that the germinated amaranth protein in GA25 dough formed small molecules of lower elasticity. On the other hand, this significant variation in E was not reflected in T1, which showed no differences with respect to the C dough with the exception of GA25 (Figure 3 b). This behavior suggests that the GA25 dough had the lowest degree of relaxation (<T1) in zone 1 with a lower E1, indicating a greater contribution of viscosity.

All doughs presented one order higher values of E2, compared to E1 and E3 moduli (Figure 3 a, c, e), due to the presence of polymeric gluten proteins that are greater in size and undergo less relaxation and therefore greatly contribute to dough elasticity. No significant differences in E2 values with respect to C were observed for dough with non-germinated amaranth seeds (A), while for GA, E2 increased with the increment of GA flour (Figure 3 c). This increase in E2 suggests the formation of a structure stabilized by polymers of higher elasticity. This could be due to the contribution of amaranth globular proteins that after germination changed their conformation, acquiring a structure that improved the interaction with water and consequently the structure of dough, which was also evidenced by a lower molecular mobility.

The behavior for relaxation time T2 (Figure 3 d) was similar to that observed for T1; a decrease for GA dough with the increase in the amount of amaranth flour was observed. The very low values of T2 for GA25 suggest a low relaxation degree of gluten polymers in the presence of amaranth proteins, in concordance with the highest value of E2.

Finally, Figure 3 e shows values of E3 that represent the energy storage in dough in a zone (zone 3) where stress does not change with the deformation applied, reaching the equilibrium state. The doughs with the higher values of E3 were those formulated with non-germinated amaranth seeds (A doughs) and GA5, with values higher than C and without significant differences between different levels of A flour. Dough GA15 and GA25 presented lower values of E3, associated with a low elastic behavior at equilibrium, after the deformation process.

In spite of the lower relaxation time T2 and the highest elastic modulus E2 of the polymeric fraction of GA25 dough, this sample presented a higher contribution of the low molecular mass molecules to viscosity (lower E1), accompanied by a very low equilibrium elastic modulus (E3). The relaxation behavior of this dough is in concordance with the low value of consistency observed in TPA (Table 1). Salinas & Puppo (2014) found the same behavior for dough formulated with calcium citrate and 13% of inulin, i.e., low values of hardness together with low values of E3.

Another way to study the viscoelasticity of dough is through dynamic rheology at low deformation. Dough was left to rest for few minutes before the measurement to favor the molecular arrangement of gluten polymers. The viscoelastic parameters obtained from mechanical spectra were storage (G’) and loss (G”) moduli and the ratio G”/G’ =

tan δ (Table 1). Values of G' and G" for amaranth dough increased with respect to sample C. Nevertheless, no significant differences were observed between dough A and GA when the amount of amaranth flour was increased. On the other hand, values of tan δ were the typical ones observed for wheat dough (Letang, Piau, & Verdier, 1999) with values around 0.3. In the case of amaranth-wheat dough, values were in the range 0.341-0.425. Dough A5 and C presented the lowest values of tan δ associated with a major elastic behavior, similar to that observed in the relaxation assay at high deformation (high E1 and E3). At equal amount of amaranth flour, GA samples exhibited higher values of tan δ, suggesting a net increase in the viscous behavior. The increase of tan δ associated with a more viscous matrix agreed with the lower value of consistency obtained by the texture assay, the effect being more pronounced for GA25 (Table 1).

Another alternative for analyzing mechanical spectra is by evaluating the dependence between G' and G" in all the frequency range studied (Figure 4). The equality of the G' and G" moduli (tan δ = 1) is evidenced by a red line at 45º. The relationship between these two moduli was evidenced by a curve. The proximity of this curve to the red line suggests a more viscous behavior of the sample. In turn, the slope of curve G' versus G" has been used as an indicator of changes in the morphology of the different polymers (Ahmed et al., 2013). A superposition of the curves indicates that there are no differences in the morphology of the polymers, while no superposition suggests the formation of a heterogeneous matrix. Dough with curves with a high slope refers to a more elastic network (Salinas et al., 2015). At low frequencies (<0.05 Hz), at which practically there is no deformation of dough and the changes observed are attributable to the nature of the dough structure, the curve of dough C was the highest one, while curves of A5, A15 and A25 were placed below, A5 being the lowest one (Figure 4 a). At low level of amaranth flour (5%) a softening of the gluten matrix would be produced, while at high levels this weakening effect would be compensated by a reinforcement of the gluten structure probably due to the contribution of the globular amaranth proteins. For this reason, at the highest level (25%) the amaranth-wheat dough (A25) presented a G' vs. G" behavior similar to that obtained for the control dough. In contrast, dough with GA (Figure 4 b) showed a similar tendency to that observed for the dough with non-germinated amaranth seed flour at low frequencies (<0.03 Hz). The main differences for the GA dough are that the curve furthest from that of C was GA25. This behavior suggests that with a high amount of GA, the content of low molecular mass molecules present in dough is higher, due to de-polymerization during germination that leads to a less structured matrix with a more viscous rheological performance. For all the doughs assayed, at high values of G' and G" a total superimposition of the curves was observed, suggesting an equal behavior of both moduli at high deformation frequencies.

4. Conclusions

The incorporation of amaranth flour (up to 25%) produced relatively minor changes in the physicochemical and rheological properties of wheat dough, including higher water absorption and presence of water-insoluble proteins. In addition, the flour obtained from germinated seeds had a different behavior in the parameters studied with respect to the flour obtained from the non-germinated seeds. The GA25 dough had the highest water content and also a lower molecular mobility associated with a certain degree of structure of dough. The GA25 dough presented the same hardness although a greater elasticity (TPA) than the control dough, due to the modification of the globular amaranth proteins as a consequence of seed germination. However, the dough was more viscous (greater tan δ and smaller E3), possibly due to morphological changes in the gluten structure with respect to wheat dough. Overall, wheat flour supplemented with up to 25% amaranth flour obtained from germinated or non-germinated seeds produced changes in water absorption, it was possible to obtain dough of acceptable rheological properties for breadmaking.

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FIGURE CAPTIONS

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

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

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

Fig. 4 Elastic modulus (G’) as a function of viscous modulus (G’’) of wheat flour dough with: non-germinated (**a**) or germinated (**b**) amaranth flours.

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 ($p < 0.05$).

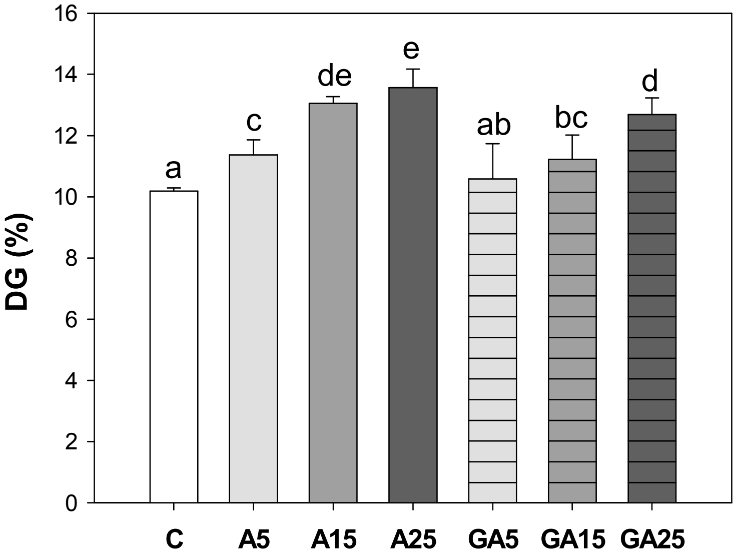

Figure 1

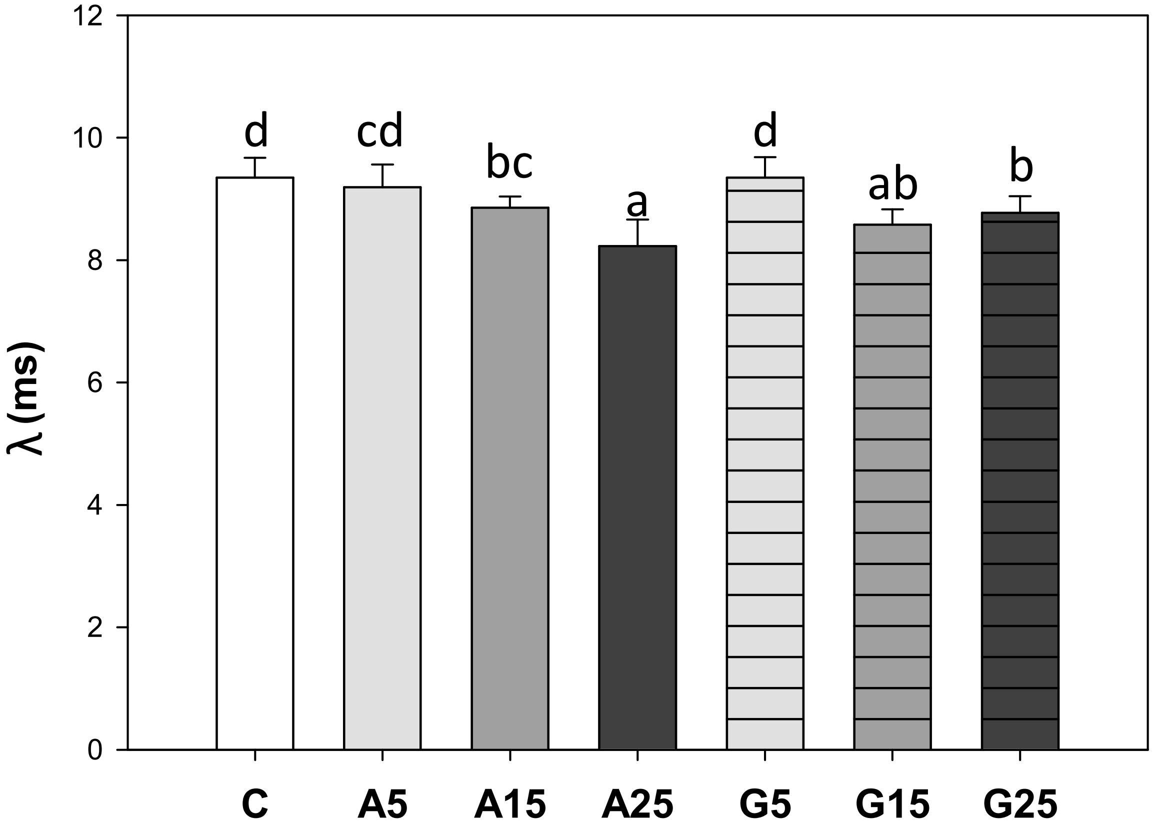

Figure 2

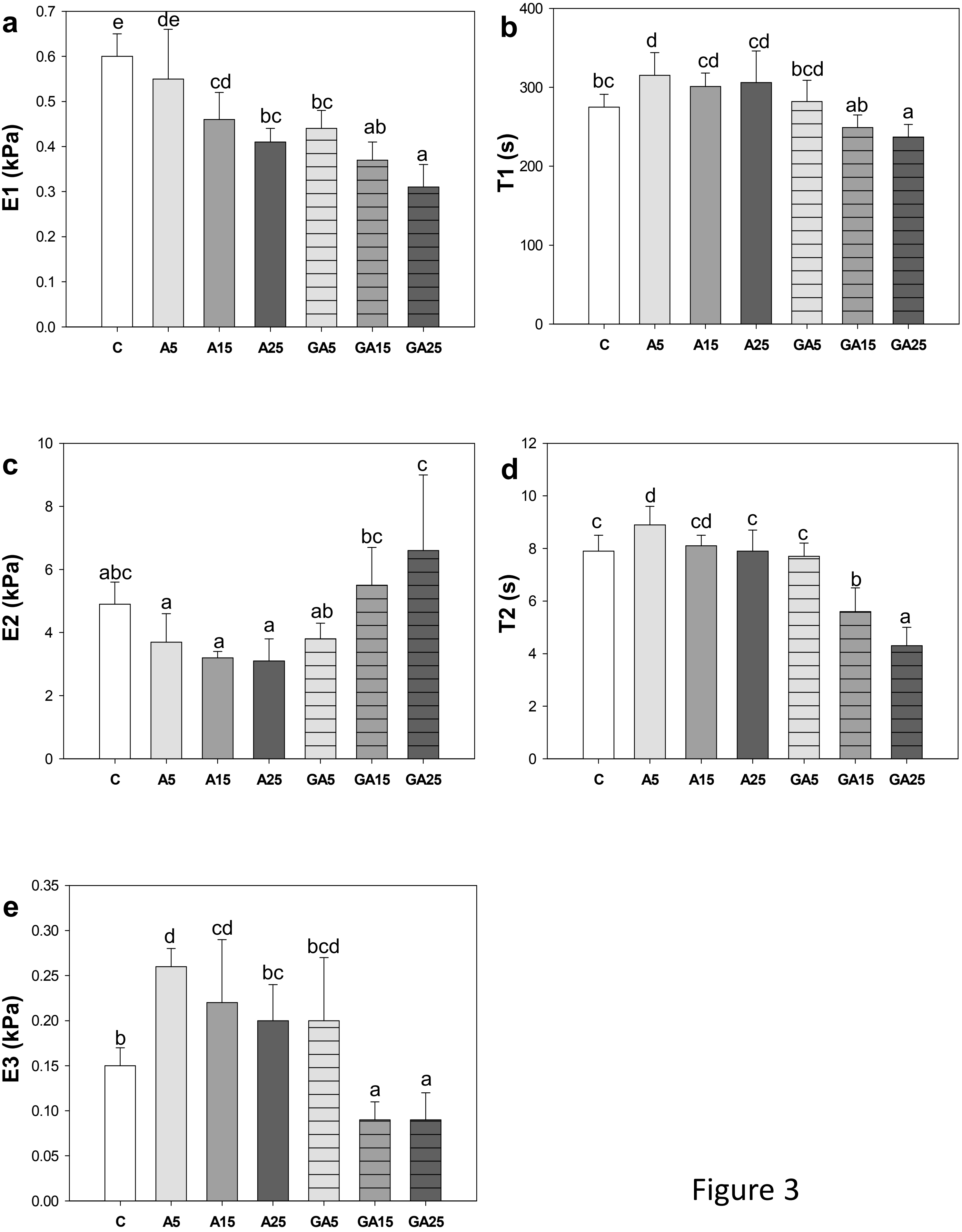
a
E1 (kPa)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
e
de
cd
bc
bc
ab
a
C
A5
A15
A25
GA5
GA15
GA25
b
T1 (s)
0
100
200
300
400
bc
d
cd
cd
bcd
ab
a
C
A5
A15
A25
GA5
GA15
GA25
c
E2 (kPa)
0
2
4
6
8
10
abc
a
a
a
ab
bc
c
C
A5
A15
A25
GA5
GA15
GA25
d
T2 (s)
0
2
4
6
8
10
12
c
d
cd
c
c
b
a
C
A5
A15
A25
GA5
GA15
GA25
e
E3 (kPa)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
b
d
cd
bc
bcd
a
a
C
A5
A15
A25
GA5
GA15
GA25

Figure 3

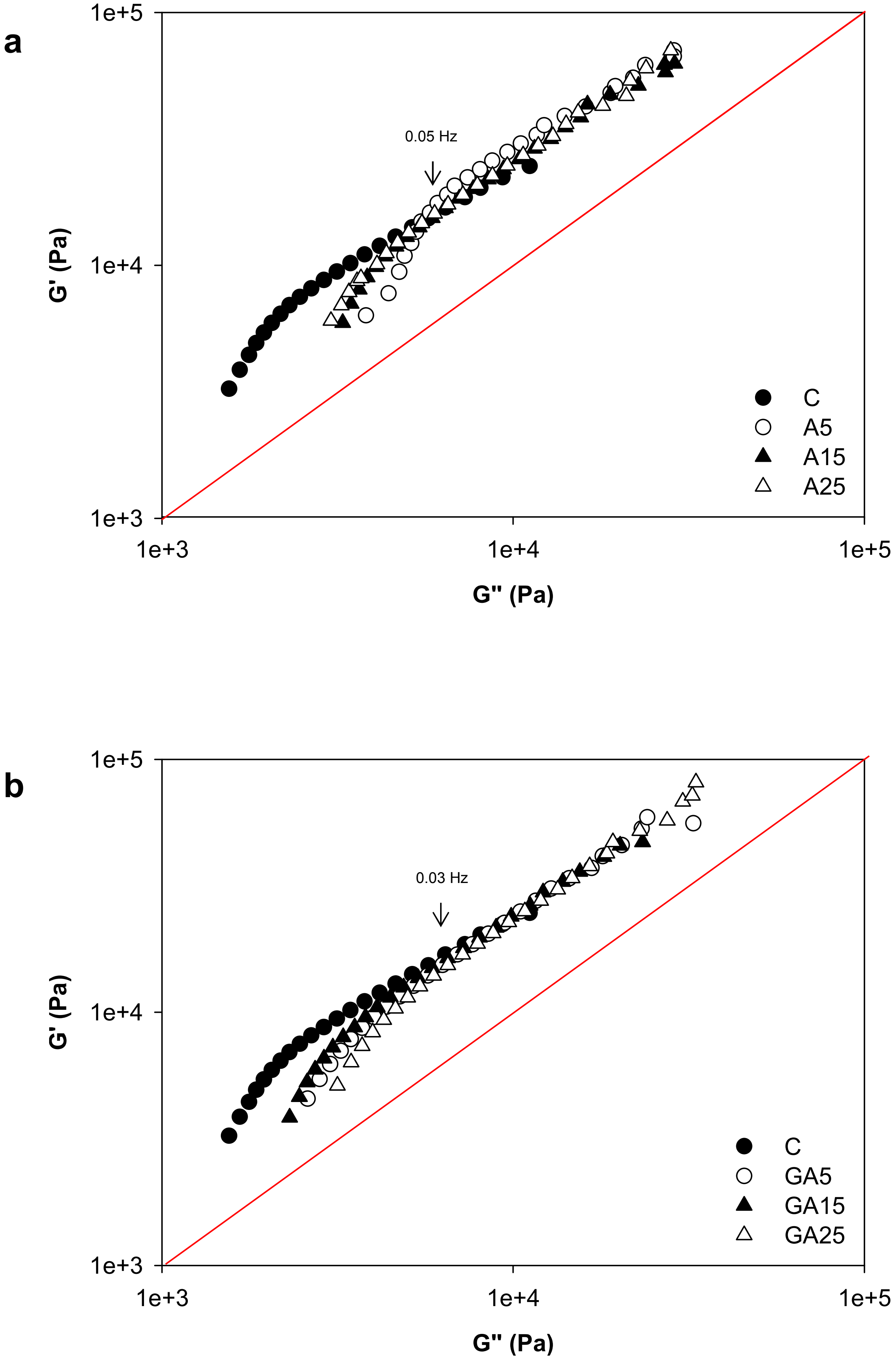
a
1e+5
1e+4
1e+3
G' (Pa)
1e+3
1e+4
1e+5
G" (Pa)
0.05 Hz
● C
○ A5
▲ A15
△ A25
b
1e+5
1e+4
1e+3
G' (Pa)
1e+3
1e+4
1e+5
G" (Pa)
0.03 Hz
● C
○ GA5
▲ GA15
△ GA25

Figure 4