



Effect of freezing and frozen storage on mesquite–wheat dough for panettone-like breads

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Abstract

The aim of this work was to study the viability of freezing preservation of composite wheat–mesquite dough of panettone-like bread. Three levels of replacement of wheat by mesquite flour were assayed: 15, 25 and 35% (w/w). Texture profile analysis, stress relaxation assays and dynamic oscillatory measurements were conducted to characterize the rheological behavior of thawed dough after different times of frozen storage at $-20\text{ }^{\circ}\text{C}$. Moisture, water activity and freezable water by DSC were measured. The damage by freezing on dough structure was analyzed by ESEM. Composite wheat–mesquite dough could undergo frozen storage without drastic rheological changes when 15–25% level of replacement was used. With the maximum level of mesquite, 35%, a less regular and crosslinked network than the control without mesquite could be observed in fresh dough. This network resulted more susceptible to damage by ice formation leading to lesser cohesiveness and elasticity of dough after thawing.

Keywords Algarrobo · Frozen dough · Texture · Dynamic moduli · ESEM

Introduction

Bread is one of the most widely consumed foods all over the world, but it has a short shelf life due to staling. During storage the occurrence of different physicochemical phenomenon such as starch retrogradation [1] and moisture loss that increases bread hardness [2], contribute to the loss of quality. Therefore, the breadmaking industry has searched alternative technologies for being able to supply fresh breads at any moment. Frozen dough technology can effectively allow having fresh breads at the moment of consumption; this process is now being widely used and is gradually replacing the traditional production of bread [3]. However, during preparation and frozen storage, dough can undergo several phenomena as inhibition of yeast activity and damage of dough structure that can result in deterioration of dough. In fact, when dough is subjected to below zero temperatures,

free water forms ice crystals that may grow and damage the gluten network during storage [4, 5].

Gluten is mainly damaged by freezing as demonstrated by Wang et al. [6] in dough of steamed breads undergoing frozen storage or freeze–thaw cycles. These treatments produced gluten macropolymer depolymerization and weight loss of dough leading to a reduction of bread volume. Angioloni et al. [7] have attributed changes in viscoelastic behavior of dough to the damage on the gluten cross-linking, mainly produced by ice crystallization. Frauenlob et al. [8], examined the influence of flour quality on the properties of bread made from pre-fermented frozen dough, reported that flours with stronger gluten networks were most suitable for frozen dough production.

The effect of freezing rate on frozen sweet dough was analyzed by Meziani et al. [9] who found a significant effect on dough viscoelastic and structural parameters. Air blast freezing increased significantly the elastic modulus (G') values and decreased $\tan \delta$ (G''/G') parameters with respect to fresh dough. Slow freezing caused protein aggregation manifested by a decrease in the amount of α -helix and the increase in β -sheet structure. Rheological changes in frozen sweet dough were attributed to the formation and the mechanical action of ice crystals, leading to dehydration of the dough components, mainly at low freezing rates. Fast

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freezing rate by cryogenic freezing immersion gave the global best results with regard to the rheological properties of frozen/thawed dough. Öhgren et al. [10] studied the quality of bread prepared with sweet frozen dough varying the time of kneading. These authors found that a longer dough kneading time was necessary to obtain a well-distributed and homogeneous gluten network at higher sugar contents. As frozen storage conducted to the contraction of gluten network leading to a less well-distributed gluten structure, the increase in kneading time of dough, especially for the sweet dough, could help to improve the quality of bread.

The incorporation of certain ingredients, such as leguminous flours seems to have a protective effect on dough structure when it undergoes a freezing process. Simmons, Smith and Vodovotz [11] found that defatted soy flour and soy milk powder produced a stabilization of bread dough during frozen storage. Comparing with wheat bread, soy bread was denser and chewier, with higher moisture content. In addition, soy-dough better retained quality characteristics during frozen storage.

Chickpea flour was also reported to contribute to prevent damage of dough structure during frozen storage. Ozulku and Arici [12] characterized rheological and technological properties of frozen sourdough ($-35\text{ }^{\circ}\text{C}$, 28 days) added with chickpea flour (25 and 50%). The elastic modulus (G') of chickpea–wheat sourdough did not change along frozen storage; while for wheat dough decreased. Besides, specific volume after frozen storage of dough was higher in chickpea–wheat breads, with respect to wheat breads.

Mesquite (algarrobo) flour is obtained by grinding the whole pod of *Prosopis* spp. tree, which belongs to the leguminous family. This flour is pleasant flavored and sweet and it is used in many traditional food products in different countries of the American continent. In a previous study on mesquite–wheat flour blends [13], the effect of applying part-baking technology on panettone-like breads with different contents of *Prosopis alba* (mesquite) flour was reported. Using this technology, after eight weeks of frozen storage at $-18\text{ }^{\circ}\text{C}$, no changes were observed in the texture parameters of breads in comparison with non-frozen bread.

The aim of the present work was to apply frozen storage for the preservation of sweet mesquite–wheat dough and to study the damage that freezing produced on dough structure with the consequent changes in rheological and water binding properties of dough.

Materials and methods

Materials

Commercial wheat flour (WF) (type 000 according to CAA) was provided by Molino Campodónico SA (La Plata,

Argentina) and the flour from algarrobo (mesquite) pods (MF) was obtained from INTI ATUN farm (Santiago del Estero, Argentina). Values of moisture were 13.7% for WF and 10.3% for MF. Wheat and mesquite flours contained (w/w) 12.1 and 7.8% of proteins, 1.3 and 1.3% of lipids, 0.7 and 2.7% of minerals (measured as total ash), 3.7 and 21.9% of total dietary fiber, respectively. Sugar composition of MF was: 44.8% sucrose, 3.2% fructose and 1.0% glucose.

Methods

Formulations

Four formulations (Table 1) of sweet panettone-like bread were prepared with increasing partial replacement of WF by MF (0, 15, 25 and 35%). Concomitantly with the addition of MF, the amount of sugar in the formulations was reduced, so as to maintain a constant value of 25 g sugar/100 g total flour blend. This correction was done to balance sugar content and flavor, because MF naturally presents a high content of carbohydrates (approximately 49%).

Dough preparation and frozen storage

Doughs were prepared according to the procedure described by Bigne et al. [13] but without adding yeast. Firstly, solid components along with the melted margarine were mixed during 1 min in a planetary kneader (Kenwood, Treviso, Italy). After that, liquid egg and water were incorporated and mixed during 30 min at 90 rpm. Dough was divided into 200 g portions and pieces were wrapped in individual plastic bags suitable for freezing and frozen in a chamber with force air displacement at $-30\text{ }^{\circ}\text{C}$ during 2 h. The time selected was enough for achieving a temperature close to $-15\text{ }^{\circ}\text{C}$ in the center of each piece. Bags containing dough were stored in a freezer at $-20\text{ }^{\circ}\text{C}$ during 90 days. For each formulation two independent batches of dough were prepared. After

Table 1 Formulations utilized for obtaining composite wheat–mesquite sweet doughs

Component	Formulations (g)			
	MF0	MF15	MF25	MF35
Wheat flour	1000	850	750	650
Mesquite flour	0	150	250	350
Water	240	240	240	240
Sugar	250	168	112	57
Margarine	200	200	200	200
Liquid egg	170	170	170	170
Salt	10	10	10	10

MF0, MF15, MF25, MF35: formulations with 0, 15, 25 and 35% of replacement of wheat flour by mesquite flour

15, 30, 60 and 90 days, samples were thawed in a chamber at 25 °C during 120 min. Thawed doughs were analyzed with the methods described in “[Rheological measurements](#)”, “[Water properties](#)”, and “[Microstructure characterization by environmental scanning electron microscopy \(ESEM\)](#)”.

Rheological measurements

The effect of freezing time on dough rheology was evaluated by different rheological assays on thawed dough, at low and high deformations. Doughs without freezing were used as control samples.

Texture profile analysis (TPA) This assay consisted in a double compression up to 40% of the original height of dough disc (height: 2 cm, diameter: 3 cm). A Texture Analyzer TA.XT2i (Stable Micro Systems, Godalming, UK) equipped by a flat probe (SMSP/75) was utilized. Dough pieces were compressed at a rate of 1 mm/seg. From force versus time curves different parameters were obtained: hardness (maximum force of the first peak), adhesiveness (total area of the negative peak), cohesiveness (ratio between the area corresponding to the second positive peak and the area of the first one) and springiness (ratio between the distance between the beginning and the maximum of the second peak and the same distance measured in the first peak). Sixteen measurements were performed for each formulation.

Stress relaxation assays A Texture Analyzer TA.XT2i (Stable Micro Systems, Godalming, UK) with a flat probe (SMSP/75) was used to perform a single compression up to 40% during 20 min on dough discs. Solid-vaseline was used to cover dough edges to prevent dehydration during test. Force was registered as function of time and stress was calculated. Stress decay curves were fitted with the Maxwell model (Eq. 1) to characterize the systems [14].

$$\sigma = A_1 e^{-\frac{t}{\lambda_1}} + A_2 e^{-\frac{t}{\lambda_2}} + \sigma_0 \quad (1)$$

In Eq. 1: σ is the stress (Pa); A_1 and A_2 are pre-exponential factors (Pa); t is time (min); λ_1 and λ_2 are the relaxation times and σ_0 is the equilibrium stress (Pa).

Small amplitude oscillatory tests Dynamic rheological parameters of dough were analyzed in a RheoStress RS600 (Haake, Waltham, MA, USA) equipment using a probe of parallel plates with rough surface and a gap of 1.5 mm. Elastic (G') and viscous (G'') moduli were registered as function of frequency (0.01–100 Hz) within the linear viscoelastic range (5 Pa) at 25 °C. Assays were performed at least by triplicate. Solid-vaseline was used to cover dough edges to prevent dehydration during testing.

Water properties

Moisture content The moisture content was determined on dough pieces by drying samples until constant weight in oven at 105 °C according to de official AACC method 44-15A [15]. Measurements were done by triplicate.

Water activity The water activity (a_w) of dough was determined with a Meter Aqualab series 3 equipment (Decagon Devices Inc., Washington, USA). Measurements were performed by triplicate.

Total freezable water Total freezable water of dough was determined using a differential scanning calorimeter equipment Q100 (TA Instruments, New Castle, DE, USA). Dough samples (10–15 mg) were introduced in aluminum capsules that were hermetically sealed. Capsules were kept 5 min at 5 °C, cooled to – 32 °C at a freezing rate of – 0.33 °C/min. Then an isotherm during 45 min and an ulterior freezing to – 40 °C at – 2 °C/min was applied. Finally, samples were subjected to a heating ramp at 2 °C/min up to 5 °C. From thermograms, glass transition temperatures of the maximum concentrated matrix (T_g'), temperatures and enthalpies of ice melting were obtained. From values of enthalpies the amount of freeze water and the percentage of freezable water of dough were calculated. Assays were performed by triplicates on control dough without MF (MF0) and dough with the maximum level of MF (MF35).

Microstructure characterization by environmental scanning electron microscopy (ESEM)

Samples corresponding to day 0 (fresh doughs) and frozen stored during 90 days were unfrozen and qualitatively analyzed by ESEM. Portions of approximately 0.1 g were placed onto a specific supports for observation in an FEI Quanta 200 microscope (Thermo Fisher Scientific, Waltham, MA, USA) at 10 °C and 4.14 Torr of pressure. Micrographs of several fields of each specimen at different magnifications (between $\times 800$ and $\times 5000$) were acquired.

Statistical analysis

The software Statgraphics Centurion XV v15.2.06 (Stat-Point Inc.) was used for the statistical analysis. A two-way ANOVA was performed to discriminate the effect and interactions of the both factors analyzed. Fisher LSD tests were applied for determining the significant differences among means at a confidence level of 95%.

Results and discussion

Rheological properties

Doughs prepared with MF were soft and sticky, particularly those with higher levels of MF. The amount of water used for these formulations (Table 1) was considerably lower, almost the half than that used in other pastry products [16]. Nevertheless, it must be taken into account that the liquid egg contributes not only with water but also with lipids that jointly with the high content of margarine account for the rheological properties of dough. The plasticizer effect of fat in dough has been previously described by several authors [17, 18].

Figure 1 shows the changes in texture parameters of frozen/thawed doughs as a function of frozen storage time. There was a significant ($p < 0.05$) effect of the type of formulation and the length of frozen storage period on hardness, adhesiveness and cohesiveness. Control dough without mesquite (MF0) presented values of 1.5 ± 0.1 N, 0.93 ± 0.01 , 0.72 ± 0.01 and 2.4 ± 0.3 N s (mean \pm SD) for hardness, springiness, cohesiveness and adhesiveness,

respectively. These results show that the freezing/thawing procedure reduced hardness and springiness and increased adhesiveness after two weeks of frozen storage. Several authors have suggested that the ice crystals formed during freezing and their subsequent recrystallization along the storage period can severely damage the dough structure leading to poor rheological properties and the subsequent loss of breadmaking quality [5, 7, 19]. In spite that the moisture content did not change during storage (data not shown), the increase in adhesiveness indicates that water migrated to surface, thus leading to stickier dough.

At intermediate levels of wheat replacement with MF (MF15 and MF25) hardness, springiness and cohesiveness of the composite dough remained almost invariant during storage (Fig. 1). For MF15, hardness varied between 1.2 ± 0.1 and 1.4 ± 0.1 N, the elasticity ranged from 0.90 ± 0.01 to 0.92 ± 0.01 and cohesiveness varied between 0.70 ± 0.01 and 0.73 ± 0.01 . For MF25 hardness ranged between 1.0 ± 0.2 and 1.5 ± 0.1 N, the springiness, from 0.89 ± 0.03 and 0.91 ± 0.01 and the cohesiveness, from 0.70 ± 0.01 to 0.72 ± 0.01 . MF35 dough exhibited a significant ($p < 0.05$) decrease in hardness after 90 days of storage but cohesiveness and springiness were much less affected. Adhesiveness

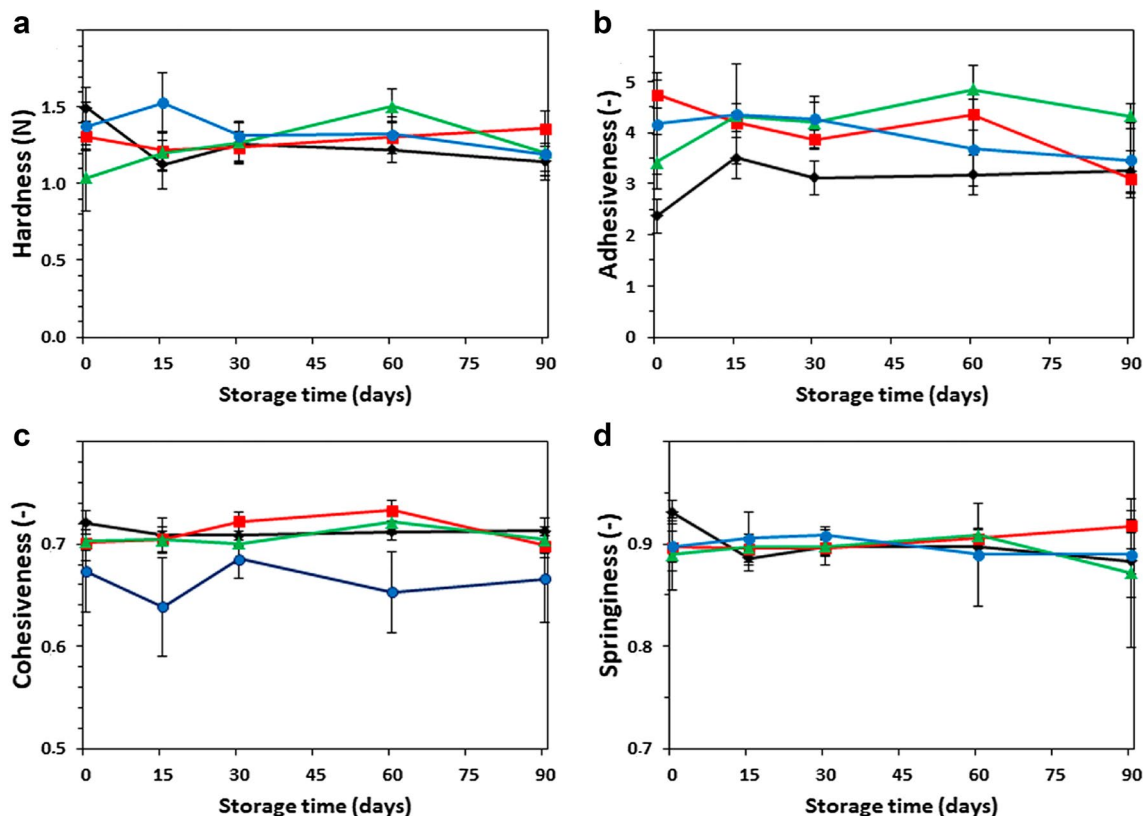


Fig. 1 Evolution of hardness (a), adhesiveness (b), cohesiveness (c) and springiness (d) during the frozen storage of 90 days for doughs with 0% (black filled diamond), 15% (red filled square), 25% (green

filled triangle) and 35% (blue filled circle) of replacement of WF by MF. Mean values from at least 15 replicates are reported. Bars indicate standard deviations (Color figure online)

was slightly affected by frozen storage but it significantly decreased after 90 days of frozen storage for MF35. The minor changes on texture attributes that were observed along frozen storage suggest that the main damage was produced by ice formation during freezing.

In Table 2 the coefficients obtained by modeling the relaxation curve for fresh and frozen doughs (90 days storage) are shown. Comparing the values of equilibrium stress (σ_0) obtained for fresh samples, no significant differences ($p > 0.05$) were found. For relaxation times (t_1 and t_2), the trend was the same in both cases: when MF content was increased, lower relaxation times were obtained. Higher relaxation times are associated to a slower decay during relaxation, a typical behavior of solid-like viscoelastic systems, which are more ordered matrices [20]. These higher relaxation times usually correspond to the relaxation of polymers like gluten in the case of dough [21]. The decrease in relaxation times with the increase of MF content suggests that the system is less elastic, which is in accord with the changes caused by gluten network dilution when WF is replaced by MF, as described in previous works [14, 22]. With freezing and frozen storage, relaxation times (t_1) exhibited a decreasing tendency, though differences were significant only at the highest level of replacement (MF35). This behavior is also in agreement with the changes observed in hardness and springiness (TPA) for MF0 and MF35.

In Fig. 2, the mechanical spectra for all formulations (without freezing or fresh and after 90 days storage) are shown. In all cases, the spectra correspond to viscoelastic systems, where the storage (G') and the loss (G'') moduli are strongly dependent on frequency. The behaviors observed in rheometric dynamic assays are in agreement with those

described previously by Bigne et al. [22] in composite doughs WF–MF.

For samples MF0, MF15 and MF25 no significant modifications ($p > 0.05$) on mechanical spectra were observed before and after frozen storage (Fig. 2a–c). On the other hand, the dynamic moduli of MF35 significantly decreased ($p < 0.05$) after the frozen storage (Fig. 2b).

When changes in G' (measured at 1 Hz) were analyzed along all the storage period (Fig. 3a) significant effects ($p < 0.05$) of both factors (the storage time and the level of replacement) were found. In the case of MF35 G' continuously diminished along storage period (Fig. 3a). Only the level of replacement had a significant effect on $\tan \delta$ (Fig. 3b). In all cases, the values of $\tan \delta$ remained almost constant along storage (Fig. 3b), indicating that the relative contributions of elastic and viscous behaviors did not change. Significant differences ($p < 0.05$) in $\tan \delta$ were found between samples MF35 and MF0. The decrease in elastic modulus for MF35 due to frozen storage is in accord with the changes observed in hardness.

Changes in frozen/thawed dough can be related to the rupture of the gluten network due to freezing along with progressive dehydration. Yeast destruction by freezing releases some compounds as glutathione than can decrease dough resistance and increase dough extensibility, nevertheless gluten damage is also found in non-yeasted dough. Fat, emulsifiers, milk components, egg, additional gluten, enzyme transglutaminase are some of the ingredients used for improving frozen/thawed dough performance [23].

Water properties and thermal transitions

Moisture and water activity (a_w) of the different formulations are shown in Table 3. As a constant level of water was used and the added sugar decreased when MF level increased (as reported in Table 1), a higher ratio of water/solid components was obtained for composite dough. Water activity is related to the type of water interaction with the other components of the flours; MF contributes mainly with soluble and insoluble fiber, proteins, sucrose and other monosaccharides leading to an increased interaction with water and water linking. Thus, values of a_w were slightly lower than control (0.935) in formulations with MF and significantly lower for MF35 (0.931). Along freezing storage no significant changes were detected in moisture and a_w values of thawed dough respect to fresh dough (data not shown). This is a positive result because one of the major problems during frozen storage of dough is the exudate produced after defrosting related to the loss of water retention by dough [24].

Sub-zero thermal transitions of the formulations MF0 and MF35 were evaluated. The glass transition temperature (T_g') and the ice melting temperature (T_m') of the maximally concentrated frozen matrix were significantly higher for

Table 2 Fitting parameters from stress relaxation curves for composite wheat–mesquite doughs

Treatment	Formulation	t_1	t_2	σ_0
Fresh dough	MF0	283 ^d	10 ^{bc}	231 ^a
	MF15	250 ^{bcd}	9 ^{ab}	233 ^a
	MF25	256 ^{bcd}	8 ^a	291 ^a
	MF35	249 ^{bc}	8 ^a	258 ^a
90 days freeze stored dough	MF0	276 ^{cd}	10 ^b	196 ^a
	MF15	227 ^{ab}	8 ^a	183 ^a
	MF25	250 ^{bcd}	9 ^{abc}	270 ^a
	MF35	202 ^a	8 ^a	203 ^a
	PSD	28	1	77

MF0, MF15, MF25, and MF35: formulations with 0, 15, 25 and 35% of replacement of wheat flour by mesquite flour; T_1 , T_2 and σ_0 : fitting parameters from stress relaxation curves, corresponding to relaxation times and equilibrium stress. Mean values from measurements of at least four replicates are reported. Different letters within the same column indicate significant differences by LSD test ($p < 0.05$)

PSD pooled standard deviation

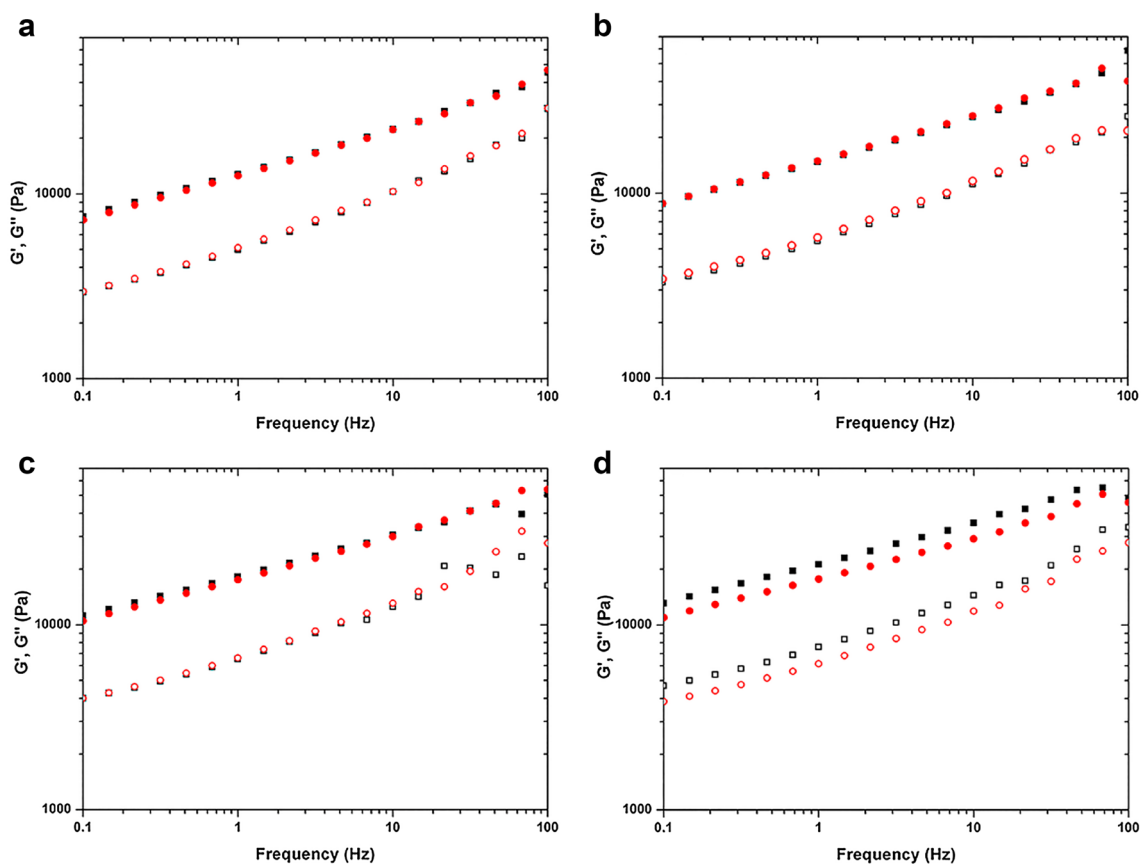


Fig. 2 Typical curves of dynamic moduli G' and G'' as function of frequency for fresh (black filled square, open square) and frozen stored during 90 days (red filled circle, open circle) doughs with 0% (a), 15% (b), 25% (c) and 35% (d) of replacement of WF by MF (Color figure online)

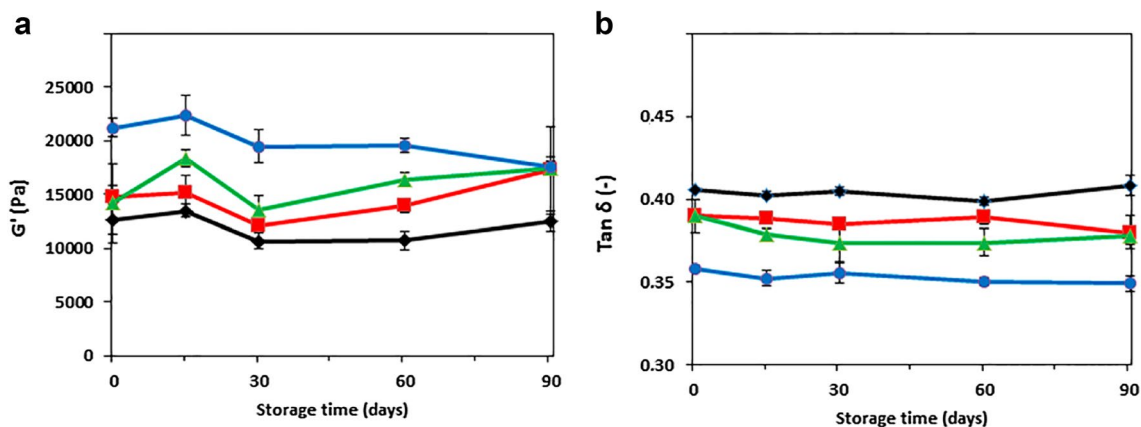


Fig. 3 Evolution of elastic modulus (a) and $\tan \delta$ (b) during the frozen storage of 90 days for doughs with 0% (black filled diamond), 15% (red filled square), 25% (green filled triangle) and 35% (blue

filled circle) of replacement of WF by MF. Mean values from at least triplicates are reported. Bars indicate standard deviations (Color figure online)

MF0 ($-30.9\text{ }^{\circ}\text{C}$ and $-8.6\text{ }^{\circ}\text{C}$, respectively) than for MF35 ($-31.7\text{ }^{\circ}\text{C}$ and $-9.0\text{ }^{\circ}\text{C}$) but no significant differences ($p > 0.05$) were found in the relative amount of freezable water for both dough (mean = $42 \pm 1\%$). According to this, the ratio unfreezable water / amount of solids were 0.24 g

water/g solids for MF0 and 0.29 g water/g solids for MF35. As the sample MF35 has a higher unfreezable water/solids ratio, it would be indicating that the lesser amount of solids is capable of linking more water and this fact is in agreement with the significantly lower a_w observed for MF35.

Table 3 Water related and DSC parameters for composite wheat–mesquite sweet doughs

Formulation	Moisture (g/100 g)	a_w	Tg' (°C)	Tm' (°C)	Freezable water (%)
MF0	29.5 ^a	0.935 ^b	−30.9 ^b	−8.6 ^b	41 ^a
MF15	31.3 ^b	0.932 ^{ab}	ND	ND	ND
MF25	32.3 ^c	0.932 ^{ab}	ND	ND	ND
MF35	32.9 ^d	0.931 ^a	−31.7 ^a	−9.0 ^a	43 ^a
PSD	1.2	0.002	0.4	0.3	2

MF0, MF15, MF25, and MF35: formulations with 0, 15, 25 and 35% of replacement of wheat flour by mesquite flour. Mean values from measurements by triplicates are reported. Different letters within the same column indicate significant differences by LSD test ($p < 0.05$)

a_w , water activity measured at 25 °C; Tg', glass transition temperature of the matrix maximally concentrated; Tm, fusion temperature of ice; PSD, pooled standard deviation; ND, no determined

Microstructure

Figure 4 shows representative images of fresh control and composite dough microstructure obtained by ESEM. It is possible to appreciate some structural differences among doughs with different levels of replacement, MF0, MF15 MF25 and MF35 (Fig. 4a–d, respectively). When MF content was increased, the gluten network resulted less uniform, with less proportion of gluten films (GF) and more proportion of gluten strands (GS). The development of an adequate gluten network, characterized by film formation and a high degree of protein crosslinking is the cause of the elastic properties of wheat dough [25]. On the other hand, the structural changes and the dilution of gluten proteins caused by MF incorporation can explain the decrease in springiness and cohesiveness described in a previous section.

In micrographs of Fig. 4e–h, dough corresponding to MF0–MF35 after 90 days of frozen storage are shown. For MF0, protein aggregation under the form of thick strands can be observed (Fig. 4e) in contrast with the fresh dough appearance (Fig. 4a) where films covered a great part of starch granules surface. Similar thick strands are also visible in composite dough after freezing (Fig. 4f–h) especially in

MF35. MF35 was characterized by a coarse strand structure after frozen storage (90 days) and these observations are in agreement with the lesser tolerance to freezing (higher modifications of G' and textural parameters) with respect to the other samples. These dissimilar structures can be related to the drastic changes in water distribution during frozen storage and after thawing. Esselink and van Duynhoven [26] demonstrated that gluten resulted dehydrated during freezing and frozen storage leading to changes in water distribution after thawing. In the same trend, Baier-Schenk et al. [27] also concluded that growth of ice crystals led to the redistribution of frozen water affecting the properties of gluten and consequently the baking performance of dough.

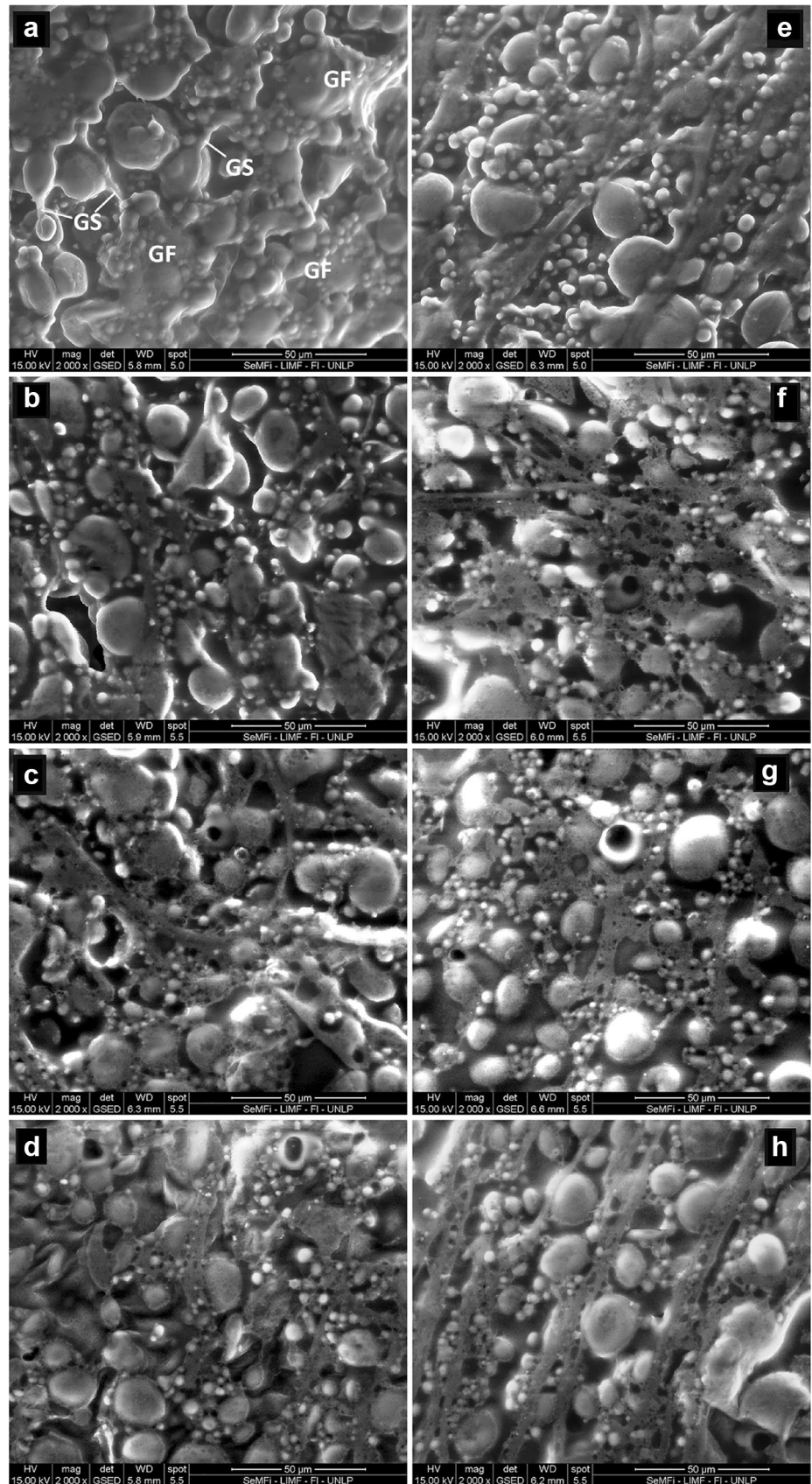
Conclusions

In the present work, mesquite flour, a valuable nutritional ingredient for breadmaking, was assayed in the formulation of panettone-like dough to be preserved by freezing and stored at −20 °C. Being the panettone a typical sweet bread whose demand is seasonally increased, the possibility of preserving dough by freezing until the moment of its baking could facilitate the supply of this product.

The effect of freezing and frozen storage on dough rheology and microstructure varied depending on the level of replacement with mesquite. At intermediate levels of replacement (15 and 25%), the relative stability of the rheological parameters associated to dough integrity (cohesiveness) and its viscoelasticity (elasticity and $\tan \delta$) as well as moisture and water activity values during frozen storage indicate an acceptable tolerance respect to the freezing process.

On the other hand, the increased susceptibility to frozen storage observed for the composite dough with the maximum level of replacement (35%) could be attributed to a more pronounced damage in gluten network due to ice formation. In this case, the presence of a high amount of mesquite flour led to a poorer development of the gluten network and thus, weaker dough was obtained. This network was even more weakened during frozen storage thus suggesting an enhanced susceptibility to damage by freezing.

Fig. 4 Micrographs obtained by ESEM with magnification of $\times 2000$ for no frozen and after 90 days of frozen storage doughs with 0% (**a**, **e**), 15% (**b**, **f**), 25% (**c**, **g**) and 35% (**d**, **h**) of replacement of wheat flour by mesquite flour, respectively. *GS* gluten strand, *GF* gluten film



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