Texture development in gluten-free breads: Effect of different enzymes and extruded flour

Mario M. Martínez, Pablo Marcos, Manuel Gómez*

Food Technology Area, E.T.S. Ingenierías Agrarias. Valladolid University, Ed. La Yutera, Avd. Madrid 44, 34004 Palencia, Spain.

[*]Corresponding author e-mail: pallares@iaf.uva.es

Abstract

One of the main problems with gluten-free breads is their texture and their rapid staling. In this work the influence of different enzymes (one protease, one lipase and two amylases) and of extruded rice flour on rice-bread texture and texture development was studied. For this purpose, the development of firmness, cohesiveness, resilience, springiness and chewiness was modelled and the parameters that define the initial values and the development of these characteristics were measured. The addition of lipase and extruded flour increased bread volume and reduced the initial firmness and hardening of breads. There was an early fall in cohesiveness and resilience, with minimum values reached a few days after elaboration. There were 99.9% significant correlations between bread density and firmness, springiness and chewiness development.

PRACTICAL APPLICATIONS

This methodology simplifies the study of gluten free breads textural parameters during storage and the result interpretation. Moreover, the correlation analysis has demonstrated that the number of textural parameters of gluten free breads to study can be reduced. Finally, the results obtained show that use of enzymes and extruded flours decreases bread staling.

Keywords: rice bread, staling, lipase, amylase, pre-gelatinized flour.

1. Introduction

In recent years there has been increasing interest in the development of high-quality gluten-free products and this has led to an increase in the volume of research and in the number of scientific publications on this subject. Gluten-free breads are characterized by a deficient quality and high cost compared to traditional bread, leading to a low turnover of products and hence the need to obtain breads with a long shelf-life. The staling of baked products is usually defined as an increase in crumb firmness and a loss of product freshness. The retrogradation of starch, a major component of flour, plays an important role in these changes. In the case of gluten-free bread and, in particular, when rice flour is used, these staling phenomena may be augmented because retrogradation has been found to be greater with rice starch than with wheat starch (Baker and Rayas-Duarte, 1998). It has also been observed that rice bread is drier and crumblier than wheat bread and that it shows greater retrogradation during storage than wheat bread (Kadan et al., 2001).

Although there are fewer studies of staling and of its prevention with gluten-free breads than with wheat bread, the interest in this subject has increased in recent years. Ziobro et al. (2012) observed that the staling of breads made with potato and corn starch can be reduced during the first 48 hours by the addition of modified starches such as hydroxypropyl distarch phosphate. Purhagen et al. (2012) showed that the addition of emulsifiers could decrease bread hardening over the first three days. That finding coincides with the observations of Nunes et al. (2009) after the addition of sodium stearoyl lactylate (SSL) and monoglycerides, but not with other emulsifiers, such as lecithin or DATEM. The addition of gums with a high water-holding capacity, such as oat β-glucan (Hager et al., 2011), xanthan gum (Sciarini et al., 2010a) or small quantities of high-pressure treated sorgum (Vallons et al., 2010) or oat (Huettner et al., 2010), may also reduce bread hardening. In breads made with different types of starch, the addition of protein sources, such as soya flour (Sciarini et al., 2010b) or gluten (Every et al., 1998), also increase shelf-life. Witczak et al. (2010) studied the effect of adding maltodextrins and reported that low-dextrose-equivalent maltodextrins produced poorer bread whereas high-dextrose-equivalent maltodextrins improved volume and reduced hardening and amylopectin recrystallisation enthalpy.

Physically-modified flours, such as extruded flours, are being used more every time in bread-making due to the particularities they show. Extruded flours have a lower retrogradation and a higher water retention capacity than native flours (Camire et al. 1990). These flours have been used in the manufacture of gluten free bread improving volume and texture (Clerici et al., 2009; Clerici and El-Dash, 2006; Sanchez et al., 2008). However there is few studies about the influence of extruded flour on the development of the bread texture.Enzymes are widely used in the manufacture of wheat bread. They enable various chemical additives to be substituted because they catalyze certain reactions and they are considered to be a good alternative to additives as “clean label compounds”. Furthermore, the protein structure of enzymes is denaturalized during baking and the enzymes are therefore not active in the final product. The use of enzymes in gluten-free bread making is not so widespread and studies are now focussing on increasing bread volume through the addition of transglutaminase, glucose oxidase (Rosell, 2009) or protease (Hamada et al., 2013). The use of enzymes to delay hardening in gluten-free breads has not been extensively investigated and only Gujral et al. (2003) observed that the use of mid-stability amylases and cyclodextrin glycosyltransferase can help to minimize bread hardening by reducing the retrogradation capacity of amylopectin during storage.

The modelling of texture development in gluten-free breads has been already studied. The Avrami model has been used to investigate starch retrogradation phenomena (Zhang and Jackson, 1992) and the development of bread hardness (Armero and Collar, 1998), although, with short storages times, some authors have found better correlations using a linear model (Jekle and Becker, 2012). Gomez et al. (2008) proposed simple models with two variables to study the development of different texture parameters over time. They observed that the most suitable models were different for white breads and whole-grain breads. In cakes, in which the gluten matrix is not developed, the modelling of texture parameters also differs from the models used for wheat bread (Gomez et al., 2010); thus, the most suitable model for gluten-free breads may also be different.

Most of the studies performed on the staling of gluten-free bread are based on firmness and they have not taken other parameters, such as cohesiveness or springiness, into account. In addition, those studies looked at firmness on specific days or differences between those days and the initial firmness. In the present study, we have investigated the development of the different texture parameters of gluten-free breads over time by using a number of simple mathematical models. We have also looked at the possibility of reducing the changes through the use of enzymes and extruded flour.

2. Materials and methods

2.1 Materials

The rice flours used in this study were supplied by Harinera Los Pisones (Zamora, Spain). Both the raw and the extruded flour used in the study were obtained from the fraction between the 132 and 200µm sifts. Extruded rice flour was prepared using an industrial single-screw extruder (Buhler S.A., Uzwil, Switzerland) with 10% of additional humidity and with a maximum flour temperature of 140ºC in the final section of the extruder. The product obtained was then milled in a compression-roller flour mill and sieved. Four commercial enzymes were used: a protease (Grindamyl PR59), a glycolipase (Powerbake 4070), an amylase (Max-Life P15) and a G-4 amylase (Powersoft 7001), all supplied by Danisco, Denmark.

The other ingredients used in the bread making included Saf-Instant yeast (Lesaffre, Lille, France) as the leavening agent, refined dry salt (Esco European Salt Company, Niedersachsen, Germany), tap water, white sugar (Acor, Valladolid, Spain), refined sunflower oil (Coosur, Vilches, Spain) and Methocel K4M Food-grade hydroxymethylcellulose (Dow Wolf Celullosics, Bitterfeld, Germany).

2.2 Methods

2.2.1 Bread making

A straight-dough process was employed, prepared using a Kitchen-Aid Professional mixer (KPM5, KitchenAid, St. Joseph, Michigan, USA) with a dough hook (K45DH). The following ingredients (as % on flour basis) were used in all formulas: water (80%), sunflower oil (6%), sucrose (5%), salt (1.8%), instant yeast (3%) and hydroxypropyl methylcellulose (2%). Instant yeast was first rehydrated in half the amount of water. The dough was kneaded for 8 minutes using a dough hook at speed 2. The doughs were moulded into aluminium pans of 232 x 108 x 43.5 mm, placing 300 g into each pan, and were then proofed for 90 minutes in a chamber at 30ºC and 70% relative humidity. After proofing, the breads were baked in an electric oven for 40 minutes at 190ºC; they were then demoulded, cooled for 60 minutes at room temperature and packed into sealed polyethylene bags to prevent dehydration. Two batches per manufacture were made with five loaves per batch.The enzymes PROT, LIP, AMYL and G-4AMYL were added at dosages selected according to the supplier's recommendations: 0.3 g, 0.08 g, 0.4 g, and 0.9 g/1000 g of flour, respectively. When extruded flour was used, 100g/1000g of untreated flour was substituted by extruded rice flour. Enzymes and extruded rice flour were added according to the experimental 2^5 factorial design shown in Table 1. Each additive was tested at two levels: 0 (absence) and 1 (presence). Before the tests, the flour and enzymes (when added) were mixed for one hour using a Rotary Mixer MR 2L (Chopin, Tripette et Renaud, France).

2.2.2 Bread characteristics

After cooling for 1 hour, baked breads were weighed and loaf volume was measured using a laser sensor with the BVM-L 370 volume analyser (TexVol Instruments, Viken, Sweden). Bread density was calculated dividing bread weight by bread volume.. Measurements were run in triplicate. Crumb texture was determined by a Texture Analyzer TA-XT2i (Stable Microsystems, Surrey, UK) running the "Texture Expert" software and equipped with a 25 mm diameter cylindrical aluminium probe. Slices with a thickness of 3 cm were compressed to 50% of their original height in a "Texture Profile Analysis" (TPA) double compression test at a test speed of 1 mm/s, with a 30 second delay between first and second compressions. Primary parameters (firmness, cohesiveness, springiness and resilience) and chewiness (a secondary mechanical characteristic) were calculated from the TPA graph. Measurements were made on two central slices from two breads made with each dough on days 0 (two hours after baking), 3, 6, 9 and 12.

2.2.3 Statistical analysis

Changes in each texture parameter were adjusted to different curvilinear models for each combination of factors studied. Mean values of the coefficients of determination for each model are shown in Table 1. After selecting the most suitable models for each texture parameter, we determined the "a" and "b" values that defined each test.

Data were analysed using analysis of variance (ANOVA). Fisher's least significant difference (LSD) test was used to describe means with 95% confidence. The analyses were performed using the Statgraphics Plus V5.1 statistical software (Statpoint Technologies, Warrenton, USA).

3. Results and Discussion

3.1 Modelling of texture parameters

Table 2 shows the means of the correlation coefficients (r^2) obtained when the trends of the different texture parameters over time in each test were adjusted to different simple curvilinear models. The means of the correlation coefficients obtained when adjusting to a second degree polynomial equation are also included. Firmness is the most extensively studied texture parameter in research into staling in both wheat and gluten-free breads. In the case of wheat bread, the Avrami equation has been used in several studies to model the development of firmness because of its relationship with starch retrogradation phenomena (Armero and Collar, 1998; Collar and Bollain, 2005). High r^2 values were obtained in those cases, though it is important to take into account that curves with only 5 or 7 points were adjusted to equations more complex that those applied in the present study. In our study, the highest mean correlation coefficient corresponded to the polynomial equation. This would appear to be a logical result as this is an equation with three variables, unlike the other equations with only two variables. Furthermore, the interpretation of the values obtained in the polynomial equation can be more complex than with other equations (except for the “a” parameter, which is equivalent to the initial value of the curve). The linear model is easier to interpret and the correlation coefficient is not significantly different from the coefficient of the polynomial equation. Some authors have stated that the development of bread firmness adequately fitted the linear model over the initial days of storage, although there was subsequently a fall in the rate of increase of firmness (Rasmussen and Hansen, 2001). In previous studies on bread (Gómez et al., 2008) and sponge cakes (Gómez et al., 2010), this model was proposed to study firmness development because of the high correlation coefficients that were obtained. In the present study, the mean correlation coefficients in the linear model were lower than those obtained in the study of wheat bread but higher than those obtained for sponge cakes. In the case of the linear model, the “a” parameter corresponds to the initial value of the variable studied and the “b” parameter indicates the rate of change of the variable.

With regard to cohesiveness, resilience and springiness, the model with the highest mean correlation coefficients was the reciprocal-x model, followed by the S-curve model. With the reciprocal-x model, these correlations exceeded 97% for cohesiveness and resilience and 94% for springiness. In previous studies on the changes in these parameters in wheat bread, Collar and Bollain (2005) proposed the multiplicative model to study the development of cohesiveness and resilience, but Gomez et al. (2008) proposed the logarithmic-x model for cohesiveness, the reciprocal-x model for resilience and the square root-x model for springiness. In our study the multiplicative and logarithmic-x models also obtained good correlation coefficients for cohesiveness and resilience, and thus the changes in these parameters in gluten-free breads did not differ greatly from those observed in wheat breads. However, the reciprocal-x model showed significantly higher values than the others models and, compared with the S-curve model, the reciprocal-x is easier to interpret, and was thus the model chosen. In our study, the changes in the texture parameters were characterised by an abrupt fall in the initial values and stabilization of the values after the second day of measurement. In contrast to other models, such as the linear model, the "a" parameter did not indicate the initial value but rather the value at which the studied parameter was going to stabilize over time; it thus indicated the change. In all cases, we analysed the initial value obtained from the mathematical model and the "a" parameter as the indicator of change.

In the case of chewiness, the correlation values were very low in the curvilinear models. The reason for the low correlation observed in the present study is the abrupt fall in chewiness observed in the initial stages of the experiment, followed by a slow recovery after the second day of measurement. The behaviour thus seemed to fit better to a 2nd degree polynomial model, and this was the reason for the inclusion of this model in the study. The mean correlation coefficient with the 2nd degree polynomial model for chewiness was over 80%, noticeably higher than with the other models. In this case the "a" value indicated the initial value of the parameter, whilst b/2c defined the time to reach the minimum value, and a-(b2/2c)+(b2/4c) defined the minimum value of the slope.

It is important to note that although the models used to describe the changes in the texture parameters of gluten-free breads are similar to those of gluten-containing breads, the values obtained were very different. The firmness values of gluten-free breads were lower than those obtained for gluten-containing breads in the study by Gomez et al. (2008), both initially and during the course of the study; this could be considered as a positive finding. In the case of cohesiveness, resilience and springiness, the values obtained for gluten-free breads were lower than those obtained for wheat breads in the study by Gómez et al. (2008), both initially and after a few days of storage, and the changes in these parameters progressed more rapidly in the case of wheat breads. Low values of these parameters should be considered as a negative characteristic and one of the most important defects of gluten-free breads (Matos and Rosell, 2012). These values do not show an adequate fit to the proposed models for firmness and their development is therefore not closely related to starch retrogradation phenomena. However, these changes may be related to the redistribution of the internal water, which hydrates the gluten, the structural matrix that gives cohesion to the dough in the case of wheat gluten (Hathorn et al. 2008), or hydrocolloids in the case of gluten-free breads (Matos and Rosell 2012).

3.2 Effect of enzymes and extruded flour on texture

The effects of the addition of the different enzymes and extruded flour on the texture variables are shown individually for each product in Table 3. The lipase and the extruded flour positively affected bread volume, whereas the protease had a negative effect and the amylases showed no significant effect. Lipases are capable of acting as emulsifiers by hydrolyzing the lipids in dough, and Nunes et al. (2009) have already reported that the emulsification of certain substances, such as the monoglycerides, lecithin or SSL, increases the volume of gluten-free breads. This effect may be related to an increased capacity of dough to incorporate air or to stabilization of the bubbles formed in the dough and the prevention of coalescence phenomena (Sahi and Alava, 2003). In our study, the effect of adding extruded flour was similar to the effect reported in other studies using extruded rice flours, whether acidified (Clerici et al., 2009), non-acidified (Clerici and El-Dash, 2006) or waxy (Sanchez et al., 2008). Defloor et al. (1991) also observed that the addition of extruded starches improved the volume of breads made with a mixture of tapioca and soya flours. These effects may be attributed to the partial gelatinization of starch during the extrusion process and to the increased consistency of the dough; these changes improve gas retention. The negative effect of proteases on volume may be explained by the effect of these enzymes on the rheology of gluten-free doughs, as they reduce dough viscosity (Renzetti and Arendt, 2009) and, therefore, the capacity of the dough to retain the gas generated during fermentation.

We also observed that a higher bread volume was associated with a lower weight after baking. This fact can be explained by the increased surface area of the loaves and the consequent increase in the area for moisture exchange with the exterior. In Table 3, an inverse correlation can be observed between bread volume and bread weight (r=0.55). As expected, there is a high inverse correlation between volume and density (r=0.985).

Firmness is the most extensively studied parameter in texture research. We observed a decrease in the initial firmness of breads with the lipase, with both amylases and with the extruded flour. However, only lipase and extruded flour were significantly associated with a reduced hardening during storage. The most intense effect on initial firmness and firmness development was observed with the addition of extruded flour, with a 43% decrease in initial firmness and a 52% decrease in firmness development. The effect of the addition of lipase and extruded flour may be related to the increase in the volume observed after the addition of these two products. An inverse correlation was found between volume and initial firmness (r=0.670), which would seem logical, but we also detected an inverse correlation between volume and firmness development (r=0.774), which was somehow higher than the correlation with initial firmness. These values are higher when the firmness parameters are correlated with the density of the loaves. The correlation between firmness development and volume or density has already been demonstrated in other studies on wheat bread (Axford et al., 1968; Gomez et al., 2008), although the correlation coefficients with wheat bread were higher with initial firmness than with firmness development, and were higher than those obtained in our study. However, Gomez et al. (2010) performed a study of sponge cakes—a product made with flour but which, like rice bread, does not show development of the gluten matrix—and found correlations with similar values. In that case, however, in contrast to gluten-free bread, the correlation coefficients were higher with initial firmness than with firmness development. Those authors also included layer cakes in the same study and found that volume and density showed significant correlations with firmness development but not with initial firmness. Initial firmness and firmness development can therefore be partly explained by differences in the volume or density of the loaves, but this is by no means the only factor. The addition of amylases reduces initial firmness but does not have any significant effect on the volume or density of the loaves. In gluten-free breads, contradictory effects have been observed after the addition of amylases. Although no differences in the initial firmness or in firmness development compared to controls were detected in some studies (Sciarini et al., 2012), other authors have reported a reduction in initial firmness, though with a firmness development very similar to the control product (Gujral et al., 2003). In that latter study, with similar results to those found in our study, the authors used an amylase with intermediate stability, as we did, and the enzymatic activity could therefore have continued during the early stages of baking, when starch also starts to gelatinize and becomes more accessible to the action of enzymes. Some authors have stated that the low-molecular-weight dextrins produced in this hydrolysis may interfere with the retrogradation phenomena of amylopectin (Lin and Lineback, 1990; Defloor and Delcour, 1999; Rojas et al., 2001; León et al., 2002) and, therefore, alter bread hardening. However, in the present study it would appear that increased amylose retrogradation was the main factor responsible for the initial firmness. The effect of the lipases, apart from their influence on volume, may be related to the generation of emulsified substances and the interaction between those substances and starch, reducing retrogradation phenomena through the formation of an amylose-lipid complex, as has already been observed after the addition of lipases to wheat breads (León et al., 2002; Purhagen et al., 2011). Emulsifiers have been used to reduce hardening in gluten-free breads in a number of studies (Nunes et al., 2009; Purhagen et al., 2012). As it has been mentioned extruded flour breads present a higher volume, which can be negatively correlated either with initial firmness of the breads or their development. Furthermore, during flour extrusion at high temperatures and with sufficient humidity, starch undergoes partial gelatinization (Camire, et al., 1990) and the molecules of amylopectin can be fragmented (Mason, 2009). These phenomena can also have an influence on staling of breads.

As already mentioned, the low values of cohesiveness, resilience and springiness compared with typical wheat breads are one of the main problems of gluten-free breads (Matos and Rosell, 2012). Cohesiveness characterizes the degree to which a material can be deformed before breaking, reflecting the internal cohesion of the material. Breads with high cohesiveness values are desirable as they allow a bolus to be formed during mastication instead of breaking up. Low cohesiveness values indicate an increased susceptibility of breads to break up or crumble

(Onyango et al. 2011). Low springiness values are also indicative of greater bread fragility, leading to crumbling during slicing (McCarthy et al. 2005). Table 4 shows the high correlation between the initial cohesiveness and resilience values (r=0.972) and between their development indices (r=0.908); in contrast to firmness, the initial cohesiveness and resilience values did not correlate with bread volume or density. However, a correlation was found between the development index and bread weight.. The factors studied did not generally show any effect on the initial cohesiveness and resilience, except the addition of amylase, which had a negative effect as it reduced the initial resilience. In the case of the development of these parameters, the extruded flour reduced the fall in the values of cohesiveness and resilience, and thus it had a positive effect, whereas the protease increased the fall in cohesiveness and thus had a negative effect. The enzymes that affected firmness development, which is related to starch retrogradation (León et al., 2002) did not show any effect on cohesiveness or resilience development, and therefore this development may not be related to starch retrogradation. In contrast, there was a correlation with bread humidity, and both the extruded flour, which has high water retention capacity (Hagenimana et al., 2006), and the proteases, which reduce water retention capacity (Primo-Martin et al., 2006), had a detectable effect. It can therefore be assumed that the fall in cohesiveness and resilience are related to the water redistribution phenomena within the bread structure, as previously suggested Hathorn et al. (2008); to minimize this, it would be necessary to resort to additives with a high water retention capacity, such as certain hydrocolloids (Matos and Rosell, 2012).

The initial values of springiness showed a degree of correlation with the volume and density of the breads, and with the initial values of cohesiveness and resilience, as may be seen in Table 4. Only the addition of the protease showed a significant effect on springiness, producing an increase in the value. However, springiness development showed a higher correlation with bread volume, initial firmness and firmness development. We may therefore assume that springiness development can be partially explained by starch retrogradation phenomena, as occurred with firmness. However, the higher the volume and the lower the firmness of breads, the lower the value of springiness stabilization over time. Both kinds of amylase and the extruded flour, which reduced the initial firmness of breads, were also found to reduce the values of springiness stabilization. The values of initial chewiness, in particular the lowest ones, showed a high correlation with initial firmness, and thus with bread volume and density. This result would appear logical as chewiness is calculated from firmness (Chewiness = Firmness * Cohesiveness * Springiness). The amylase and the extruded flour, which had a significant effect on bread firmness, showed a significant effect on the minimum chewiness values during storage, and lipase, extruded flour and amylase produced a significant decrease in initial chewiness. The changes in chewiness, as already commented for firmness, can therefore be explained, at least partially, by starch retrogradation phenomena.

Table 5 shows the interactions of combinations of two of the factors studied. In general only a few interactions are observed; the most evident were those that occurred between the amylases and the extruded flour. It is important to note that the extruded flour presented high levels of damaged starch (Yeh et al. (1999)) Chao-Chi-Chuang & Yeh (2002)), which is therefore accessible to enzyme action (Tipples, 1969).

4. Conclusion

The model of the texture parameters has enabled us to study the influence of different factors on the staling of gluten-free breads. The addition of lipases and extruded flour can improve the quality of rice breads by increasing the volume, reducing initial firmness and delaying hardening. Extruded flour can also minimize the loss of cohesiveness and resilience of these breads. However, the degrees of cohesiveness and resilience of rice breads are still very low in comparison with those of wheat breads and are not related to other texture parameters, such as firmness. Further studies are therefore required to establish methods to increase these values.

Acknowledgements

This study was supported financially by Junta de Castilla y León (VA054A12-2), Spain. The authors are also grateful to Harinera Los Pisones in Zamora, Spain, for supplying the rice flours and to Dupont Nutrition and Biosciences Ibérica for supplying the enzymes.

References

AACC (2010). Approved methods of the American Association of Cereal Chemists, methods 10-91.01 (cake symmetry and volume index), 11st ed. St. Paul: American Association of Cereal Chemists.

AXFORD, D.W.E., COLWELL, K.H., CORNFORD, S.J. and ELTON, G.A.H. (1968). Effect of loaf specific volume on rate and extent of staling in bread. J Sci Food Agric, 19, 95-101.

ARMERO, E. and COLLAR, C. (1998). Crumb firming kinetics of wheat breads with anti-staling additives. J Cereal Sci, 28, 165-174.

BAKER, L.A. and RAYAS-DUARTE, P. (1998). Retrogradation of amaranth starch at different storage temperatures and the effects of salt and sugars. Cereal Chem, 75, 308-314.

CAMIRE , M., CAMIRE, A. and KRUMHAR, K. (1990). Chemical and nutritional changes in foods during extrusion. Crit Rev Food Sci Nutr, 29, 35-57.

CHAO-CHI-CHUANG, G. and YEH, A. (2002). Effects of product temperature and moisture content on viscoelastic properties of glutinous rice extrudate. Cereal Chem, 79, 36-40.

CLERICI, M. and EL-DASH, A. (2006). Extruder rice flour as a gluten substitute in the production of rice bread. Arch Latinoam Nutr, 56, 288-294.

CLERICI, M., ARIOLDI, C. and EL-DASH, A. (2009). Production of acidic extruder rice flour and its influence on the qualities of gluten free bread. LWT-Food Sci Technol, 42, 618-623.

COLLAR, C. and BOLLAIN, C. (2005). Impact of microbial transglutaminase on the staling behaviour of enzyme-supplemented pan breads. Eur Food Res Technol, 221, 298-304.

DEFLOOR, I., DE GEEST, C., SCHELLEKENS, M., MARTENS, A. and DELCOUR, J. (1991). Emulsifiers and/or extruded starch in the productions of breads from cassava. Cereal Chem, 68, 323-327.

DEFLOOR, I. and DELCOUR, J.A. (1999). Impact of malto dextrins and antistaling enzymes on the differential scanning calorimetry staling endotherm of wheat flour-water doughs. J Agric Food Chem. 47, 737-741.

EVERY, D., GERRARD, J.A., GILPIN, M.J., ROSS, M. and NEWBERRY, M.P. (1998). Staling in starch bread: the effect of gluten additions on specific loaf volume and firming rate. Starch-Starke, 50, 443-446.

GOMEZ, M., OLIETE, B., PANDO, V., RONDA, F., and CABALLERO, P. A. (2008). Effect of fermentation conditions on bread staling kinetics. Eur Food Res Technol, 226, 1379-1387.

GOMEZ, M., RUIZ-PARIS, E., OLIETE, B. and PANDO, V. (2010). Modeling of texture evolution of cakes during storage. J Texture Stud, 41, 17-33.

GUJRAL, H.S., HAROS, M. and ROSELL, C.M. (2003). Starch hydrolyzing enzymes for retarding the staling of rice bread. Cereal Chem, 80, 750-754.

HAGENIMANA, A., DING, X. and FANG, T. 2006. Evaluation of rice flour modified by extrusion cooking. J Cereal Sci 43, 38-46.

HAGER, A.S., RYAN, L.A.M., SCHWAB, C., GAENZLE, M.G., O'DOHERTY, J.V. and ARENDT, E.K. (2011). Influence of the soluble fibres inulin and oat beta-glucan on quality of dough and bread. Eur Food Res Technol, 232, 405-413.

HAMADA, S., SUZUKI, K., AOKI, N. AND SUZUKI, Y. (2013). Improvements in the qualities of gluten-free bread after using a protease obtained from Aspergillus oryzae. J Cereal Sci, 57, 91-97.

HATHORN, C.S., BISWAS, M.A., GICHUHI, P.N. and BOWELL-BENJAMIN, A.C. (2008). Comparison of chemical, physical, micro-structural, and microbial properties of breads supplemented with sweet potato flour and high gluten dough enhancers. LWT-Food Sci Technol, 41, 803-815.

HUETTNER, E.K., DAL BELLO, F. and ARENDT, E.K. (2010). Fundamental study on the effect of hydrostatic pressure treatment on the bread-making performance of oat flour. Eur Food Res Technol, 230, 827-835.

JEKLE, M. and BECKER, T. (2012). Effects of acidification, sodium chloride, and moisture levels on wheat dough: II. Modeling of bread texture and staling kinetics. Food Biophys, 7, 200-208.

KADAN, R.S., ROBINSON, M.G., THIBODEUX, D.P. and PEPPERMAN, A.B. (2001). Texture and other physio-chemical properties of whole rice bread. J Food Sci, 66, 940-944.

LEON, A.E., DURAN, E. and DE BARBER, C.B. (2002). Utilization of enzyme mixtures to retard bread crumb firming. J Agric Food Chem, 50, 1416-1419.

LIN, W. and LINEBACK, D.R. (1990). Changes in carbohydrate fractions in enzyme-supplemented bread and the potential relationship to staling. Starch, 42, 385-394.

MASON, W.R. (2009). Starch use in foods. In J. BeMiller and R. Wristler (Eds.), Starch chemistry and technology, pp 745-795. Academic Press, Oxford, UK.

MATOS, M.E. and ROSELL, C.M. (2012). Relationship between instrumental parameters and sensory characteristics in gluten free breads. Eur Food Res Technol, 235, 107-117.

MCCARTHY, D.F., GALLAGUER, E., GORMLEY, T.R., SCHOBER, T.J. and ARENDT, E.K. (2005). Application of response surface methodology in the development of gluten free bread. Cereal Chem, 82, 609-615.

NUNES, M.B., MOORE, M.M., RYAN, L.A.M. and ARENDT, E.K. (2009). Impact of emulsifiers on the quality and rheological properties of gluten-free breads and batters. Eur Food Res Technol, 228, 633-642.

ONYANGO, C., MUTUNGI, C., UNBEHEND, G. and LINDHAUER, M.G. (2011). Modification of gluten free sorghum batter and bread using maize, potato, cassava or rice starch. LWT-Food Sci Technol, 44, 681-686.

PRIMO-MARTIN, C., DE PIJPEKAMP, A.V., VAN VLIET, T., DE JONGH, H.H.J., PLIJTER, J.J. and HAMER, R.J. (2006). The role of the gluten network in the crispness of bread crust. J Cereal Sci, 43, 342-352.

PURHAGEN, J.K., SJOO, M.E. and ELIASSON, A.C. (2011). Starch affecting anti-staling agents and their function in freestanding and pan-baked bread. Food Hydrocolloid, 25, 1656-1666.

PURHAGEN, J.K., SJOO, M.E. and ELIASSON, A.C. (2012). The anti-staling effect of pre-gelatinized flour and emulsifier in gluten-free bread. Eur Food Res Technol, 235, 265-276.

RASMUSSEN P.H. and HANSEN, A. (2001). Staling of wheat bread stored in modified atmosphere. LWT-Food Sci Technol, 34, 487-491.

RENZETTI, S. and ARENDT, E.K. (2009). Effects of oxidase and protease treatments on the breadmaking functionality of a range of gluten-free flours. Eur Food Res Technol, 229, 307-317.

ROJAS, J.A., ROSELL, C.M., and BENEDITO DE BARBER, C. (2001). Role of maltodextrins in the staling of starch gels. Eur Food Res Technol, 212, 364-368.

ROSELL, C. M. (2009). Enzymatic manipulation of gluten-free breads. In E. Gallagher (Ed.), Gluten-free food science and technology, pp 83-98. Willey-Blackwell, Chichester, UK.

SAHI S. and ALAVA, J. (2003). Functionality of emulsiers in sponge cake production. J Sci Food Agric, 83, 1419–1429.

SANCHEZ, H., GONZALEZ, R., OSELLA, C., TORRES, R. and DE LA TORRE, M. (2008). Elaboration of bread without gluten from extruded rice flour. Cienc Tecnol Aliment, 6, 109-116.

SCIARINI, L.S., RIBOTTA, P.D., LEON, A.E. and PEREZ, G.T. (2010a) Effect of hydrocolloids on gluten-free batter properties and bread quality. Int J Food Sci Technol, 45, 2306-2312.

SCIARINI, L.S., RIBOTTA., P.D., LEON, A.E. and PEREZ, G.T. (2010b). Influence of gluten-free flours and their mixtures on batter properties and bread quality. Food Bioprocess Technol, 3, 577-585.

SCIARINI, L.S., RIBOTTA, P.D., LEON, A.E. and PEREZ, G.T. (2012). Incorporation of several additives into gluten free breads: Effect on dough properties and bread quality. J Food Eng, 111, 590-597.

TIPPLES, H. (1969). The relation of starch damage to the baking performance of flour. The Baker's Digest, 43.

VALLONS, K.J.R., RYAN, L.A.M., KOEHLER, P. and ARENDT, E.K. (2010). High pressure-treated sorghum flour as a functional ingredient in the production of sorghum bread. Eur Food Res Technol, 231, 711-717.

WITCZAK, M., KORUS, J., ZIOBRO, R. and JUSZCZAK, L. (2010). The effects of maltodextrins on gluten-free dough and quality of bread. J Food Eng, 96, 258-265.

YEH, A., WU, T. and JAW, Y. (1999). Starch transitions and their influence on flow pattern during single-screw extrusion cooking of rice flour. Food Bioprod Process, 77, 47-54.

ZHANG, W. and JACKSON, D.S. (1992). Retrogradation behavior of wheat-starch gels with differing molecular profiles. J Food Sci, 57, 1428-1432.

ZIOBRO, R., KORUS, J., WITCZAK, M. and JUSZCZAK, L. (2012). Influence of modified starches on properties of gluten-free dough and bread. Part II: Quality and staling of gluten-free bread. Food Hydrocolloid, 29, 68-74.

Table 1. 2^5 Factorial design for sampling

Sample nº	Factors[a]				
	A	B	C	D	E
1	1	1	0	0	0
2	1	0	0	0	0
3	1	1	1	0	0
4	0	1	1	0	0
5	0	0	0	0	0
6	1	1	1	0	1
7	1	0	0	0	1
8	0	1	1	1	1
9	1	1	1	1	0
10	1	0	1	1	1
11	1	1	0	1	1
12	1	1	0	0	1
13	0	1	1	1	0
14	0	0	0	1	0
15	1	1	1	1	1
16	0	0	1	0	1
17	1	0	1	1	0
18	0	0	1	0	0
19	0	1	0	1	0
20	1	0	1	0	1
21	0	1	0	1	1
22	0	0	0	0	1
23	1	0	0	1	0
24	1	1	0	1	0
25	1	0	0	1	1
26	1	0	1	0	0
27	0	0	1	1	0
28	0	0	1	1	1
29	0	1	1	0	1
30	0	1	0	0	1
31	0	0	0	1	1
32	0	1	0	0	0

[a]Levels (0,1) of factors (A to E): A = Lipase (LIP): none (0), 0.08 g/1000g flour (1); B = Protease (PROT): none (0), 0.3 g/1000 g flour (1); C = G-4 amylase (G-4AMYL): none (0), 0.9 g/1000 g flour (1); D = Amylase (AMYL): none (0), 0.4 g/1000 g flour (1); E = Extruded flour (EXT): none (0), 100 g/1000 g flour (1).

Table 2. Mean of R^2 values of the different curvilinear models used to describe the relationship between different texture parameters and the storage time in rice breads.

Model	Firmness (N)	Cohesiveness	Resilience	Springiness	Chewiness (N)
Linear y=a+b*x	**87.55±10,34ab**	55.16±8,34f	51.24±8,90e	58.94±12,91e	23,08±19,20gh
Multiplicative y=a*xb	79.76±16,17bc	92.23±6,20bc	90.39±6,85c	88.83±8,93b	47,77±25,13de
Square root-x $y=a+b*x^{1/2}$	83.08±10,88b	80.28±5,14e	77.49±5,97d	80.82±8,33d	39,07±24,25ef
Square root-y $y=(a+b*x)^2$	87.54±10,63ab	55.66±9,33f	50.71±10,10 e	58.82±13,75e	19,67±18,84ghi
Exponential y=exp(a+b*x)	86.54±11,10ab	56.11±10,75f	49.95±11,76e	58.52±14,71e	16,47±17,96hi
Logarithmic-x y=a+b*ln(x)	69.99±17,00de	93.51±4,04abc	92.54±3,47bc	91.36±6,48ab	59,63±25,81bc
Double reciprocal y=1/(a+b/x)	73.80±21,92cd	89.81±10,54cd	90.22±10,37c	87.76±11,38bc	49,98±22,31cd
S-curve y=exp(a+b/x)	63.89±21,47e	94.51±8,75ab	95.54±7,57ab	91.79±8,56ab	62,45±22,94b
Reciprocal-y y=1/(a+b*x)	81.28±14,31bc	56.50±14,73f	47.52±16,26e	57.56±17,00e	15,24±24,16hi
Reciprocal-x y=a+b/x	52.24±20,69f	**97.19±6,66a**	**98.07±5,39a**	**94.79±6,25a**	72,95±24,16a
Square of X $y=a+b*x^2$	80.27±17,34bc	30.55±8,18g	27.18±8,32f	35.72±14,39f	12,83±14,26i
2nd order polynomial regression $y=a+b*x+c*x^2$	91.18±11,34a	85.60±9,09d	82.05±13,43d	82.88±7,66cd	**79,07±14,43a**

Different letters in the same column mean significantly different ($p<0.05$)

In bold, the models selected for the data analysis.

Table 3. Significant individual effects of design factors on bread quality and staling kinetics parameters of rice breads

			LIP		PROT		G-4AMYL		AMYL		EXT	
	Media	SE	0	1	0	1	0	1	0	1	0	1
Volume	973.4	15.2	930.5	1016.3**	1009.7	937.1**					915.7	1031.1***
Weight	244.4	0.8	245.7	243.0*	242.7	246.1**					246.1	242.7*
Density	0.254	0.004	0.266	0.242**	0.244	0.264**					0.270	0.238***
Initial hardness (a)	1.87	0.12	2.23	1.50***			2.20	1.53**	2.17	1.56**	2.38	1.35***
Hardness development (b)	0.272	0.026	0.351	0.193***							0.368	0.176***
Initial cohesiveness	0.434	0.014										
Cohesiveness development (a)	0.156	0.004			0.161	0.150*					0.148	0.164**
Initial resilience	0.239	0.012							0.256	0.221*		
Resilience development (a)	0.067	0.002									0.064	0.070*
Initial springiness	0.737	0.023			0.701	0.774*						
Springiness development (a)	0.415	0.010					0.432	0.397*	0.440	0.390**	0.446	0.383***
Initial chewiness (a)	0,543	0,047	0,645	0,440***					0,629	0,456*	0,653	0,433***
Chewiness development 1[a]	6,53	0,528										
Chewiness development 2[b]	0,190	0,023							0,230	0,149*	0,258	0,122**

[a] Time to reach the minimum value

[b] Minimum value

Levels (0,1) of factors (A to E): A = Lipase (LIP): none (0), 0.08 g/1000g flour (1); B = Protease (PROT): none (0), 0.3 g/1000 g flour (1); C = G-4 amylase (G-4AMYL): none (0), 0.9 g/1000 g flour (1); D = Amylase (AMYL): none (0), 0.4 g/1000 g flour (1); E = Extruded flour (EXT): none (0), 100 g/1000 g flour (1).

*p<0.05; **p<0.01; ***p<0.001

Table 4. Coefficients of correlation of the linear relationship between weight, density and texture parameters of rice breads

	Weight	Density	Initial hardness (a)	Hardness development (b)	Initial cohesiveness	Cohesiveness development (a)	Initial resilience	Resilience development (a)	Initial springiness	Springiness development (a)	Initial chewiness (a)	Chewiness development 1[a]	Chewiness development 2[b]
Volume	-0.550**	-0.985***	-0.670***	-0.774***		0.411*			-0.422*	-0.635***	-0.662***		-0,369***
Weight		0.641***		0.597***		-0.494**		-0.475**		0.477**	0.389*		0,359*
Density			0.677***	0.819***		-0.422*			0.422*	0.688***	0.677***		0,663***
Initial hardness (a)				0.696***						0.757***	0.741***	-0,397*	0,818***
Hardness development (b)						-0.471**		-0.401*		0.699***	0.659***		0,789***
Initial cohesiveness							0.972***		0.463**				
Cohesiveness development (a)								0.908***					
Initial resilience									0.504**				
Resilience development (a)													
Initial springiness										0.511**			
Springiness evolution (a)											0.646***		0,799***
Initial chewiness (a)													0,704***
Chewiness development 1[a]													

[a] Time to reach the minimum value
[b] Minimum value
*p<0.05; **p<0.01; ***p<0.001

Table 5. Significant second-order interactive effects of design factors on bread quality and staling kinetics parameters of rice breads

			Density		Initial hardness (a)		Hardness development (b)		Initial cohesiveness		Cohesiveness development (a)		Initial resilience		Resilience development (a)		Initial springiness		Springiness development (a)		Initial chewiness(a)		Chewiness development 1[a]		Chewiness development 2[b]	
			Media	SE	Media	SE	Media	SE	Media	SE	Media	SE	Media	SE	Media	SE	Media	SE	Media	SE	Media	SE	Media	SE	Media	SE
	0	0			2.35	0.17*																				
LIP-PROT	0	1			2.11	0.17																				
	1	0			1.20	0.17																				
	1	1			1.81	0.17																				
PROT-G4AMYL	0	0													0.067	0.002*										
	0	1													0.071	0.002										
	1	0													0.068	0.002										
	1	1													0.061	0.002										
G4AMYL-AMYL	0	0																								
	0	1																								
	1	0																								
	1	1																								
G4AMYL-EXT	0	0							0.381	0.020*			0.200	0.016*												
	0	1							0.465	0.020			0.265	0.016												
	1	0							0.447	0.020			0.253	0.016												
	1	1							0.443	0.020			0.237	0.016												
AMYL-EXT	0	0			3.056	0.17**																				
	0	1			1.286	0.17																				
	1	0			1.706	0.17																				
	1	1			1.43	0.17																				

[a] Time to reach the minimum value
[b] Minimum value
Levels (0,1) of factors (A to E): A = Lipase (LIP): none (0), 0.08 g/1000g flour (1); B = Protease (PROT): none (0), 0.3 g/1000 g flour (1); C = G-4 amylase (G-4AMYL): none (0), 0.9 g/1000 g flour (1); D = Amylase (AMYL): none (0), 0.4 g/1000 g flour (1); E = Extruded flour (EXT): none (0), 100 g/1000 g flour (1).

*p<0.05; **p<0.01; ***p<0.001