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Effect of maize resistant starch and transglutaminase: A study of fundamental and empirical rheology properties of pan bread dough

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Professor Da-Wen Sun
Editor-in-Chief,
Food and Bioprocess Technology

Date: 2013-12-09
Subject: Online Manuscript Submission – Revised Version (FBT-D-13-01200)

Dear Professor Sun,

Thank you for your e-mail concerning the manuscript we submitted for your consideration for publication in *Food Bioprocess and Technology*, Ref. No. FBT-D-13-01200 and entitled: *Effect of maize resistant starch and transglutaminase: A study of fundamental and empirical rheology properties of pan bread dough*.

We are now submitting a revised version of the manuscript in which we have considered and responded/justified to all *Reviewer*s' remarks. Accordingly, we introduced some changes in the manuscript and all of them are identified in red color.

We are thankful for the constructive comments of *Reviewer*s, which have certainly contributed for the improvement of this manuscript. We hope that, with these changes, we have met all of the *Reviewer*s' requirements. Let me close by thanking you for the time spent dealing with this subject.

Best regards,

C.C. Tadini

***Reviewer* #3:**
Please find enclosed file and my few comments for your manuscript.

Please see below the corrections:

Abstract
Line 22: corrected
Line 27: corrected

Results and discussion
Lines 46-52 Page 10: the range values were inserted.

Conclusion
Line 25: the text was modified.

Table 1
There was a change between blends 2 and 3, between 3 and 4 and so on. Therefore all data were revised. Many thanks.

We would like to thank again the *Reviewer* #3 for the thoughtful comments: the paper will certainly benefit from the changes introduced in order to meet their requirements. We believe that the manuscript was modified accordingly.

***Reviewer* #4:**

The insertion of different wheat flour, ingredients commonly affects the characteristics of retrogradation certain types of breads. What would happen with the addition of MRS and TG?

There would be greater the less water retention in the end produced bread?
It could be argued that even hypothetically?

New phrases were inserted. Please see lines 25-36, Page15.

Finally able would recommend adding the RMS and TG? Could be considered in this conclusion?

This information was inserted in the conclusion.

We would like to thank again the *Reviewer* #4 for the thoughtful comments: the paper will certainly benefit from the changes introduced in order to meet their requirements. We believe that the manuscript was modified accordingly.

Effect of maize resistant starch and transglutaminase: A study of fundamental and empirical rheology properties of pan bread dough

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Keywords: pan bread dough, uniaxial extension, biaxial extension, thermal properties

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Abstract

The effect of maize resistant starch (MRS) and transglutaminase (TG) on rheological and thermal properties of pan bread dough was studied. The MRS was added as an alternative to increase the fiber ingestion while TG supplies the gluten dilution, catalyzing protein bonds. A second order Central Composite Design (2^2) with three central and four star points was applied and the results were compared to those of pan bread dough prepared without MRS and TG, as control. The presence of MRS and TG significantly ($P<0.05$) influenced the maximum resistance to extension achieving the highest value for the dough formulated with 8.8 g/100g of MRS and 0.12 g/100g of TG. A modified power-law model was fitted to the stress-strain data obtained from biaxial test, indicating that partial substitution of wheat flour by MRS resulted high n index (degree of strain hardening). Only starch gelatinization enthalpy significantly changed ($P<0.05$) by MRS and TG contents, increasing with the increase of TG. The temperatures obtained from thermograms are compared to those obtained from DSC curves from aqueous suspensions of MRS, indicating gelatinization temperatures above 100 ºC.

Keywords: pan bread dough, uniaxial extension, biaxial extension, thermal properties

Introduction

Bread is generally prepared from wheat flour, water and yeast that after kneading the mixture forms an elastic dough (Stauffer, 1999), and it is the one of most consumed food around the world. However, its consumption does not supply enough fiber of human diet. To increase the fiber ingestion, pan bread produced with resistant starch from maize (MRS) could be a good alternative for human diet.

Resistant starches have drawn broad interest worldwide recently both for their potential health benefits and functional properties. The term "resistant starch" (RS) refers to the portion of starch that resists digestion in the small intestine within 120 min of being consumed, but it can act in the large intestine as a substrate for microbial fermentation for colonic microflora (prebiotical effect). The products of intestinal fermentation are hydrogen, carbon dioxide, methane, and short chain fatty acids (SCFAs) (Fuentes-Zaragoza et al., 2010; Nugent, 2005; Roberts, 2000).

Extensive studies have shown that Resistant Starch (RS) has physiological functions similar to those of dietary fiber (Eerlingen & Delcour, 1995), such as calorie reduction and colonic health benefits including increased stool bulk, decreased transit time of stool, and generation of volatile free fatty acids, especially butyrate, propionate and acetate, that can increase colonic blood flow, lower luminal pH and help to prevent the development of abnormal colonic cell populations (Gelencsér et al., 2008; Xie & Liu, 2004). Thus, RS reduces the risk of colon cancer, and high-resistant starch content in the diet may improve glucose and lipid metabolism (Liljeberg et al., 1999; Nugent, 2005). They can also reduce the risk of type II diabetes mellitus, obesity, coronary diseases, gastrointestinal disorders, and inflammatory bowel diseases (Meyer et al., 2000; Tungland & Meyer, 2002).

In general, starches rich in amylose are naturally more resistant to digestion and more susceptible to retrogradation than starches rich in amylopectin. RS is found naturally in all starch containing foods, but some factors influence the net amount of RS. These are food processing, cooking, storing, and the method of ingestion (Muir et al., 1995; Nugent, 2005).

Up to five classes of RS are quoted in the literature (from RS1 to RS5) as proposed by Englyst and Cummings, 1987; Englyst et al., 2007; Regina et al., 2007; Sajilata et al., 2006. They include physically inaccessible sites to α-amylase (RS1), ungelatinized granular starch with B-type crystallinity (RS2), retrograded amylose (RS3), chemically modified starch (RS4), and lipid-complexed amylose (RS5).

Many cereal foods have been fortified with RS2 and RS3, including bread, pasta, noodles, tortillas, cakes, waffles, brownies, cookies, snacks, muffins and breakfast cereals (Baixauli et al., 2008; Brown, 2004; Gómez et al., 2013; Nugent, 2005; Sajilata et al., 2006; Sharma et al., 2008; Sozer et al., 2007; Yeo & Seib, 2009). Food applications of resistant starch are of interest to product developers and nutritionists mainly due to fiber-fortification and the potential physiological benefits as well as unique technological properties. RS provides better appearance, texture, color, flavor, and mouthfeel than conventional fibers in cereal products (Fuentes-Zaragoza et al., 2010; Haralampu, 2000; Yue & Waring, 1998). However, the replacement of wheat flour by RS affects bread quality due to gluten dilution (Michniewicz et al., 1991).

Maize Resistant Starch (MRS), as compare to others fibers, presents benefits such as appearance, taste and texture that stimulate people to consume this functional ingredient. It is commercialized as a fine powder with white appearance and bland flavor (Sajilata et al., 2006).

Bread staling is an important factor to evaluate the quality of bread and the retrogradation of starch is believed to be responsible for this phenomenon. Hung and Morita (2004) studied the effects of cross-linked cornstarches (CLCSs) and vital gluten as substitutes of wheat flour at level (5 – 15) %, on dough rheological properties and bread quality. The CLCSs improved the resistance and the extensibility of dough against mixing and stretching. The onset and peak temperatures obtained from DSC curves of the flours substituted with CLCSs were significantly lower than control.

Ozturk et al. (2009) evaluated rheological and baking properties of breads that were supplemented with three different commercial resistant starch at contents between (0 and 30) %. The authors reported that commercial starches with high content of RS did not have substantial deteriorative effect on the crumb color values, external appearance and symmetry of bread loaves.

More recently, Sanz-Penella et al. (2010) studied the addition of resistant starch (0 – 30) % from modified pea starch on dough and bread performance and they found that up to 20 % the flour substituted by modified pea starch did not affect the dough machinability, and produced bread with acceptable changes in sensory properties.

Gluten is the main protein of the wheat, which is responsible for the structure, and consequently, for the bread quality. To minimize the detrimental effect of MRS on gluten strength, the enzyme transglutaminase (TG) can be also added into mixture. This enzyme has been used to obtain higher product quality in a wide variety of foods, including wheat products (Yokoyama et al., 2004). It improves the crosslinking of gluten proteins, increasing the gluten strength and water holding capacity (Collar & Bollaín, 2004).

The rheological behavior of dough can be determined by two distinct types of measurements, fundamental and empirical tests. Studies on the fundamental rheology of dough and gluten are usually carried out using small deformation while the empirical measurements are obtained using large deformation. Nonetheless, fundamental dough and gluten rheological testings using large deformation, which are associated with bread quality, are growing popularity with the presence of newer techniques and equipment (Zaidel et al., 2010).

The objective of this work was to study the influence of Maize Resistant Starch (MRS) as partial substitution of wheat flour and transglutaminase enzyme (TG) addition on fundamental and empirical rheological and thermal properties of pan bread dough. A second order Central Composite Design (2^2) with three central and four star points was applied and the results were compared to those of pan bread dough, prepared without RMS and TG, as control.

Materials and Methods

Materials

Commercial wheat flour (water content 13 %, protein 10 %, ash 0.54 % and farinograph water absorption 61 % according to AACC methods (2000)) was provided by a local milling company (Tirani SA, La Plata, Argentina); commercial Maize Resistant Starch (MRS), Hi-maize® 260 (62.5 % of fibers and gelatinization temperature about 120 ˚C) was provided by National Starch (Brazil); Transglutaminase Activa™ TG (100 U/g activity) was obtained from Ajinomoto (Japan); and sodium chloride (Synth, Brazil).

Bread dough preparation

The dough formulation was based on wheat flour and maize resistant starch (based on 100 g of mixture), 60 g of MiliRO water, 2 g of salt, being MRS and TG added in each formulation according to a second order Central Composite Design (2^2), with three central and four star points (Table 1).

The dough was prepared according to the tests:

For uniaxial extension and Texture Profile Analysis (TPA), the ingredients were mixed for 2.5 min in a mixer HR 1495 (Philips, Argentina) and rested for 10 min. Then, it was separated in two parts: about 40 g was put in a form and Teflon press to precisely shape and rested for 40 min; the second part was laminated (height 8 mm) and cut in two pieces, covered with plastic film and rested for 20 min until the analysis.

For biaxial extension, after mixing, the dough was sheeted and cut into five circular samples of 55 mm diameter and 8 mm thickness, coated with paraffin oil to prevent moisture loss and drying and place securely in the sample holder, according to method D/R Dobrasczcyk (SMS, 1995) and rested for 30 min until analysis.

For rheofermentometer assays, dough was prepared by mixing raw materials (wheat flour, MRS and TG quantities according to experimental design; 0.2 g ascorbic acid; 0.0176 g α-amylase; 20 g of sodium chloride and 3 g of DATEM; basis 1000 g of mixture) for 1 min at 840 rpm in a spiral mixer (SUPREMAX AL-25 IM, Brazil) at 25 °C (Matuda, 2008; Matuda et al., 2008). Water was added according to the farinographic absorption of dough and 28 g of compressed yeast was incorporated to the mixtures. Dough was kneaded for 12 min at 1,700 rpm at 25 °C and rested for 15 min.

For DSC experiments, the dough was prepared in a micro-farinograph® (Brabender, Germany) (basis 10 g of mixture).

Dough rheology

Empirical methods

The Texture Profile Analysis (TPA) were applied in two dough sheets of 8 mm thickness and were compressed up to 60 % of their original height, using a TA.XT2i texture analyzer (SMS, UK), with the 50 mm diameter (P/50) aluminium probe, at 1 $mm \cdot s^{-1}$ of speed pre-test, $1 mm \cdot s^{-1}$ of speed test, $1 mm \cdot s^{-1}$ of speed pos-test, a trigger type auto of 0.2 N, with a 75 s delay between first and second compression. Parameters such as hardness (H), springiness (S), resilience (R_{es}) and adhesiveness (Ad) were calculated from the TPA curves using the software Texture Expert version 1.22 (SMS, UK).

The fermentation process was monitored using a rheofermentometer (F3, Chopin, France). A piece of dough (250 g) was placed in the rheofermentometer basket and a piston with 2-kg resistance weight was placed on top. The temperature was maintained at 28.5 °C and the test was conducted for 3 h. Two curves were obtained from this test. The dough development curve provides information about the maximum height reached by the piston (H_m) and the time taken to reach maximum height (t_1). The gaseous release curve presents the maximum pressure (P_m) developed by fermentation, the time taken to reach that pressure (t'_1) and the time when porosity appears (t_x, related to CO_2 loss).

The farinograph assays were conducted in a Brabender® Farinograph (Germany) according to constant flour weight AACC 54-21 procedure (AACC, 2000). The following parameters were obtained from curves: dough development time, tolerance index and stability.

Fundamental methods

Uniaxial extension measurements were performed using a TA.XT2i texture analyzer (SMS, UK) with the Kieffer dough and gluten extensibility rig. The analyses were carried out according to the extensibility of dough and measure of gluten quality method (SMS, 1995). The test was conducted under the following conditions: pre-test speed 2 mm·s^{-1}, test speed 3.3 mm·s^{-1}, post-test speed 10 mm·s^{-1}, distance 75 mm and trigger type auto of 0.2 N. Ten strips of each formulation were tested and the parameters maximum resistance to extension ($R_{\max}$) and maximum extensibility at rupture ($E_{\max}$) were obtained from load-extension curves. They were calculated using the software Texture Expert 1.22 (SMS, UK). From $R_{\max}$ and $E_{\max}$, the maximum stress or fracture stress ($\sigma_{\max}$) and the Hencky strain (ε_H) at fracture were calculated according to Dunnewind et al. (2004) as follows:

$$\sigma_{\max} = \frac{F_d l_t}{V} \qquad (1)$$

wherein: $\sigma_{\max}$ is the maximum stress [kPa]; F_d is the force acting on the dough [N], assuming that $R_{\max}$ is divided equally over both stretches of dough at each side of the hook during the extension; l_t is the length of the dough correspondent to the $E_{\max}$ [mm]; and V is the dough volume [cm^3]. F_d and l_t are calculated according to:

$$F_d = \frac{R_{\max} l_t}{4\left(y_t + y_0\right)} \qquad (2)$$

$$l_t = 2\sqrt{w^2 + \left(y_t + y_0\right)^2} \tag{3}$$

wherein: $R_{\max}$ is the maximum resistance to extension obtained from load-extension curves [N]; y_t is the displacement of the hook from the point at which the actual extension starts [mm]; y_0 is the distance which hook has to travel from the surface of the lower plate to the point where the actual extension starts [mm]; and w is half the width of the gap in the lower plate through which hook passes [mm].

The Hencky strain (ε_H) at fracture was calculated as follows:

$$\varepsilon_H = \ln\left(\frac{l_t}{l_0}\right) \tag{4}$$

wherein: l_0 is the initial length [mm], calculated according to Equation 5:

$$l_0 = 2\sqrt{w^2 + y_0^2} \tag{5}$$

Biaxial extension (inflation) was performed using the TA.XT2i texture analyzer (SMS, UK) with the Dobraszczyk–Roberts dough inflation system in five replicates of each dough formulation. The parameters determined from curves obtained were maximum pressure required (P), baking strength (W), and extensibility (L), calculated by the software D/R Dough Inflation System version 1.05 (SMS, UK). From the pressure, volume and time data recorded during the inflation, stress and strain data were calculated, according to Chin and Campbell (2005). The stress (σ) and the Hencky strain (ε_H) were calculated as follows:

$$\sigma = \frac{P}{4}\frac{\left(a^2 + h^2\right)^3}{h\Delta_0 a^4} \tag{6}$$

$$\varepsilon_H = \ln\left(1 + \frac{h^2}{a^2}\right) \tag{7}$$

wherein: σ is the stress (MPa); ε_H is the Hencky strain (dimensionless); P is the pressure (kPa); a is the initial sample radius (2.75 cm); h is the bubble height (cm); and Δ_0 is the initial sample thickness (0.267 cm). The bubble height (h) was calculated using the scaled volume (SV) and the inversion length (IL) according to following equations:

$$SV = \frac{3}{\pi}V \tag{8}$$

$$IL = \left(SV + \sqrt{a^6 + \left(SV\right)^2}\right)^{1/3} \tag{9}$$

$$h = IL - \frac{a^2}{IL} \quad (10)$$

wherein: SV is the scaled volume [cm^3]; V is the bubble volume [cm^3]; and IL is the inversion length [cm].

Thermal analyses

DSC experiments were conducted for each formulation using a differential scanning calorimeter DSC Q100 (TA Instruments, USA) calibrated with indium (melting point = 156.61 °C; $\Delta_{fus}H$ = 28.54 J·g^{-1}). To avoid water condensation, nitrogen gas was continuously flushed through the calorimeter head at a rate of 45 mL·min^{-1}. Dough samples (15 – 20) mg were placed in a hermetic aluminum pan (20 μL), which was placed inside the calorimeter and frozen with liquid nitrogen to a temperature of -40 °C. After that, the samples were to 20 °C at a heating rate of 5 °C·min^{-1}, followed by heating to 150 ºC at a heating rate of 10 °C·min^{-1}. An identical empty pan was used as reference and all experiments were conducted in triplicate. The onset and peak temperatures, as well as water fusion and transition enthalpies were calculated by Universal Analysis 2000 v 4.3E software provided by the manufacturer (TA Instruments, USA) (Matuda et al., 2005).

Aqueous suspensions of maize resistant starch were prepared at different concentrations (0 – 70) % and submitted to the same temperature program.

Gluten quantity

This analysis was performed according to AACC 38-12A in replicates (AACC, 2000), using a Glutomatic System consisting of Glutomatic 2020, Gluten Index Centrifuge 2015 and Glutork 2020 (Pertens Instruments, Sweden). The wet gluten, gluten index, dry gluten, and water binding capacity were calculated.

Water and ash contents

The water content of the dough produced from blends without yeast was measured according to method AACC 44-15A (AACC, 2000), using an air oven (Marconi, MA-030, Brazil), in triplicate.

The ash content of dough without yeast was also measured according to method AACC 8-01 (AACC, 2000), using an electric muffle furnace (QUIMIS, model 25079, Brazil), in triplicate.

Statistical analyses

Using the statistical program Statgraphics Centurion XV (StatPoint, Inc., USA), analysis of variance (ANOVA) was applied to the results obtained within a 95 % of confidence interval. A response surface methodology was applied and the suitability of the fitted models was evaluated by the determination coefficient (r^2), the significance level ($P < 0.05$), and residual analysis.

Results and Discussion

Dough rheology

Empirical methods

Significant increase ($P<0.05$) of hardness (H) and resilience (R_{es}) obtained from TPA was noticed in the dough as increased the flour substitution by MRS (Table 1), whereas other parameters such as adhesiveness (Ad) from (58.6 to 116.1) N·s, springiness (S) from (0.984 to 0.995) and cohesiveness (C) from (0.43 to 0.54) were not significantly influenced ($p>0.05$) by MRS and TG, if compared to the control dough (without MRS and TG), 128.0 N·s, 0.992, 0.61, respectively. The presence of TG in the dough at different quantities did not affect the hardness (H) and the resilience (R_{es}) of the dough. Low values of dough resilience indicated lower capability to recover its shape after deformation, corresponding to a plastic flowable material, and therefore harder doughs recovered their shape more quickly (Armero & Collar, 1997).

From rheofermenter assays, only partial substitution of wheat flour by maize resistant starch (MRS) significantly diminished the maximum height (H_m), as expected. However, the weakness coefficient (W_{coef}), correspondent to the relation between the maximum height (H_m) and the height at the end of the test, was not significantly different among formulations, presenting 3.8 % as the maximum value for dough formulated with 3.5 g/100g of MRS and 0.05 g/100gof TG. In addition the time taken to reach H_m was (173 ± 7) min, value around by the end of the test. Therefore, it is expected that breads obtained in these conditions would present similar volumes.

The results obtained from the CO_2 release curves showed a positive influence of partial substitution of wheat flour by maize resistant starch (MRS) on maximum pressure (Table 1), whereas TG increased t'_1 (time taken to reach P_m), from 136.5 min for dough without the enzyme to 162.0 min for dough with 0.15 g/100g of TG. In relation to t_x, the MRS and TG had opposite effects, suggesting that although the dough with partial substitution of wheat flour by maize resistant starch (MRS) began to lose CO_2 in less time from (88.5 to 82.5) min, ANOVA indicated that there was not significant differences between formulations, because of the positive effect of TG on the cross linking of gluten proteins, increasing the gluten strength. It is confirmed by the highest value of the retention coefficient (97.2 %) of the dough formulated with 8.5 g/100g of MRS and 0.10 g/100g of TG (Table 1). These results showed that the incorporation of TG to dough formulated with partial substitution of wheat flour by maize resistant starch (MRS) would develop bread with enhanced rheological properties in comparison with the control (R_{coef} = 93.8 %; t_x = 88.5 min).

ANOVA applied on farinograph results indicated that both MRS and TG did not influence significantly the water absorption and varied from (62.4 to 65.2) %. However, the MRS decreased significantly the dough development time from (2.0 ± 0.2) min for dough without MRS to (1.3 ± 0.2) min for dough formulated with 15.5 g/100g of MRS. The dough development time, or peak time, is commonly believed to be the point at which the dough is optimally developed and best able to retain gas and can be correlated with the t_x obtained from release gas curve of rheofermentometer.

The same tendency was observed with the stability, varying from (4.3 ± 0.3) min for dough without MRS to (1.7 ± 0.3) min for dough formulated with 15.5 g/100g of MRS, suggesting a low tolerance of flour to kneading. The mixing tolerance index (*MTI*) of dough without MRS presented a lowest value (36 BU) and increased markedly with MRS (84 BU for dough formulated with 15.5 g/100g). The *MTI* (the dough breakdown rate) and stability parameters indicate how well a mixture resists additional work after it has been mixed to optimum. Low mixing tolerance index values are representative of mixtures that can be over mixed with little change in the consistency of the dough. Therefore, these results indicate that maize resistant starch conduces to a greater degree of dough softening during kneading. The same tendencies were found by Sanz-Penella et al. (2010) studying the effects of wheat flour substitution by modified pea starch with high level of RS on dough rheological properties.

Fundamental methods

The parameters obtained from uniaxial extension of dough formulations are presented in Table 2. The force-deformation curves were recalculated into stress–strain data, taking into account changes in the sizes of the extended specimen. The maximum stress or fracture stress ($\sigma_{\max}$) and the Hencky strain (ε_H) at fracture were calculated according to Equations 1 and 4, considering the initial length l_0 = 19.54 mm; the width half the gap w = 9 mm; the distance which the hook has to travel from the surface y_0 = 2.8 mm; and for the dough volume the following values were considered: density ρ = 1.175 g·cm^{-3} (Matuda, 2008); average specimen weight m = 0.345 g.

Analysis of variance, within 95 % of confidence interval, applied to the results of parameters obtained from uniaxial tests showed that the partial substitution of wheat flour by maize resistant starch (MRS) influenced the maximum resistance to extension ($R_{\max}$) and maximum extensibility at rupture ($E_{\max}$) of dough (Table 2). As can be observed in Figure 1a, the value of $R_{\max}$ achieved the maximum at a level of MRS substitution of 9 %. The opposite effect occurred with the $E_{\max}$, as expected. The addition of TG up to approximately 0.15 % increased $R_{\max}$ of the dough and decreased the $E_{\max}$. These results are comparable with those of Basman et al. (2002), who studied the effect of addition of TG from (0 to 1.5) % on two kinds of wheat flour produced from USA.

The multiple regression analysis was performed to obtain the combined effect of partial substitution of MRS and TG on $R_{\max}$ obtained from uniaxial extension of dough as shown in Figure 1a and the fitted model is (r^2 = 0.72):

$$R_{\max} = \begin{pmatrix} 152.0 + 56.4 \times \mathrm{MRS} + 4.2 \times 10^{3} \times \mathrm{TG} - 3.9 \times \mathrm{MRS}^{2} \\ -2.1 \times 10^{3} \times \mathrm{TG}^{2} + 108.9 \times \mathrm{MRS} \times \mathrm{TG} \end{pmatrix} \pm 63.3 \qquad (11)$$

$$1.5 \le \mathrm{MRS} \le 15.5 \text{ g/100 g} \qquad 0.03 \le \mathrm{TG} \le 0.17 \text{ g/100 g}$$

The optimized response correspondent to the maximum $R_{\max}$ over the indicated region in Figure 1a was $R_{\max}$ = 653.2 mN, for the dough formulated with 8.8 g/100 g of MRS and 0.12 g/100g of TG, value superior that obtained from control ($R_{\max}$ = 490.9 ± 46.1) mN.

This result shows the dough behavior known as strain hardening, in which the force that extends the material increases in order for additional strain to occur. The phenomenon of strain hardening occurs when the stress increases more than proportional with the strain and can be related to baking performance (Uthayakunaran et al., 2002).

Rheological properties of dough and gluten during mixing are affected greatly by the flour composition (protein content), processing parameters (mixing time, energy, temperature) and ingredients (water, salt, yeast, fats and emulsifiers).

The biaxial extension produced during dough bubble inflation is well linked, from a physical viewpoint, with the process of dough rising. Analysis of variance within 95 % of confidence interval, applied to the results of parameters obtained from biaxial extension tests, showed that both the partial substitution of wheat flour by maize resistant starch (MRS) and the addition of transglutaminase enzyme (TG) influenced the maximum pressure required (*P*) and the extensibility of the dough (*L*) (Table 2). However, only TG influenced significantly the baking strength of the dough (*W*). In Figure 1b, it is clear to observe that the partial substitution of wheat flour by MRS increased the maximum pressure required to inflate the bubble, whereas the TG increased it up to a level of 0.06 g/100g.

The multiple regression analysis was performed to obtain the combined effect of partial substitution of MRS and TG on *P* obtained from biaxial extension of dough as shown in Figure 1b and the fitted model is (r^2 = 0.67):

$$P = \begin{pmatrix} 2.0 + 4.2 \times 10^{-2} \times \mathrm{MRS} - 1.4 \times \mathrm{TG} + 8.8 \times 10^{-3} \times \mathrm{MRS}^{2} \\ -74.2 \times \mathrm{TG}^{2} + 2.0 \times \mathrm{MRS} \times \mathrm{TG} \end{pmatrix} \pm 0.2 \qquad (12)$$

$$1.5 \le \mathrm{MRS} \le 15.5 \text{ g/100 g} \qquad 0.03 \le \mathrm{TG} \le 0.17 \text{ g/100 g}$$

As previous works (Dobraszczyk and Roberts, 1994; Dobraszczyk et al., 2003; Chin and Campbell, 2005), a power-law model was fitted (Eq. 13) to the stress-strain data, considering the failure strain (Hencky strain value) and stress when the dough bubble material failed to sustains the inflation and ruptured.

$$\sigma_B = k\exp(n\varepsilon_{HB}) \tag{13}$$

wherein: σ_B is the stress [MPa]; ε_{HB} is the Hencky strain [dimensionless]; k is the coefficient related to the dough viscosity [MPa]; and n is the index that measures the degree of strain hardening [dimensionless]. The stress and the Hencky strain were calculated according to Equations 6 and 7. Applying non-linear regression to stress-strain data for each MRS quantity, using the Marquardt estimation method, and minimizing the sum of the normalized errors to find the coefficient k and the index n, a modified power-law model was fitted for the failure stress ($r^2 = 0.94$):

$$\begin{gathered}\sigma_B = \left(6.8\times10^{-3}\times\exp\left(2.41\times\varepsilon_{HB}\right)+1.94\times10^{-2}\times\mathrm{MRS}\right)\pm0.15\\ 1.5\le\mathrm{MRS}\le15.5\ \mathrm{g/100\ g}\end{gathered} \tag{14}$$

wherein k = 6.8 Pa and n = 2.41.

Values of n = 1 indicates that the original stress-strain curve is linear, whilst n = 2 signifies a parabolic stress-strain relationship and the greater the value of n, the greater the curvature. It can be observed in Figure 1c, for each MRS quantity, a parabolic stress-strain relationship demonstrating a non-linearity of dough rheological properties with strain, highlighting the dangers in extrapolating rheological measurements made at low to high strains experienced by dough gas cell walls during expansion (Dobraszczyk and Roberts, 1994).

As described before, higher value of n (greater strain hardening) gives a greater strain at which instability occurs, and hence greater bubble failure occurs and ultimately greater loaf volume is obtained. Moreover, the extensional strain hardening can well correlate with the gas retention. In fact, in the current study the high value of retention coefficient (R_{coef}) obtained from dough formulated with 8.8 g/100g of MRS and 0.12 g/100g of TG confirms this criterion.

Thermal analyses

Starch gelatinization is an important phenomenon occurring in various food processing operations because it provides unique textural and structural characteristics for the products. It can be observed in Figure 2 that there were not thermal changes between (60 and 85) ºC when the aqueous suspensions of MRS were submitted to temperature program. However, it can be observed an endotherm occurring of a peak temperature around 130 ºC and 120 ºC for aqueous suspensions of 50 g/100g and 30 g/100g of MRS, respectively, and there was also an increase of the enthalpy values as increased the water content, as expected. Gelencsér et al. (2008) comparing the physical and chemical properties of different resistant starches observed that suspensions resistant starch from maize (30 g/100 g) have shown thermal changes beyond 90 ºC. Moreover, when a mixture of 50 % of native maize starch with 50 % of resistant starch RS2 from maize was submitted to the same DSC program, it was observed two peak temperatures T_{p1} = 71.87 ºC and T_{p2} = 101.81 ºC.

Only wheat starch gelatinization enthalpy was significantly ($P<0.05$) influenced by MRS and TG contents of the dough (Table 3). Experimental design analysis indicated that as TG content increased, the gelatinization enthalpy increased; whereas this property decreased as increased the MRS up to 8.5 g/100g, confirming that the only starch that gelatinized was the wheat starch. Therefore, less water could be absorbed by the starch granules, with a consequent decrease in their gelatinization, as well as, there would be less gelatinized starch that could be retrograded during storage. In fact, Gómez et al. (2013) studying the quality of bread formulated with a blend of wheat flour and maize resistant starch, incorporated with a mixture of emulsifiers, found an optimum proportion which has presented the lowest retrogradation level after 7-day ambient storage. At temperatures above the gelatinization temperature range, at excess-water conditions, an endothermic transition was detected in the DSC thermograms of the dough (Table 3). This transition is related to a transition of the amylose-lipid complex and the enthalpy value (ΔH) is related to the amylose content. In some formulations, which the maize resistant starch was higher (MRS ≥ 8.5 g/100g), the third endothermic transition was found with an average onset temperature of (130.5 ± 1.1) ºC and an average peak temperature of (140.1 ± 1.4) ºC, that can be compared those obtained from DSC thermograms of aqueous suspensions of MRS, therefore these temperatures corresponds to MRS gelatinization. This finding has important implications for process, since the temperatures of loaves during the baking do not achieve values above 100 ºC; therefore water evaporation and consequently the MRS content are preserved.

Gluten quantity

The wet gluten (*WG*), dry gluten (*DG*), water binding capacity (*WBC*) and ash content decreased significantly ($P<0.05$) as MRS increased, as expected (Table 3). The key protein complex which contributes to dough functionality is gluten and the Gluten Index (GI) characterizes as being weak, normal or strong, therefore the GI is a method of analyzing wheat protein that provides simultaneous determination of gluten quality and quantity, and consequently allows a reliable prediction of bread making quality. The values of GI found in this work varied from 79.3 ± 2.9 to 98.6 ± 0.8. According to Ćurić et al. (2001), tests carried out in many countries indicate that flours for the production of bakery products have the gluten index value from 60 to 90. Flours with the gluten index exceeding 95 are too strong and those with the index value less than 60 are too weak for bread production. Costa et al. (2008) analyzed the technological quality of samples of national and imported wheat grains, in Brazil, as well as of flour samples obtained from them. They found that wheat flour from imported grains presented higher values for wet gluten from (28.0 to 33.4) %, whereas the national varied from (22.6 to 29.2) %. The values of wet gluten found in this work are similar varying from (22.9 ± 1.2) % for dough produced with higher MRS quantity to (29.0 ± 1.2) % for dough produced with lesser MRS quantity.

Conclusions

The purpose of the current study was to evaluate the rheological and thermal properties of pan bread dough formulated with partial substitution of wheat flour by Maize Resistant Starch (MRS) and transglutaminase (TG) in order to overcome the gluten dilution.

This paper has shown that the partial substitution of wheat flour by MRS increased the maximum resistance to extension of the dough, $R_{\max}$ = 664.9 mN, value superior to that obtained from control ($R_{\max}$ = 490.9 mN).

Also significant increase of hardness of the dough was noticed, whereas the springiness and adhesiveness were not significantly influenced by MRS and TG, if compared to the control.

A modified power-law model was fitted to the stress-strain data obtained from biaxial extension test and the most important finding to emerge from this analysis is that the partial substitution of wheat flour by MRS (8.8 g/100g) and addition of 0.12 g/100g of TG, presented a high value of strain hardening in accordance with a higher value of gas retention in comparison with dough formulated without MRS and TG.

The second major finding was that the third endotherm found from DSC curves probably relates to the MRS gelatinization, which occurred around 130 ºC; because it is only possible to reach this temperature at the bread surface, while inside is around 100 ººC, it can be concluded that the maize resistant starch remains after baking.

Further research should be done to investigate the effect of MRS on pan bread performance and it is necessary to determine the RS concentration in the pan bread after baking.

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Figure captions

Figure 1. Maximum resistance to extension (R_{max}) obtained from uniaxial extension test [a]; maximum pressure required (P) to inflate the bubble of the dough [b] and stress (σ_B) as a function of Hencky strain (ε_{HB}) [c]; prepared with different quantities of maize resistant starch (MRS) and transglutaminase enzyme (TG).

Figure 2. Typical DSC thermograms of aqueous suspensions of maize resistant starch (MRS) at different concentrations: MRS:W 100:0 (100 g of MRS/100g of suspension); MRS:W 70:30 (70 g of MRS/100g of suspension); MRS:W 50:50 (50 g of MRS/100g of suspension); MRS:W 30:70 (30 g of MRS/100g of suspension).

Figure 1

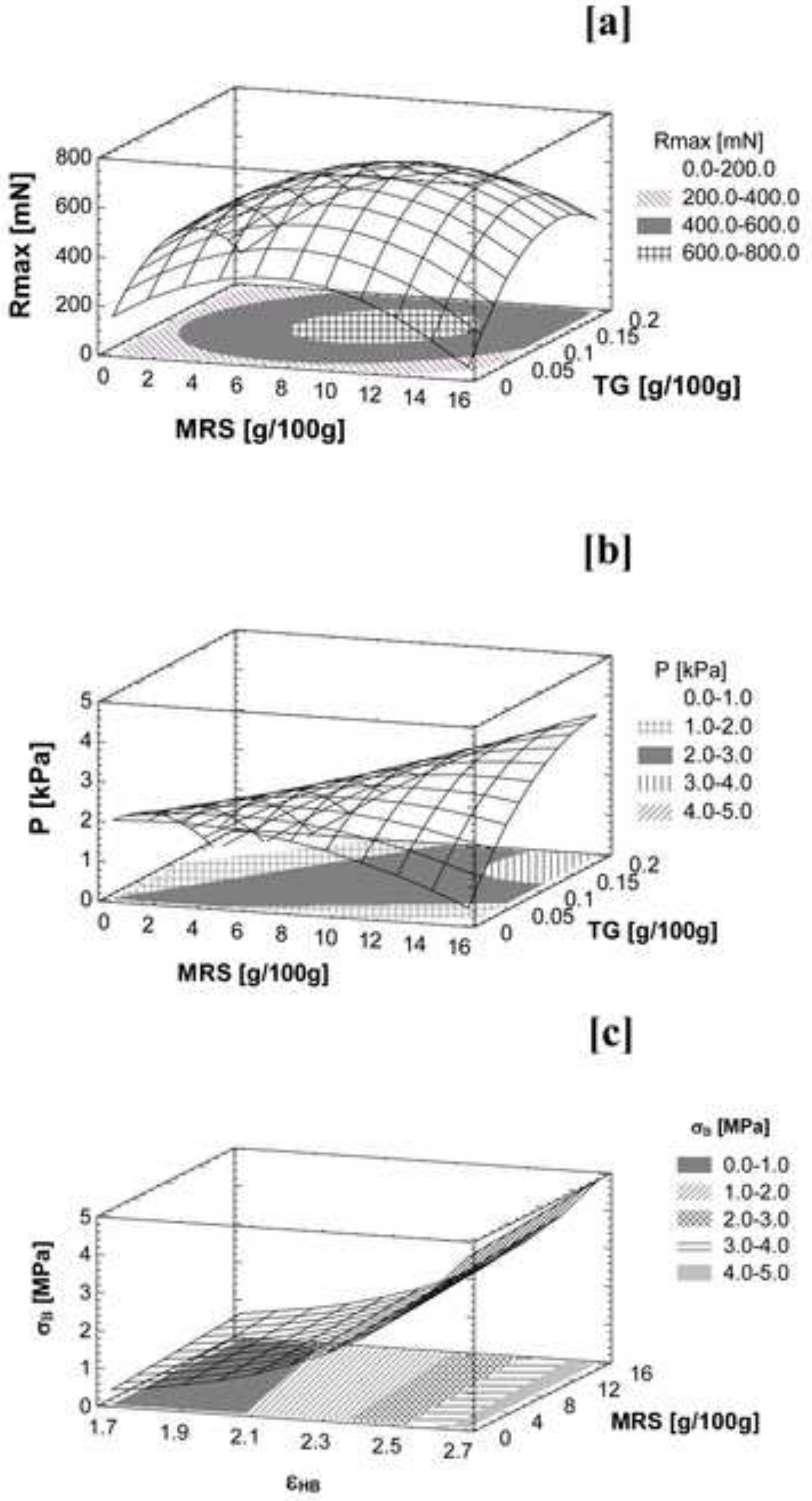
[a]
Rmax [mN]
800
600
400
200
0
0 2 4 6 8 10 12 14 16
MRS [g/100g]
0 0.05 0.1 0.15 0.2
TG [g/100g]
Rmax [mN]
0.0-200.0
200.0-400.0
400.0-600.0
600.0-800.0
[b]
P [kPa]
5
4
3
2
1
0
0 2 4 6 8 10 12 14 16
MRS [g/100g]
0 0.05 0.1 0.15 0.2
TG [g/100g]
P [kPa]
0.0-1.0
1.0-2.0
2.0-3.0
3.0-4.0
4.0-5.0
[c]
σB [MPa]
5
4
3
2
1
0
1.7 1.9 2.1 2.3 2.5 2.7
εHB
0 4 8 12 16
MRS [g/100g]
σB [MPa]
0.0-1.0
1.0-2.0
2.0-3.0
3.0-4.0
4.0-5.0

Figure 2

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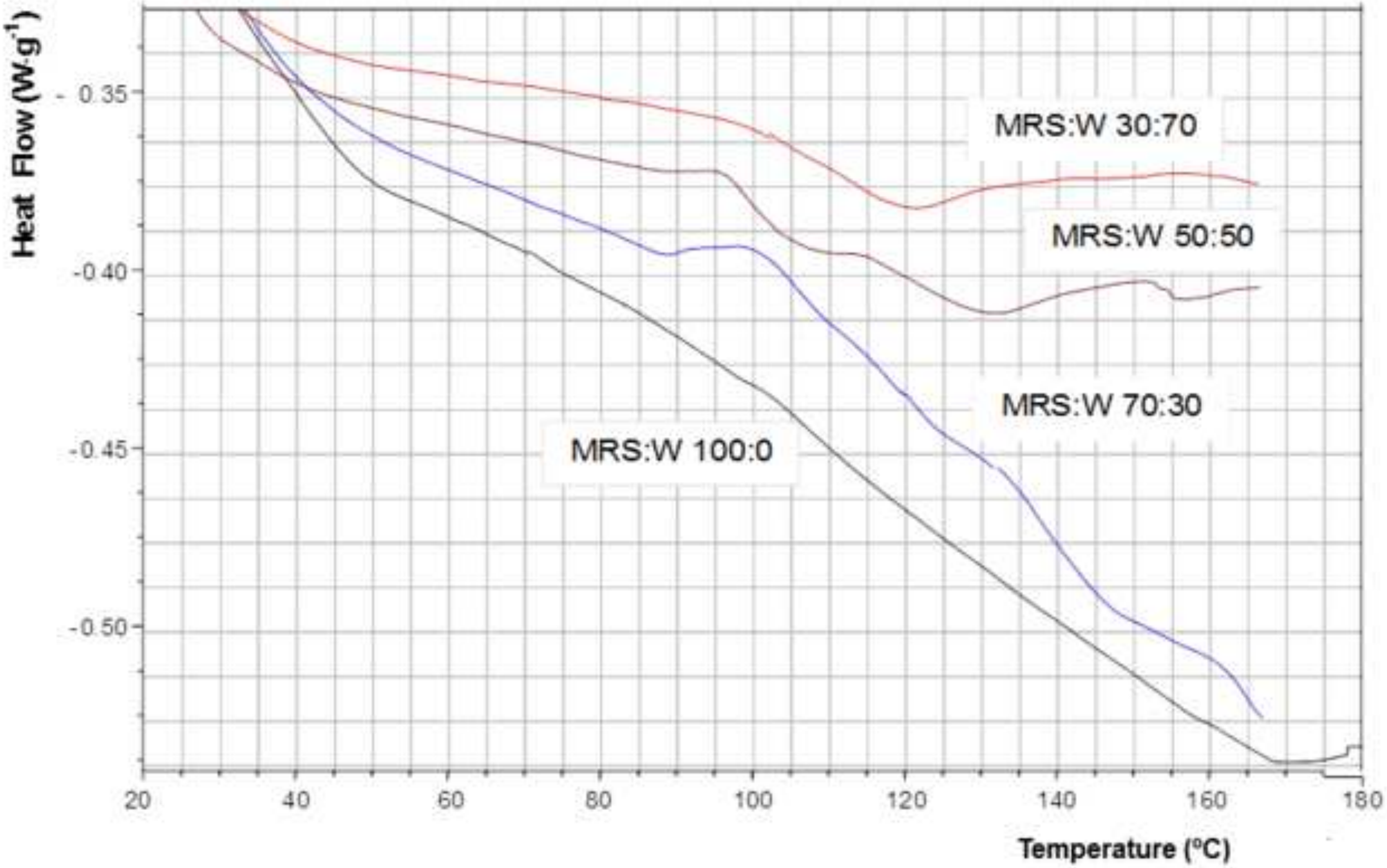

Table 1.

Hardness (H) and resilience (R_{es}) obtained from Texture Profile Analysis (TPA); maximum height (H_m), maximum pressure (P_m) and retention coefficient (R_{coef}) obtained from rheofermentometer assays of dough prepared according to a second order Central Composite Design (2^2), with three central and four star points, in relation to maize resistant starch (MRS) and transglutaminase (TG) quantities.

Blend	MRS	TG	MRS[1] [g/100g]	TG[1] [g/100g]	H [N]	R_{es} [%]	H_m [mm]	P_m [mmH_2O]	R_{coef}[4] [%]
1	-1	-1	3.5	0.05	99.57 ± 3.4^{abcA}	0.090 ± 0.002^{bcA}	42.1 ± 0.9^{cdA}	59.9 ± 3.1^{aA}	89.5 ± 3.1^{aA}
2	1	-1	13.5	0.05	107.01 ± 3.6^{bcA}	0.090 ± 0.001^{bcA}	34.6 ± 0.9^{abA}	62.7 ± 3.1^{aA}	86.5 ± 3.1^{aA}
3	-1	1	3.5	0.15	94.63 ± 3.4^{abcA}	0.084 ± 0.000^{bcA}	40.6 ± 0.9^{cdA}	53.8 ± 3.1^{aA}	94.6 ± 3.1^{aA}
4	1	1	13.5	0.15	102.79 ± 8.4^{bcA}	0.086 ± 0.001^{bcA}	33.9 ± 0.9^{aA}	67.9 ± 3.1^{aA}	95.2 ± 3.1^{aA}
5^2	0	0	8.5	0.10	99.13 ± 3.0^{bcA}	0.087 ± 0.002^{bcA}	38.7 ± 0.6^{bcA}	58.1 ± 2.0^{aA}	89.9 ± 2.0^{aA}
6^2	0	0	8.5	0.10	106.68 ± 5.0^{bcA}	0.091 ± 0.004^{bcA}	36.7 ± 0.6^{bcA}	62.6 ± 2.0^{aA}	85.3 ± 2.0^{aA}
7^2	0	0	8.5	0.10	122.05 ± 3.0^{bcA}	0.097 ± 0.002^{bcA}	40.3 ± 0.6^{bcA}	69.8 ± 2.0^{aA}	97.2 ± 2.0^{aA}
8	$-\sqrt{2}$	0	1.5	0.10	79.70 ± 1.9^{aA}	0.075 ± 0.004^{aA}	41.2 ± 1.3^{cdA}	62.6 ± 4.3^{aA}	83.9 ± 2.0^{aA}
9	0	$\sqrt{2}$	8.5	0.17	103.53 ± 3.5^{bcA}	0.088 ± 0.001^{bcA}	38.2 ± 0.6^{bcA}	65.3 ± 2.0^{aA}	83.2 ± 4.4^{aA}
10	$\sqrt{2}$	0	15.5	0.10	117.86 ± 3.5^{cAA}	0.094 ± 0.001^{cA}	32.2 ± 1.3^{aA}	60.8 ± 4.3^{aA}	86.6 ± 2.0^{aA}
11	0	$-\sqrt{2}$	8.5	0.03	87.08 ± 6.5^{bcA}	0.085 ± 0.004^{bcA}	36.7 ± 0.6^{bcA}	67.0 ± 2.0^{aA}	82.6 ± 4.4^{aA}
12^3			0.0	0.00	81.86 ± 1.9^{abA}	0.079 ± 0.001^{abA}	45.2 ± 1.3^{dA}	54.4 ± 4.3^{aA}	93.8 ± 4.4^{aA}
			Tukey HSD 5 %		24.51	0.008	2.1	14.4	14.7

[1] based on 100 % mixture

[2] central points

[3] control

[4] $$R_{\mathrm{coef}} = \left(\frac{V_{\mathrm{Total}} - V_{\mathrm{Loss}}}{V_{\mathrm{Total}}}\right)_{CO_2} \times 100$$

Different letters and different capital letters mean significant differences ($p<0.05$) amongst values in relation to MRS and TG, respectively.

Table 2.

Maximum resistance to extension (R_{max}) and maximum extensibility at rupture (E_{max}) obtained from uniaxial extension test; fracture stress (σ_{max}) and the Hencky strain (ε_H) at fracture; maximum pressure required (P), extensibility (L), baking strength (W), stress (σ_B) and the Hencky strain (ε_{HB}) obtained from biaxial extension test of dough prepared according to a second order Central Composite Design (2^2), with three central and four star points.

Blend	MRS[1] [g/100g]	TG[1] [g/100g]	R_{max} [mN]	E_{max} [mm]	σ_{max} [kPa]	ε_H	P [kPa]	L [mm]	$W \times 10^{-4}$ [J]	σ_B [MPa]	ε_{HB}
1	3.5	0.05	442.7 ± 54.4^{aA}	38.3 ± 8.0^{cC}	64.9 ± 11.7^{bB}	1.4 ± 0.2^{cC}	1.9 ± 0.2^{aAB}	102.9 ± 35.5^{bAB}	511.8 ± 89.0^{aAB}	2.6 ± 1.3^{abAB}	2.4 ± 0.3^{bA}
2	13.5	0.05	442.3 ± 79.0^{abA}	34.7 ± 9.2^{abC}	59.6 ± 8.4^{abB}	1.4 ± 0.2^{bC}	2.6 ± 0.3^{bcAB}	59.2 ± 7.6^{aAB}	535.0 ± 64.4^{aAB}	1.3 ± 0.2^{aAB}	2.1 ± 0.1^{aA}
3	3.5	0.15	446.6 ± 39.0^{aA}	43.4 ± 5.6^{cBC}	72.9 ± 3.6^{bB}	1.6 ± 0.1^{cBC}	2.9 ± 0.2^{aA}	79.7 ± 15.1^{bA}	477.8 ± 90.1^{aA}	1.8 ± 0.7^{abA}	2.3 ± 0.1^{bA}
4	13.5	0.15	555.1 ± 60.6^{abA}	23.6 ± 4.3^{abBC}	55.6 ± 6.3^{aB}	1.0 ± 0.1^{bBC}	2.3 ± 0.2^{bcAB}	57.8 ± 18.6^{aA}	441.5 ± 114.8^{aA}	1.2 ± 0.7^{aA}	2.0 ± 0.3^{aA}
5[2]	8.5	0.10	657.7 ± 119.6^{cB}	24.1 ± 6.6^{aAB}	66.9 ± 4.2^{abB}	1.1 ± 0.2^{aAB}	2.5 ± 0.3^{abB}	76.5 ± 20.2^{abAB}	563.3 ± 77.1^{aB}	1.9 ± 0.7^{abAB}	2.2 ± 0.2^{bA}
6[2]	8.5	0.10	608.0 ± 88.5^{cB}	20.4 ± 4.2^{aAB}	55.3 ± 10.8^{abB}	0.9 ± 0.2^{aAB}	2.0 ± 0.3^{abB}	88.4 ± 18.0^{abAB}	513.3 ± 105.1^{aB}	2.0 ± 0.7^{abAB}	2.4 ± 0.1^{bA}
7[2]	8.5	0.10	667.4 ± 102.1^{cB}	27.9 ± 9.7^{aAB}	75.7 ± 9.0^{abB}	1.2 ± 0.3^{aAB}	2.1 ± 0.1^{abB}	96.5 ± 21.1^{abAB}	568.7 ± 70.6^{aB}	2.6 ± 0.9^{abAB}	2.4 ± 0.2^{bA}
8	1.5	0.10	550.8 ± 91.4^{bc}	30.4 ± 12.1^{abAB}	66.8 ± 15.2^{abB}	1.2 ± 0.4^{bcAB}	2.1 ± 0.2^{abAB}	96.0 ± 27.4^{bAB}	585.1 ± 72.4^{aB}	2.6 ± 1.2^{abAB}	2.4 ± 0.2^{bA}
9	8.5	0.17	648.5 ± 57.1^{cB}	21.6 ± 2.4^{aA}	61.2 ± 6.2^{abAB}	1.0 ± 0.1^{bA}	2.2 ± 0.2^{abB}	61.6 ± 9.0^{abA}	489.0 ± 56.3^{aAB}	1.2 ± 0.3^{abA}	2.1 ± 0.1^{bA}
10	15.5	0.10	432.2 ± 53.5^{aB}	33.5 ± 11.8^{bcAB}	56.6 ± 14.8^{abB}	1.3 ± 0.4^{bcAB}	2.8 ± 0.3^{cAB}	73.7 ± 13.8^{abAB}	681.6 ± 128.2^{aB}	2.0 ± 0.5^{abAB}	2.2 ± 0.2^{abA}
11	8.5	0.03	486.9 ± 40.2^{cA}	23.9 ± 3.5^{aA}	49.3 ± 6.7^{abA}	1.1 ± 0.1^{bA}	2.3 ± 0.4^{abB}	71.1 ± 10.0^{abAB}	525.8 ± 68.4^{aAB}	1.5 ± 0.4^{abAB}	2.2 ± 0.1^{bA}
12[3]	0.0	0.00	490.9 ± 46.1^{abA}	30.1 ± 6.5^{abABC}	59.1 ± 5.1^{abAB}	1.2 ± 0.2^{bABC}	2.0 ± 0.1^{abA}	114.3 ± 38.0^{bB}	580.9 ± 139.4^{aAB}	3.3 ± 1.8^{bB}	2.5 ± 0.3^{bA}
	Tukey HSD 5 %		63.20	9.30	9.4	0.2	0.4	31.5	108.2	0.1	0.2

[1] based on 100 % mixture

[2] central points

[3] control

Different letters and different capital letters mean significant differences ($p<0.05$) amongst values in relation to MRS and TG, respectively.

Table 3.

Gelatinization and transition endotherm parameters obtained from DSC analyses; wet gluten (*WG*), dry gluten (*DG*), gluten index (*GI*), water binding capacity (*WBC*) and ash content of dough prepared according to a second order Central Composite Design (2^2), with three central and four star points.

Blend	MRS[1]	TG[1]	Gelatinization			Transition endotherm			Gluten quantity				Ash
	[g/100g]	[g/100g]	T_{onset} [ºC]	T_{peak} [ºC]	ΔH $[J \cdot g^{-1}]$[4]	T_{onset} [ºC]	T_{peak} [ºC]	ΔH $[J \cdot g^{-1}]$[4]	*WG* [%]	*DG* [%]	*GI*	*WBC* [%]	[g/100g]
1	3.5	0.05	62.1 ± 1.6^{aA}	79.9 ± 2.9^{aA}	6.5 ± 0.9^{abA}	107.3 ± 2.5^{aA}	118.1 ± 5.2^{aA}	1.2 ± 0.3^{aA}	27.0 ± 0.1^{dA}	9.2 ± 0.1^{abA}	89.2 ± 3.0^{aA}	17.8 ± 0.2^{bA}	1.6 ± 0.0^{aA}
2	13.5	0.05	60.8 ± 1.4^{aA}	77.6 ± 1.1^{aA}	6.8 ± 0.7^{abA}	107.0 ± 0.9^{aA}	115.4 ± 6.4^{aA}	0.7 ± 0.3^{aA}	24.9 ± 0.6^{abA}	7.4 ± 1.2^{aA}	89.3 ± 4.5^{bA}	17.4 ± 0.4^{abA}	1.5 ± 0.0^{bA}
3	3.5	0.15	60.3 ± 1.6^{aA}	77.5 ± 2.9^{aA}	7.9 ± 0.9^{abA}	112.1 ± 2.5^{aA}	112.3 ± 5.2^{aA}	2.1 ± 0.3^{aA}	29.6 ± 0.8^{dA}	10.7 ± 1.1^{abA}	79.3 ± 2.9^{aA}	17.5 ± 0.6^{bA}	1.6 ± 0.0^{aA}
4	13.5	0.15	62.8 ± 1.4^{aA}	79.2 ± 1.1^{aA}	7.7 ± 0.7^{abA}	108.3 ± 0.9^{aA}	116.7 ± 6.4^{aA}	0.7 ± 0.3^{aA}	24.9 ± 0.4^{abA}	8.8 ± 0.1^{aA}	98.5 ± 0.2^{bA}	17.8 ± 0.2^{abA}	1.6 ± 0.0^{bA}
5[2]	8.5	0.10	62.9 ± 1.6^{aA}	78.5 ± 1.9^{aA}	5.6 ± 0.7^{aA}	106.3 ± 5.4^{aA}	116.1 ± 3.7^{aA}	0.9 ± 0.2^{aA}	26.1 ± 0.2^{bcA}	8.2 ± 1.0^{abA}	98.6 ± 0.8^{bA}	19.0 ± 1.9^{abA}	1.6 ± 0.0^{abA}
6[2]	8.5	0.10	61.1 ± 1.6^{aA}	77.6 ± 1.9^{aA}	6.5 ± 0.7^{aA}	119.4 ± 5.4^{aA}	119.6 ± 3.7^{aA}	1.0 ± 0.2^{aA}	24.9 ± 0.3^{bcA}	8.8 ± 0.2^{abA}	92.1 ± 1.4^{bA}	$18.4 \pm 2.8_{abA}$	1.5 ± 0.0^{abA}
7[2]	8.5	0.10	61.5 ± 1.6^{aA}	78.0 ± 1.9^{aA}	6.7 ± 0.7^{aA}	106.3 ± 5.4^{aA}	116.0 ± 3.7^{aA}	1.0 ± 0.2^{aA}	25.4 ± 0.3^{bcA}	9.9 ± 0.6^{abA}	91.4 ± 1.0^{bA}	$16.2 \pm 0.3_{abA}$	1.6 ± 0.0^{abA}
8	1.5	0.10	61.0 ± 1.3^{aA}	78.8 ± 3.8^{abA}	9.3 ± 1.1^{bA}	107.2 ± 3.6^{aA}	116.8 ± 9.0^{aA}	1.4 ± 0.4^{aA}	29.0 ± 1.5^{dA}	9.6 ± 0.5^{abA}	93.3 ± 3.8^{abA}	16.5 ± 0.8^{bA}	1.6 ± 0.0^{aA}
9	8.5	0.17	62.6 ± 1.6^{aA}	79.5 ± 1.9^{aA}	6.4 ± 0.7^{aA}	107.2 ± 5.4^{aA}	116.6 ± 3.7^{aA}	0.9 ± 0.2^{aA}	25.8 ± 0.5^{bcA}	10.1 ± 0.1^{abA}	95.9 ± 0.3^{bA}	17.9 ± 1.2^{abA}	1.6 ± 0.0^{abA}
10	15.5	0.10	61.8 ± 1.3^{aA}	77.6 ± 3.8^{aA}	5.8 ± 1.1^{abA}	104.8 ± 3.6^{aA}	115.1 ± 9.0^{aA}	0.9 ± 0.4^{aA}	22.9 ± 0.2^{aA}	7.9 ± 0.3^{abA}	93.4 ± 0.5^{abA}	17.3 ± 2.1^{aA}	1.5 ± 0.0^{cA}
11	8.5	0.03	61.3 ± 1.6^{aA}	77.6 ± 1.9^{aA}	4.9 ± 0.7^{aA}	106.0 ± 5.4^{aA}	115.9 ± 3.7^{aA}	0.9 ± 0.2^{aA}	26.7 ± 0.3^{bcA}	9.2 ± 0.0^{abA}	89.4 ± 0.7^{bA}	16.1 ± 0.4^{abA}	1.6 ± 0.0^{abA}
12[3]	0.0	0.00	61.8 ± 1.0^{aA}	87.4 ± 1.4^{bA}	6.5 ± 0.5^{abA}	108.2 ± 2.4^{aA}	118.5 ± 6.4^{aA}	1.2 ± 0.3^{aA}	29.7 ± 3.9^{cdA}	9.9 ± 1.5^{bA}	92.9 ± 2.2^{abA}	15.6 ± 1.1^{abA}	1.6 ± 0.0^{abA}
	Tukey HSD 5 %		3.7	5.0	1.8	10.6	22.6	1.1	1.8	1.6	7.6	2.2	0.04

[1] based on 100 % mixture
[2] central points
[3] control
[4] dry basis

Different letters and different capital letters mean significant differences ($p<0.05$) amongst values in relation to MRS and TG, respectively.