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Effect of a combination of enzymes on the fundamental rheological behavior of bread dough enriched with resistant starch

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Abstract

The effect of three enzymes on the fundamental rheological parameters of bread dough with high content of resistant starch (RS) was studied. The RS was added as an alternative to increase the fiber ingestion while the enzymes, to overcome the gluten dilution. Optimum dough was formulated with partial substitution of wheat flour by RS (12.5 g/100 g) and enzymes transglutaminase (4 mg/100 g), glucose oxidase (2.5 mg/100 g) and xylanase (0.5 mg/100 g). Dough produced with RS and without enzymes was considered as control and dough without RS or enzymes was considered as regular for comparison. Fundamental rheological parameters were obtained from uniaxial extension, biaxial extension and oscillatory tests. Also, starch gelatinization and retrogradation were studied by differential scanning calorimetry. The partial replacement of WF by RS resulted in less extensible dough, whereas the addition of enzymes increased the strain hardening index allowing higher dough expansion. The addition of enzymes reduced the elastic modulus resulting in a behavior similar to the regular dough. RS was not gelatinized during baking, hence it can be considered as dietetic fiber. Wheat starch retrogradation after 7 days of storage was observed, indicating bread aging.

Keywords: transglutaminase; glucose oxidase; xylanase; rheology; starch gelatinization.

1. Introduction

[1]Dietary fiber provides health benefits such as the decrease of intestinal transit time, increase of stools bulk, being fermentable by colonic microflora, reduction of total and/or LDL cholesterol levels of blood and reduction of post-prandial blood glucose level (FAO/WHO, 2009), what makes it an interesting ingredient for the development of functional foods in response to the epidemic of non-communicable diseases like cardiovascular diseases, cancer and diabetes (WHO, 2011). Resistant starch (RS), which is not digested allowing fermentation in the colon, can be considered a kind of dietary fiber. Four types of RS have been described: RS_1, that is physically inaccessible to digestion as the starch found in grains or seeds; RS_2, which its granules are structured in a way that does not allow enzymes to hydrolyze it; RS_3 which is the retrograded starch formed when foods are cooked and cooled; RS_4 which is the chemically-modified starch (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata & Pérez-Álvarez, 2010). High-amylose maize starch,

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[1] **Abbreviations**

RS Resistant Starch

Gox Glucose oxidase

HE xylanase

TG transglutaminase

WF wheat flour

SSL sodium stearoyl lactylate

DATEM diacetyl tartaric acid ester of mono- and diglycerides

PS80 Polysorbate 80

HSD Honest significant difference

DSC differential scanning calorimetry

defined as RS_2, is a fine white powder, obtained from a specific hybrid of corn naturally rich in amylose content. Its addition to bread dough produces gluten dilution yielding dough with poor rheological properties and baking performance (Sanchez, Puppo, Añón, Ribotta, León & Tadini, 2014), and bread with poor texture properties (Almeida, Chang & Steel, 2013), which limits its application. So, additives such as enzymes need to be used to minimize these effects.

Enzymes transglutaminase (TG), glucose oxidase (Gox) and fungal xylanase (HE) have wide application in the bakery industry. TG is a strong protein cross-linking enzyme, improving the dough strength and bread volume (AB Enzymes, 2014). Gox catalyzes the oxidation of glucose to gluconic acid with simultaneous formation of hydrogen peroxide (Bankar, Bule, Singhal & Ananthanarayan, 2009). Hydrogen peroxide is capable of oxidizing free sulfhydryl groups forming disulfide bonds within the gluten network, resulting in its strengthening (Novozymes, 2014). HE breaks down the hemicellulose in wheat flour helping the redistribution of water and leaving the dough softer and easier to knead (Polizeli, Rizzatti, Monti, Terenzi, Jorge & Amorim, 2005).

When studying bread dough, rheological measurements (fundamental or empirical and of large or small deformation) constitute an important approach, which can be correlated to bread quality as reported by many authors (Dobraszczyk & Salmanowicz, 2008; Janssen, Van Vliet & Vereijken, 1996; Kenny, Wehrle, Dennehy & Arnedt, 1999). Empirical measurements are the most used in the bread industry; however, by their nature are dependent of the equipment used. Otherwise, fundamental measurements provide physical parameters like force, deformation, torque, energy, and the results are independent of the test equipment and can theoretically be used to model the flow conditions encountered by the dough during mixing, proofing and baking (Stojceska, Butler, Gallagher & Keehan, 2007). Small deformation tests provide fundamental parameters, but they are not directly related to the baking process in which the dough is submitted to large deformation. During kneading, dough is stretched and stressed and a small amount of air is occluded in the dough, forming small spherical gas cells whose size increases during the fermentation, stage in which part of the carbon dioxide produced by the yeast migrates into them. For that reason, authors like Bloksma (1957), Dunnewind, Sliwinski, Grolle, and Van Vliet (2004) and Launay, Buré, and Praden (1977) proposed approaches that allow obtaining fundamental parameters in large deformation tests. Moreover, Dobraszczyk (2003) in his review, suggested that existing studies show better relationships between rheological properties with large deformation extensional and relaxation properties and baking performance.

In a previous work, enzymes TG, Gox and HE were added to bread dough with RS in different concentrations and an optimum formulation was found which presented baking performance similar to regular dough without RS (Altuna, Ribotta & Tadini, 2015).

The objective of this work was to study the effect of a combination of the enzymes TG, Gox and HE on the fundamental rheological properties of bread dough with high content of RS submitted to small and large deformation tests. Dough formulated with RS and enzymes (optimum) was compared to dough formulated without RS or enzymes (regular) and dough formulated with RS and without enzymes (control).

2. Materials and Methods

2.1. Materials

Wheat flour (WF) with 13.9 g/100 g of moisture, 29 g/100 g of wet gluten, 9.1 g/100 g of dry gluten and 0.43 g/100 g of ash was supplied by AB Brasil (Brazil). The Brabender Farinograph parameters were: water absorption (500 BU) of 59.1 g/100 g, stability of 24.3

min, development time of 13.4 min and mixing tolerance of 0 UB; resistant starch Hi-maize® 260 containing 60 g/100 g of resistant starch (insoluble dietary fiber) and 40 g/100 g of digestible (glycemic) starch was supplied by Ingredion (Brazil); transglutaminase (TG) obtained from specific cultures of S*treptoverticilium mobarense* with enzyme activity of 100 TGU/g was supplied by AB Enzymes (Brazil); glucose oxidase (Gox) produced by submerged fermentation of a selected strain of *Aspergillus niger* with enzyme activity of 10,000 GOD/g and fungal xylanase (HE) produced by submerged fermentation of *Aspergillus oryzae* with enzyme activity of 60,000 FXU/g from Novozymes were supplied by Granotec (Brazil); emulsifiers sodium stearoyl lactylate (SSL) and diacetyl tartaric acid ester of mono- and diglycerides (DATEM) and enzyme α-amilase were supplied by DuPont (Brazil). Polysorbate 80 (PS80) from Oxiteno was supplied by AB Brasil (Brazil). Sodium chloride (Cisne®, Brazil) was purchased from the local market and distilled water was used.

2.2. Experimental procedure

Dough was formulated according to Table 1. The blend of emulsifiers SSL, PS80 and DATEM used was found as optimum in a previous work (Gómez, Buchner, Tadini, Añón & Puppo, 2013) and enzyme α-amilase was added to correct the Falling Number. A mixture of WF and RS was used in control and optimum dough while regular dough was produced without RS. The concentrations of enzymes used in optimum dough formulation was chosen according to the results found by Altuna et al. (2015) in a previous work. The content of RS in the mixture was about 7.5 g/100 g based on the content of RS in the Hi-maize® 260 added to the dough. It is expected that no significant changes are produced on the RS content during baking due to the temperatures reached in the process as verified by Sanchez et al. (2014) and Matsuda (2007).

Dough was mixed and kneaded using a Stand Mixer Professional (Kitchen Aid, Brazil) equipped with dough hook. All dry ingredients except for salt were mixed for 2 min at low speed, after that, water was added during 2 min while mixing at low speed, then sodium chloride was added and dough was mixed for additional 3 min. Finally, dough was kneaded for 12 min at medium speed.

2.3. Uniaxial extension tests

Uniaxial extension tests were performed using a TA.XT*plus* Texture Analyser (SMS, UK) equipped with the accessory Kieffer Dough & Gluten Extensibility Rig and following the protocol described by the manufacturer (SMS, 1995).

The mold was covered with a thin layer of mineral oil and Teflon® strips were placed in the mold to aid sample removal. Immediately after kneading, a portion of dough was pressed in the mold, the excess was trimmed, and then the mold was closed and placed in a plastic bag to rest for 45 min at 25 °C. The dough strips in the three first and last positions of the mold were discarded and the remaining strips (at least 7 for each formulation) were submitted to the uniaxial extension test 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. From the force-time curves, the fundamental parameters: force normal to the sample section (F_d), uniaxial tension (σ_u), uniaxial deformation (ε_u) and uniaxial extensional viscosity (μ_{eu}) were calculated according to the equations proposed by Dunnewind et al. (2004). At the point of maximum force the following parameters were obtained: maximum force normal to the sample section ($F_{d\max}$), uniaxial tension at maximum force (σ_{uf}), uniaxial deformation at maximum force (ε_{uf}) and uniaxial extensional viscosity at maximum force (μ_{euf}). The tension (σ_u) vs. deformation (ε_u) curves were adjusted to the Power Law model and the strain hardening index (n_u) and the viscosity index (K_u) were obtained according to eq.(1).

$$\sigma_u = K_u \varepsilon_u^{\,n_u} \quad (1)$$

wherein σ_u is the uniaxial tension [kPa], K_u is the viscosity index [kPa], ε_u is the uniaxial deformation [dimensionless] and n_u is the uniaxial strain hardening index [dimensionless].

2.4 Biaxial extension tests

Biaxial extension tests were performed using a TA.XT*plus* Texture Analyser (SMS, UK) equipped with the accessory D/R Dough Inflation System and following the protocol described by the manufacturer (SMS, 1995).

Dough was left to rest for 15 min after kneading and then placed between two Teflon® bars of 8 mm height and sheeted with a Teflon® roll until it reached the same height of the bars (8 mm of thickness). Five disks of dough of 65 mm diameter were cut with a pastry cutter, placed in the molds and pressed for 30 s to a thickness of 2.67 mm. The disks were left to rest for 30 min inside the molds covered with a Perspex® lid before the tests were performed. Dough was inflated with air at a growing flow rate with the aim of maintaining the strain rate constant at 0.1 s^{-1}, until bubble rupture. Air volume and pressure inside the bubble were registered along the assay.

The bubble volume (V_b), biaxial tension (σ_b), biaxial deformation (ε_b), and biaxial extensional viscosity (μ_{eb}) were calculated using the equations proposed by Bloksma (1957) and Launay and Buré (1977) and at the point of bubble rupture, the following parameters were obtained: $V_{b\text{rup}}$, $\sigma_{b\text{rup}}$, $\varepsilon_{b\text{rup}}$, $\mu_{eb\text{rup}}$.

The tension (σ_b) vs. deformation (ε_b) curves were adjusted to the Power Law model and the strain hardening index (n_b) and the viscosity index (K_b) were obtained.

2.5 Oscillatory tests in rheometer

Tests were performed in a dynamic rheometer AR 550 (TA, USA) equipped with two parallel plates of 40 mm diameter covered with sandpaper to avoid dough slipping and a 1.5 mm gap between plates was used. The rheometer used includes a water container around the sample that provided a moisture saturated atmosphere to avoid sample drying. Samples were placed in the equipment right after kneading and left to rest for 15 min. Then, a stress sweep between (0.5 and 200) Pa was performed at a frequency of 1 Hz to determine the linear region of viscoelasticity of the material. The frequency sweep was carried out between (0.005 and 40) Hz at a fixed maximum stress equal to 5 Pa. The storage (elastic) modulus (*G'*) and the loss (viscous) modulus (*G"*) as a function of the frequency were calculated by the software Data Analysis (TA, USA). Tests were performed in duplicates.

2.6 Starch gelatinization and retrogradation

Starch gelatinization was measured by differential scanning calorimetry (DSC) in a Q2000 (TA, USA) calibrated with indium. Tests were performed using DSC high pressure capsules, made of stainless steel and of 35 μL maximum capacity, hermetically sealed with gold-plated copper seals.

Suspensions of WF or RS with different levels of hydration were prepared inside the capsules adding (0, 25, 60 and 233) g/100 g of deionized water (WF or RS basis). Samples weighing between (4 and 11) mg were left to rest for one hour before they were stabilized at 15 °C and then heated to 180 °C at 10 °C min^{-1}.

Dough was prepared as described in section 2.2 and samples weighing between (10 and 14) mg were placed in the capsules and heated to 120 °C at 10 °C min^{-1}. This heating rate was chosen because it is close that occurring during the baking process (Ribotta, León & Anón, 2003).

After the tests, the samples were stored inside the sealed capsules at room temperature for 7 days and then submitted to the same temperature program to quantify the wheat starch retrogradation, which is an indirect measurement of bread aging. Tests were performed in duplicates.

2.7 Statistical analyses

Data obtained from all tests were analyzed to determine if there were honest significant differences (HSD) between the three formulations, by the Tukey test within the 95 % of confidence interval. All the analyses were performed using the statistics software Statgraphics Centurion XVI (Statpoint Technologies, USA).

3. Results and Discussion

3.1 Uniaxial extension

In Table 2, from uniaxial extension test, it can be observed that the control dough showed higher maximum force ($F_{d\max}$) and lower deformation (ε_{uf}) compared to regular and optimum doughs. The partial replacement of WF by RS resulted in harder dough, more difficult to extend while the addition of enzymes minimized this effect due to the protein crosslinking by TG and Gox. This can be related to a reduced extensibility of the dough due to gluten dilution, since its rheological characteristics are attributed to the gluten network developed during kneading (Masi, Cavella & Piazza, 2001). Ktenioudaki, Butler, and Gallagher (2011) have correlated specific volume of bread with deformation during uniaxial extension performed with the Kieffer rig, showing the importance of these measurements regarding bread quality.

As can be observed in Figure 1a, as deformation increases, the dough becomes more resistant and higher tension is necessary to deform it. This phenomenon is known as strain hardening and prevents the dough to collapse while being extended, allowing higher expansion during fermentation. Data were fitted to the Power Law model ($r^2 > 0.98$) from which strain hardening index (n_u) and viscosity index (K_u) were determined (Table 2). Regular and optimum doughs had higher n_u and lower K_u compared to control dough, in agreement with Altuna et al. (2015) who observed that dough expansion during fermentation was reduced with the addition of RS and this undesired effect was overcame when enzymes TG, Gox and HE were added to the formulation.

3.2 Biaxial extension

Baking is about the growth and stability of bubbles and their failure cause great impact on the final quality of the bread, both in terms of its appearance (texture) and final volume. Therefore the rheological properties of the bubble walls are important in relation to gas cell stabilization and baking, and thus to the final structure and volume of the baked product (Dobraszczyk, 2003). Chin and Campbell (2005) studied the relationship of aeration and rheology of dough using biaxial extension and found that dough produced from strong flour had high peak pressure and further drum distance before bubble rupture. Regular, control and optimum doughs were submitted to the biaxial extension test and the results were analyzed by a fundamental approach, shown in Table 2. Partial substitution of WF by RS reduced the bubble volume at rupture ($V_{b\mathrm{rup}}$) and the biaxial deformation at rupture ($\varepsilon_{b\mathrm{rup}}$) indicating that the gluten dilution resulted in less expansion of the bubbles which is directly related to their stability. The addition of enzymes TG, Gox and HE increased the biaxial tension at rupture ($\sigma_{b\mathrm{rup}}$) indicating dough strengthening.

The values of biaxial tension at bubble rupture were higher than those obtained in the uniaxial extension tests, indicating that dough is more resistant to this type of deformation, which is related to the growth of the gas bubbles inside the dough during fermentation responsible for dough expansion. With respect to the deformation, values obtained in both tests were comparable and presented the same tendency. The advantage of this test is that it resembles practical conditions experienced by the cell walls within the dough during the proof and oven rise.

The curves of biaxial tension vs. biaxial deformation (Fig. 1 b) had the same shape of those obtained for the uniaxial extension test, again showing the strain hardening characteristic of the dough. From the Power Law model fitting ($r^2 > 0.93$) the parameters n_b and K_b were obtained (Table 2). The addition of enzymes to dough formulated with RS increased the strain hardening index, probably due to the raise of the number of disulfide bonds, that is, the greater the strain hardening, the greater the deformation allowed before failure, and consequently better baking performance (Altuna et al., 2015).

3.3 Oscillatory tests

Viscoelastic behavior of regular, control and optimum dough was measured in oscillatory tests. The results of the stress sweep between (0.5 and 200) Pa at 1 Hz (Fig. 2 a) show that all doughs tested presented linear viscoelastic behavior between (0 and 10) Pa. For tension higher than 10 Pa, a decrease in the elastic modulus (*G'*) was observed indicating structural changes. Therefore, the frequency sweep tests were carried out at 5 Pa of maximum tension. In all the tests, both the elastic (*G'*) and viscous (*G"*) contributions to the complex modulus increased with the increase of frequency, i.e., with reducing time of observation. Although the relative contribution of each parameter varies along the frequency interval (0.01 - 40

Hz), the elastic character of the dough dominates, indicating that the gluten network behaves like a cross-linked polymer (Fig. 2 b).

As observed by Petrofsky and Hoseney (1995), dough with higher content of gluten (regular) shows lower *G*' and *G*" values, indicating more expansible dough, if compared to the control dough. This result is in agreement with Ahmed, Almusallam, Al-Salman, Abdulrahman and Al-Salem (2013) who observed an increase in *G'* and *G"* when adding date fiber to dough. The addition of enzymes (optimum dough) reduced the elastic modulus resulting in a behavior similar to regular dough, probably due to the action of the HE, which produced water redistribution softening the dough (Roccia, Ribotta, Ferrero, Pérez & León, 2012). The same tendency was observed regarding *G"* (Fig. 2 b).

3.4 Starch gelatinization and retrogradation

Natural starch resists human digestion, however, when heated in the presence of water it overcomes a transformation known as gelatinization, which leaves it easily digestible. The gelatinization temperature and enthalpy depend on the proportion of water, the presence of other solutes and the process conditions (Sablani, 2009). With the aim of determining the temperatures and enthalpies of gelatinization of the starch present in the WF and the maize RS, aqueous suspensions of WF and of RS at different levels of hydration were analyzed by differential scanning calorimetry. Results obtained for WF show that starch gelatinization takes place only in the presence of water and at temperatures around 60 °C (Fig. 3 a). Furthermore, for some suspensions a second peak was observed around 100 °C corresponding to the fusion of the amylo-lipid complex. Regarding the RS suspensions, the gelatinization peak was observed in temperatures above 140 °C (Fig. 3 b) confirming that the RS is not gelatinized during bread baking, in which the product reaches temperatures around 100 °C (Purlis & Salvadori, 2009). These results are in agreement with Sanchez et al. (2014) who also observed that RS gelatinization occurred at temperatures above 100 °C. Besides, thermograms exhibit some spikes that do not correspond to thermal transformations of the material, but might be due to power supply disturbances.

Regular, control and optimum doughs were also tested by DSC (Fig. 4) and the following parameters were calculated from the curves obtained: onset temperature (T_{onset}), peak temperature (T_{peak}) and starch gelatinization enthalpy (ΔH) (Table 3). No significant differences were found between the three formulations tested. However, it was observed that starch gelatinization takes place at a higher temperature in the dough, compared to the WF suspensions and that the gelatinization peak has a flat shape, which means a slower transformation. A possible explanation to this could be the presence of sodium chloride and emulsifiers (Sablani, 2009). Moreover, in our previous work, 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. When the samples were submitted to the same program of temperature after 7 days of storage, a peak corresponding to the fusion of the retrograded starch crystals was observed. Therefore, there was wheat starch retrogradation, indicating bread aging, which explains the results found by Altuna et al. (2015) who observed an increase in crumb firmness at the 7th day of storage. There was not significant difference between formulations and the temperatures and enthalpy were lower than those observed in the first heating (Table 3).

4. Conclusions

The present study was designed to analyze the rheological behavior of dough enriched with resistant starch by a fundamental approach. The results have enhanced our understanding of dough rheology and predicting baking performance, providing information that could be important for the bakery industry.

The major finding was that the addition of resistant starch reduces the dough expansion during fermentation and the enzymes overcame this undesirable effect. The second finding was that the fundamental approach used in this study offered information about dough responses at the same conditions experienced during the proof and oven rise.

It was concluded that the enzymes TG, Gox and HE improved the rheological behavior of dough with RS.

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Figure captions

Fig. 1 Uniaxial tension (σ_u) as a function of uniaxial deformation (ε_u) obtained from the uniaxial extension test for regular dough without resistant starch or enzymes, fitted to the Power Law model (········ experimental; —— fitted) **(a)**; biaxial tension (σ_b) as a function of biaxial deformation (ε_b) obtained from the biaxial extension test for regular dough without resistant starch or enzymes, fitted to the Power Law model (········ experimental; —— fitted) **(b)**.

Fig. 2 Elastic modulus (*G'*) as a function of tension (σ) obtained in oscillatory tests for dough formulated without resistant starch (RS) (■ regular), with RS and without enzymes (● control) and with RS and 2.5 mg/100 g of Gox, 4 mg/100 g of TG and 0.5 mg/100 g of HE (▲ optimum) **(a);** elastic modulus (*G'*), viscous modulus (*G"*) and loss tangent (tan δ) as a function of frequency (*f*) obtained in oscillatory tests for dough formulated without resistant starch (RS) (■; □; ▣; regular), with RS and without enzymes (●; ○; ◉; control) and with RS and 2.5 mg/100 g of Gox, 4 mg/100 g of TG and 0.5 mg/100 g of HE (▲; △; ◭; optimum) **(b)**.

Fig. 3 Heat flow as a function of temperature (*T*) obtained by DSC during heating of wheat flour (WF) suspensions in water (W) at different levels of hydration (········ WF:W=100:0; - - - - WF:W=100:25; —— WF:W=100:60; – – – WF:W=100:233) **(a);** heat flow as a function of temperature (*T*) obtained by DSC during heating of resistant starch (RS) suspensions in water (W) at different levels of hydration (········ RS:W=100:0; - - - - RS:W=100:25; —— RS:W=100:60; – – – RS:W=100:233) **(b)**.

Fig. 4 Heat flow as a function of temperature (*T*) obtained by DSC during heating of bread dough (—— first heating; - - - - second heating after 7 days of storage), formulated without RS (regular) **(a)**, with RS and without enzymes (control) **(b)** and with RS and 2.5 mg/100 g of Gox, 4 mg/100 g of TG and 0.5 mg/100 g of HE (optimum) **(c)**.

Table 1

Ingredients used in the formulation of dough (regular, control and optimum).

Ingredients [g/100g][a]	**Formulation**		
	Regular	**Control**	**Optimum**
Wheat flour	100	87.5	87.5
Resistant starch		12.5	12.5
Water	59.1	59.1	59.1
Sodium chloride	2	2	2
Yeast	1.2	1.2	1.2
SSL	0.245	0.245	0.245
DATEM	0.075	0.075	0.075
PS80	0.18	0.18	0.18
α - amilase	0.0152	0.0152	0.0152
TG			0.004
Gox			0.0025
HE			0.0005

[a] Concentrations expressed in mixture (wheat flour + resistant starch) basis

Table 2

Maximum force normal to the sample section ($F_{d\mathrm{max}}$), uniaxial tension at maximum force (σ_{uf}), Hencky uniaxial deformation at maximum force (ε_{uf}), uniaxial extensional viscosity at maximum force (μ_{euf}), strain hardening index (n_u) and viscosity index (K_u) obtained from uniaxial extension of dough ($\mathrm{U_{ext}}$) and bubble volume at rupture ($V_{b\mathrm{rup}}$), biaxial tension at rupture ($\sigma_{b\mathrm{rup}}$), Hencky biaxial deformation at rupture ($\varepsilon_{b\mathrm{rup}}$), biaxial extensional viscosity at rupture ($\mu_{eb\mathrm{rup}}$), biaxial strain hardening index (n_b) and biaxial viscosity index (K_b) obtained from biaxial extension ($\mathrm{B_{ext}}$): regular, control and optimum formulations.

Formulation			**Regular**	**Control**	**Optimum**	**HSD**
U$_{\mathrm{ext}}$	$F_{d\mathrm{max}}$	[mN]	130 ± 14^{b}	174 ± 10^{a}	140 ± 14^{b}	16
	σ_{uf}	[kPa]	58.16 ± 10.38^{a}	56.12 ± 5.89^{a}	54.45 ± 6.30^{a}	9.46
	ε_{uf}	[-]	1.89 ± 0.10^{b}	1.61 ± 0.10^{a}	1.75 ± 0.14^{b}	0.15
	μ_{euf}	[kPa s]	836.2 ± 211.2^{b}	586.5 ± 115.2^{a}	689.5 ± 153.5^{ab}	201.7
	n_u	[-]	1.78 ± 0.14^{b}	1.45 ± 0.13^{a}	1.89 ± 0.22^{b}	0.22
	K_u	[kPa]	16.65 ± 1.66^{a}	23.87 ± 6.36^{b}	17.38 ± 3.19^{a}	5.93
B$_{\mathrm{ext}}$	$V_{b\mathrm{rup}}$	[cm^3]	329.1 ± 50.3^{b}	212.4 ± 52.5^{a}	245.5 ± 34.0^{a}	78.4
	$\sigma_{b\mathrm{rup}}$	[MPa]	0.99 ± 0.10^{a}	0.64 ± 0.20^{a}	1.50 ± 0.45^{b}	0.50
	$\varepsilon_{b\mathrm{rup}}$	[-]	2.17 ± 0.11^{b}	1.84 ± 0.17^{a}	1.96 ± 0.10^{ab}	0.22
	μ_{ebrup}	[MPa s]	4.21 ± 0.41^{ab}	2.44 ± 0.92^{a}	6.03 ± 2.06^{b}	2.24
	n_b	[-]	2.44 ± 0.10^{a}	2.51 ± 0.29^{a}	3.53 ± 0.33^{b}	0.44
	K_b	[kPa]	163.5 ± 41.6^{a}	154.8 ± 27.6^{a}	165.3 ± 21.3^{a}	53.1

Means in the same row with the same letters are not statistically different ($p>0.05$).

HSD: Honest Significant Differences.

Regular: dough formulated without resistant starch (RS).

Control: dough formulated with RS and without enzymes.

Optimum: dough formulated with RS and a blend of enzymes (2.5 mg/100 g of Gox, 4 mg/100 g of TG and 0.5 mg/100 g of HE).

Table 3

Onset temperature (T_{onset}), peak temperature (T_{peak}) and gelatinization enthalpy (ΔH) obtained during the first heating of the dough and after 7 days of storage, by differential scanning calorimetry: regular, control and optimum formulations.

		Regular	**Control**	**Optimum**	**HSD**
First heating					
T_{onset}	[°C]	60.89 ± 0.87^{aB}	61.23 ± 0.70^{aB}	62.44 ± 0.88^{aB}	4.19
T_{peak}	[°C]	83.30 ± 0.31^{aB}	76.15 ± 0.12^{aB}	74.44 ± 9.79^{aB}	8.58
ΔH	[J/g] d.b.	3.17 ± 0.74^{aB}	3.24 ± 0.11^{aB}	3.70 ± 1.22^{aB}	1.15
Second heating after 7 days of storage					
T_{onset}	[°C]	53.84 ± 4.09^{aA}	51.39 ± 2.72^{aA}	52.23 ± 1.57^{aA}	4.19
T_{peak}	[°C]	58.22 ± 0.83^{aA}	55.52 ± 1.06^{aA}	57.60 ± 3.56^{aA}	8.58
ΔH	[J/g] d.b.	0.68 ± 0.36^{aA}	1.01 ± 0.40^{aA}	0.89 ± 0.27^{aA}	1.15
HSD		2.77	5.66	0.76	

Means in the same row with the same lowercase letters are not statistically different ($p>0.05$).

Means in the same column with the same uppercase letters are not statistically different ($p>0.05$).

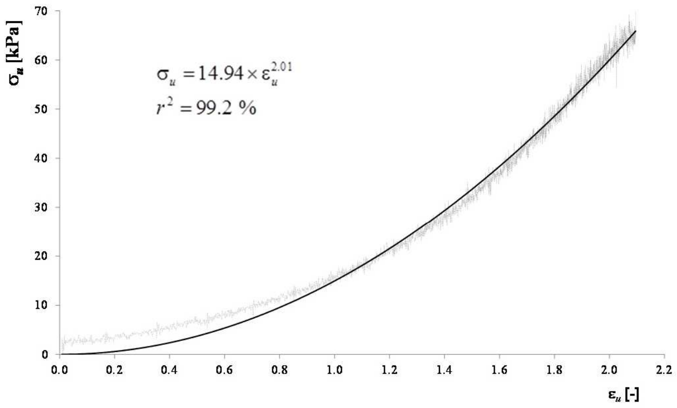
HSD: Honest Significant Differences.

Regular: dough formulated without resistant starch (RS).

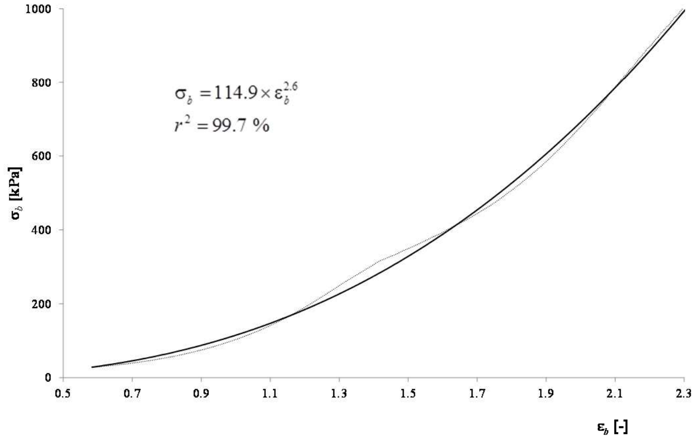
Control: dough formulated with RS and without enzymes.

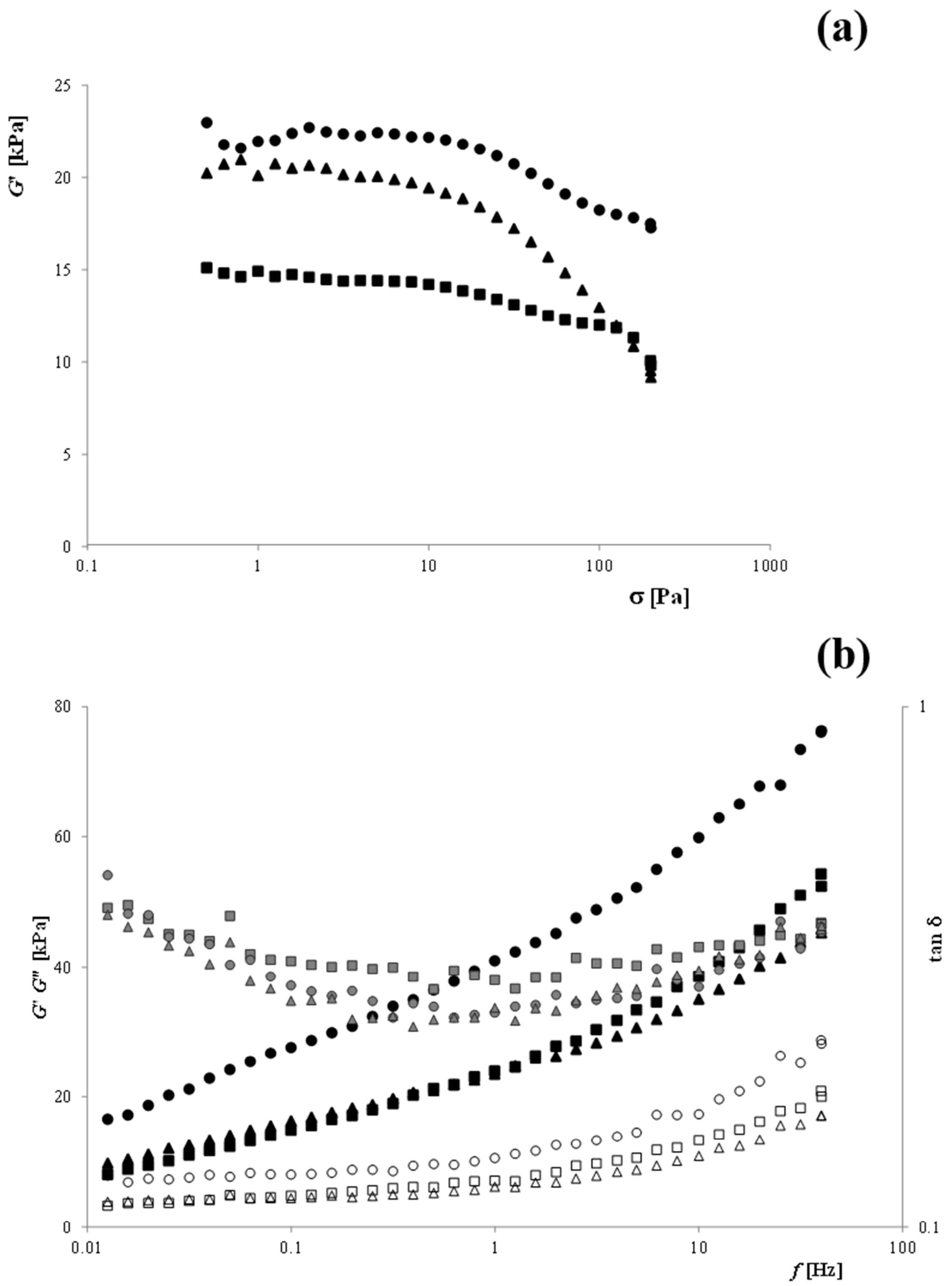
Optimum: dough formulated with RS and a blend of enzymes (2.5 mg/100 g of Gox, 4 mg/100 g of TG and 0.5 mg/100 g of HE).

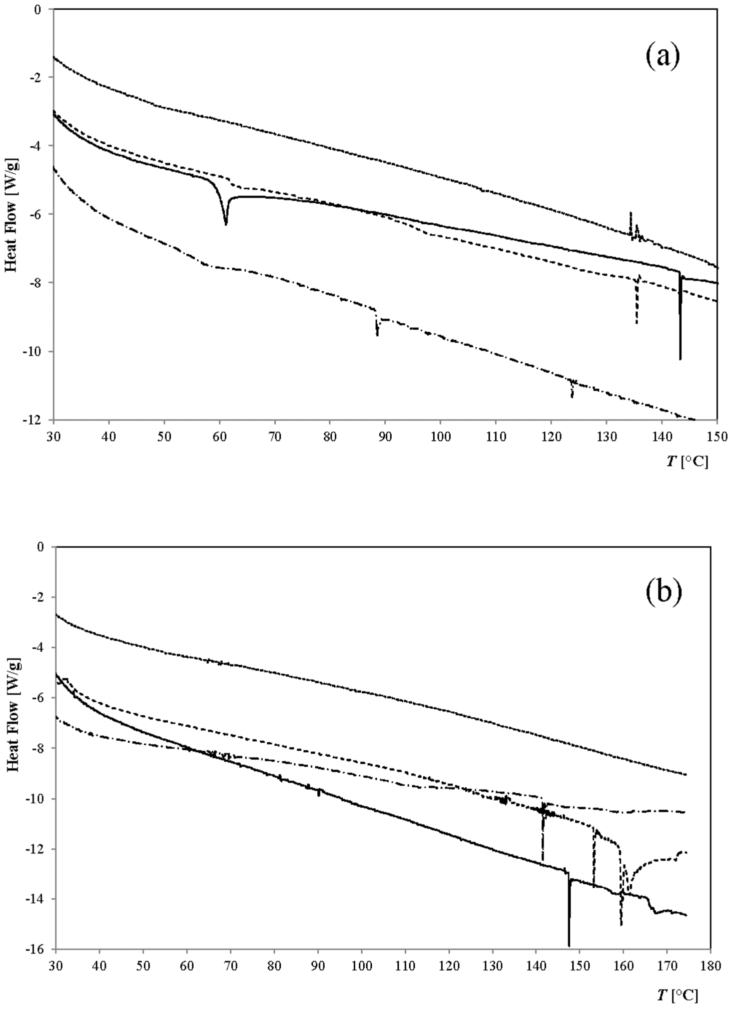
(a)

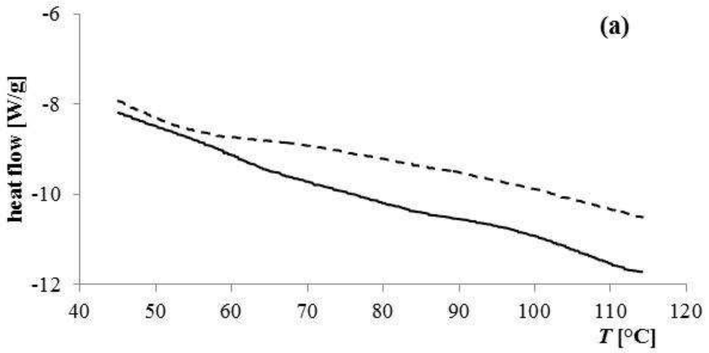

$\sigma_u = 14.94 \times \varepsilon_u^{2.01}$
$r^2 = 99.2$ %
σ_u [kPa]
70
60
50
40
30
20
10
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
ε_u [-]

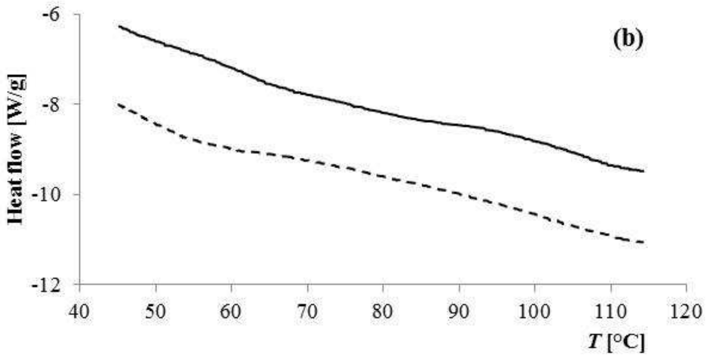
(b)

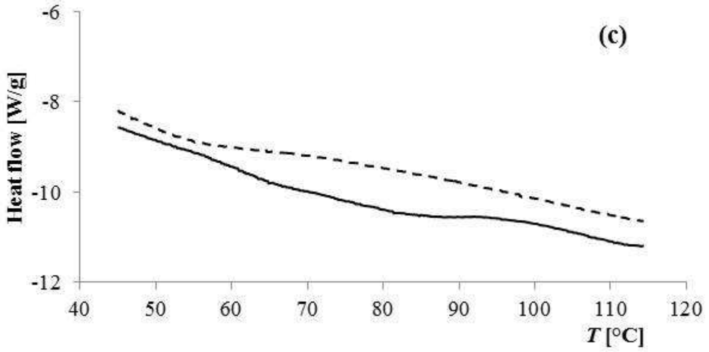

$\sigma_b = 114.9 \times \varepsilon_b^{2.6}$
$r^2 = 99.7$ %
σ_b [kPa]
1000
800
600
400
200
0
0.5
0.7
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
ε_b [-]


(a)
25
20
15
10
5
0
G' [kPa]
0.1
1
10
100
1000
σ [Pa]
(b)
80
60
40
20
0
G' G'' [kPa]
1
0.1
tan δ
0.01
0.1
1
10
100
f [Hz]


(a)
Heat Flow [W/g]
0
-2
-4
-6
-8
-10
-12
30
40
50
60
70
80
90
100
110
120
130
140
150
T [°C]
(b)
Heat Flow [W/g]
0
-2
-4
-6
-8
-10
-12
-14
-16
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
T [°C]


(a)
heat flow [W/g]
-6
-8
-10
-12
40
50
60
70
80
90
100
110
120
T [°C]


(b)
Heat flow [W/g]
-6
-8
-10
-12
40
50
60
70
80
90
100
110
120
T [°C]


(c)
Heat flow [W/g]
-6
-8
-10
-12
40
50
60
70
80
90
100
110
120
T [°C]

Highlights

- Wheat flour was partially replaced by maize resistant starch (MRS).
- Enzymes were used as additives to improve baking performance.
- Fundamental rheological parameters were obtained.
- MRS reduced extensibility and enzymes increased the strain hardening.
- MRS was not gelatinized during baking continuing indigestible.