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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.

1. Introduction

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, 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 et al., 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

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.

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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 *Streptoverticilium 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

Table 1
Ingredients used in the formulation of dough (regular, control and optimum).

Ingredients [g/100 g][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
$\alpha -$ 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.