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Effect of a combination of enzymes on dough rheology and physical and sensory properties of bread enriched with resistant starch

Luz Altuna [a], Pablo D. Ribotta [b], Carmen C. Tadini [a, c, *]

[a] University of São Paulo, Escola Politécnica, Department of Chemical Engineering, Main Campus, 05508-010, São Paulo, SP, Brazil
[b] Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba-CONICET, CC 509, 5000 Córdoba, Argentina
[c] University of São Paulo, FoRC/NAPAN — Food Research Center, São Paulo, Brazil

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A B S T R A C T

The effect of three enzymes on the rheology and the baking performance 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 make up the gluten dilution. A full factorial 2^3 design with central point in triplicate was applied with dough formulated with partial substitution of wheat flour by RS (12.5 g/100 g) and the enzymes: transglutaminase (0–8 mg/100 g), glucose-oxidase (0–5 mg/100 g) and xylanase (0–1 mg/100 g). Dough produced without RS or enzymes was considered as regular and dough produced with RS and without enzymes was considered as control for comparison. Bread was produced from regular, control and optimum formulations and the quality was assessed concerning specific volume, firmness and preference by sensory panel. Enzymes had significant effect ($p < 0.05$) on the weakening coefficient obtained by fermentation monitoring indicating the central point as optimum formulation. From bread quality tests it was observed that the RS delayed, whereas the enzymes accelerated the aging process. No significant difference of attribute preference was detected by sensory panel, while the bread produced with the optimum dough had the highest value.

1. Introduction

The addition of functional ingredients to different kinds of food like bread is being studied as an approach to the current epidemic of non-communicable diseases taking place worldwide. While cardiovascular diseases, cancer and diabetes accounted for 72.6% of the deaths in 2008 (WHO, 2011), the risk factors associated to these conditions continue spreading in a worrying way. Raised blood pressure, raised cholesterol, raised fasting blood glucose, overweight and obesity have reached high proportions of the global adult population (40%, 39%, 9.5%, 35% and 12% respectively) (WHO, 2011, 2013).

In relation to the risk factors mentioned, dietary fiber appears as an interesting ingredient providing benefits such as decrease of intestinal transit time and increase of stools bulk, being fermentable by colonic microflora, reduction of total and/or LDL cholesterol levels of blood, reduction of post-prandial blood glucose level (FAO/WHO, 2009). Resistant starch (RS) is not digested allowing fermentation in the colon, so it can be considered a kind of dietary fiber. Four types of RS have been described: RS_1, that is physically inaccessible to digestion such as in grains or seeds; RS_2, which its granules are structured in a way that does not allow enzymes to hydrolyze it; RS_3 is the retrograded starch formed when foods are cooked and cooled; RS_4 is a chemically-modified starch (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010). High-amylose maize starch, defined as RS_2, can be obtained from a specific hybrid of corn which naturally has high amylose content. Since it is a fine white powder, its sensory attributes like color and taste are more appreciated by consumers when compared with traditional sources of dietary fiber (Fuentes-Zaragosa et al., 2010). However, 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

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; PS 80, Polysorbate 80; HSD, Honest significant difference; ANOVA, analysis of variance; TPA, Texture profile analysis.

* Corresponding author. Food Engineering Laboratory, Escola Politécnica, University of São Paulo, Av. Prof. Luciano Gualberto, travessa 3 n° 380, 05508-010, São Paulo, SP, Brazil. Tel.: +55 11 3091 2258.

E-mail address: catadini@usp.br (C.C. Tadini).

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limits its application. To minimize this effect additives such as enzymes are used.

Three enzymes with application in the bakery industry are transglutaminase (TG), glucose oxidase (Gox) and fungal xylanase (HE). TG is a strong protein crosslinking enzyme present in most animal tissues and body fluids that can be industrially obtained from microorganisms (Yokoyama, Nio, & Kikuchi, 2004). In bakery it is used for weak flour, its action is irreversible and yields dough with increased elasticity and fermentation tolerance (AB Enzymes, 2014). Gox is an enzyme produced by fungi, with wide technological application. It 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 in gluten protein forming disulfide bonds within the gluten network and resulting in gluten strengthening (Novozymes, 2014). HE degrades xylan, a kind of hemicellulose very abundant in nature. In bread making, it breaks down the hemicellulose in wheat flour helping in the redistribution of water and leaving the dough softer and easier to knead (Polizeli et al., 2005).

The objective of this work was to study the effect of TG, Gox and HE on the baking performance of bread dough with high content of RS and find a formulation with adequate rheological and texture properties compared to regular bread dough without RS. Quality parameters of bread prepared with optimum, regular and control formulations were also assessed.

2. Materials and methods

2.1. Materials

Wheat flour (WF) with 13.9% of moisture, 29% of wet gluten, 9.1% of dry gluten and 0.43% 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% of resistant starch (insoluble dietary fiber) and 40% 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) and dried yeast *Saccharomyces cerevisiae* (Dr. Oetker, Brazil) were purchased from the local market and distilled water was used.

2.2. Experimental procedure

Dough was formulated with a mixture of WF and RS (87.5 g/100 g and 12.5 g/100 g respectively, mixture basis), 59.1 g/100 g of water, 2 g/100 g of sodium chloride, 1.2 g/100 g of dried yeast, 0.5 g/100 g of a blend of emulsifiers (245 mg of SSL, 180 mg of PS80 and 750 mg of DATEM) found as optimum in a previous work (Gómez, Buchner, Tadini, Añón, & Puppo, 2013) and 15 mg/100 g of enzyme α-amilase to correct Falling Number. The enzymes transglutaminase, glucose-oxidase and xylanase were added at concentrations varying between (0 and 8) mg/100 g, (0 and 5) mg/100 g and (0 and 1) mg/100 g, respectively, according to a factorial 2^3 design of experiments with central point in triplicate (Table 1). The formulation produced without enzymes (F1) was considered as control. The maximum concentration of each enzyme was defined taking into account the United States Food and Drug Administration (FDA, 2000a, 2000b and 2002) and manufacturer's recommendations. Besides the factorial design of experiments, regular dough formulated without RS or enzymes was tested for comparison. All the concentrations above are expressed on mixture basis (WF + RS). 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).

For the texture assays, three formulations were tested without yeast and prepared as described above: the optimum dough obtained from baking performance, the control dough with RS and without enzymes (F1) and the regular dough without enzymes or RS. Dough was mixed and kneaded using a Stand Mixer Professional (Kitchen Aid, Brazil). 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.

Table 1
Maximum height (H_m), time at maximum height (t_1), and weakening coefficient (W) obtained from dough development curves; maximum pressure (H'_m), time at maximum pressure (t'_1), time at gas release (t_x), and retention coefficient (R) obtained from gas curves; and the calculated adjusted maximum height (H_m^{adj}) of dough formulated with a blend of enzymes transglutaminase (TG), glucose-oxidase (Gox) and xylanase (HE), according to a factorial 2^3 design of experiments.

Assay	TG	Gox	HE	TG	Gox	HE	H_m [mm]	t_1 [min]	W [%]	H'_m [mmH$_2$O]	t'_1 [min]	t_x [min]	R [%]	H_m^{adj} [mm]
	Level			[mg/100 g]										
F1 (control)	−1	−1	−1	0	0	0	27.2	111.0	8.8	47.8	151.5	96.0	92	27.2
F2	1	−1	−1	8	0	0	31.5	133.5	7.6	45.4	144.0	97.5	92	32.8
F3	−1	1	−1	0	5	0	29.7	180.0	0.0	48.9	157.5	87.0	89	27.8
F4	1	1	−1	8	5	0	32.4	180.0	0.0	47.9	169.5	91.5	92	33.0
F5	−1	−1	1	0	0	1	29.6	142.5	44.6	44.7	154.5	105.0	94	30.8
F6	1	−1	1	8	0	1	26.3	127.5	50.2	47.2	156.0	106.5	93	26.0
F7	−1	1	1	0	5	1	40.5	180.0	0.0	46.2	175.5	124.5	98	44.1
F8	1	1	1	8	5	1	29.7	151.5	9.4	46.1	163.5	102.0	94	31.1
F9	0	0	0	4	2.5	0.5	43.3	166.5	3.0	48.1	166.5	108.0	96	44.2
F10	0	0	0	4	2.5	0.5	42.1	171.0	4.8	47.5	151.5	114.0	96	42.4
F11	0	0	0	4	2.5	0.5	49.5	180.0	0.0	49.0	175.5	123.0	97	49.8
Regular[a]				0	0	0	37.0	138.0	12.2	41.1	157.5	100.5	93	43.7

[a] Dough formulated without partial substitution of wheat flour by resistant starch.