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Torque Measurement in Real Time during Mixing and Kneading of Bread Dough with High Content of Resistant Maize Starch and Enzymes

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Abstract: In this work, a methodology to measure torque during dough mixing in large scale was developed and the baking performance of bread dough formulated with resistant starch (RS) and enzymes was evaluated. Dough was formulated with 12.5 g/100 g of RS and 4 mg/100 g of a mixture of enzymes, glucose-oxidase (Gox), tranglutaminase (TG) and xylanase (HE) in proportions according to a three-component mixture design of experiments. Dough was mixed in a large-scale dynamic rheometer measuring instant torque and speed in real time through a personal computer (PC) interface. Maximum torque during mixing and parameters of the dough development curves obtained from rheofermentometer were fit to mathematical models within 95 % of confidence. Gox and TG showed positive effects on the maximum height of dough, while HE showed a negative one. Also, it was found that Gox and TG in the presence of HE could be important for reducing dough weakening.

Keywords: torque, transglutaminase, xylanase, glucose-oxidase, baking performance

1 Introduction

Bread is a widely consumed food in most western cultures. Nutritionally, it is a source of carbohydrates since its main ingredient is wheat flour (WF). Bread can be turned into a functional food by adding special ingredients as seeds and dietary fiber. Dietary fiber consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants [1]. The generally accepted benefits of dietary fiber are reduction of intestinal transit time and increase of stools bulk; it is fermentable by colonic microflora, reduces blood total and/or low-density lipoprotein (LDL) cholesterol levels and the postprandial blood glucose and/or insulin levels [2], which are relevant regarding noncommunicable diseases such as cardiovascular disease, diabetes and colon cancer [3]. Resistant starch (RS) can be considered a kind of dietary fiber due to the fact that it is not digested allowing fermentation in the colon, and it has been added to food products such as muffins, biscuits and bread to produce functional foods with the potential to become accessible, far reaching products with desired effects regarding risk factors of the mentioned diseases [4–6].

There are four types of RS: RS_1 is starch that is physically inaccessible to digestion such as in grains or seeds; RS_2 is starch with its granules structured in a way that does not allow enzymes to digest it; RS_3 is retrograded starch formed when starch-containing foods are cooked and cooled; RS_4 is chemically modified starch [7]. High-amylose maize starch (defined as RS_2) is isolated from a hybrid of corn that is naturally high in amylose content. Since it is a fine white powder, its addition to bread formulation is interesting as its sensory attributes like color and taste are more appreciated by consumers when compared with traditional sources of dietary fiber [7]. However, the application of RS in bread formulations is limited by the resulted gluten dilution. When added in high proportions, RS yields dough with poor rheological properties and baking performance [8]. To minimize this effect, additives such as enzymes are used.

Transglutaminase (TG) (EC 2.3.2.13) is a strong protein cross-linking enzyme present in most animal tissues and body fluids. In bakery, it is used for weak flour, its action being irreversible, and yields dough with increased elasticity and fermentation tolerance [9].

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Glucose-oxidase (Gox) (EC 1.1.3.4) 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 [10]. Hydrogen peroxide is capable of oxidizing free sulfhydryl groups in gluten protein-forming disulfide bonds within the gluten network and resulting in gluten strengthening [11].

Xylanolytic enzymes are the enzymes that degrade xylan, a kind of hemicellulose very abundant in nature. In bread making, fungal xylanase (HE) (EC 3.2.1.8) breaks down the hemicellulose in WF helping in the redistribution of water and leaving the dough softer and easier to knead [12].

When studying bread dough, mixing parameters constitute an important approach, since gluten development (GD) is detected by the raise of resistance to mixing [13]. The traditional equipment that study dough mixing, such as farinograph, mixograph and consistograph, uses a very little quantity of flour; therefore, the test conditions are very different from the bread-making process. Measuring torque during mixing in large scale has been studied [14–16] since it allows obtaining a fundamental rheological measurement in conditions very similar or even equal to the mixing process.

The objective of this work was to develop a methodology to measure torque in real time during dough mixing and kneading in large scale and to compare the data obtained with baking performance of bread dough formulated with resistant maize starch and enzymes TG, Gox and HE.

2 Materials and methods

2.1 Materials

WF, with 13.9/100 g of moisture, 29/100 g of wet gluten, 9.1/100 g of dry gluten and 0.427/100 g of ash and the following Brabender Farinograph parameters: water absorption equal to 59.1/100 g, stability equal to 24.3 min, development time equal to 13.4 min and mixing tolerance equal to 0 UB, was supplied by AB Brasil (Brazil); RS Hi-maize® 260 containing 60/100 g of RS (insoluble dietary fiber) and 40/100 g of digestible (glycemic) starch was supplied by Ingredion (Brazil); TG obtained from specific cultures of *Streptoverticilium mobarense* with enzyme activity equal to 100 TGU/g was supplied by AB Enzymes (Brazil); Gox produced by submerged fermentation of a selected strain of *Aspergillus niger* with enzyme activity equal to 10,000 GODU/g and fungal HE produced by submerged fermentation of *Aspergillus oryzae* with enzyme activity equal to 60,000 FXU-W/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 and dried yeast were purchased from the local market and distilled water was used.

2.2 Dough formulation

Dough formulated with a mixture of WF and RS (87.5/100 g and 12.5/100 g, mixture basis), 59.1/100 g of water (amount determined by farinograph absorption), 2/100 g of sodium chloride, 1.2/100 g of dried yeast and 0.5/100 g of a blend of emulsifiers (49/100 g SSL, 36/100 g PS80 and 15/100 g DATEM) was found to be optimum in a previous work [17], with enzyme α-amilase to correct falling number and 4 mg/100 g of a mixture of enzymes TG, Gox and HE varying according to a three-component mixture design of experiments with three replicates of the central point (Table 2). The maximum concentration of each enzyme was defined taking into account the United States Food and Drug Administration [18–20] and manufacturer's recommendations. Besides the mixture design of experiments, two formulations were tested for results comparison: a regular formulation equal to the one described above but without RS or enzymes and a control formulation with WF replacement by RS but without enzymes. All the concentrations above are expressed on mixture basis (WF + RS). For each run, approximately 6.4 kg of dough was prepared.

2.3 Torque measurement during dough mixing

Dough was mixed using a dynamic rheometer designed and assembled by the Materials and Components Laboratory (Civil Engineering Department, Escola Politécnica, University of São Paulo) which allowed measuring instant torque during mixing in large scale with a sample of dough of 6.4 kg, in conditions more similar to the bread-making process compared to traditional methods like the farinograph. The equipment consists of a motor, a reductor and a planetary mixer

in which it is possible to control torque and rotation speed. Instant motor speed and electrical current were measured in real time and were acquired by the software especially developed, through a PC interface. Torque was calculated by converting the electrical current by a constant ($K_1 = 24.91$ N m/mA) determined by measuring torque in the planetary with a torquimeter. Motor speed was transformed into planetary speed by another constant ($K_2 = 0.253$). The equipment, designed for working with concrete, was adapted to work with bread dough. Dough-mixing hook and bowl from a commercial mixer were mechanically modified to assemble the rheometer.

For the purposes of this work, rotation speed of mixer arm (ω) was controlled and torque (τ) was measured as a response. The program of time (t) and speed of mixing applied were determined after preliminary assays. These assays were performed with the objective of familiarizing with the equipment and observing the behavior of dough in the rheometer at different conditions. For the tests, a minimum of 4 kg of WF or mixture (WF + RS) was used in order to observe the maximum mixing performance. After that, the need of mixing in three steps was identified: homogenization (low speed), mixture (medium speed) and kneading (high speed). Finally, two protocols of different process conditions were tested (Table 1) and the dough was evaluated by the baking performance test in a rheofermentometer. The second protocol was chosen and the process conditions used for all the subsequent assays are described as follows:

Table 1: Mixing time (t) and angular speed (ω) conditions tested in preliminary assays with dynamic rheometer.

Protocol	Homogenization		Mixture		Kneading	
	t (min)	ω (rad/s)	t (min)	ω (rad/s)	t (min)	ω (rad/s)
1	1	2.62	4	10.58	11	26.39
2	1	5.24	4	10.58	6.7	26.39

Dry ingredients except salt were homogenized at low speed (5.24 rad/s) for 1 min. Water was added at constant flow (10 mL/s) during mixing at medium speed (10.58 rad/s) for 4 min. Finally, salt was added and dough was kneaded for 6.7 min at high speed (26.39 rad/s).

A curve of torque (τ) as a function of time (t) was obtained for each assay and from data, a smoother curve was calculated using the 20 s moving mean. Two parameters were obtained (Figure 1(A)):

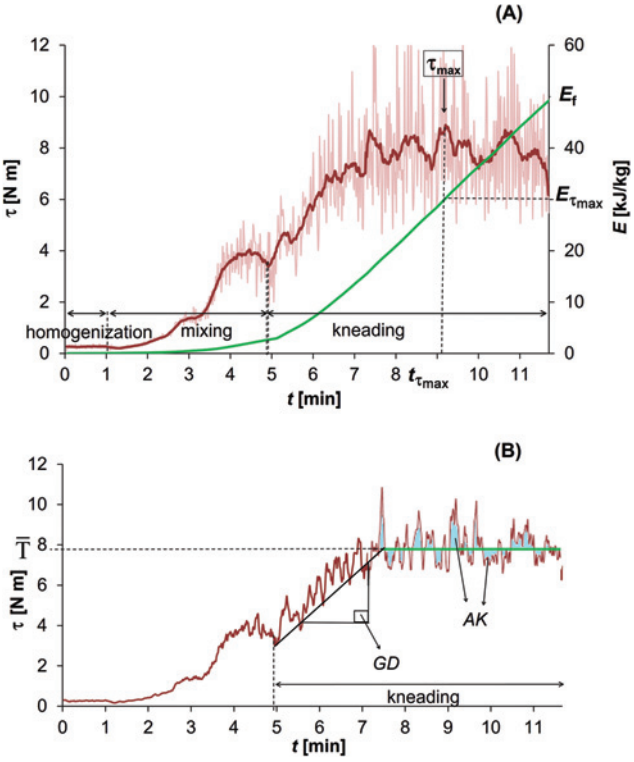

Figure 1: Results obtained in rheometer as a function of time (t) during mixing of dough formulated according to assay 10 (Table 2), measured torque (τ), torque 20 s moving mean, specific mechanical energy input (E) (A). Torque 5 s moving mean as a function of mixing time (t) of dough formulated according to assay 10 (Table 2) (B).

- τ_{max}: maximum torque (N m).
- $t_{\tau_{max}}$: time to maximum torque (s).

The instant power (P) was calculated as the following equation:

$$P = \tau \times \omega \quad (1)$$

wherein, τ is the instant torque (N m) and ω is the angular speed of the mixer arm (rad/s).

The specific mechanical energy input (E) can be obtained by the integration of power in time divided by the mass. Two parameters were obtained from the curve of E as a function of time (Figure 1(A)):

- $E_{\tau_{max}}$: specific mechanical energy input at maximum torque (kJ/kg);
- E_f: specific mechanical energy input at the end of kneading (kJ/kg).

With the aim of extracting more information from the data, a 5-s moving mean curve was drawn and the following parameters were obtained (Figure 1(B)):

- mean torque ($\bar{\tau}$) calculated as the mean of the torque between the highest peak and the end of kneading (N m);
- action of kneading (AK), which is the area formed by the instant torque curve around the mean torque (J s);
- GD rate, which is the slope of the line drawn between the start of kneading and the maximum peak (W).

2.4 Baking performance test

Baking performance test was conducted using a Rheofermentometer F3 (CHOPIN, France). A portion of 250 g of dough was fermented for 3 h at 28.5 °C with 2 kg of weight over it according to the Chopin protocol.

From the test, two curves were obtained: the dough development curve by an optical sensor, which shows the variation of dough height as a function of time during fermentation and the gas production and retention curves by a pressure sensor. The following parameters are from the dough development curve: maximum height (H_m), time at maximum height (t_1), final height (h) and the weakening coefficient (W) calculated according to the following equation:

$$W = \frac{(H_m - h)}{H_m} \tag{2}$$

The following parameters were obtained from the gas curves (production and retention of gas as a function of time): maximum pressure (H'_m), time at gas release (t_x), total volume of gas produced (V_t), volume of gas retained (V_r) and the retention coefficient (R) calculated as the following equation:

$$R = \frac{V_r}{V_t} \tag{3}$$

An additional parameter, adjusted maximum height (H_m^{adj}), was calculated (eq. [4]) in order to identify the dough development independently from gas production which is due to yeast activity instead of dough properties:

$$H_m^{adj} = \frac{H_m}{V_t} \tag{4}$$

2.5 Statistical analyses

Results were fitted to mathematical models, linear (eq. [5]), quadratic (eq. [6]) and linear with inverse terms (eq. [7]) and statistically analyzed performing analysis of variance (ANOVA) at 95 % of confidence, using the statistics software Statgraphics Centurion XVI (Statpoint Technologies, EUA).

$$\hat{y} = b_1 \times x_1 + b_2 \times x_2 + b_3 \times x_3 \tag{5}$$

$$\hat{y} = xb_1 \times x_1 + b_2 \times x_2 + b_3 \times x_3 + b_{12} \times x_1 \times x_2 + b_{13} \times x_1 \times x_3 + b_{23} \times x_2 \times x_3 \tag{6}$$

$$\hat{y} = b_1 \times x_1 + b_2 \times x_2 + b_3 \times x_3 + b_{-1} \times x_1^{-1} + b_{-2} \times x_2^{-1} + b_{-3} \times x_3^{-1} \tag{7}$$

wherein, $\hat{y}$ is the variable estimated by the model; x_i is the proportion of each mixture component; b_i, b_{-i} and b_{ij} are the model coefficients which represent the estimated effects of the components and their interactions.

In this work, the mixture components are the three enzymes, TG, Gox and HE, and the model equations are valid in the ranges expressed in the following equations:

$$0 \le x_{TG} \le 1 \tag{8}$$

$$0 \le x_{Gox} \le 1 \tag{9}$$

$$0 \le x_{HE} \le 0.25 \tag{10}$$

$$x_{TG} + x_{Gox} + x_{HE} = 1 \tag{11}$$

3 Results and discussion

3.1 Torque during dough mixing

Typical results for torque (τ) during dough mixing are shown in Figure 2. The first minute corresponds to the homogenization of the dry ingredients at low speed, showing very low torque and little mechanical energy dissipation, this stage was equal for all the assays. The second stage, between 1 and 5 min, corresponds to the addition of water and mixing of the dough. As water is added, τ raises since hydration of the flour components takes place, which is a prerequisite for subsequent operations since water is required as a medium within which molecules and enzymes become mobile and through which interactions may take place [21]. During the kneading stage (from 5 min to the end of mixing), the gluten proteins become under tensile and shear stress increases their contact surface leading to interactions that contribute to form a continuous branched network. As a consequence of this, a dynamic situation ensues in the dough in which new interactions begin, while others cease. The progressive increment of torque during the dough development obtained in the curve (Figure 1(A)) reflects that at any specific time increment, the net

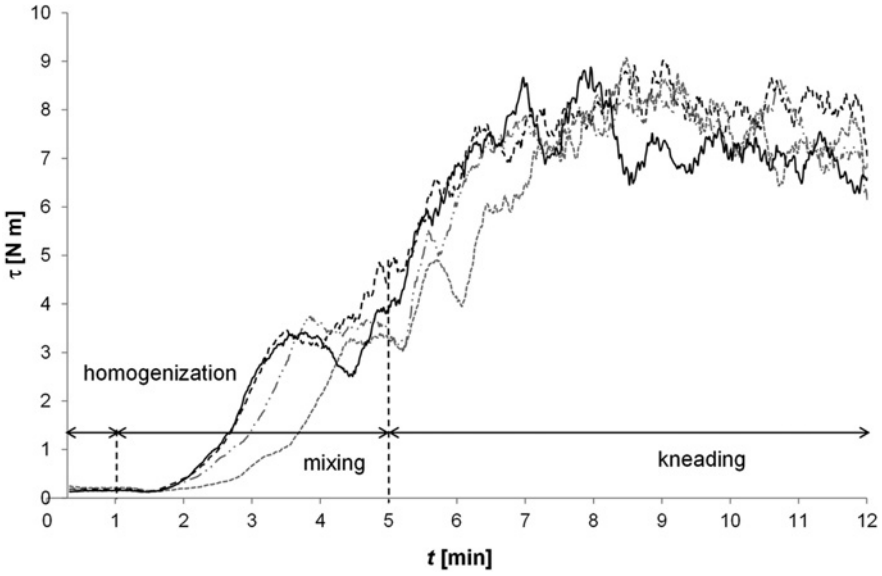

Figure 2: Torque 20 s moving mean (τ) as a function of time (*t*) obtained in rheometer for the formulations —— regular, ––– control and two replicates of the central point of the design corresponding to assays ——12a and ——12c (Table 2).

balance between these two mechanisms favors the increased number of link formation [22]. After achieving a maximum, τ started to decrease with time indicating the full development of the dough. Similar curves were obtained for all the formulations tested and parameters obtained from these curves are shown in Table 2. The highest value of τ_{max} was 9.38 N m corresponding to the mixture with 2.875 mg/100 g of TG, which means high proportion of that enzyme (assay 5, Table 2). However, the lowest τ_{max} (8.36 N m) was obtained for the dough produced with Gox being predominant (2.375 mg/100 g). If compared with the dough produced with a mixture of

Table 2: Maximum torque (τ_{max}), time corresponding to maximum torque ($t_{\tau_{max}}$), specific mechanical energy input corresponding to maximum torque ($E_{\tau_{max}}$), specific mechanical energy input at the end of mixing (E_f), mean torque ($\bar{\tau}$), action of kneading (AK) and gluten development (GD) obtained in a dynamic rheometer, during mixing of dough formulated with enzymes: transglutaminase (TG), glucose-oxidase (Gox) and xylanase (HE), according to a three-component mixture design of experiments.

Assay	TG	Gox	HE	τ_{max}	$t_{\tau_{max}}$	$E_{\tau_{max}}$	E_f	$\bar{\tau}$	AK	GD
			(mg/kg)	(N m)	(min)	(kJ/kg)	(kJ/kg)	(N m)	(J s)	(W)
Control	0.00	0.00	0.00	9.01	8.7	7.63	13.70	8.06	145.9	0.027
1	40.00	0.00	0.00	9.19	8.4	5.97	12.31	7.75	190.9	0.021
2	0.00	40.00	0.00	9.35	8.5	6.21	12.36	7.90	225.0	0.021
3	30.00	0.00	10.00	8.69	9.0	7.08	12.23	7.51	119.4	0.017
4	0.00	30.00	10.00	8.89	7.8	5.23	12.45	7.35	154.2	0.030
5	28.75	8.75	2.50	9.38	9.1	6.70	11.91	8.05	159.8	0.023
6	8.75	28.75	2.50	8.56	8.0	5.39	12.59	7.63	155.6	0.027
7	8.75	23.75	7.50	8.36	11.1	11.26	12.51	7.18	33.6	0.013
8	20.00	20.00	0.00	9.16	10.3	9.81	12.65	7.62	85.2	0.014
9	35.00	0.00	5.00	8.77	8.7	6.32	12.35	7.91	130.8	0.016
10	0.00	35.00	5.00	8.89	9.2	7.68	12.66	7.83	182.0	0.032
11	15.00	15.00	10.00	9.19	9.9	8.90	12.54	8.13	167.1	0.019
12a	17.50	17.50	5.00	8.61	10.4	10.42	12.84	7.45	125.4	0.018
12b	17.50	17.50	5.00	9.11	8.7	7.50	13.24	7.73	157.7	0.019
12c	17.50	17.50	5.00	9.07	8.2	5.39	12.08	7.66	168.9	0.026
Regular*	0.00	0.00	0.00	8.87	7.7	5.46	12.59	7.16	156.8	0.015
*SD***				0.28	1.2	2.53	0.59	0.15	22.6	0.004

Note: *Without resistant starch or enzymes.
**Standard deviation of the central point of the design of experiments.

the three enzymes according to the central point of the design which had τ_{max} equal to (8.93 ± 0.28) N m, regular dough showed τ_{max} 1% lower and control dough showed τ_{max} 1% higher.

The specific mechanical energy (E) input to the dough, determined by the integration of the power spent by the mixer, is also shown in Figure 1(A). As the mixer speed increases, the rate of energy input also raises. This mechanical energy is turned into thermal energy resulting in the temperature raise of the dough, and heat losses with the environment, as modeled in a previous work [16]. However, it has been stated that dough stores some of the mechanical energy expended during kneading as elastic potential energy, since the rate of deformation of the dough in this process is faster than the rate of relaxation to its equilibrium state [23]. The specific mechanical energy input at maximum torque ($E_{\tau_{max}}$) for regular dough was 5.46 kJ/kg at 7.7 min of kneading ($t_{\tau_{max}}$), whereas for the control dough, it was 7.63 kJ/kg at 8.7 min, indicating that the gluten dilution as a consequence of partial substitution of WF by RS is evident. It took longer time of mixing and therefore higher quantity of energy to develop the gluten network when proteins were diluted, meaning that more movement of the molecules was necessary to enable the interactions that allow link formation. The maximum value of $E_{\tau_{max}}$ (11.26 kJ/kg) was obtained at 11.1 min of kneading for the assay 7, in which Gox is the predominant enzyme. Formulations that presented $E_{\tau_{max}}$ similar to regular dough corresponded to assay 4 (5.23 kJ/kg at 7.8 min) and assay 6 (5.39 kJ/kg at 8.0 min), both with Gox as the predominant enzyme in the mixture. In a previous work, a higher quantity of energy necessary to achieve peak torque and optimum GD on strong flours when compared to weak flours was reported [14]. Also, it was stated that there exists a nexus between mixing requirements such as work input and mixograph development time, and dough strength measured as extensigraph resistance to extension [24].

The specific mechanical energy input at the end of mixing (E_f) depends on the shape of the curve of torque, since all the dough was mixed at equal speed for the same time. Results obtained for this parameter varied from 11.91 to 13.70 kJ/kg. The highest value corresponded to control dough, while E_f of the central point was 7% lower and for the regular dough, E_f was 8% lower than the control, and the same tendency was observed for the mean torque during kneading ($\bar{\tau}$). When flour properties are very different, the same amount of energy will result in arrival at very different positions in a mixing curve. Furthermore, variability in flour properties could mean difference in water absorption; thus, variations in dough consistency will occur when fixed levels of water are used [21]. The lower value of E_f for regular dough could be attributed to the high water absorption of maize RS compared to wheat starch [25]. With respect to the central point of the design, enzymes Gox and TG have been reported to increase water absorption in bread dough [26, 27]; however, HE addition may have resulted in water redistribution from xylan to gluten since it decreases the water-binding capacity of the xylan [28].

Concerning GD, it was 0.015 W for regular dough and 43% higher for the central point and 84% higher for control dough. The highest AK corresponded to dough formulated only with Gox followed by dough formulated only with TG. This means that when comparing dough without RS, dough with RS and without enzymes and dough with RS and enzymes according to the central point of the design, the same tendency was observed for τ_{max}, $\bar{\tau}$, E_f and GD and the opposite tendency was observed for AK.

Data corresponding to the mixture design of experiments were statistically analyzed. τ_{max} showed significant ($p < 0.05$) fit to the linear model with inverse terms. This model is useful when there is a change in the response behavior as the factors get near their limits. In this case, the effect of the Gox on τ_{max} varied with the enzyme concentration. The model coefficients indicate that the three enzymes have a positive influence on τ_{max} (Table 3). The most important effect was of TG, which had a strengthening effect on dough due to its protein cross-linking capacity, followed by HE. For Gox, there is a positive effect when the enzyme is added in medium or high quantities, while this effect disappears when less than 0.014 mg/100 g of the enzyme is used. As Gox promotes the hydrogen-peroxide formation, which is capable of oxidizing the sulfhydryl group, its presence in the mixture resulted in gluten strengthening by the cross-linking of proteins by disulfide bonds [29].

For the other mixing parameters, data could not be fit to the linear, quadratic and linear with inverse terms models; hence, the influence of the enzymes on those parameters could not be explained by any of the mathematical models. This result is in agreement with other researchers [30] who found no significant difference in water absorption, development time and stability measured in farinograph when adding TG or HE to bread dough, whereas the cited authors found that development time increased for concentrations of Gox equal to 1 mg/100 g and 10 mg/100 g and stability increased when adding 10 mg/100 g of Gox. Furthermore, in another work, tolerance was measured in consistograph for

Table 3: Parameters of the models fitted to values of maximum height (H_m), time corresponding to maximum height (t_1), weakening coefficient (W) and adjusted maximum height (H_m^{adj}) obtained from rheofermentometer curves and maximum torque (τ_{max}) obtained from dynamic rheometer.

	H_m^{adj}	H_m	t_1	W	τ_{max}
Model	**Linear**	**Linear**	**Linear**	**Quadratic**	**Linear with inverse terms**
R_a^2	0.831	0.634	0.724	0.833	0.542
SE	1.73	2.41	10.52	0.0746	0.21
P	0.0000	0.0016	0.0003	0.0003	
b_{TG}	28.20*	25.24*	161.35*	0.008	9.82*
b_{Gox}	30.22*	28.71*	191.24*	0.027	8.03*
b_{HE}	−13.63*	−8.10	9.06	5.066*	8.92*
$b_{TG\text{-}Gox}$					
$b_{TG\text{-}HE}$				−3.510	
$b_{Gox\text{-}HE}$				−6.164	
$b_{\text{-}TG}$					0.00008*
$b_{\text{-}Gox}$					−0.00009*
$b_{\text{-}HE}$					0.00004

Note: *Coefficients statistically significant.

dough with concentrations of Gox varying from 0 to 15 mg/100 g and significant increment was found only for 15 mg/100 g of Gox [26]. However, other authors found that TG along with DATEM and/or the hydrocolloid hydroxypropylmethylcellulose influenced mixing parameters measured in Brabender farinograph [27].

3.2 Baking performance test

Parameters obtained from the baking performance test are shown in Table 4. The regular dough showed the highest maximum height of development (H_m), while this parameter was 32% lower for the control dough. Height developed by dough during fermentation is a measurement of the dough expansion, which takes place thanks to the rheological characteristics of dough, as a result of the CO_2 production by the yeast. This parameter is important since it is correlated with specific volume of bread.

The results show that gluten dilution as a consequence of partial substitution of WF by RS resulted in less expansible dough, prejudicing height development. The central point of the design developed a maximum

Table 4: Maximum height (H_m), time corresponding to maximum height (t_1), weakening coefficient (W), maximum pressure (H'_m), time corresponding to gas release (t_x), retention coefficient (R) and adjusted maximum height (H_m^{adj}) obtained in rheofermentometer during fermentation of dough formulated with enzymes transglutaminase (TG), glucose-oxidase (Gox) and xylanase (HE), according to a three-component mixture design of experiments.

Assay	H_m **(mm)**	t_1 **(min)**	W	H'_m **(mmH_2O)**	t_x **(min)**	R	H_m^{adj} **(mm)**
Control	31.3	178.5	0.0064	36.5	129.0	0.980	31.30
1	25.8	166.5	0.0310	33.8	129.0	0.987	28.44
2	25.5	178.5	0.0000	32.4	144.0	0.985	28.98
3	16.7	102.0	0.7066	35.5	120.0	0.973	16.84
4	19.2	147.0	0.1875	37.1	129.0	0.979	18.69
5	25.3	157.5	0.0830	35.1	136.5	0.982	26.88
6	29.3	168.0	0.0443	38.1	120.0	0.984	28.33
7	25.3	160.5	0.0593	38.3	126.0	0.977	24.02
8	27.7	180.0	0.0000	33.9	138.0	0.985	29.86
9	20.9	154.5	0.1722	31.8	130.5	0.985	24.28
10	25.2	180.0	0.0000	34.9	129.0	0.985	26.16
11	17.2	142.5	0.2558	32.0	132.0	0.985	19.46
12a	21.1	156.0	0.0948	33.3	142.5	0.988	22.95
12b	19.4	147.0	0.1804	33.4	142.5	0.982	20.85
12c	20.5	151.5	0.1854	35.2	132.0	0.982	21.28
Regular*	46.2	180.0	0.0000	40.3	129.0	0.980	41.81
*SD***	0.9	4.5	0.0509	1.1	6.1	0.003	1.11

Note: *Without resistant starch or enzymes.
**Standard deviation of the central point of the design of experiments.

height 56 % lower than the regular dough and the formulations containing only Gox and TG developed maximum heights close to the control dough. The same tendency was observed for H_m^{adj} and for t_1. The final height (h) was lower than maximum height H_m in most of the assays indicating that at some point during fermentation, dough started to lose height. This phenomenon was described by the weakening coefficient (W) calculated according to eq. (2) which has to be minimized to obtain better baking performance. For W, control and regular dough and dough formulated only with TG and Gox had a weakening coefficient close to zero, while the central point had a W equal to 0.15.

Regarding the gas curve parameters, regular dough showed the highest H'_m, control dough had an H'_m 9 % lower and the central point was 16 % lower than regular dough, in agreement with the values obtained from the dough development curves. Since the maximum pressure was reached after t_x in all the assays, the parameter corresponded to the gas produced by yeast which was not completely retained by dough, considering that CO_2 loss started at t_x. Therefore, this parameter was more related to yeast activity than to the rheological behavior of dough. For that reason, the parameter H_m^{adj} was calculated, to isolate the effect of yeast activity. Control and regular dough showed the same t_x, while dough formulated according to the central point of the design took 10 min longer to start releasing gas. The retention coefficient was 0.1 % higher for regular dough when compared to control dough and the central point.

Data corresponding to the mixture design of experiments were statistically analyzed. H_m, t_1 and H_m^{adj} showed significant ($p < 0.05$) fit to linear model and W showed significant ($p < 0.05$) fit to quadratic model (Table 3), the last being chosen since it considers the interaction effects between the factors, which was important in the weakening coefficient for the enzyme HE. The parameters best predicted by the models, with high adjusted coefficient of determination (R_a^2), were H_m^{adj} (0.831) and W (0.833). The model equations are formed by the estimated effects of the enzymes and in the case of the quadratic model, also the interaction effects. The coefficients of each model are shown in Table 3 and the contour lines of the response surfaces for $H_m^{adj}$and W are shown in Figure 3.

TG and Gox had a positive effect on H_m, while HE had negative effect on that parameter. The same tendency was observed in H_m^{adj}, with all the effects accentuated and a better fit to the model. The parameters H_m and H_m^{adj} measure dough expansion during fermentation. As gas is produced by yeast, dough expands increasing its height which is measured by an optical sensor. The

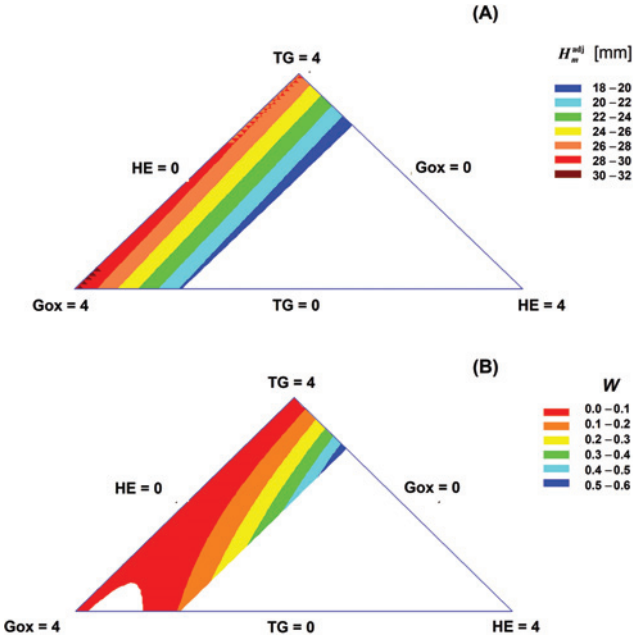

Figure 3: Contour regions of the response surfaces of adjusted maximum height (H_m^{adj}) (A) and weakening coefficient (W) (B) corresponding to the models fitted to data obtained from the rheofermentometer test.

maximum height reached during fermentation is related to bread volume, an important quality parameter. Since the amount of gas produced by yeast influences the height developed, the adjusted maximum height was calculated in this work to normalize variations in yeast activity. The positive effects of TG and Gox on H_m and H_m^{adj} indicate that the enzymes increased dough extensibility allowing for higher expansion, probably due to the protein linking produced by the enzymes which strengthened the gluten network in the dough. This result is in agreement with previous work [26] in which it was found that Gox increased loaf-specific volume in concentrations between 1 mg/100 g and 5 mg/100 g. Also, other researchers found that addition of 0.5 g/100 g of TG significantly improved sensory and texture profiles as well as loaf-specific volume in bread formulated with low-rate extraction flours [31]. High levels of Gox and TG also have been found to reduce loaf-specific volume due to excessive dough strengthening suggesting that these enzymes could be useful for supplementing weak flours [30].

Regarding W, the linear effects of TG and Gox on this parameter were negligible when compared to the effect of HE, which had a weakening effect. However, interaction between HE and the other two enzymes showed a strengthening effect of the same magnitude order, indicating that Gox and TG in the presence of HE could be important to reduce weakening. The addition of HE improved the

expansion capacity of the gas cells without CO_2 loss, resulting an increase of bread specific volume [30]. Moreover, improving effects of HE were reported in soy-supplemented wheat bread [28]. When adding HE from *Aspergillus foetidus* to whole-wheat bread, higher expansion of dough during fermentation and higher specific volume of bread loaf were reported [32].

Data obtained from the gas production and gas retention curves did not show significant fit to the models tested; hence, the effect of the enzymes on those parameters could not be mathematically explained. This result is in agreement with previous work [8] in which baking performance was tested in rheofermentometer for dough with WF replacement by maize RS varying from 0 to 15.5 g/100 g and enzyme TG in concentrations between 0 and 0.17 g/100 g and it was reported that there was no significant difference in the maximum pressure and retention coefficient between formulations.

Substituting b_i in eq. (5) by the coefficients shown in Table 3 for H_m^{adj}, the optimum formulation that maximizes dough development would be 4 mg/100 g of Gox and 0 mg/100 g of the other two enzymes. The adjusted maximum height predicted for this formulation is 30.44 ± 1.52 mm. This result is quite similar that obtained for assay 2, with the adjusted maximum height of 28.98 mm, indicating the accurace of the fitted model. Control formulation had H_m^{adj} equal to 31.3 mm and for regular dough, H_m^{adj} was 41.81 mm; thus, addition of enzymes did not allow improvement of dough performance. However, results obtained in this work show that TG and Gox help to minimize the effects of gluten dilution, since both enzymes enhance protein cross-linking, improving the gluten network responsible for dough elasticity. HE, which was reported to improve loaf volume due to its softening effect [28, 30, 32], was expected to compensate the strengthening effect of the other two enzymes helping to improve dough extensibility, and this was seen in the interaction effect of Gox and HE regarding *W*. Therefore, it is recommended for future studies to test higher concentrations of Gox and TG combined with little concentrations of HE.

4 Conclusions

A new methodology was successfully developed for measuring torque during mixing and kneading of dough in an equipment specially adapted for this work. The studied enzymes positively influenced the dough maximum torque, indicating that the TG had an important effect on the dough strength, predicted by a fitted mathematical model. Concerning the baking performance test, it was observed that TG and Gox had a positive effect on dough development, whereas HE had a negative one. Regarding weakening coefficient, it was found that TG and Gox have a favorable effect when used along with HE.

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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

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