Research Article

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Use of enzymes to minimize the rheological dough problems caused by high levels of damaged starch in starch–gluten systems

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Abstract

BACKGROUND: During wheat milling, starch granules can experience mechanical damage, producing damaged starch. High levels of damaged starch modify the physicochemical properties of wheat flour, negatively affecting the dough behavior as well as the flour quality and cookie and bread making quality. The aim of this work was to evaluate the effect of α-amylase, maltogenic amylase and amyloglucosidase on dough rheology in order to propose alternatives to reduce the issues related to high levels of damaged starch.

RESULTS: The dough with a high level of damaged starch became more viscous and resistant to deformations as well as less elastic and extensible. The soluble fraction of the doughs influenced the rheological behavior of the systems. The α-amylase and amyloglucosidase reduced the negative effects of high damaged starch contents, improving the dough rheological properties modified by damaged starch. The rheological behavior of dough with the higher damaged-starch content was related to a more open gluten network arrangement as a result of the large size of the swollen damaged starch granules.

CONCLUSION: We can conclude that the dough rheological properties of systems with high damaged starch content changed positively as a result of enzyme action, particularly α-amylase and amyloglucosidase additions, allowing the use of these amylases and mixtures of them as corrective additives. Little information was reported about amyloglucosidase activity alone or combined with α-amylase. The combinations of these two enzymes are promising to minimize the negative effects caused by high levels of damaged starch on product quality. More research needs to be done on bread quality combining these two enzymes.

Keywords: damaged starch; enzymes; dough; rheological properties

INTRODUCTION

Wheat is one of the most important cereals since wheat flour is the principal ingredient of many products. During wheat milling, the starch granules can experience mechanical damage, producing what is called damaged starch (DS). The level of damage is related to the wheat hardness and the milling conditions.[1] The granular integrity of starch is affected by mechanical damage, changing the structure of the granule which, in turn, affects the rheological behavior and functional properties of the starch systems.[2–5] The damage to the starch granule facilitates its swelling.[6] Consequently, DS granules have the ability to absorb more water than native granules and are more readily hydrolyzed by amylases.[1] The DS content of the regular wheat flour is around 5–13%.[7] Therefore, high DS levels modify the physicochemical properties of wheat flour and cause a higher water absorption capacity, negatively affecting the dough characteristics as well as the flour quality and cookie and bread-making quality.[8]

Many additives are nowadays used to manipulate the structural and physicochemical characteristics of the flour constituents. Amylases are extensively used as additives in the baking industry. Different amylolytic enzymes are employed to improve dough behavior and quality of products,[9] especially α-amylase and amyloglucosidase to a lesser degree. Various kinds of amylase have been used in the baking industry to prevent staling and improve the texture and flavor of baked goods.[1] In this way, maltogenic amylase is one of the most effective amylases to reduce the bread staling effects.[10–12]

Damaged starch and solubilized starch polymers are the main substrates for the amylases in dough. Taking this into consideration, the aim of this work was to evaluate the effect of the high levels of DS and of the α-amylase, maltogenic amylase and amyloglucosidase on the dough rheology, in order to (1) understand the changes of the starch–gluten structure caused by swollen DS granules and the starch polymers liberated from these, and (2) propose alternatives to reduce the issues related to high levels of DS. With the purpose of becoming independent of the milling process

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and protein influence and of making the interpretation of results simpler, a model system containing starch and gluten proteins was used in this study.

MATERIALS AND METHODS

Samples

Unmodified (native) wheat starch (S5127, Sigma-Aldrich, Buenos Aires, Argentina) and commercial wheat gluten (CBH, Quingdao, China) were used for samples preparation. Damaged starch was produced as described by Barrera *et al.*[4] Two systems with 4.4% (H1) and 14.7% (H2) DS were obtained by mixing native and partially damaged wheat starch and commercial wheat gluten (85:15 starch:gluten). The DS content was determined according to the AACC 76-30A.[13] Analyses were performed in triplicate.

The enzymes used were α-amylase (AMY) (Fungamyl-2500BG), maltogenic amylase (MAMY) (Novamyl-10000BG) and amyloglucosidase (AMG) (AMG-800BG), and mixtures of them. The enzymes come with an activity of 2500 FAU g^{-1} (AMY), 10 000 MANU g^{-1} (MAMY) and 800 AGU g^{-1} (AMG). The enzymes doses used were 0.002 g 100 g^{-1} flour to AMY (5 FAU g^{-1} flour), 0.02 g 100 g^{-1} flour to MAMY (200 MANU g^{-1} flour), and 0.04 g 100 g^{-1} flour to AMG (32 AGU g^{-1} flour). Three food-grade enzymatic additives were used. For experimental testing, the doses used were 50% higher than the maximum doses recommended.

The prepared systems with and without the addition of enzymes were kneaded while adding water (farinograph water). The dry ingredients were blended before the addition of water. The ingredients were mixed at constant speed for 2 min. The dough samples were allowed to stand for 15 min at room temperature before the determinations.

Farinograph test

The Farinograph determinations were performed according to AACC method 54–21.[13] The flour water absorption and the properties related to the mixing process were evaluated using a Brabender Farinograph (Brabender Instruments, Inc., Duisburg, Germany). Water absorption values are based on dough consistency at the 500 BU (Brabender Units) line. The parameters determined from the farinographic curves were dough development time (DDT, the time interval from the first addition of water required to reach the maximum consistency), mixing tolerance index (MTI, the consistency difference between the point of maximum consistency and the consistency point after 12 min of the maximum) and stability (*S*, the time interval over which the dough consistency remains at 500 BU). Analyses were performed in duplicate.

Analysis by high-performance liquid chromatography

The molecular profiling of the starch polymers in the freeze-dried dough was analyzed using high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan). A PL-Hi-Plex Na 10 µm ligand exchange chromatography column (Varian, Inc., Palo Alto, CA, USA) was used for the analyses. The operation conditions were: eluent, micropore filtered water; flow rate, 0.3 mL min^{-1}; column temperature, 80 °C; and detector, refractive index. The dough samples for the analysis were prepared as described previously; however, immediately after standing for 15 min at room temperature them were frozen at −40 °C and subsequently lyophilized (L-T8 Rificor, Bs.As, Buenos Aires, Argentina) and stored at 4 °C until the analysis. The extraction of water-soluble dextrins and sugars from lyophilized dough was performed with 85% (v/v) aqueous ethanol at 65 ± 2 °C. The suspensions were maintained at 65 ± 5 °C for 45 min. After centrifugation, the supernatants were evaporated to dryness at 60 °C. The dry extracts were redissolved in water and filtered on a membrane filter (0.45 µm).[14]

The maltoheptose (DP7, Supelco, Buenos Aires, Argentina), maltopentose (DP5, Supelco), maltose (DP2, Sigma-Aldrich) and glucose (Sigma-Aldrich) were used as standards. The peaks were identified with the retention times of the standards. The concentration of sugars in each sample was performed by an external-standard method. The proportion of oligosaccharides larger or equal to 8 glucose units (≥DP8) present in the chromatographic profiles was estimated by area ratios (A_c/A_x) due to the standards of these dextrins were not available for quantification, where A_c is the total peak area of ≥DP8 dextrins of the control sample and A_x is the total peak area of ≥DP8 dextrins of the each sample with enzymes. Analyses were performed in triplicate.

Dough stickiness

The stickiness properties were determined with a TA.XT2i texture analyzer (Stable Micro-Systems Ltd, Godalming, UK). The dough stickiness was evaluated using a 25 mm Perspex cylinder probe (P/25P) and a SMS/Chen–Hoseney dough stickiness cell (A/DSC). The dough was extruded about 1 mm thick above the screen surface and allowed to rest for 30 s. The extruded dough was compressed at 0.4 N and 2 mm s^{-1}. The force required to separate the probe from the dough surface was recorded. Analyses were performed in triplicate.

Dough extensibility

Dough extension properties were determined with a TA.XT2i texture analyzer (Stable Micro-Systems). Uniaxial extension measurements were made using the Kieffer dough & gluten extensibility rig (A/KIE). Dough fragments (20 g) were pressed and cut into strips with the grooved base of the mold. At least five dough strips were extended at 3.3 mm s^{-1} until their elasticity was exceeded. Resistance to extension (R_m, maximum resistance), extensibility (*E*, maximum extensibility) and area under the curve (*A*, deformation work required to extend the dough until rupture) were provided by Texture-Expert 1.22 software (Stable Micro-Systems). Analyses were performed in quadruplicate.

Dough visco elasticity

The visco elastic properties were evaluated by a compression test with a TA.XT2i texture analyzer (Stable Micro-Systems). Cylindrical dough pieces of 10 mm height and 25 mm diameter, approximately, were cut and a 30 mm cylinder probe was used for the test. The rheological measurements were made under a normal stress of 1.39 kPa, which was applied during 45 s (minimum time experimentally determined necessary to reach the system equilibrium). Triplicate determinations were performed. The strain data were described in terms of compliance, *J*:

$$J = f(t) = \frac{\varepsilon}{\sigma}$$

where ε is the strain and σ is the stress applied during test.[15] The compression test results were analyzed using the Burgers model:

$$J(t) = J_0 + J_1\left[1 - \exp\left(-t/\lambda_{ret}\right)\right] + \left(\frac{t}{\eta}\right)$$

where J_0 is the instantaneous compliance, J_1 is the retarded compliance, λ_{ret} is the retardation time of the Kelvin component and

η is the Newtonian viscosity. Instantaneous compliance is related to the elastic properties of the material. The retarded compliance and Newtonian viscosity represent the elasticity and viscosity of the visco elastic element of the model, respectively. The retardation time is the time required for the system to reach a 63.2% of final deformation value.[15] The Burgers model parameters were estimated by fitting the experimental data with SIGMAPLOT-10 (Systat Software, Inc., Erkrath, Germany). The determination coefficients (r^2) of the fitted curves were higher than 0.90 in all cases. The fitting parameters were used to calculate the maximum compliance (J_{max}) reached at the end of the test, frequently used to describe dough stiffness.[16]

Statistical analysis

The data were statistically treated by variance analysis (ANOVA) and multivariate analysis of variance (MANOVA). The ANOVA analysis evaluates the average responses of treatments and compares between them. However, the MANOVA analysis simultaneously evaluates the means of several variables. Therefore, the MANOVA analyzes the global differences between the samples considering more than one variable. The means were compared by the LSD Fisher test at a significance level of 0.05, and the relationship between the measured parameters was assessed by Pearson test (significant levels at $P \leq 0.05$) using the Infostat Statistical Software.[17]

RESULTS AND DISCUSSION

Mixing properties

The high DS level increased the flour water absorption (H1 = 60.4 ± 2.4%, a; H2 = 74.8 ± 2.3%, b) as was expected, due to the high capacity to absorb water of the DS granules. The DDT and MTI parameters calculated from the farinograph curves increased significantly by DS effect, while S was not modified significantly (Table 1). These results indicated that high DS levels are related to more levels of energy and time to produce dough with a standard consistency (500 BU) and promoted weakening of the system as a result of over-mixing. It is probable that the high water absorption capacity of the DS granules decreased water availability in the system during the early stages of mixing process, interfering in the gluten proteins hydration and resulting in an increase in the time required to achieve the 500 BU. The reductions in the over-mixing tolerance could be related to a lower resistance of the swollen DS granules to the shear and extension deformations produced during the mixing process.[18] The more fragile swollen DS granules associated to smaller water holding capacity of the system reduced the dough consistency since the higher plasticizing effect. In addition, the mechanical depolymerization of the starch granules[5,19] during the milling process could be associated to the reductions in over-mixing tolerance. In this way, Miyazaki *et al.*[20] have shown that higher proportions of dextrins longer than 20 glucose units caused reductions in the over-mixing tolerance, which is consistent with the results recorded.

The enzymes incorporations to the H1 and H2 flour samples did not modify the flour hydration in respect of their respective controls. It could be related to the fact that the enzymatic activity did not reach a significant level of degradation of DS granules due to the short time and the low temperatures during the first part of the test. The results published by Indrani and Venkateswara Rao[21] support our results since it was demonstrated that the sugars incorporation until 1.5% did not produce modifications in the farinograph parameters; however, higher sugars concentrations until 9% produced water absorption diminutions. In addition, Duedahl-Olesen *et al.*[22] reported that dextrins between one and four glucose units did not produce significant variations in water absorption.

Table 1. Mixing parameters, sugar profile, extension and visco elastic properties of control dough samples

Parameter	Sample H1	Sample H2
DDT (min)	3.27 ± 0.4^a	6.27 ± 0.6^b
MTI (BU)	0.0 ± 0.0^a	50.0 ± 22.0^b
S (min)	24.0 ± 2.5^a	19.8 ± 2.2^a
≥DP8 (area ratio)	1.00 ± 0.02^a	0.99 ± 0.07^a
DP7	0	0
DP5 (mg 100 g^{-1} freeze-dried dough)	0	0
Maltose	385.8 ± 18.7^a	2498.4 ± 440.7^b
Glucose	23.8 ± 1.2^a	26.0 ± 4.1^a
R_m (g)	46.3 ± 7.5^a	64.7 ± 9.3^b
E (mm)	21.6 ± 2.1^b	17.3 ± 1.6^a
A (g mm^{-1})	788.7 ± 74.2^b	692.1 ± 13.5^a
$J_0 \times 10^{-5}$ (Pa^{-1})	7.3 ± 0.5^b	2.4 ± 0.1^a
$J_1 \times 10^{-5}$ (Pa^{-1})	7.5 ± 0.8^b	1.3 ± 0.1^a
$J_{max} \times 10^{-4}$ (Pa^{-1})	1.7 ± 0.1^b	0.4 ± 0.03^a
λ_{ret} (s)	2.5 ± 0.1^a	2.9 ± 0.2^a
$\eta \times 10^6$ (Pa s)	1.8 ± 0.2^a	6.6 ± 0.7^b

Values followed by different letters in the same line are significantly different ($P \leq 0.05$) (ANOVA).
DDT, Dough development time; MTI, mixing tolerance index; *S*, stability; DP, polymerization degree; A_{H1}, totaling peaks area ≥DP8 of the H1 sample; A_{H2}, totaling peaks area ≥DP8 of the H2 sample; R_m, maximum resistance; *E*, extensibility; *A*, deformation work required to extend the dough until rupture; J_0, instantaneous compliance; J_1, retarded compliance; J_{max}, maximum compliance; η, Newtonian viscosity; λ_{ret}, retardation time of the Kelvin component.

The enzymatic additions to the H1 dough did not modify DDT; however, *S* and MTI were influenced. In this regard, the AMY presence in the enzymatic incorporations promoted *S* reduction and MTI increases and the AMG incorporations did not affect the dough mixing properties. In regard to the H2 dough, enzyme additions decreased DDT and S (Table 2). The AMY addition caused the same effect recorded to H1 dough, regarding *S* and MTI parameters, and AMG incorporation reduced *S* and increased MTI. In general, the MAMY presence did not modify significantly the mixing parameters. Duedahl-Olesen *et al.*[22] have demonstrated that dextrins between DP17 and DP20 and lower polymerization degree decreased DDT and MTI and increased *S*, while, small sugars promoted MTI increase. Miyazaki *et al.*[20] reported that the flour substitution with 2.5% of dextrins between DP3 and DP19 caused DDT reductions, while, the opposite effect was observed with dextrins between DP25 and DP40. In relation to the *S* parameter, these authors reported that dextrins between DP3 and DP19 increased *S*, while, dextrins between DP25 and DP40 produced the opposite effect. Additionally, they observed that dextrins between DP8 and DP29 promoted a MTI increase, while the rest of the dextrins substitutions decreased this parameter.

The results suggested that the dextrin production from enzymatic degradation of DS granules reduces the mixing resistance and the tolerance to over-mixing, indicating that the starch hydrolysis products would have not a positive influence on the dough structure. The hydrolysis of the swollen DS granules decreased

Table 2. Effect of enzymatic additions on the mixing dough properties

Sample	DDT (min)	*S* (min)	MTI (BU)	
H1	3.3 ± 0.4	24.0 ± 2.5	0.0 ± 0.0	*F*
H1 + AMY	3.5 ± 0.3	18.8 ± 2.0	50.0 ± 18.3	*C*
H1 + MAMY	3.3 ± 0.5	50.0 ± 1.9	10.0 ± 21.0	*B*
H1 + AMG	3.3 ± 0.2	24.0 ± 2.6	20.0 ± 17.0	*F*
H1 + AMY + MAMY	3.3 ± 0.3	11.4 ± 2.3	50.0 ± 19.5	*D*
H1 + AMY + AMG	3.5 ± 0.2	12.0 ± 1.8	50.0 ± 20.0	*E*
H1 + AMY + MAMY + AMG	3.3 ± 0.4	6.3 ± 1.9	40.0 ± 18.0	*A*
H2	6.3 ± 0.3	19.8 ± 2.4	50.0 ± 18.0	E
H2 + AMY	1.8 ± 0.2	3.0 ± 2.5	140.0 ± 21.0	C
H2 + MAMY	5.8 ± 0.4	14.3 ± 2.0	60.0 ± 19.0	B
H2 + AMG	6.5 ± 0.3	9.0 ± 1.7	60.0 ± 18.5	A
H2 + AMY + MAMY	4.5 ± 0.5	5.6 ± 2.2	60.0 ± 20.0	G
H2 + AMY + AMG	1.5 ± 0.4	1.8 ± 2.5	130.0 ± 20.4	F
H2 + AMY + MAMY + AMG	3.8 ± 0.3	5.3 ± 1.9	55.0 ± 21.5	D

Different letters indicate significant differences ($P \leq 0.05$) (MANOVA).
The statistical test was realized between H1samples and H2 samples separately. The letters show the global differences between the samples in each group of samples, considering the three variables at the same time.
DDT, dough development time; MTI, mixing tolerance index; *S*, stability; AMY, α-amylase; MAMY, maltogenic amylase; AMG, amyloglucosidase.

the water-holding capacity, causing an increase of the free water proportion and a change on the water plasticizer capacity of the system. The mixing properties modifications were mainly produced by AMY activity. The DDT and *S* reductions and the MTI increase caused by the α-amylase action are consistent with the results published by Kim *et al.*,[23] who also reported DDT, *S* and over-mixing tolerance reductions. These authors proposed that the changes in mixing properties could be attributed to a weakened dough structure produced by low molecular dextrins presence, which derived from the DS hydrolysis by α-amylase action. The highest *S* reductions caused by AMY in H2 could be related to high DS levels of this system, which was associated with higher starch hydrolysis products presence. Regarding the amyloglucosidase influence on dough mixing properties, no reports have been found to compare with the obtained results. However, taking into account that β-D-glucose is the main amyloglucosidase product, Duedahl-Olesen *et al.*[22] have found similar results. They showed that the incorporation of maltose and glucose resulted in small reductions in DDT and small increments in *S*. Additionally, they reported no modification of MTI with maltose presence but increments in this parameter were produced by glucose addition. As a consequence of enzymatic degradation of DS granules, a combined effect between the water plasticizer capacity and the structure modifications could explain the changes in the dough characteristics.

Water-soluble dextrins and sugar profiles

The sugar profiles of the lyophilized dough samples without enzymes (H1 and H2) showed that the proportion of dextrins ≥DP8 was higher than the dextrin fractions of DP5 and DP7 and glucose (Table 1). These results are consistent with those published by Leman *et al.*,[24] who showed a higher proportion of dextrins between DP10 and DP24 and a smaller contribution of dextrins with a lower polymerization degree in amylopectin fractions extracted from gelatinized wheat-starch suspensions. The proportion of ≥DP8 dextrins and glucose was similar in the H1 and H2 dough samples without enzymes (Table 1). However, the maltose concentration significantly increased in the H2 dough sample. The presence of DP7 and DP5 was not detected in these samples (Table 1). Leman *et al.*,[25] Morrison and Tester[19] and Morrison *et al.*[5] have demonstrated that the milling process produces low molecular weight fragments of starch polymers (DP50–80 and DP20–30). They suggested that the shear forces produced during the milling process cause a mechanical damage to the starch, which promotes the breaking of the α-1,4-glycosidic bonds of starch polymers.

Enzymatic additions to the H1 and H2 dough samples increased the level of dextrins, maltose and glucose, as expected (Table 3). These results are related to the enzymatic degradation of starch polymers from spontaneously gelatinized damaged granules and the hydrolysis of the fragments produced as a consequence of the mechanical depolymerization of starch molecules.[4–6] As expected, the dough sample prepared with H2 flour showed higher level of dextrins, maltose and glucose than H1 flour.

The incorporation of MAMY did not affect the sugar profile in any case. However, AMY and AMG and the combinations of them modified the ≥DP8, DP7, DP5, maltose and glucose proportions. The lack of activity of MAMY could be associated with the fact that this enzyme presents an optimum activity between 50 and 80 °C and, therefore, a reduced hydrolytic activity at room temperature.[10] The presence of AMY in the H1 and H2 dough samples caused an increase in the ≥DP8 dextrins proportion and DP5 and maltose concentration. The α-amylase is an endo-enzyme and produces mostly a mixture of dextrins, maltotriose and maltose,[26] which is consistent with the results obtained in this study. The results showed that H1 and H2 dough containing only AMG exhibited a significantly higher proportion of glucose compared to the controls dough, as was observed by Diler *et al.*[27] In addition, the result demonstrated that the amount of glucose in the presence of AMG was related to the amount of DS level in the system, which is in agreement with Diler *et al.*[27] with regard to amyloglucosidase incorporation and Potus *et al.*[28] with regard to α-amylase addition. The AMG promoted the increase of the DP7 dextrin in the H1 dough sample. However, in the H2 dough sample this fraction was not detected.

Table 3. Effect of enzymatic additions on the sugar profile

Sample	≥DP8	DP7	DP5	Maltose	Glucose	
	Area ratios (A_x/A_c)	(mg 100 g^{-1} freeze-dried dough)				
H1	1.00 ± 0.01	0	0	385.8 ± 18.7	23.8 ± 1.2	*B*
H1 + AMY	1.37 ± 0.03	0	5.9 ± 0.4	777.8 ± 12.6	34.4 ± 0.2	*D*
H1 + MAMY	0.99 ± 0.02	0	0	448.4 ± 6.0	24.2 ± 0.2	*B*
H1 + AMG	1.13 ± 0.01	2.4 ± 0.1	1.5 ± 0.3	88.5 ± 8.0	1310 ± 104.4	*A*
H1 + AMY + MAMY	1.45 ± 0.03	0	7.7 ± 0.8	762.1 ± 20.5	41.1 ± 2.1	*D*
H1 + AMY + AMG	1.18 ± 0.02	2.7 ± 0.4	19.1 ± 0.04	133.5 ± 2.1	1902.2 ± 68.3	*C*
H1 + AMY + MAMY + AMG	1.18 ± 0.02	3.9 ± 0.01	19.5 ± 1.9	106.6 ± 12.8	1950.7 ± 156.6	*C*
H2	1.00 ± 0.02	0	0	2498.4 ± 470.7	26.0 ± 4.1	F
H2 + AMY	2.23 ± 0.01	0	1.3 ± 0.1	4516.7 ± 2.7	48.7 ± 0.2	D
H2 + MAMY	1.06 ± 0.03	0	0	2808.8 ± 78.5	29.2 ± 0.6	F
H2 + AMG	1.16 ± 0.02	0	0	1465.4 ± 11.3	2532.2 ± 3.2	A
H2 + AMY + MAMY	2.15 ± 0.03	0	1.3 ± 0.2	4012.7 ± 679.8	40.9 ± 5.9	C
H2 + AMY + AMG	2.37 ± 0.01	0	7.5 ± 0.1	2936.3 ± 81.2	3038.3 ± 103.4	E
H2 + AMY + MAMY + AMG	2.46 ± 0.02	0	10.6 ± 0.5	3009.2 ± 136.9	3027.6 ± 135.8	B

Different letters indicate significant differences ($P \leq 0.05$) (MANOVA).
The statistical test was realized between H1samples and H2 samples separately. The letters show the global differences between the samples in each group of samples, considering the five variables at the same time.
DP, polymerization degree; A_c, totaling peaks area ≥DP8 of the control sample; A_x, totaling peaks area ≥DP8 of the each sample with enzymes; AMY, α-amylase; MAMY, maltogenic amylase; AMG, amyloglucosidase.

The results demonstrated that the main changes of the sugar profile were produced by AMY and AMG activity. As regards these results, a synergistic effect between both enzymes was recorded, which has been reported before by Fujii *et al.*[29] The presence of α-amylase caused an increase in the maltose concentration in the H1 and H2 dough samples. However, when the amyloglucosidase was incorporated in the system, the level of this disaccharide decreased while the glucose proportion increased. The function of α-amylase in the mixture of AMY + AMG was to provide new substrates to amyloglucosidase while the amyloglucosidase liberates monomers and dimmers from the non-reducing end of the substrate molecules. Therefore, it is possible to consider α-amylase and amyloglucosidase incorporation as a positive contribution on the fermentation process in the production of yeast-leavened bakery products, as a consequence of higher glucose and maltose concentrations producing.

Table 4. Effect of enzymes on the stickiness properties

Sample	Stickiness (g)	
	H1	H2
Control	34.6 ± 2.3^{p,ab}	56.5 ± 0.5^{s,ab}
AMY	42.7 ± 1.1^{c}	54.5 ± 3.8^{a}
MAMY	38.4 ± 1.6^{bc}	56.1 ± 1.35^{ab}
AMG	35.1 ± 3.5^{ab}	65.5 ± 6.6^{b}
AMY + MAMY	33.2 ± 2.8^{ab}	58.6 ± 2.2^{ab}
AMY + AMG	32.4 ± 2.0^{a}	60.6 ± 4.3^{ab}
AMY + MAMY + AMG	34.4 ± 3.3^{ab}	57.9 ± 7.6^{ab}

The letters p and s indicate the statistical difference between controls ($P \leq 0.05$).
Values followed by different superscript letters in the same column are significantly different ($P \leq 0.05$) (ANOVA).
AMY, α-amylase; MAMY, maltogenic amylase; AMG, amyloglucosidase.

Stickiness properties

Increments in DS caused an increase in dough stickiness, which indicates a dough with a more adhesive and less cohesive structure (Table 4). In agreement with these observations, Ghodke *et al.*[30] have shown that the dough adhesiveness was higher in systems with high levels of DS. The higher levels of energy and time to reach the standard consistency recorded to H2 sample could be associated with dough stickiness, making dough kneading more difficult. In our study, the H1 and H2 doughs were prepared according to farinograph-water absorption, and under this condition, the values of wet (H1 = 32.4%, H2 = 31.2%) and dry (H1 = 13.9%, H2 = 13.5%) gluten content (AACC 38-10 [13]) did not show significant differences. Taking into account that protein proportion and quality were the same in both systems and that the gluten content were similar in the H1 and H2 dough samples, we assumed an optimal developed gluten network during mixing in both cases. Therefore, the high stickiness of the H2 dough sample suggests that the internal structure of this system did not provide enough restraining arrangement to hold the free water. Consequently, the free water could become more accessible to the dough surface, resulting in an increase of adhesiveness, despite the H2 soluble fraction rich in maltose. Additionally, it is possible that the higher stickiness of the H2 dough was influenced by a lower resistance of the swollen DS granules to the shear and extension deformations produced during the kneading, which could be associated with lower water-holding capacity, as already suggested. The most adhesive structure of the H2 dough sample could be associated with a more open gluten network arrangement as a result of the large size of the swollen DS granules[3] immersed in it. The formation of this structure suggests a change in the internal interactions in the system, resulting in a less cohesive structure.

The enzyme addition did not succeed in reducing the dough stickiness caused by the DS presence. However, the AMY incorporation in the H1 dough sample caused an increase in this parameter (Table 4). Autio and Laurikainen[31] have stated that α-amylase produces sticky doughs, which presented handling

Table 5. Effect of enzymatic incorporations on the extension properties

Sample	R_m (g)	E (mm)	A (g mm^{-1})	
H1	46.3 ± 7.5	21.6 ± 2.1	788.7 ± 74.2	*C*
H1 + AMY	86.4 ± 12.3	15.0 ± 1.3	657.5 ± 102.6	*E*
H1 + MAMY	66.4 ± 1.4	17.8 ± 0.6	786.2 ± 33.6	*D*
H1 + AMG	85.8 ± 14.4	18.5 ± 3.1	946.8 ± 78.9	*A*
H1 + AMY + MAMY	55.8 ± 10.0	17.3 ± 1.3	808.3 ± 118.4	*D*
H1 + AMY + AMG	94.9 ± 8.9	14.4 ± 1.8	934.8 ± 23.9	*B*
H1 + AMY + MAMY + AMG	89.7 ± 12.9	15.2 ± 0.5	730.9 ± 38.0	*E*
H2	64.7 ± 9.3	17.3 ± 1.8	692.1 ± 13.5	A
H2 + AMY	48.1 ± 3.4	17.6 ± 1.9	570.3 ± 75.8	CD
H2 + MAMY	40.5 ± 2.6	18.8 ± 1.6	529.0 ± 32.4	CD
H2 + AMG	41.8 ± 1.3	16.2 ± 0.4	582.8 ± 59.6	D
H2 + AMY + MAMY	47.7 ± 4.6	15.7 ± 1.4	487.9 ± 66.0	B
H2 + AMY + AMG	43.5 ± 5.3	18.5 ± 1.5	472.2 ± 29.3	C
H2 + AMY + MAMY + AMG	42.4 ± 2.3	17.1 ± 0.6	584.1 ± 60.6	D

Different letters indicate significant differences ($P \leq 0.05$) (MANOVA).
The statistical test was realized between H1samples and H2 samples separately. The letters show the global differences between the samples in each group of samples, considering the three variables at the same time.
R_m, maximum resistance; E, extensibility; A, deformation work required to extend the dough until rupture; AMY, α-amylase; MAMY, maltogenic amylase; AMG, amyloglucosidase.

Table 6. Effect of enzymatic incorporations on the visco elastic properties

Sample	J_0 (Pa^{-1}) × 10^{-5}	J_1 (Pa^{-1}) × 10^{-5}	J_{max} (Pa^{-1}) × 10^{-4}	λ_{ret} (s)	η (Pa s) × 10^6	
H1	7.3 ± 0.5	7.5 ± 0.8	1.7 ± 0.1	2.5 ± 0.1	1.8 ± 0.2	*A*
H1 + AMY	5.8 ± 0.3	5.9 ± 0.3	1.4 ± 0.7	2.9 ± 0.2	2.1 ± 0.1	*B*
H1 + MAMY	6.3 ± 0.01	6.5 ± 0.3	1.5 ± 0.03	2.8 ± 0.01	1.8 ± 0.02	*AB*
H1 + AMG	5.7 ± 0.4	5.9 ± 0.2	1.4 ± 0.08	2.8 ± 0.2	2.3 ± 0.2	*B*
H1 + AMY + MAMY	6.0 ± 0.2	6.6 ± 0.3	1.5 ± 0.06	3.0 ± 0.3	1.9 ± 0.03	*B*
H1 + AMY + AMG	5.7 ± 0.5	5.8 ± 0.8	1.3 ± 0.2	2.8 ± 0.01	2.3 ± 0.2	*B*
H1 + AMY + MAMY + AMG	5.0 ± 0.1	5.4 ± 0.4	1.2 ± 0.07	2.9 ± 0.01	2.5 ± 0.3	*B*
H2	2.4 ± 0.1	1.3 ± 0.1	0.44 ± 0.03	2.9 ± 0.2	6.6 ± 0.7	AB
H2 + AMY	4.4 ± 0.4	2.9 ± 0.3	0.86 ± 0.06	2.2 ± 0.1	3.8 ± 0.3	C
H2 + MAMY	2.2 ± 0.02	1.3 ± 0.07	0.41 ± 0.07	3.2 ± 0.4	7.0 ± 0.4	B
H2 + AMG	3.8 ± 0.3	1.5 ± 0.08	0.60 ± 0.04	2.6 ± 0.1	6.6 ± 0.8	B
H2 + AMY + MAMY	4.2 ± 0.3	3.8 ± 0.06	0.93 ± 0.03	2.3 ± 0.04	3.3 ± 0.1	C
H2 + AMY + AMG	4.7 ± 0.6	3.1 ± 0.1	0.89 ± 0.08	2.1 ± 0.08	4.0 ± 0.05	C
H2 + AMY + MAMY + AMG	3.2 ± 0.2	2.2 ± 0.07	0.63 ± 0.04	2.7 ± 0.4	4.7 ± 0.03	AC

Different letters indicate significant differences ($P \leq 0.05$) (MANOVA).
The statistical test was realized between H1samples and H2 samples separately. The letters show the global differences between the samples in each group of samples, considering the five variables at the same time.
J_0, instantaneous compliance; J_1, retarded compliance; J_{max}, maximum compliance; η, Newtonian viscosity; λ_{ret}, retardation time of the Kelvin component; AMY, α-amylase; MAMY, maltogenic amylase; AMG, amyloglucosidase.

problems during the bread baking process. This effect was related to the excessive DS granules degradation as a consequence of the enzymatic action. Therefore, the α-amylase proportion in a formulation must be carefully optimized. Taking into account the results obtained, the enzyme effect on the H2 dough sample was probably hidden due to the high proportion of water in this system. Additionally, the presence of a higher proportion of soluble sugars as a consequence of enzymatic activity did not significantly modify the adhesive properties of the system.

Dough extension and visco elastic properties

High levels of DS changed the dough extension and visco elastic properties. The H2 dough sample recorded an increase in the maximum resistance, and a decrease in the extensibility and deformation work required to extend the dough until rupture (Table 1). These results indicated that increments in the DS content produce more resistance to extension and less extensible doughs, regardless of the high water proportions added to this system. The reduction in deformation work suggested a less cohesive dough structure. The Burger model parameters indicated that the increase in DS content causes a reduction on the instantaneous compliance (J_0), retarded compliance (J_1) and maximum compliance (J_{max}) and an increase in the Newtonian viscosity (η) of the system (Table 1). The retardation time of the Kelvin component (λ_{ret}) did not show modifications by the DS effect. The results indicated that the H2 dough sample had a less elastic capacity

and was stiffer and more viscous than the H1 dough structure. In agreement with these results, Dexter *et al.*,[32] Rao *et al.*[33] and El-Porai *et al.*[34] proved that the degree of damage of the starch granules reduced the extensibility and increased the resistance of dough. The referenced results were obtained from doughs prepared with the same water proportion for all flours in each case. Therefore, the differences found by these authors could be related to the change in the water distribution of the system caused by higher DS levels. It is probable that the systems tend to develop less gluten as a result of the high absorption capacity of the DS granules and, consequently, the extension properties are modified. Unlike previous studies, in our study the water added to the dough formation was sufficient to ensure the complete gluten development (farinograph absortion). Creep-compliance values are mainly associated with softness.[35] In relation to this, materials exhibiting low creep values over time are representative of strong or stiff structures. Taking this into account, the changes in the rheological behavior of H2 could be related to a stiffer gluten arrangement, as a result of the presence of higher size DS granules embedded in the gluten network. Water plays an important role in the visco elastic behavior of these systems. The water content in the H2 sample was higher than H1 (H2 = 43.98%; H1 = 39.94%) due to the increment in the flour water absorption of the H2 system. However, the H2 dough sample showed a higher resistance to flow. This effect could be related to a lower water plasticizing capacity in the system with a higher DS level, possibility due to lack of the lubrication effect of the H2 soluble fraction from the higher maltose proportion recorded. In relation to these results, Trinh *et al.*[36] reported that the dough became less extensible as the saccharose content increased.

The dough extension and visco elastic properties were modified by enzymatic incorporations. In general, the enzymes in the H1 dough sample promoted an increase in the maximum resistance, stiffness and viscosity of the structure and a decrease in the extensibility and elastic properties, while the deformation work was not modified. However, in the H2 dough sample, the enzymatic additions mainly decreased the maximum resistance, deformation work, stiffness and viscosity of the system and increased the elastic parameters, but did not modify the extensibility (Table 5 and Table 6). The opposite mechanical behavior showed by the H1 and H2 dough samples, as a consequence of the enzymatic incorporation, could be related to the differences in the structural characteristics of the matrix in each system and the water plasticizing properties associated to the soluble fraction in each system. In the H1 dough sample, the swollen DS granules are hydrolyzed by amylases, resulting in a higher proportion of dextrins in the dough. Consequently, the water plasticizing properties are reduced in these conditions because of dextrins reduced the water mobility in the system. On the other hand, the free water and dextrin levels in the H2 dough are higher than the H1 dough, as a result of the higher proportion and enzymatic degradation of the hydrated DS granules. In the H2 system the water released as a consequence of a much higher number of damaged granules, which were enzymatically hydrolyzed (loss of granular integrity), probably resulted to be more significant than the reduction of the water plasticizing properties produced by the soluble fraction of sugars.

The results showed that the enzymatic additives promoted an improvement in the rheological properties of systems with higher levels of DS. In general, the enzymatic additions had a positive effect on the H2 dough resistance. The α-amylase and the combination of α-amylase and amyloglucosidase helped to improve the visco elastic properties of the H2 dough sample. The α-amylase and amyloglucosidase addition caused a higher disintegration of the damaged granules, which appears to contribute to a greater alteration in the system structure. In addition, the combination of higher levels of hydrolyzed products and their molecular size could be responsible for the changes in the free water levels in the system, which might cause differences in the dough properties. In agreement with our findings, Kim *et al.*[23] have reported that the α-amylase addition causes a reduction in extensibility and an increase in resistance. In addition, Diler *et al.*[27] have proved that the amyloglucosidase incorporation reduce the wheat-dough visco elastic properties as well as the α-amylase additions.

CONCLUSION

Previous studies have suggested that higher levels of DS produced dough with a weakened structure due to competition for water between DS and gluten proteins during the dough development. In this sense, the deterioration of the dough rheological properties has been attributed to less developed gluten. Considering the characteristics of the system studied and the data obtained, a complementary new hypothesis can be proposed. The results suggested that the rheological behavior of dough with higher DS content is related to the modifications of the gluten network structure and water plasticizing capacity. Although the role played by starch granules in dough has been minimized, it is clear that their properties affect the structure conformation of the dough system, indicating a more open gluten network arrangement as a result of the large size of the swollen DS granules.

The α-amylase and amyloglucosidase changed the sugar profile, reducing the dough resistance and improving the visco elastic properties of systems with high levels of DS. On the other hand, reductions of the mixing resistance and the tolerance to over-mixing were also recorded as a consequence of the production of dextrins from enzymatic degradation. The incorporation of maltogenic amylase, in the dose used, did not cause relevant modifications on the dough sugar profile and the rheological characteristics. Hence, we can conclude that the dough rheological properties of systems with high DS content changed positively as a result of enzymatic action, particularly α-amylase and amyloglucosidase additions, allowing the use of these amylases and mixtures of them as corrective additives. Previous results have shown the effects of α-amylase; however, little information was reported about amyloglucosidase activity alone or combined with α-amylase. The combinations of these two enzymes are promising to minimize the negative effects caused by high levels of DS on product quality and more research investigating the effects of a combination of these two enzymes on bread quality needs to be done.

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