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Improvement of Flour Quality through Carbohydrases Treatment during Wheat Tempering

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Wheat flour is obtained by the milling process, which includes several steps such as cleaning, tempering, and milling. In the tempering the moisture content of wheat grains is increased to 15.5% by adding an adequate amount of water. The addition of different enzymes (cellulase, xylanase, and β-glucanase) to the tempering solution has been tested in order to modify the quality of the resulting flour. Rheological and fermentative properties were measured by the farinograph, amylograph, and rheofermentometer. The data show that the technological parameters of the resulting flours were greatly modified by the addition of enzymes to the tempering solution. The quality of the fresh bread obtained from the carbohydrase-treated wheat was improved with regard to specific bread volume, bread shape, and crumb firmness. This method is revealed as an excellent tool to ensure a good distribution of the enzymes in the resulting flour, to control dosage during milling, and to obtain flour of specific characteristics according to their final use.

KEYWORDS: Enzymes; carbohydrases; wheat; tempering; bread-making; dough; fresh bread; quality

INTRODUCTION

Highly hydratable substances in cereals are mostly present in the cell walls and mainly consist of β-glucans (in the aleurone layer) and pentosans (in the endosperm). Their high water binding capacity affect milling and flour quality (*1*), the most important compounds being those that determine the water economy through the bread processing. Modifications of initial structures of wheat carbohydrates by enzyme addition usually bring positive changes on dough and bread characteristics (softness, texture, whiteness, and shelf life).

In fact, several enzymes have been proposed as technological aids. Among them could be cited amylases (*2*−*5*), cellulases or β-glucanases (*6*−*8*), hemicellulases (*3*, *9*, *10*), and xylanases or pentosanases (*4*, *6*, *8*, *11*−*13*), which are able to partially solve the technological problems; in addition they are inactivated during baking and do not leave residues in the final product (*3*).

Pentosans are a minor component of wheat flour, and they play an important role in dough rheology and bread quality. They can be found in a free form or associated with glycopeptides, and that determines their water solubility. Soluble pentosans are implicated in the dough elasticity, and the hydrolysis of the insoluble pentosans promotes changes on dough rheology due to the release of water, which can be used to form the gluten network (*14*). The hydrolysis of pentosans can be accomplished by some cellulases, which also catalyze the breakdown of cellulose into glucose and cellobiose. The activity of xylanases is more related to soluble pentosans, and β-glucanases hydrolyze β-glucans. The literature about the mechanism of each carbohydrase in bread-making is very confusing due to their complex specificity and the wheat flour composition. However, it has been described that enzyme hydrolysis of nonstarch polysaccharides led to an improvement of the rheological properties of dough, bread specific volume, and crumb firmness (*10*, *13*).

Enzymes as technological aids are usually added to flour, during the mixing step of the bread-making. However, this could sometimes cause some problems due to overdosage and nonuniform mixing. Recently, Rosell et al. (*15*) reported an alternative method for improving wheat gluten by using enzymes through the milling process. The addition of glucose oxidase or transglutaminase to the tempering solution led to a wheat flour with improved gluten-forming capacity.

Therefore, the addition of enzymes that degrade cell walls such as cellulase (CEL), xylanase (XYL), and β-glucanase (GLUC) to the tempering solution of wheat may provide a new method to obtain flour of specific characteristics.

The objective of this study was to modify wheat flour characteristics by adding enzymes to the tempering solution in order to change or improve the rheological characteristics of the resulting wheat flour and, consequently, the quality of the fresh bread.

MATERIALS AND METHODS

Materials. Wheat kernels from the Bolero cultivar, grown in a northern area in Spain, were used in this study. Wheat characteristics

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Table 1. Summary of Commercial Enzyme Characteristics Supplied by the Manufacturer

code	main activity	EC no.	secondary activities	dosage (units/g of kernel)	enzyme activity[a] (munits/g of flour)
CEL	cellulase	3.2.1.4		280.5	85.5
XYL	xylanase	3.2.1.8		14.6	2.9
GLUC	multi-β-glucanase	3.2.1.73	cellulase; xylanase; pentosanase; arabanase	3.5	1.2

[a] Remaining enzyme activities in the resulting flour.

were as follows: test weight, 81.3 kg/hL; thousand kernel weight, 32.2 g; and protein, 12.4% (based on 12% moisture content). Compressed yeast was used as a starter.

Enzymes were a generous gift from Novo Nordisk A/S (Madrid, Spain). Characteristics of the commercial enzymes used are summarized in **Table 1**. The enzyme activities are expressed as micromoles of the specific substrate per minute and gram of the commercial preparation, and they were measured by using the specific Megazyme kits with colored substrates.

Milling Procedure. Wheat (600 g), after appropriate cleaning, was tempered by adding the adequate amount of water (control) to 15.5% moisture in a Chopin conditioner or by adding different enzyme solutions (dosage of enzyme indicated in **Table 1**). Tempering was carried out at 20 °C during 16 h. The milling test was performed on a Chopin laboratory mill (Tripette et Renaud, France). Flour yields were calculated from the scale weights of the total recovered product. Four samples were milled for each treatment. The wheat flour obtained after milling [0.68% ash content, 10.16% protein (based on 12% moisture content), 188 $\times$ 10^{-4} J energy of deformation] was used to prepare dough samples for rheological assays and breads. The remaining enzyme activities in the resulting flour are detailed in **Table 1**.

Determination of Mixing Behavior of the Flour. A farinograph (Brabender, Duisburg, Germany) with a 50 g mixer was used to evaluate the impact of the enzyme addition on the water of tempering procedure by following the official standard method (*16*). The effect of the enzyme addition was also analyzed by adding the same enzyme activity remaining in the flour directly to the mixer.

The following parameters were determined in the farinograph analysis: water absorption [percentage of water required to yield a dough consistency of 500 Brabender units (BU)], dough development time (DDT, time to reach maximum consistency), stability (time dough consistency at 500 BU), mixing tolerance index (MTI, consistency difference between height at peak and that 5 min later), and elasticity (bandwidth of the curve at the maximum consistency). The standard deviations in all of these measurements were <4% of the mean and, therefore, are not presented with the data.

Determination of the Amylogram Parameters. The pasting properties of wheat flour and the effect of enzyme addition in the tempering water were investigated with a Brabender viscograph A/RH. The amylographic procedure was in accordance with ICC Method 126/1 (*17*), using a heating−cooling cycle, described by Rojas et al. (*18*). The amylograph characteristics included pasting temperature (at the intersection point of the horizontal and vertical tangential lines of the amylograph curve during the heating) and viscosity peak. Additionally, the bump area or peak area, measured with a planimeter connecting the baseline of the bump from the starting point to the ending point of the peak area as described Xu et al. (*19*), and the setback, the difference between the viscosity at 50 °C and the viscosity after the first holding period (*20*), were determined. All amylograph tests were replicated twice for a given sample.

Determination of the Proofing Behavior of the Modified Flours. The behavior of dough during fermentation was determined using a Rheofermentometer F2 (Tripette et Renaud) following the supplier specifications. Dough was mixed using the farinograph (at constant consistency) and was placed (315 g) in the fermentation vessel at 28.5 °C for 3 h; a weight constraint of 2.0 kg was applied. The rheofermentometer measured and recorded simultaneously the parameters related to dough development, gas production, and gas retention.

Table 2. Effect of Enzyme Treatment on the Farinograph Characteristics of Flour

enzyme	WA[a] (%)	DDT[b] (min)‘	stability (min)	MTI[c] (BU)	elasticity (BU)
control	51.4	3.50	17.5	45	160
CEL	50.7	3.33	5.5	170	170
XYL	50.9	3.25	4.5	95	140
GLUC	51.7	3.75	14.5	50	160

[a] WA, water absorption. [b] DDT, time to reach maximum consistency. [c] MTI, consistency difference between height at peak and that 5 min later.

Bread-Making Procedure. The bread dough formula consisted of wheat flour (300 g), compressed yeast (3.0%, flour basis), salt (2.0%, flour basis), and water (up to optimum absorption). The ingredients were mixed for 4 min, rested for 10 min, divided (50 g), kneaded, and then rested again (10 min); doughs were mechanically sheeted and rolled, proofed (up to 3 times the initial dough volume, at 29 °C and 80% relative humidity), and baked (170 °C for 20 min) according to the method of Haros et al. (*21*). After baking, the resulting rolled breads were cooled for 2 h at room temperature. Three sets of samples were prepared for each treatment.

For studying the effect of enzyme addition, the same enzyme activities measured in the treated flours were added to the flour with the rest of the ingredients.

Technological Evaluation. During the proofing stage, the dough volume increase was followed in a graduated cylinder, and dough pH was potentiometrically determined. Physicochemical characteristics of breads including weight, volume (seed displacement), width/height ratio of the central slice, specific volume index (specific volume of the control was taken as 1, and the samples were relative to the control), and texture (texture profile analysis, TPA) were determined. TPA was performed using a Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, U.K.). A bread slice of 2 cm thickness was compressed twice by using a stainless steel 1.0 cm diameter plunger, moving at 1.0 mm/s to a penetration distance of 50%, with an interval of 50 s between compressions. The following parameters were evaluated: relative hardness (hardness of the control was taken as 1, and the samples were relative to the control), springiness, cohesiveness, chewiness, and resilience (*22*).

RESULTS AND DISCUSSION

Effect of Enzyme Treatments on Milling and Rheological Parameters. The different enzyme treatments of the wheat kernels during tempering did not affect flour extraction, giving total flour yields between 70.1 and 70.4%. After the differently treated flours had been obtained, a characterization of their rheological properties was carried out.

The farinograph parameters are summarized in **Table 2**. Enzyme addition to the tempering water barely modified the water absorption capacity of the resulting treated wheat flours. Girhammar (*11*) obtained similar results when adding xylanase directly to flour in the farinograph assay. However, to compare the effect of the carbohydrases at the same activity level as that of the treated flours, a study of the direct addition of the carbohydrases to the flour was made, and the same tendency was found (**Table 3**). The dough development time or time required to reach 500 BU of dough consistency (DDT) was slightly affected by the presence of enzymes in the tempering solution, obtaining a decrease in the CEL- and XYL-treated flours and an increase in the GLUC-treated flour. Conversely, in the direct enzyme addition (**Table 3**), a decrease of the DDT was obtained in all cases.

Table 3. Effect of Direct Enzyme Addition on the Farinograph Characteristics of Flour

enzyme	WA[a] (%)	DDT[b] (min)	stability (min)	MTI[c] (BU)	elasticity (BU)
control	51.4	3.50	17.5	45	160
CEL	51.6	3.22	11.4	90	146
XYL	51.6	3.22	12.3	82	146
GLUC	51.8	3.22	9.2	74	160

[a] WA, water absorption. [b] DDT, time to reach maximum consistency. [c] MTI, consistency difference between height at peak and that 5 min later.

Table 4. Effect of Enzyme Addition in Tempered Solution on the Pasting Properties of Wheat Flour

enzyme	pasting temp (°C)	viscosity peak (BU)	setback (BU)	bump area (cm^2)
control	82.6	565	510	4.1
CEL	83.8	480	438	4.6
XYL	84.4	450	430	4.5
GLUC	83.6	544	447	4.3

On the other hand, all added enzymes decreased the dough stability; this value is an indication of the flour strength, with higher values suggesting stronger dough. The same trend was observed when the same enzyme activity was directly added to the flour, although the extent of the effect was different (**Table 3**). Similar results were found by Laurikainen et al. (*23*) and McCleary et al. (*24*), who investigated the effect of the direct addition of xylanase to flour on the farinograph parameters. In addition, Al-Suaidy et al. (*25*) pretreated wheat with cellulase and hemicellulase, but farinograph data showed little variation among treatments.

Enzyme supplementation to the tempering water promoted a pronounced increase in the MTI, with the exception of the sample treated with GLUC. In addition, an increase in the degree of softening was produced by all of the enzyme treatments, which agrees with the results obtained by direct addition of carbohydrases (**Table 3**). According to Laurikainen et al. (*23*), addition of xylanases causes softening of the wheat dough because they break down soluble pentosans, whereas Al-Suaidy et al. (*25*) did not find significant modifications in the wheat treated with cellulase. The elasticity of dough was reduced with the incorporation of XYL.

The effect of the wheat enzyme treatment on the paste behavior was also analyzed, because the polysaccharides are mainly responsible for the wheat hydration and, consequently, the final viscosity. The addition of enzymes to the tempering water changed the pasting properties of the final flours, although the extent of the modification was dependent on the enzyme added (**Table 4**). The enzyme-treated flours showed higher pasting temperatures than the control. The addition of enzymes that degrade cell walls and membranes, such as cellulase, hemicellulase, β-glucanase, xylanase, pentosanase, and arabanase, increased the amount of soluble oligosaccharides and monosaccharides, which may interfere with the gelatinization by hindering the swelling of starch granules, increasing the temperature of the onset of paste. Bean and Osman (*26*) and Savage and Osman (*27*) reached the same conclusion when studying the effect of different sugars pastes (various monosaccharides, disaccharides, and mixtures such as corn syrups) on the consistency of starch. In addition, Hester et al. (*28*) found markedly higher onset temperature of wheat flour paste when sucrose was added.

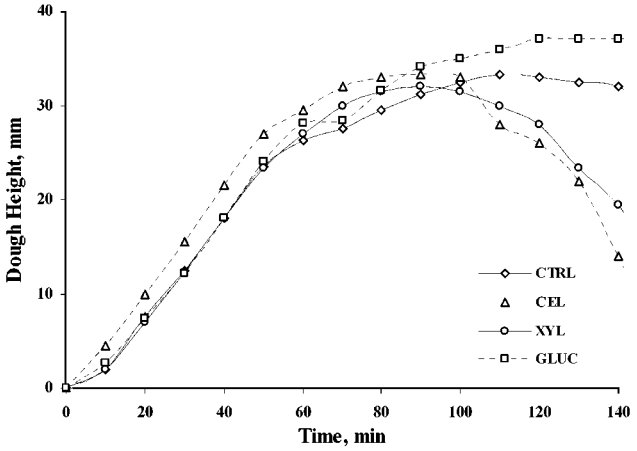

Figure 1. Dough development curves obtained using the rheofermentometer showing dough height during proofing.

Enzyme-treated flours yielded pastes with a decreased maximum viscosity. The presence of the nonstarch polysaccharides is related to an increase in the viscosity, as was stated previously by Delcour et al. (*29*), particularly the water soluble pentosans (*20*). Therefore, from this it is clear that the breakdown of pentosans (arabinoxylan and arabinogalactan) and β-glucans (cellulose and glucans) by enzymes should promote the reduction of viscosity, as was found in this study. Treatment with XYL (high specificity toward the soluble pentosan fraction) showed the highest effect in the drop in maximum viscosity. A decrease of viscosity has been previously observed with the addition of glucanase to barley flour slurries (*30*), of glucanase to oat extract (*31*), of xylanase in water extractable arabinoxylans to wheat flour (*32*), and of xylanase to wheat flour slurry (*33*).

Enzyme-treated flours led to pastes with a decreased setback, which describes the tendency of amylose to retrograde after diffusing outside the starch granules during the cooking stage (*18*). The phenomenon of amylose retrogradation is responsible for the firming of the bread crumb during the first hours after baking. Therefore, with regard to the reduction effect on the setback, carbohydrase treatment during the wheat tempering could be considered a useful method to yield flours with antistaling properties in the bread-making process.

The bump area, a parameter related to the extent of the formation of amylose−lipid complex during the cooling phase (*34*), was barely modified in the wheat pastes from enzyme-treated flours.

Effect of Enzyme Treatments on Dough Proofing Measured by a Rheofermentometer. The proofing behavior of the enzyme-treated flours was tested with the rheofermentometer. In **Figure 1** can be observed the dough development from flours submitted to the different enzyme treatments. The first glance indicates that the enzyme treatment during tempering modifies the flour characteristics. Dough from cellulase-treated flour showed an early dough development compared to the control, likely due to the breakdown of cellulose into glucose (fermentable sugar) and other glucose polymers, accelerating CO_2 production. With regard to the dough height after 90 min of fermentation, the enzyme-treated flours yielded higher values than the control, although the reduced stability of the dough from CEL- and XYL-treated flours would make them suitable for short-term proofing processes. In contrast, the dough from the GLUC-treated flour led to both higher maximum dough height and greater stability than the control; therefore, this flour would be appropriate for long-term fermentation.

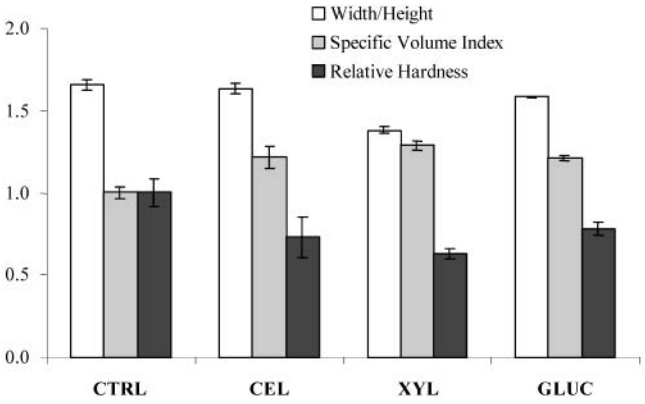

Figure 2. Effect of enzyme treatment during wheat tempering on fresh bread quality. Errors bars are the mean of at least three replicates ± standard deviation. See **Table 1** for codes.

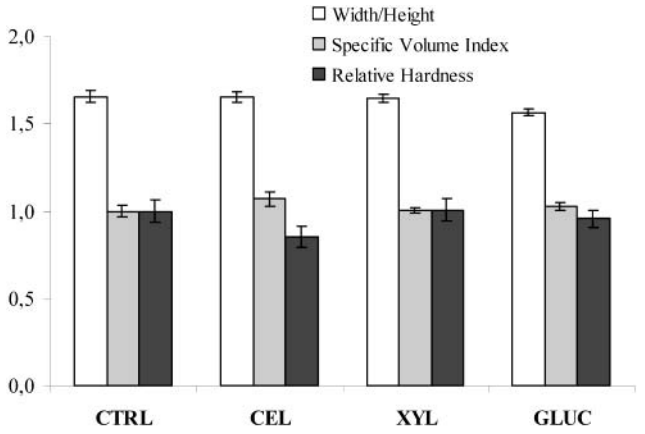

Figure 3. Effect of the carbohydrases addition on fresh bread quality. Errors bars are the mean of at least three replicates ± standard deviation. See **Table 1** for codes.

Direct addition of carbohydrases to the flour showed the same trend, although less pronounced effects were observed, likely due to the shorter period allowed for enzyme action when enzymes are added in the proofing assay than in the tempering solution (results not showed).

Effect of Wheat Enzyme Treatment on Fresh Bread Quality. Flours obtained from the enzyme-treated wheat were used for bread-making, and the bread quality characteristics were determined. During bread-making it was observed that shorter proofing was required when cellulase- and xylanase-treated flours were used (results not showed), compared to the control. The pH during proofing decreased from 5.6 to 5.4 in all cases, without and with enzyme treatment.

Addition of enzymes in the tempering solution improved the quality parameters of the resulting fresh bread (**Figure 2**). An improvement of the bread shape (width/height ratio) was obtained with the XYL- and GLUC-treated flours. The maximum effect was obtained with the XYL treatment, with an increase of 17% compared to the control sample. In the direct addition of the enzymes, only a slight improvement was observed with the addition of glucanase, but no effect was obtained with the other two carbohydrases tested.

On the other hand, the specific volume of the bread increased with all enzymatic treatments, rising up to 29% with the xylanase treatment (XYL). When the same enzyme activity was added directly to the flour (**Figure 3**), only the addition of cellulase led to a slight increase in the specific volume index. The different results observed between the enzyme treatment in the tempering solution and the direct addition of enzymes might be attributed to hydrolysis products released during the tempering and further milling of the wheat.

Laurikainen et al. (*23*) also obtained an increase of 18−19% in the bread specific volume by adding hemicellulases and cellulases directly to flour. Martinez-Anaya and Jimenez (*13*) also observed greater specific volume of bread when the dough was supplemented with pentosanase.

According to Matt et al. (*35*) the effect of xylanase on bread volume improvement results from the redistribution of water from the pentosan phase to the gluten phase. The increase in the gluten volume fraction gives the gluten more extensibility, which eventually results in a better oven-spring. The improving effects of pentosanases/hemicellulases on bread volume also have been associated with a better gas retention during proofing, probably due to the action of enzymes in reducing the viscosity of the gelling starch (*36*).

Enzymatic treatments during wheat tempering also promoted a pronounced effect on the texture properties of the bread crumb (**Figure 2**). In all samples the hardness or firmness of bread crumb was reduced; thus, softer crumbs were obtained in the breads from enzyme-treated wheat than from the control. Other parameters from the texture profile analysis, such gumminess and chewiness, were also decreased in all analyzed samples (results not shown). In the firmness analysis of the bread obtained with the direct enzyme addition (**Figure 3**), again the results were less evident than when the enzymes were used in the tempering solution.

Rouau et al. (*37*) and Krishnarau and Hoseney (*12*) also reported that the addition of hemicellulases during mixing decreased crumb firmness, probably by increasing loaf volume, although Gil et al. (*4*) reported that the addition of pentosanase had no detectable effect on the firmness.

The results show the improvement of fresh bread quality promoted by the presence of carbohydrase activities; in all cases the carbohydrases tested led to better bread shape, an increase of the bread specific volume, and a reduced crumb firmness, xylanase showing the most positive effects. The results were more pronounced than the ones obtained by the direct addition of the enzyme to the flour, which might be explained because in the enzyme-treated flour during tempering the overall effect should be due to the enzyme activities and the hydrolysis products present in the resulting flour.

Conclusions. Addition of enzymes that degrade cell walls, such as cellulase, xylanase, and β-glucanase, to the tempering solution may provide a new technique to improve the rheological and baking characteristics of flour. Diffusion of enzymes through the kernel during tempering, into the endosperm, is revealed as an excellent tool for obtaining flours of specific characteristics according to their final use.

Addition of carbohydrases into the tempering solution modifies the rheological characteristics of flours and improves the dough proofing and the quality of the resulting fresh bread. The results obtained by this method are similar to the ones obtained by adding these enzymes with the other ingredients during mixing, but in the enzyme-treated wheat through tempering the distribution and overdosage problems are overcome.

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