JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 4673−4679 **4673**

Effect of Pentosanase and Oxidases on the Characteristics of Doughs and the Glutenin Macropolymer (GMP)

C. PRIMO-MARTÍN, R. VALERA, AND M. A. MARTÍNEZ-ANAYA*

Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Pol. La Coma s/n, 46980 Paterna, Valencia, Spain

Rheological characteristics of dough and glutenin macropolymer (GMP) extracted thereof were investigated. Three single enzymes, pentosanase (PP), glucoseoxidase (GLZ), and laccase (LAC), and their combinations were used. GLZ gave the least extensible and most resistant dough, and pentosanase/glucoseoxidase (PPGLZ) resulted in dough with improved extensibility. The enzymes improved gluten quality. The glutenin macropolymer (GMP) was characterized in terms of wet weight, protein content, pentosan association, and dynamic rheological properties. Enzymatic addition decreased the wet weight of GMP but increased the protein content. PP decreased the content of pentosans on the GMP, but single oxidases increased the content of pentosans associated with GMP. PP did not modify the elastic modulus (G') of the GMP, whereas GLZ increased G' by increasing the polymerization of proteins and LAC diminished G'. The combination PPGLZ produced a synergic increase of G'.

KEYWORDS: Pentosans; pentosanase; glucoseoxidase; laccase; rheology; glutenin macropolymer

INTRODUCTION

Gluten proteins, responsible for the unique viscoelastic properties of wheat, have been extensively researched, the quantity and quality of proteins being strongly related to the bread-making quality of flour (*1*). The glutenin subfraction of flour unextractable in sodium dodecyl sulfate (SDS) is called the glutenin macropolymer (GMP). GMP is highly related to quality parameters, such as loaf volume and physical dough properties (*2*, *3*). GMP studies have shown its evolution during bread-making (*4*−*6*). During mixing GMP is partly depolymerized, rendering more glutenin extractable in SDS, whereas during resting these extractable polymers repolymerize (*4*, *7*, *8*), thus increasing the GMP fraction. The mixing process (*9*) leads to the formation of a gluten matrix and the disaggregation of the glutenin macropolymer, probably due to shear stress and oxidoreduction reactions.

The existence of associations between wheat pentosans and gluten have been evidenced in different studies (*8*, *10*−*13*), although the possible existence of covalent bonding remains undetermined. It has been suggested that mixing induces cross-linking between feruloylated arabinoxylans and side chains of amino acids (*14*−*16*); however, studies in model solutions of arabinoxylans (AX), using laccase or manganese peroxidase, have not detected the formation of such a covalent bonding (*9*, *17*−*18*). Some authors (*12*, *19*) reported that most of the polysaccharide associated with gluten is simply entrapped in the gluten network. Recently, the existence of cross-linking between AX and proteins has been shown (*20*), but there is controversy about the possibility of glycosylation of the glutenin (*11*, *21*−*23*) and the nonglycoproteic nature of the high molecular weight glutenin subunits (*24*, *25*).

Pentosans associated with wheat gluten could influence dough-handling properties, hindering gluten aggregation due to steric hindrance (*26*). Thus, the use of pentosanases would improve gluten quality by counteracting the chemical aggregation of gluten (*27*).

Oxidative enzymes such as glucoseoxidase (*28*−*30*) and laccase (*30*, *31*) are increasingly used in bread-making. Glucoseoxidase catalyzes the oxidation of β-D-glucose to D-gluconic acid and H_2O_2. The mechanism of bread improvement has not been fully established, although the liberated H_2O_2 has been hypothesized to be responsible for this improvement (*28*, *32*−*33*). Laccase catalyzes the conversion of benzenodiols to benzenoquinones. In wheat flour, laccase catalyzes the oxidative gelation of feruloylated arabinoxylans by dimerization to their ferulic esters (*17*), and when it is added to dough, it can besides exert an oxidant effect on dough constituents and improve the resistance of the gluten network (*31*).

Dynamic rheological experiments generate information about the viscoelastic properties of dough. Large deformation rheological tests were performed on dough, as the stress applied by these methods is comparable to stresses experienced during dough mixing and proofing (*34*). Small deformation rheology can be related to molecular weight distribution (*35*, *36*). The nonlinear behavior of starch masks the effect of small deformation assays on gluten proteins (*37*); thus, in this study small deformation tests will be done directly over GMP.

The aim of this study is to evaluate changes in the quantity, quality, and viscoelastic properties of the glutenin macropolymer

* Address correspondence to this author at P.O. Box 73, 46100 Burjasot, Valencia, Spain (telephone 34 6 390 00 22; fax 34 6 363 63 01; e-mail manaya@iata.csic.es).

10.1021/jf0257695 CCC: $25.00
Published on Web 07/08/2003

Table 1. Characteristics of Wheat Flour

	flour
chemical composition	
protein (% dw[a])	14.94
ash (% dw)	0.60
moisture (% dw)	15.27
farinogram	
water absorption (%)	54.5
alveogram	
deformation energy (J)	389 E–4
curve deformation ratio P/L	1.48
gluten index	
gluten index (%)	99.6
dry gluten (%)	10.2
wet gluten (%)	29.8
falling number	
time (s)	528.6

[a] Dry weight.

Table 2. Sample Design[a]

	enzyme		
	Pentopan Mono BG	Gluzyme	laccase
code	PP	GLZ	LAC
control	0	0	0
PP	1	0	0
PPGLZ	1	1	0
PPLAC	1	0	1
PGL	1	1	1
GLZ	0	1	0
LAC	0	0	1

[a] 0, presence; 1, absence.

caused by the addition of enzymes—pentosanase, glucoseoxidase, laccase, and their combinations—and determine possible associations with pentosans. Dough characteristics and their relationships with GMP will also be reported.

MATERIALS AND METHODS

Materials. Wheat (Farak variety from Spain) was milled on a CD1 Chopin mill to an extraction rate of 68%. ICC standard methods (*38*, *39*) were used to determine flour characteristics (**Table 1**).

Three commercial enzymes were used: a pentosanase (endo-1,4-β-xylanase) [Pentopan Mono, BG (PP)] containing 2500 fungal xylanase units/g (equivalent to 3341 units/g enzyme, following the Megazyme endo-xylanase assay procedure, XYL9/95); a glucoseoxidase [Gluzyme (GLZ)] containing 500 glucoseoxidase units/g; and a laccase (LAC) containing 1552 laccase units/mL, all of them manufactured by Novo Nordisk. These enzymes did not contain significant side activities, like protease (universal protease substrate, Boehringer Mannheim) or amylase (Ceralpha method, Megazyme) (*40*), and they were used individually and in 1:1 mixes. Selected dosages of the enzymes were, following the supplier's recommendations, 4 mg/100 g, 2 mg/100 g, and 50 μL/100 g of flour, respectively, which led to beneficial effects on technological properties (*30*).

All chemicals used for analyses were of analytical grade.

Methods. *Dough Preparation.* Doughs were made in a Brabender farinograph using the 50 g mixing bowl. Dough formulation, based on 100 g of flour, included 54.5 mL of water, 2 g of salt, and the amount of enzyme indicated in the previous section. To samples containing glucoseoxidase was added glucose (0.5 g) as well. Doughs were mixed for 10 min and allowed to rest for 60 min (rested dough) at 30 °C. Six doughs containing enzymes and a control dough without enzymes were prepared (**Table 2**).

Kieffer Microextensibility Test. The Kieffer dough extensibility test was carried out using a Texture Analyser TA-XT2i with the Kieffer dough and gluten extensibility rig (Stable Microsystems) and a 25 kg load cell. Microextension tests were performed on dough following the methodology developed by Kieffer and others (*37*). Maximum resistance (R_{MAX}) to the extension and extensibility at R_{MAX} (E at R_{MAX}) and at break point (E at break) were determined. Four extension tests were performed for each sample.

Gluten Index (GI). Fifteen grams of rested dough was used for the determination of gluten, by duplicate, in a Glutomatic system (Perten) following the ICC 155 standard procedure (*38*).

GMP Characterization: (a) Extraction. GMP was obtained following the procedure reported by Graveland and others (*41*). Suspensions of freeze-dried doughs (1:20 w/v) in 1.5% SDS were centrifuged during 30 min at 75000*g* and 20 °C in a Kontron ultracentrifuge. After the supernatant had been decanted, the GMP was obtained as the gel-like layer on top of the starch.

(b) Determination of Wet Weight. GMP wet weight (grams of GMP per gram of freeze-dried dough) was measured as the difference in weight before and after removal of the gel layer by chemical reduction with Na_2SO_3 (0.01 g).

(c) Protein Content of GMP. The whole residue, gel layer plus starch, obtained from the GMP extraction was freeze-dried, and protein content was determined with the ICC standard Kjeldahl method 105/2 (N × 5.7) (*39*).

(d) Total and Water-Soluble Pentosans Associated with GMP. Quantification of total pentosans (TP) and water-soluble pentosans (WSP) from GMP was performed following the orcinol−hydrochloric acid−ferric method as described previously (*42*, *43*) with the modifications made by Jiménez and Martínez-Anaya (*44*, *45*). Freeze-dried GMP (125 and 100 mg, respectively) was used for TP and WSP quantification.

(e) Dynamic Rheological Properties of GMP. The dynamic rheological properties of GMP were characterized in a Bohlin VOR rheometer (Germany) following the methodology described previously (*46*) and modified by Don and others (*47*). A plate−plate geometry (PP30) and a torsion bar of 1.7 g·cm were used. The GMP was scraped off from the top of the gel, and 1 g was carefully transferred into the cell with caution so as not to introduce interfering starch. Measurements were done with a gap of 1 mm at 20 °C. A strain sweep measurement was done at 0.15 Hz from 0.002 to 0.209 strain (1−100% strain amplitude). An oscillatory test with a frequency sweep from 0.05 to 5 Hz with a fixed strain (2% strain, 9% amplitude), selected from the linear viscoelastic region obtained from the strain sweep, was performed.

The dynamic rheological properties of a material are determined by the storage modulus G' (elastic modulus) and the loss modulus G'' (viscous modulus); from both moduli the complex modulus G^* [$G^* = (G''^2 + G'^2)^{1/2}$] and the phase angle δ ($\tan \delta = G''/G'$) are derived (*48*). The phase angle, δ, is the ratio of elastic to viscous behavior and varies between 0 and 90°, a phase angle of 0° indicating a completely elastic behavior (solid) and a phase angle of 90° indicating a completely viscous behavior (liquid). The complex modulus represents the stiffness of a sample (*48*) or total energy to induce changes.

Statistical Analysis. Univariate analysis of variance (ANOVA) and multivariate factorial analysis were performed to study possible interactions between processing factors using the bio-medical package (BMDP) (2V, 7D, and 4M programs).

RESULTS AND DISCUSSION

Microextensibility Test. The extensibility of doughs, at maximum resistance and at break point, in the Kieffer microextensibility test on rested dough was increased (6.35%) by the addition of pentosanase (**Table 3**). Pentosanase modified the amount and composition (structure) of pentosans (*30*) associated with gluten, producing a dough of greater extensibility and lower resistance to extension. On the other hand, glucoseoxidase strengthened the gluten network, causing stiffer and less extensible doughs (41.85%). Laccase produced a smaller diminution of extensibility without significant changes in resistance to extension.

Table 3. Characterization of Dough Properties[a]

	R_{MAX} (g)	E at R_{MAX} (mm)	E at break (mm)
control	49.10 ± 4.00	92.68 ± 0.50	100.46 ± 0.99
PP	46.74 ± 4.30	98.57 ± 5.00	109.96 ± 5.84
PPGLZ	37.66 ± 4.95*	58.87 ± 4.14**	74.61 ± 3.57**
PPLAC	46.21 ± 1.26	81.90 ± 3.12	95.66 ± 5.64
PGL	35.40 ± 2.58**	68.77 ± 1.18**	81.33 ± 2.13**
GLZ	67.64 ± 2.98**	53.89 ± 1.10**	65.28 ± 3.11**
LAC	51.94 ± 4.25	83.92 ± 4.73	94.42 ± 3.87

[a] R_{MAX} = maximum resistance to the extension. E at R_{MAX} = extensibility at R_{MAX}. E at break = extensibility at break. Data represent means ± standard deviation. *, **, statistical significance of differences with respect to control within each column at 0.01 and 0.05% level, respectively. See **Table 2** for identification of samples.

Table 4. Effect of Enzymatic Addition on Gluten Index[a]

sample	GI (%)	sample	GI (%)
control	91.39	PGL	99.34**
PP	97.17**	GLZ	98.46**
PPGLZ	99.04**	LAC	96.11**
PPLAC	97.28**		

[a] *,**, statistical significance of differences with respect to control at 0.01 and 0.05% level, respectively. See **Table 2** for identification of samples.

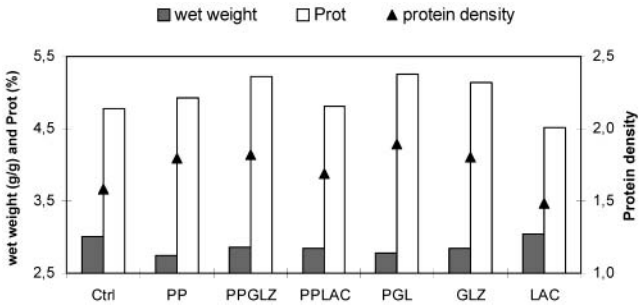

Figure 1. Effect of enzyme addition on wet weight, protein (prot), and protein density of GMP. See **Table 2** for identification of samples.

Pentosanase plus glucoseoxidase led to a more equilibrated extensibility than glucoseoxidase alone. Pentosanase palliated the strong effect of glucoseoxidasee; thus, PPGLZ and PGL presented greater extensibility and were less resistant to extension than GLZ alone. The addition of GLZ led in all cases to a dough significantly different from the control (**Table 3**).

Gluten Index. The GI as determined in the Glutomatic system (*49*) gives a measure of gluten protein quality. The presence of protein subunits of high molecular weight leads to a better quality of the gluten network and as a consequence a higher GI. The enzymatic addition significantly improved gluten quality (5.17−10.76%) (**Table 4**). Pentosanase modified the pentosans associated with gluten, producing a greater quality gluten network probably by interfering in protein−pentosan interaction. On the other hand, glucoseoxidase caused an increase in GI by strengthening the gluten (*33*). Laccase and all enzymatic combinations also improved the GI.

GMP Characterization. The content of SDS-unextractable protein expressed in wet weight (grams of GMP per gram of freeze-dried dough) diminished after enzymatic addition (from 3.02 to 2.74 g/g after enzymatic treatment), with the exception of laccase (**Figure 1**). The protein content of GMP followed the opposite tendency. Pentosanase produced an increase of protein content by releasing pentosans associated with proteins, which might interfere with protein aggregation. On the other hand, glucoseoxidase raised the protein content of GMP. Consequently, the GMP obtained from PPGLZ and PGL presented a larger increase of the protein content by combining both different enzymatic actions. Laccase, leading to the polymerization of the pentosans, would favor the interference of pentosans in the aggregation of the glutenins, thus resulting in a lower protein content. However, it has been also shown that laccase promotes disulfide formation by radical transference from phenoxyl radicals to sulfhydryl groups (*50*). Because effects of both oxidative enzymes are a collateral result of their catalytic activity on different substrates, it is difficult to compare them on a common dosage basis, which, on the other hand, was chosen because of their beneficial effects on bread quality (*30*).

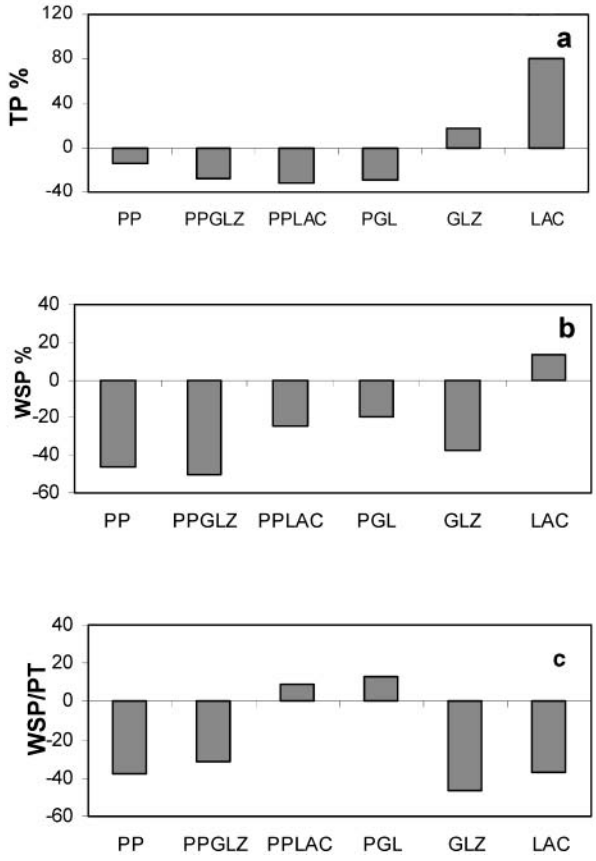

Figure 2. Effect of enzyme addition on the amount of pentosans associated with GMP (percent of variation with respect to control): (a) TP; (b) WSP; (c) WSP/TP ratio. See **Table 2** for identification of samples.

Protein density, defined as the ratio of protein content to wet weight, gives a measure of GMP quality; the enzymatic addition resulted in a GMP of improved protein quality, producing a more consistent gel, with the exception of laccase. The combinations of PPGLZ and PGL produced the greatest improvement.

Endo-xylanases rend more SDS-unextractable protein during fermentation than control doughs (*6*), confirming that AX may play a role in changes in extractability of the glutenin polymers. Breakdown of AX would lead to a less viscous dough, thus increasing the mobility of protein fragments or facilitating hydrophobic interactions between them (*6*). In both cases a faster aggregation of proteins, which may be also favored by removing the steric hindrance of AX (*6*, *12*, *51*), would take place.

Total Pentosans and Water-Soluble Pentosans Associated with GMP. With the addition of pentosanase alone or combined (**Figure 2a**) the amount of total pentosan on GMP samples was found to diminish [from 2.52 to 3.16% dry weight (dw) after enzyme treatment] as compared to the control (3.66% dw).

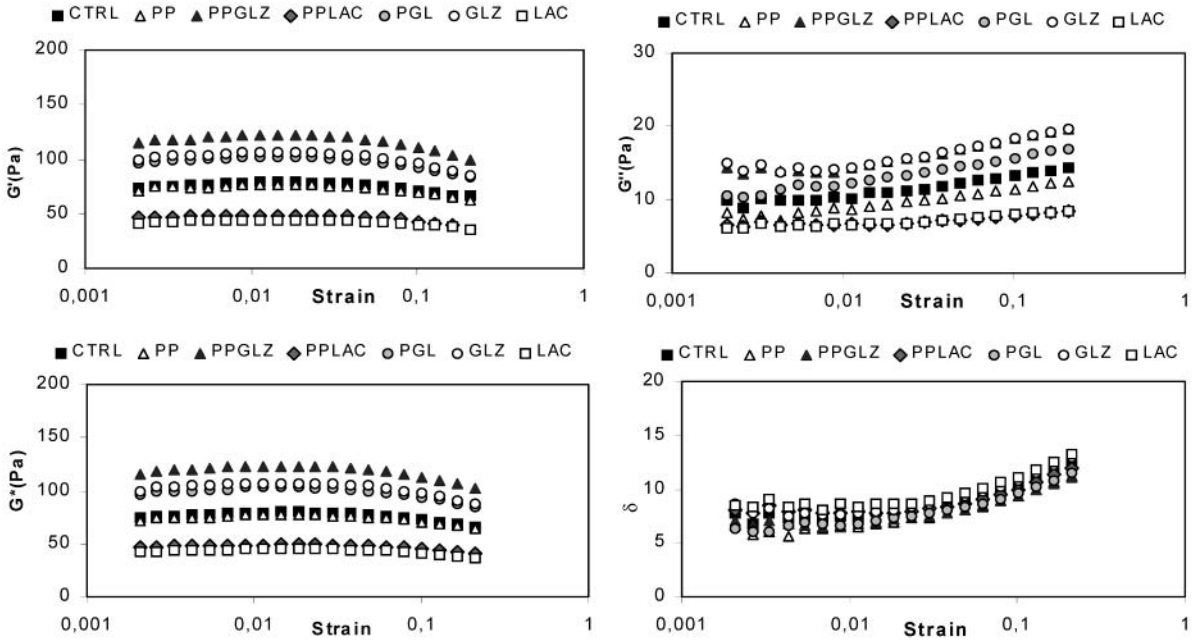

Figure 3. Changes in rheological moduli G', G'', and G^* and phase angle (δ) for control and enzyme-formulated doughs in the strain sweep (0.15 Hz). See **Table 2** for identification of samples.

The diminution of the pentoses associated with GMP by the action of cellulases and hemicellulases is strongly dependent on flour quality, the effect being more pronounced for weaker flours (*48*). Weegels et al. (*48*) suggested that the enzymes improved the coagulation of gluten by reduction of the steric hindrance of the pentosans associated with gluten. Hamer and Lichtendonk (*8*) have also hypothesized that the association of pentosans with glutenin polymers negatively affects dough properties. Pentosanases would eliminate the pentosans from the gluten matrix (*52*), improving dough properties.

Extraction of GMP from dough formulated with either glucoseoxidase or laccase alone resulted in the largest amount of total pentosan associated with GMP. This indicated a possible increase of the protein−pentosan interaction and/or the formation of pentosan fractions of higher molecular weight, which would further interfere with the aggregation of the protein network. Some studies showed that oxidants not only acted on glutenin protein but also induced the incorporation of pentosans into the insoluble glutenin protein matrix (*8*).

The unextractable fraction in SDS of gluten protein contained a higher amount of carbohydrates than the extractable fraction (*21*), indicating a possible association of the carbohydrates with the protein fractions of greater molecular weight or the presence of trapped long-chain polysaccharides that were difficult to dissolve. The use of SDS as a solvent allows the dissociation of proteins organized by non-covalent bonds (*21*); thus, the possible glycosidic bonds between protein and carbohydrates would not dissociate.

The presence in gluten of both water-extractable and water-unextractable arabinoxylans (*20*) as well as the similarity between pentosans associated with gluten and the fraction of pentosans extractable in water (*10*), has been reported. Thus, the content of the water-soluble pentosans in the GMP was determined. In control GMP the level of WSP was 1.28% (dw), this value decreasing (**Figure 2b**) when pentosanase along or combined was used (from 0.63 to 1.03% dw). Glucoseoxidase produced a diminution of the WSP, probably because its action on pentosans rends them insoluble in water. Laccase favored the presence of WSP associated with GMP, but it is possible that the rise in total pentosan content might be responsible for this increase in WSP.

Figure 2c shows the variation of WSP/TP ratio due to the enzymatic treatment. The proportion of the water-soluble pentosan increased with PPLAC and PGL and decreased with the other samples.

Dynamic Rheological Properties of GMP. The determination of a region of linear behavior for the realization of dynamic rheological measurements was done with a strain sweep at a fixed frequency of 0.15 Hz. In these conditions samples behave as three-dimensional structures in which the measurements suppose the application of nondestructive forces (*53*). The linearity range for G' and G'' within a deformation interval of strain between 0.002 and 0.209 is shown in **Figure 3**. G' was higher than G'' throughout the region of linearity. For strain values >0.037, a deviation from linearity was observed. Thus, for the frequency sweep in the oscillatory mode a strain of 2% in the linear region was used. In these conditions, an increase of G' and G^* was observed as the frequency increased (**Figure 4**). The viscous modulus and delta angle decreased from 0.05 to 0.3 Hz and then increased. Addition of pentosanase did not modify the elastic modulus with respect to control GMP. Laccase alone and combined with the pentosanase decreased both moduli, G' and G'', indicating a weakening of the gel.

Glucoseoxidase caused an increase of the elastic modulus, and a synergic effect was observed in the combination with PP. The combination PGL did not result in as strong a gel as PPGLZ because of the effect of the laccase, which induces pentosan−pentosan cross-linking, causing a gel with a G' similar to that found for the addition of GLZ alone. The phase angle did not suffer large variations with respect to the control due to the predominant elastic characteristics of the gels.

Glucoseoxidase has been reported to produce an increase in G' and a decrease in tan δ of gluten, suggesting an increase of the size of the glutenin molecules or of the number of linkages per molecule (*54*), in both cases resulting in greater elasticity.

The loss modulus G'' (**Figure 4**) was always lower than G', showing a predominant elastic behavior, and followed similar trends after enzyme treatment.

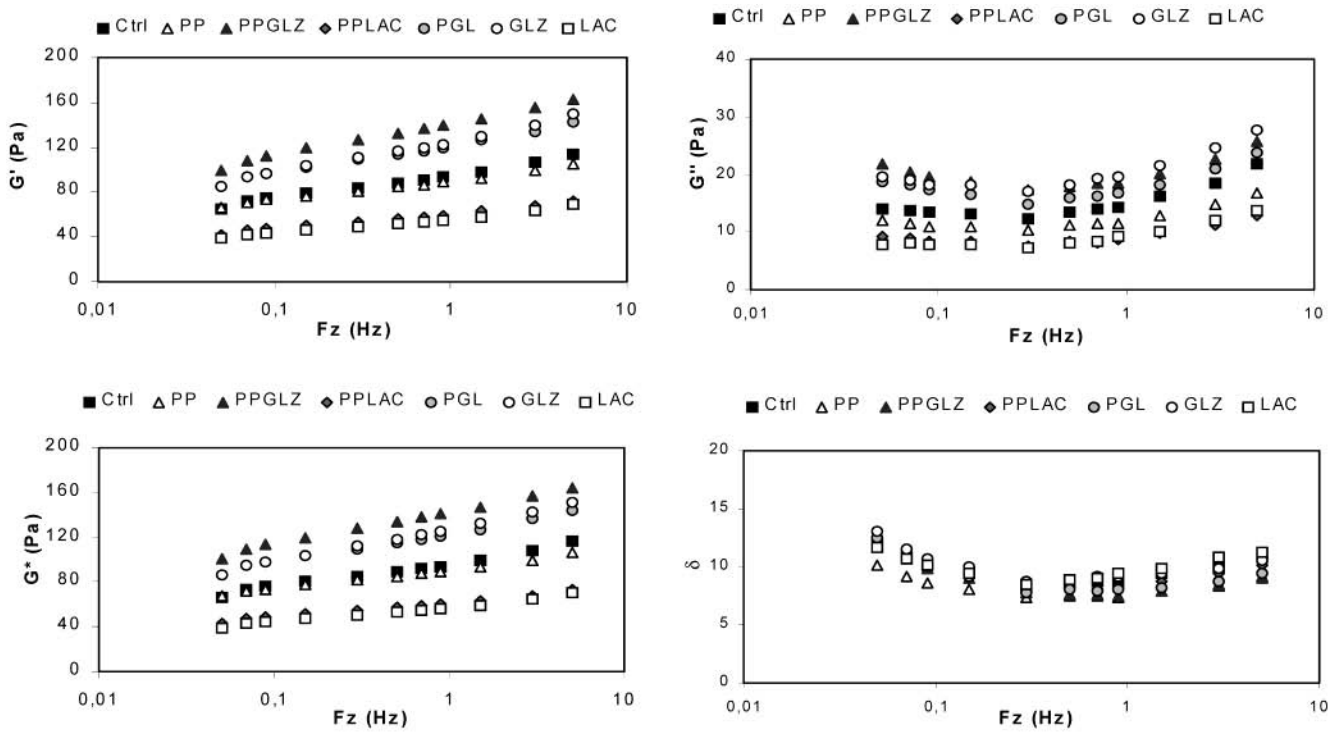

Figure 4. Changes in rheological moduli G', G'', and G^* and phase angle (δ) for control and enzyme formulated dough in the frequency sweep (2% strain). See **Table 2** for identification of samples.

Table 5. Corrrelation Matrix between Variables Associated with Dough Properties and GMP Properties (Coeficient of Correlation above 0.5)

	dynamic rheology of GMP				GMP characterization			pentosans associated with GMP			Kieffer parameters		
	G'	G''	G^*	δ	prot	wet wt (g/g)	protein density	TP	WSP	WSP/TP	R_{MAX}	E at R_{MAX}	E at break
G'	1												
G''	0.9795	1											
G^*	0.9999	0.9797	1										
δ				1									
prot	0.9131	0.8848	0.9155		1								
wet wt (g/g)				0.5224	–0.6900	1							
protein density	0.7678	0.7041	0.7700		0.9444	–0.8889	1						
TP					–0.6617	0.6983	–0.7249	1					
WSP	–0.6238	–0.5340	–0.6178		–0.7172	0.7968	–0.8048	0.6520	1				
WSP/TP								–0.5176		1			
R_{MAX}				0.5580				0.5457		–0.5601	1		
E at R_{MAX}	–0.6815	–0.7717	–0.6809	–0.5672	–0.6858							1	
E at break	–0.6751	–0.7824	–0.6749	–0.6461	–0.6529							0.9910	1
GI	0.8916	0.8817	0.8955		0.9782	–0.5792	0.8812	–0.6290	–0.5932			–0.7466	–0.7147

Values obtained from both tests, strain sweep at 2% strain (0.15 Hz) and frequency sweep at 0.15 Hz (2% strain), were very close. Thus, due to the great number of results generated from these measurements, data from the frequency sweep at 0.15 Hz were taken for statistical analysis.

Relationship between Variables Associated with Dough Properties and Variables Related to GMP. A multifactorial (4M) analysis of variables associated with dough and GMP was performed to study how GMP properties could affect dough properties.

Dynamic moduli G', G'', and G^* presented a large intercorrelation (**Table 5**). They were also significantly correlated with parameters relating to protein quality. The content of TP and WSP was negatively correlated with gluten index, protein content, and protein density of GMP and positively correlated with wet weight of the GMP. TP was also directly related to R_{MAX} of doughs, and WSP was inversely related to the dynamic moduli.

These relationships confirmed that a high content of pentosan interferes in glutenin aggregation, causing, as a consequence, a GMP with less protein content. In the same way, a higher protein content of the GMP and higher G' values indicate an increase of the size of the glutenin polymers or an increase of the number of linkages within polymers, corresponding with a less extensible dough.

A joint analysis of the variables resulted in the grouping of the samples into three factors that explained 100% of the variability of the results. The first factor [62% of variance explained (VE)] was positively related to the gluten index, dynamic moduli, and percentage of protein in GMP and negatively related to E at R_{MAX} and E at break. The second factor (26% VE) was positively influenced by the wet weight of the GMP, phase angle, and content of WSP associated with GMP. The third factor (12% VE) was directly related to the WSP/PT ratio and indirectly to the content of TP and R_{MAX}.

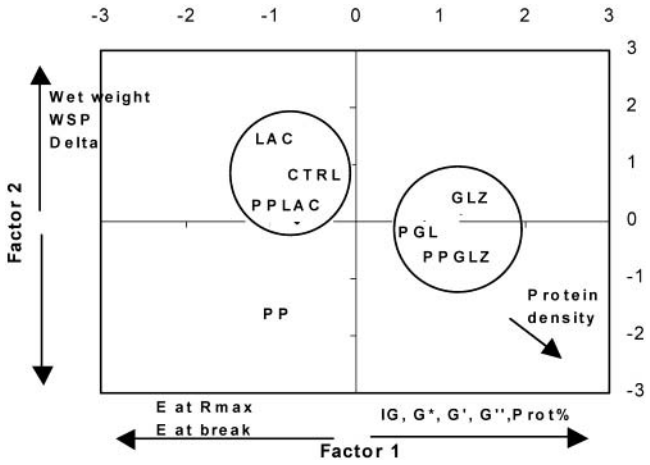

Figure 5. Multivariate factorial analysis of variables associated with dough and GMP properties as shown by distribution of samples in the planes defined by the two main factors and tendencies of the analytical variables. See **Table 2** for identification of samples.

The two first factors grouped the samples in two main groups (**Figure 5**). The first group contained the control and samples containing laccase alone and combined with the pentosanase, separated because the higher values of the variables defining the second factor and the variables defining negatively the first factor. The presence of GLZ determined a second group characterized by less extensible doughs and larger values of variables defining positively the first factor. PP was separated because it caused the most extensible dough with a lower phase angle.

ABBREVIATIONS USED

AX, arabinoxylans; dw, dry weight; *E* at R_{MAX}, extensibility at maximum resistance; *E* at break, extensibility at break point; GI, gluten index; GMP, glutenin macropolymer; δ, phase angle; G', elastic modulus; G'', viscous modulus; G^*, complex modulus; GLZ, glucoseoxidase; LAC, laccase; PP, pentosanase; PPGLZ, pentosanase/glucoseoxidase; PPLAC, pentosanase/laccase; PGL, pentosanase/glucoseoxidase/laccase; PT, total pentosans; prot, protein percentage; protein density, protein content of GMP/wet weight; R_{MAX}, maximum resistance; SDS, sodium dodecyl sulfate; VE, variance explained; WSP, water soluble pentosans; wet weight, grams of GMP per gram of freeze-dried dough.

ACKNOWLEDGMENT

We thank TNO (Zeist, The Netherlands) for allowing us to perform the rheology experiments with the Bohlin VOR rheometer and especially Ing. Wim Lichtendonk for technical assistance.

LITERATURE CITED

(1) Finney, K. F.; Barmore, M. A. Loaf volume and protein content of hard winter and spring wheats. *Cereal Chem*. **1948**, *25*, 291−312.

(2) Schofield, J. D. Wheat proteins: structure and functionality in milling and breadmaking. In *Wheat: Production, Composition and Utilization*, 1st ed.; Bushuk, W., Rasper, V., Eds.; Blackie Academic and Professional: Glasgow, Scotland, 1994; pp 73−106.

(3) Weegels, P. L.; Hamer, R. J.; Schofield, J. D. Functional properties of wheat glutenin. *J. Cereal Sci*. **1996**, *23*, 1−17.

(4) Weegels, P. L.; Hamer, R. J.; Schofield, J. D. Depolymerisation and repolymerisation of wheat glutenin during dough processing. II. Changes in composition. *J. Cereal Sci*. **1997**, *25*, 155−163.

(5) Weegels, P. L.; Hamer, R. J.; Schofield, J. D. Functional properties of low Mr wheat proteins. III. Effects on composition of the glutenin macropolymer during dough mixing and resting. *J. Cereal Sci*. **1997**, *25*, 165−173.

(6) Veraverbeke, W. S.; Courtin, C. M.; Verbruggen, I. M.; Delcour, J. A. Factors governing levels and composition of the sodium dodecyl sulphate-unextractable glutenin polymers during straight dough breadmaking. *J. Cereal Sci*. **1999**, *29*, 129−138.

(7) Graveland, A.; Bosveld, P.; Lichtendonk, W. J.; Moonen, J. H. E. Superoxide involvement in the reduction of disulphide bonds of wheat gel proteins. *Biochem. Biophys. Res. Commun.* **1980**, *93*, 1189−1195.

(8) Hamer, R. J.; Lichtendonk, W. J. Structure−function studies on gluten proteins: reassembly of glutenin proteins after mixing. In *Proceedings of the Third International Workshop on Gluten Proteins*; Lasztity, R., Bekes, F., Eds.; World Scientific: Singapore, 1987; pp 227−237.

(9) Labat, E.; Morel, M. H.; Rouau, X. Effect of laccase and manganese peroxidase on wheat gluten and pentosans during mixing. *Food Hydrocolloids* **2001**, *15*, 45−52.

(10) D'Appolonia, B. L.; Gilles, K. A. Pentosans associated with gluten. *Cereal Chem*. **1971**, *48*, 427−436.

(11) Chen, J.; Shelton, D. R.; D'Appolonia, B. L.; Khan, K. Structural characterization of the carbohydrate portion of a glycopeptide from wheat gluten. *Cereal Chem*. **1992**, *69*, 481−484.

(12) Roels, S. P.; Grobet, P. J.; Delcour, J. A. Distribution of carbohydrates in gluten fractions isolated from European wheats (*Triticum aestivum* L.) in a batter system. *J. Agric. Food Chem*. **1998**, *46*, 1334−1343.

(13) Gujska, E.; Khan, K. Characterization of the carbohydrates of nonreduced glutenin fractionated by multistacking SDS-PAGE from two hard red spring wheat flours. *Cereal Chem*. **1999**, *76*, 198−203.

(14) Neukom, H.; Markwalder, H. U. Oxidative gelation of wheat flour pentosans. A new way of cross-linking polymers. *Cereal Foods World* **1978**, *23*, 374−394.

(15) Meuser, F.; Suckow, P. Non-starch polysaccharides. In *Chemistry and Physics of Baking*; Blanshard, J. M. V., Frazier, P. J., Gaillard, T., Eds.; The Royal Society of Chemistry: London, U.K., 1986; pp 42−61.

(16) Moore, A. M.; Martínez-Muñoz, I.; Hoseney, R. C. Factors affecting the oxidative gelation of wheat water-solubles. *Cereal Chem.* **1990**, *67*, 81−84.

(17) Figueroa-Espinoza, M. C.; Rouau, X. Oxidative cross-linking of pentosans by a fungal laccase and horseradish peroxidase. Mechanism of linkage between feruloylated arabinoxylans. *Cereal Chem*. **1998**, *75*, 259−265.

(18) Figueroa-Espinoza, M. C.; More, M. H.; Suret, A.; Asther, M.; Moukha, S.; Sigoillot, J. C.; Rouau, X. Attempt to cross-link feruloylated arabinoxylans and proteins with a fungal lacasse. *Food Hydrocolloids* **1999**, *13*, 65−71.

(19) Saulnier, L.; Andersson, R.; Aman, R. A study of the polysaccharide components in Gluten. *J. Cereal Sci*. **1997**, *25*, 121−127.

(20) Oudgenoeg, G.; Hilhorst, R.; Piersma, S. R.; Boeriu, C. G.; Gruppen, H.; Hessing, M.; Voragen, A. G.; Laane, C. Peroxidase-mediated cross-linking of a tyrosine containing peptide with ferulic acid. *J. Agric. Food Chem*. **2001**, *49*, 2503−2510.

(21) Chen, J.; Khan, K.; Shelton, D. R.; D'Appolonia, B. L. Isolation and fractionation of carbohydrate-containing proteins from wheat gluten. *Cereal Chem*. **1992**, *69*, 475−480.

(22) Tilley, K. A.; Schofield, J. D. Deglycosylation of the high molecular weight glutenin subunits by β-*N*-acetylhexosaminidase and β-elimination reaction. Evidence for *O*-glycosylation. *Proceedings of the Wheat Kernel Proteins Conference*; S. Martino al Cimino: Viterbo, Italy, 1994.

(23) Tilley, K. A.; Lookhart, G. L.; Hoseney, R. C.; Mawhinney, T. P. Evidence for glsocylation of the high molecular glutenin subunits 2, 7, 8 and 12 from Chinese Spring and TAM 105 wheat. *Cereal Chem*. **1993**, *70*, 602−606.
(24) Roels, S. P.; Delcour, J. A. Evidence for the non-glycoprotein nature of high molecular weight glutenin subunits of wheat. *J. Cereal Sci.* **1996**, *24*, 227−239.
(25) Roels, S. P.; Delcour, J. A. The non-glycoprotein nature of high-molecular-weight glutenin subunits of wheat. In *Proceedings of the 6th International Gluten Workshop*; Wrighley, C. W., Ed.; RACI: Melbourne, Australia, 1996; pp 149−153.
(26) Oort van, M.; Straaten, F.; Laane, C. Pentosans and pentosanases in bread making. *Int. Food Ingredients* **1995**, *2*, 23−27.
(27) Weegels, P. L.; Hamer, R. J. Improving the bread-making quality of gluten. *Cereal Foods World* **1992**, 379−385.
(28) Vemulapalli, V.; Miller, K. A.; Hoseney, R. C. Glucose oxidase in breadmaking systems. *Cereal Chem*. **1998**, *75*, 439−442.
(29) Martínez-Anaya, M. A.; Jiménez, T. Physical properties of enzyme-supplemented doughs and relationship with bread quality parameters. *Z. Lebensm. Unters. Forsch. A* **1998**, *206*, 134−142.
(30) Primo-Martín, C.; Martínez-Anaya, M. A. Influence of pentosanase and oxidases on water-extractable pentosans during a straight breadmaking process. *J. Food Sci.* **2003**, *68*, 31−41.
(31) Si, J. Q. Use of lacasse in baking, Novo Nordisk, Denmark. Int. Patent Appl. PCT/DK94/00232, 1994.
(32) Vemulapalli, V.; Hoseney, R. C. Glucose oxidase effects on gluten and water solubles. *Cereal Chem*. **1994**, *75*, 859−862.
(33) Hilhorst, R.; Dunnewind, B.; Orsel, R.; Stegeman, P.; van Vliet, T.; Gruppen, H.; Schols, H. A. Baking performance, rheology, and chemical composition of wheat dough and gluten affected by xylanase and oxidative enzymes. *J. Food Sci.* **1999**, *64*, 808−813.
(34) Dobraszczyk, B. J. Development of a new dough inflation system to evaluate doughs. *Cereal Foods World* **1997**, *42*, 516−519.
(35) Tsiami, A. A.; Bot, A.; Agterof, W. G. M.; Groot, R. D. Rheological properties of glutenin subfractions in relation to their molecular weight. *J. Cereal Sci*. **1997**, *26*, 15−27.
(36) Tsiami, A. A.; Bot, A.; Agterof, W. G. M. Rheology of mixtures of glutenin subfractions. *J. Cereal Sci*. **1997**, *26*, 279−287.
(37) Kieffer, R.; Wieser, H.; Henderson, M. H.; Graveland, A. Correlations of the breadmaking performance of wheat flour with rheological measurements on a micro-scale. *J. Cereal Sci*. **1998**, *27*, 53−60.
(38) ICC (International Association for Cereal Chemistry). ICC Standard 155, 1994.
(39) ICC (International Association for Cereal Chemistry). Ash, 101/1; protein, 105/2; falling number, 107/1; farinograph (Brabender), 115/1; alveograph (Chopin), 121; 1995.
(40) McCleary, B. V.; Sheehan, H. Measurement of cereal α-amylase: A new assay procedure. *J. Cereal Sci.* **1987**, *6*, 237−251.
(41) Graveland, A.; Bongers, P.; Bosveld, P. Extraction and fractionation of wheat flour proteins. *J. Sci. Food Agric*. **1979**, *30*, 71−84.
(42) Hashimoto, S.; Shogren, M. D.; Pomeranz, Y. Cereal pentosans: their estimation and significance. I. Pentosans in wheat and milled wheat products. *Cereal Chem.* **1987**, *64*, 30−64.
(43) Delcour, J. A.; Vanhamel, S.; De Geest, C. Physicochemical and functional properties of rye nonstarch polysaccharides. I. Colorimetric analysis of pentosans and their relative monosaccharide composition in fractionated (milled) rye products. *Cereal Chem*. **1989**, *66*, 107−111.
(44) Jiménez, T.; Martínez-Anaya, M. A. Characterization of water soluble pentosans of enzyme supplemented dough and breads. *Food Sci. Technol. Int.* **2000**, *6*, 197−205.
(45) Jiménez, T.; Martínez-Anaya, M. A. Amylases and hemicellulases in breadmaking: degradation by-products and potential relationship with functionality. *Food Sci. Technol. Int*. **2001**, *7*, 5−14.
(46) Bekkers, A. C. A. P. A.; Lichtendonk, W. J.; Graveland, A.; Plijter, J. J. Mixing of wheat flour dough as a function of the physico-chemical properties of the SDS-gel proteins. In *Wheat Gluten—Proceedings of the 7th International Workshop on Gluten*; Shewry, P. R., Tatham, A. S., Eds.; Royal Society of Chemistry: Cambridge, 2000; pp 408−412.
(47) Don, J. A. C.; Lichtendonk, W. L.; Plijter, J. J.; Hamer, R. J. Glutenin macropolymer: a gel formed by particles. *J. Cereal Sci*. **2002**, *37*, 1−7.
(48) Weegels, P. L.; Orsel, R.; Van der Pijpekamp, A. M.; Lichtendonk, W. J.; Hamer, R. J.; Schofield, J. D. Functional properties of low Mr wheat proteins. II. Effects on dough properties. *J. Cereal Sci*. **1995**, *21*, 117−126.
(49) Ranhotra, G. S.; Gelroth, J. A.; Eisenbraun, G. J. Gluten index and bread-making quality of commercial dry glutens. *Cereal Foods World* **1992**, *37*, 261−263.
(50) Labat, E.; Morel, R. H.; Rouau, X. Effects of laccase and ferulic acid on wheat flour doughs. *Cereal Chem*. **2000**, 823−828.
(51) Weegels, P. L.; Marseille, J. P.; Voorpostel, A. M. P. Enzymes as a processing aid in the separation of wheat into starch and gluten. In *Gluten Proteins 1990*; Bushuk, W., Thachuk, R., Eds.; 1990; pp 199−203.
(52) Eijk, J. H.; Hille J. D. R. Chapter 4. Nonamylolytic enzymes. In *Baked Goods Freshness. Technology, Evaluation and Inhibition of Staling*; Hebeda, R. E., Zobel, H. R., Eds.; Dekker: New York, 1996; pp 131−150.
(53) Lindahl, L.; Eliasson, A. C. A comparison of some rheological properties of durum and wheat flour doughs. *Cereal Chem.* **1992**, *69*, 30−34.
(54) Skinner, C. V.; Tsiami, A. A.; Budolfsen, G.; Schofield, J. D. Effects of oxidoreductase enzymes on gluten rheology. In *Wheat Gluten—Proceedings of the 7th International Workshop on Gluten*; Shewry, P. R., Tatham, A. S., Eds.; Royal Society of Chemistry: Cambridge, 2000; pp 231−234.

Received for review June 27, 2002. Revised manuscript received May 7, 2003. Accepted June 5, 2003. We thank the Comisión Interministerial de Ciencia y Tecnología (CICYT, AGL2001-1273) and Consejo Superior de Investigaciones Científicas (CSIC) for financial support.

JF0257695