Eur Food Res Technol (2007) 224: 525–534
DOI 10.1007/s00217-006-0311-3

ORIGINAL PAPER

Pedro A. Caballero · Manuel Gómez · Cristina M. Rosell

Bread quality and dough rheology of enzyme-supplemented wheat flour

Received: 21 November 2005 / Revised: 26 January 2006 / Accepted: 28 February 2006 / Published online: 7 June 2006

Abstract The enzymatic treatment of wheat flours is an interesting alternative for improving their functional properties. Since enzymes with different biochemical activities could induce synergistic effects on dough behaviour or product quality, the individual and combined use of a wide range of enzymes (transglutaminase, glucose oxidase, laccase, α-amylase, pentosanase and protease) applied nowadays in bread-making processes were investigated. The blend of enzymes resulted in an improvement in the rheological behaviour of doughs and the quality of the final product. The simultaneous presence of transglutaminase (TG) and glucose oxidase (GO), as well as TG and protease (PROT) led to a synergistic effect on alveograph parameters. Polysaccharide-degrading enzymes exercised a significant effect on rheology only when used in combination with other enzymes, mainly affecting consistograph parameters. Analysis of bread-making data revealed significant interactions between TG and all the other enzymes except laccase (LAC). Significant synergistic effect on bread quality was observed by the combined use of GO and LAC, GO and pentosanase (PP), amylase (AMYL) and LAC, AMYL and PROT, and PP and PROT. Bread quality parameters showed greater correlations with alveograph parameters than with consistograph properties of dough. Tenacity (*P*) and extensibility (*L*) proved to be acceptable predictors of the height/width ratio of loaves. The duration of the alveograph test enhanced the prediction of bread quality parameters. Conversely, none of the rheological properties studied showed a high correlation with the specific volume of loaves.

Keywords Enzymes · Wheat flour · Dough rheology · Bread quality

P. A. Caballero · M. Gómez (✉)
Área de Tecnología de Alimentos, E.T.S. de Ingenierías Agrarias de Palencia,
Avda de Madrid 44, Palencia, 34004 Spain
e-mail: pallares@iaf.uva.es
Tel.: +34-979-108359
Fax: +34-979-108302

C. M. Rosell
Instituto de Agroquímica y Tecnología de Alimentos (CSIC),
P.O. Box 73, 46100-Burjassot, Valencia, Spain

M. Gómez
Escuela Técnica Superior de Ingenierías Agrarias, Universidad de Valladolid,
Avda Madrid 44, Palencia 34004, Spain

Introduction

In recent years, the baking industry has undergone very important changes in its productive processes. Some of the major changes have been brought about by an increasing mechanization in its processing unit operations. This fact has contributed to an increased demand for strong wheat flours, yielding doughs with high tolerance to handling and mixing, and able to remain stable during fermentation.

Functional properties of flours greatly depend on the gluten proteins. On the other hand, the quality of gluten is dependent on diverse factors such as wheat variety and growing conditions [1–3]. For this reason, the capacity of some countries to produce high-quality flours is limited. In this context, the treatment of flours with functional additives must be considered.

Chemical improvers have been used for decades in bread-making as a way of adjusting the variations in flour properties and baking conditions. Nowadays, the baking industry is deeply involved in research for alternatives to chemical compounds because of their potential hazards [4–7]. The enzymatic treatment of wheat flours is an interesting alternative to generate changes in the structure of the dough and in consequence, for improving functional properties of flours. They are generally recognized as safe (GRAS) and do not remain active in the final product after baking. Therefore, enzymes do not have to appear on the label, which is an additional commercial advantage.

The intentional inclusion of enzymes in bread formulas dates back to more than one century [8]. Today, a wide range of enzymes produced especially for bread-making is available for bakers. A variety of aims may be pursued by

enzyme addition, for example, to achieve a partial gluten hydrolysis for improving machinability, to obtain enough sugars for fermentation by means of starch hydrolysis, to attain a certain amount of lipid peroxidation for dough strengthening, or to reduce retrogradation and crumb firming through gelatinised starch hydrolysis.

Gluten cross-linking enzymes play an important role in current baking processes. Through different biochemical mechanisms (the oxidative coupling of thiol groups, the cross-link of tyrosine residues due to the action of intermediate reactive compounds such as hydrogen peroxide, the acyl-transfer reaction between amino acid residues), these enzymes promote the formation of covalent bonds between polypeptide chains within a protein or between different proteins, improving functional behaviour of dough during the bread-making process [9].

Transglutaminase (TG) (EC 2.3.2.13) is a transferase able to yield inter- and intramolecular ε-*N*-(γ-glutamyl)lysine cross-links [10]. Its addition causes structural changes in gluten proteins, being high molecular weight (HMW) glutenin subunits the most affected protein fraction [11–15]. TG may also lead to the formation of disulfide bridges by oxidation due to the proximity of sulphur containing amino acids [16]. Because of these effects, TG has been widely used to improve wheat dough functionality and bread quality [12, 15, 17–21]. The possibility of using this enzyme to alleviate some of the detrimental effects of frozen storage of puff pastry and croissants [22], as well as to solve the damage promoted by the insect attack of wheat [23–25] has been proposed. The results obtained with wheat flour have been also extrapolated to other cereals, allowing an improvement in the viscoelastic properties of the rice dough and therefore in the ability of rice flour to retain the carbon dioxide produced during proofing [26]. Recently, the possibility that TG in wheat-based baked products may generate the epitope associated with the coeliac response has been suggested [27], although there is no experimental evidence to support this postulate.

Glucose oxidase (EC 1.1.3.4) (GO) is an oxidative enzyme that catalyses the oxidation of β-D-glucose to δ-D-gluconolactona and hydrogen peroxide [28]. Disulfide bond interchange and the gelation of pentosans promoted by hydrogen peroxide action are the most widespread theories to explain the strengthening effect of the GO [15, 16, 29–34]. Furthermore, it has been related with the formation of non-disulfide covalent intermolecular bonds in the gluten proteins by GO treatment, either among glutenins [35, 36] or between albumins and globulins [37]. GO modifies the functional properties of dough, increasing its tenacity and elasticity [15, 38–41]. Gujral and Rosell [26] revealed even an increase in the elastic and viscous moduli of rice flour dough. As a result of such changes in dough behaviour, GO showed positive effects on bread quality, yielding improved specific volume, bread texture and crumb grain [26, 39, 42].

Through a similar oxidative mechanism, hexose oxidase (EC 1.1.3.5) (HO) has been also suggested as an efficient bread improver [43]. When this enzyme is added to dough model systems, it induces the formation of disulphide bridges between proteins and the gelation of pentosans, increasing dough strength and bread volume [44]. HO was found to be more effective than GO because of its ability for using several monosaccharides and oligosaccharides as substrates and its higher affinity for glucose.

Since Si [45] proposed laccase (LAC) (EC 1.10.3.2) as dough and bread improver as a result of its oxidant effect on dough constituents, numerous studies have been developed to analyse the effects and applications of this oxidoreductase. LAC is a type of polyphenol oxidase able to gel water soluble arabinoxylans by coupling feruloyl esters of adjacent chains into dehydrodimers [46]. The probable development of a protein–arabinoxylan network by LAC action has been hypothesized. Even though Figueroa-Espinoza et al. [47] and Labat et al. [48] have concluded that gluten and arabinoxylans form two distinct networks, Oudgenoeg et al. [49] proposed a mechanism by which tyrosine-containing proteins cross-link with arabinoxylans. Because of the simultaneous arabinoxylans gelation and oxidative action, LAC addition significantly improves gluten quality and leads to changes in the rheological properties of dough, slightly diminishing dough extensibility [34], increasing dough consistency [48], reducing time to maximum consistency and accelerating dough breakdown during mixing [50]. Improvement in the quality of bread elaborated with LAC has been also reported [51].

The functional properties of bread dough greatly depend on the proteins forming the gluten network. Strengthening enzymes affect different protein fractions (glutenins, gliadins, albumins or globulins) depending on their particular action mechanism. The type of protein being cross-linked appears to be more important than the cross-linking agent or type of cross-link formed and it is highly correlated with the character of qualitative changes in the final product. Thus, while HMW glutenin subunits are correlated with several macroscopic properties of dough and baked products (such as strength of gluten network and volume) [11, 17, 22], the albumins and globulins play an important role in textural and crumb grain properties [37]. For this reason, association of different gluten modifying enzymes could be an excellent option to improve overall quality of baked products.

Besides the gluten network, another secondary cross-links among minor compounds of flour such as arabinoxylans and pentosans can be promoted. The combined use the aforementioned enzymes with non-starch polysaccharide degrading enzymes could induce synergistic effects on dough behaviour or product quality. Combinations of hemicellulase/GO/α-amylase [30], TG/amylase/hemicellulase [52] and TG/pentosanase/α-amylase [53–55] have been reported as bread quality enhancers. Amylolytic enzymes have been also proposed as active contributors towards fresh bread quality and staling behaviour during storage.

The objective of this study was to analyse the individual and synergistic effects of a wide range of enzymes currently used in bread-making processes. In order to improve the response of some of the most representative enzymes, the effect of combined use of gluten cross-linking

Table 1 Characteristics of wheat flour

	Flour
Chemical composition	
Protein (% dry weight)	11.00
Ash (% dry weight)	0.58
Moisture (% dry weight)	12.16
Consistogram	
Water absorption (%)	52.8
Alveogram	
Deformation energy (10^{-4} J)	146
Curve configuration ratio (*P*/*L*)	0.35
Gluten index	
Gluten index (%)	94.0
Dry gluten (%)	9.0
Wet gluten (%)	26.6
Falling number	
Time (s)	405

enzymes, starch and non-starch polysaccharide degrading enzymes on dough rheology and bread quality was determined. To avoid an excessive increase in dough tenacity due to strengthening effect of gluten cross-linking enzymes, the treatment with gluten degrading enzymes (protease) is also proposed. The relationship between rheological properties of enzyme-supplemented doughs and fresh bread quality parameters was also established.

Materials and methods

Materials

A commercial blend of wheat flours provided by Harinera Castellana (Medina del Campo, Spain) was used in this study. This flour was obtained from local soft wheat (Table 1).

Six commercial enzymes were used: a glucose-oxidase (Gluzyme Mono 10000 BG (GO)), containing 10,000 glucose oxidase units per gram, a pentosanase (Pentopan Mono BG (PP)) containing 2500 fungal xylanase units per gram, a laccase (NZ 27011 (LAC)) containing 10,500 phenol oxidase units per gram, an amylase (Fungamyl SG (AMYL)) containing 2500 fungal amylase units per gram, a protease (Flavourzyme 1000 L (PROT)) containing 1000 aminopeptidase units per gram (all of them from Novozymes (Denmark)), and transglutaminase (Microbial TGM Activa WM (TG)) containing 100 transglutaminase units per gram, manufactured by Ajinomoto Co., Inc. (Tokyo, Japan). Selected dosages of the enzymes were, following the supplier's recommendations, 3 mg, 6 mg, 20 µl, 1 mg, 5 µl and 500 mg/100 g of flour, respectively. Enzymes were added according to the experimental design showed in Table 2. All of them were tested at two levels: 0 (absence of enzyme) and 1 (presence of enzyme at recommended dose). Flour and enzymes (when added) were mixed during 1 h before the tests, using a Rotary Mixer MR 2L (Chopin, Tripette et Renaud, France).

Table 2 Half fraction factorial design 2^6 for sampling

	Factors[a]					
Sample no	A	B	C	D	E	F
1	0	0	0	0	0	0
2	0	1	1	0	1	1
3	0	1	0	0	1	0
4	0	1	0	1	1	1
5	0	1	1	1	1	0
6	0	0	0	1	1	0
7	0	0	1	1	1	1
8	1	1	1	1	0	0
9	1	0	0	1	1	1
10	1	1	0	1	0	1
11	0	1	1	0	0	0
12	0	1	0	1	0	0
13	1	1	1	1	1	1
14	1	0	0	0	0	1
15	0	1	0	0	0	1
16	0	0	1	1	0	0
17	1	0	1	1	1	0
18	0	0	0	1	0	1
19	1	0	0	1	0	0
20	1	0	1	0	0	0
21	1	0	1	0	1	1
22	1	1	0	1	1	0
23	1	1	0	0	0	0
24	1	1	1	0	1	0
25	1	1	0	0	1	1
26	1	1	1	0	0	1
27	0	0	0	0	1	1
28	0	0	1	0	1	0
29	1	0	0	0	1	0
30	0	0	1	0	0	1
31	0	1	1	1	0	1
32	1	0	1	1	0	1

[a]Levels (0, 1) of factors (A–F): A, transglutaminase (TG): none (0), 500 mg/100 g flour (1); B, glucose oxidase (GO): none (0), 3 mg/100 g flour (1); C, laccase (LAC): none (0), 20 µl/100 g flour (1); D, amilase (AMYL): none (0), 1 mg/100 g flour (1); E, pentosanase (PP): none (0), 6 mg/100 g flour (1); F, protease (PROT): none (0), 20 µl/100 g flour (1)

Instant dry yeast and salt employed in bread-making process were obtained from the local market. All chemicals used for analyses were of analytical grade.

Alveograph test

The alveograph test was carried out in an Alveograph MA 82 (Chopin, Tripette et Renaud, France) following the AACC Approved Method 54-30 [56]. The parameters determined were tenacity (*P*, or resistance to extension), dough extensibility (*L*), the deformation energy (*W*), and the curve configuration ratio (*P*/*L*). A second alveograph test was performed after 3h resting period at 25 °C in order to assess the proteolytic degradation.

Consistograph test

The behaviour of the wheat flour during mixing was determined using a Consistograph NG (Chopin, Tripette et Renaud, France) following the AACC Approved Method 54-50 [56]. The parameters automatically recorded by the consistograph computer software program were water absorption (WA, water required to yield dough consistency equivalent to 1700 mb of pressure in a constant humidity measurement), dough development time (DDT, time to reach maximum consistency in an adapted humidity determination with a maximum pressure of 2200 mb), tolerance (Tol, time elapsed since dough consistency reaches its maximum until it decreases down to a 20%), decay at 250 s (D_{250}, consistency difference, in mb units, between height at peak and that 250 s later), decay at 450 s (D_{450}, consistency difference, in mb units, between height at peak and its value 450 s later). Decay at 250 s and 450 s are related with dough mixing stability. Higher stability means lower D_{250} and D_{450} values.

Bread-making procedure and evaluation of bread quality

Dough formulation, based on 100 g flour, included 57 ml water, 2 g salt, 0.83 g instant active dry yeast, 0.2 g sodium propionate and the amount of enzyme indicated previously for each sample. This basic bread formula was used to obtain roll bread. Dough was kneaded for a constant period of 12 min, divided into 315 g pieces, hand-rounded, mechanically moulded, put on trays, and proofed for 90 min at 30 °C and 75% RH. Before baking, a cut was made with a blade in the surface of the rolled pieces of dough to orientate dough expansion during the oven spring and to generate final scars on the surface, which are characteristic of this type of bread [57]. The pieces were baked into an electric oven for 35 min at 200 °C. Loaves were removed from the trays and cooled for 2 h at room temperature.

Quality analysis of fresh bread samples was carried out by measuring weight, volume (determined by seed displacement in a loaf volume meter), specific volume, and height/width ratio of the central slice.

Statistical analysis

Experimental design was conducted by means a two-level half-fractional factorial design in order to evaluate all single effects and second order interactions between factors. Resultant design is shown in Table 2. A multiple comparison analysis was performed with the program Statgraphics Plus V5.1 to assess significant differences among the samples. Fisher's least significant differences (LSD) test was used to describe means with 95% confidence.

Results and discussion

Rheological properties of enzyme-supplemented doughs

Single effects of enzymes on alveograph and consistograph parameters of doughs are showed in Table 3. Gluten cross-linking and gluten degrading enzymes had more significant ($p<0.05$) and greater effects on rheological properties than had polysaccharide degrading enzymes, surely because of the influence of gluten network on the rheological behaviour of dough. Major effects on alveograph parameters were provided by TG and PROT. The presence of TG in

Table 3 Single effects of design factors on rheological properties and bread quality of enzyme-supplemented doughs

		TG[a]		GO		LAC		AMYL		PP		PROT	
Parameter	Overall mean	0	1	0	1	0	1	0	1	0	1	0	1
P (mm H_2O)	46	37	54*									50	41*
L (mm H_2O)	119	144	94*										
W ($\times 10^{-4}$ J)	162	142	183*									173	151*
P/L	0.44	0.26	0.62*									0.52	0.36*
P_{3h} (mm H_2O)	69	31	106*									82	55*
L_{3h} (mm H_2O)	69	103	34*	86	51*								
W_{3h} ($\times 10^{-4}$ J)	132	99	166*										
P/L_{3h}	1.89	0.37	3.42*									2.54	1.26*
WA (%)	50.9												
DDT (s)	79												
Tol (s)	127	120	134*										
D_{250} (mb)	747	832	663*										
D_{450} (mb)	1199	1223	1015*									1084	1155*
Height/width ratio	0.58	0.45	0.71*	0.51	0.65*							0.60	0.56*
Specific volume (cm^3 g^{-1})	3.70	3.94	3.46*			3.58	3.81*	3.44	3.95*	3.51	3.88*	3.44	3.94*

[a]See Table 2 for levels of design factors.

*$p<0.05$ (the effect of the factor is significant with a significance level of 95%).

enzyme-supplemented doughs led to significant ($p<0.05$) increases in tenacity (P) and deformation energy (W) and decreases in extensibility (L). Hence, curve configuration ratio augmented significantly ($p<0.05$). These results were to be expected since previous studies had confirmed the strengthening effect along with dough extensibility reduction by TG addition as a result of the promotion of covalent intermolecular cross-links between gluten proteins [12, 14, 15, 19–21]. Conversely, PROT treatment significantly ($p<0.05$) diminished tenacity (P), deformation energy (W) and P/L ratio, whilst the observed increase in dough extensibility was not significant ($p<0.05$). Similar results were obtained by Wikstrom and Eliasson [40], Indrani et al. [58] and Pedersen et al. [59], who reported increases in the dough relaxation rate, and decreases in dough resistance to extension and elastic modulus by PROT action. Its weakening action on gluten network seems to be the reason of this behaviour. Proteolytic enzymes hydrolyse polypeptide chains of different protein fractions resulting in pronounced reduction in molecular mass distribution of wheat proteins, especially glutenins [60]. The micrographs of wheat dough with PROT have revealed a disruption of gluten matrix with the presence of some small pits [58].

Resting period length accentuated differences between alveograph properties of supplemented and non-supplemented doughs, with more significant ($p<0.05$) effects especially after TG and GO treatments. After a 3h period, tenacity (P), deformation energy (W) and curve configuration ratio (P/L) of dough containing TG increased 242, 68 and 824%, respectively, and extensibility decreased 65%. These percentages were comparatively more marked than those obtained previously (without resting period), which were 46, 29 and 138% for P, W and P/L increases, and 35% for L decrease. These results confirmed the findings of Gerrard et al. [17], who suggested a cumulative effect of TG with more protein cross-links being formed as the reaction time increases. The effect of GO was only significant ($p<0.05$) after incubation time, and affected to dough extensibility (L). Although Rakotozafy et al. [28] have stated important losses of GO activity during mixing, this enzyme maintained a residual activity after this operation. Vemulapalli et al. [39] have also established that GO was much more effective at improving bread quality after longer fermentation processes, suggesting a direct relation between reaction time and enzyme effect. PROT showed similar behaviours in both incubated and non-incubated samples, but its effect was less significant ($p<0.05$) in the first ones. These results can be attributed to the presence of endogenous proteolytic enzymes in the samples (deformation energy of non-treated doughs decreased during the resting period) and the subsequent masking effects on the exogenous proteases action.

The analysis of consistograph data revealed a trend similar to that of alveograph parameters. TG and PROT were the only enzymes that modified significantly ($p<0.05$) the rheological behaviour of dough during mixing. Although previous studies described the drying effect and the decrease in the dough relaxation rate when adding GO [39, 40], as well as the modification of dough consistency and stability during mixing by LAC addition [48, 50], the consistograph results showed no significant effects either of GO or of LAC. TG only improved significantly ($p<0.05$) dough tolerance and related parameters (decay at 250 and 450 s) indicating an improved dough stability when overmixing. These results totally agreed with those obtained in our previous investigations [23] but only agreed partially with the findings of Basman et al. [18] and Gerrard et al. [17], who also observed significant changes in flour-water absorption by enzyme addition at similar levels. The presence of PROT only showed a significant ($p<0.05$) effect on decay at 450 s, affecting negatively to dough tolerance to overmixing. Again it was possible to state more marked effects of these enzymes when they had more time to act, affecting to a greater extent to decay of dough consistence after 450 s.

The statistical design proposed in this study (Table 2) enabled us establish second order interactions between enzymes. As can be seen in Table 4, TG and GO had significant ($p<0.05$) effect on incubated dough rheology when added together, particularly on extensibility (L_{3h}) and deformation energy (W_{3h}). The simultaneous presence of both enzymes led to a synergistic effect on deformation energy (W_{3h}), probably because both enzymes strengthen dough through different mechanisms. Rosell et al. [15] indicated that wet gluten content slightly increased with the combined addition of TG and GO, suggesting a greater degree of polymerisation.

Addition of TG to PROT containing samples significantly increased P_{3h}, W_{3h}, and P/L_{3h}. The protein polymerisation catalysed by TG counteracted partially the hydrolytic effect of PROT, leading to improvements in rheological behaviour of doughs. The increase in the mentioned alveograph parameters was lower than that obtained for singly TG treated dough except for W_{3h}, whose values were similar in both cases. Since the addition of TG and PROT resulted in a reduction in dough tenacity maintaining deformation energy with respect to TG treatment, the simultaneous use of both enzymes could be an interesting alternative for avoiding excessive cross-linking promoted by TG and subsequent negative effects. In fact, combination of TG and PROT has been proposed as bread improver [52].

Although TG had no significant ($p<0.05$) effect on dough water absorption (WA), this consistograph parameter decreased significantly ($p<0.05$) when TG and PROT were used jointly (Table 5). Babiker et al. [61] reported an increase in the hydrophobicity of protease-treated gluten that would justify the decrease observed in WA after PROT treatment. These authors also stated that exposed hydrophobic residues were incorporated inside polymerised protein molecules by TG addition. This mechanism would also explain the dough tightness by TG action observed by Gerrard et al. [17] and Basman et al. [18] after mixing.

Polysaccharide-degrading enzymes exercised a significant ($p<0.05$) effect on rheological properties of dough only when they were used in combination with other enzymes, affecting to consistograph parameters (Table 5). In accordance with the improvement of dough tolerance (Tol) observed, a synergism between TG and PP could be

Table 4 Second-order interactive effects of design factors on alveograph parameters of dough

Parameter	Overall mean	Level[a]	TG/GO	TG/PROT
P (mm H_2O)	46	00		
		01		
		10		
		11		
L (mm H_2O)	119	00		
		01		
		10		
		11		
W ($\times 10^{-4}$ J)	162	00		
		01		
		10		
		11		
P/L	0.44	00		
		01		
		10		
		11		
P_{3h} (mm H_2O)	69	00		37*
		01		26
		10		128
		11		85
L_{3h} (mm H_2O)	69	00	140*	
		01	67	
		10	32	
		11	37	
W_{3h} ($\times 10^{-4}$ J)	132	00	107*	112*
		01	90	85
		10	154	167
		11	177	164
P/L_{3h}	1.89	00		0.45*
		01		0.30
		10		4.63
		11		2.21

[a]See Table 2 for levels of design factors

*$p<0.05$ (the effect of the factor is significant with a significance level of 95%)

concluded. The significant ($p<0.05$) increase of Tol came accompanied by a significant decrease of decay at 250 s (D_{250}). PP has been proved to diminish the amount of total pentosans associated with the gluten matrix [34] and counteract the over-aggregation of gluten [62].

As a result of the combined use of LAC and PP, the individual effects of both enzymes on the water absorption (WA), were significantly ($p<0.05$) offset. PP counteracted the negative effect of LAC on WA because of their contrary enzymatic action (the first one releases pentosans associated with proteins whereas the latter promotes polymerisation of the pentosans). The synergistic effect of these enzymes are in accordance with the findings of Primo-Martin et al. [34], who showed a more marked decrease in total pentosans associated with glutenin-macropolymer (GMP) than those obtained by the treatment with singly PP. As consequence, the combined use of PP and LAC could alter the pentosan–protein interaction implying changes in functional properties of dough.

Dough development time (DDT) and tolerance (Tol) were affected significantly by LAC and PROT combination. LAC addition to PROT containing doughs raised their DDT and Tol, but the increases were insufficient to recover the values showed by non-treated dough. It can be concluded that simultaneous arabinoxylans gelation and oxidative action promoted by LAC counteracted partially the hydrolytic activity of PROT on dough protein fraction.

AMYL and PP exhibited a significant ($p<0.05$) synergistic effect on dough water absorption (WA). Their combined use also exerted a significant ($p<0.05$) effect on tolerance (Tol). In spite of the beneficial effect of both enzymes when added individually, it was proved an antagonist effect of both enzymes on Tol. Alpha-amylase has been found to cleave long starch chains producing shorter chains or dextrins that come accompanied by a rapid loss of dough consistency and water absorption [63] and an increase in dough stickiness [64]. Dextrins may interfere with interactions between the swollen starch granules and the protein network [65] modifying dough tolerance (Tol). PP brought about a partial solubilization of water insoluble pentosans (WIP) [66], reducing also the water absorption capacity of dough by releasing the water bound to pentosans [38]. The progressive liberation of free water molecules (that aids gluten network development), along with the decrease in pentosan–protein interaction [34], could justify the improvement obtained in dough tolerance by PP treatment. In addition, the water released by PP action has been suggested as responsible of changes in selectivity of amylases, leading specific activity of amylases towards small size substrates [38], which could explain the behaviour of doughs treated with both enzymes.

Interactive effect of PP and PROT on water absorption (WA) was also significant ($p<0.05$). The decrease of WA induced by PROT was counteracted when PP was present in the samples suggesting a strengthening effect promoted by PP probably because of the diminution of associations of pentosans with glutenin polymers [34] and subsequent improvement of gluten quality.

Bread quality of enzyme-supplemented doughs

Individual effects of enzymes on bread quality parameters of doughs are showed in Table 3. Although gluten cross-linking and gluten degrading enzymes had again more significant ($p<0.05$) effects, all enzymes influenced significantly ($p<0.05$) the bread quality parameters. Addition of TG led to a significant ($p<0.05$) increase in height/width ratio and a decrease in specific volume. The particular effect of TG on bread quality has been previously studied with contradictory results, and it seems to be tied with different factors such as the quantity of water used [12, 17, 21], the dose of TG [18], and the baking quality of the flour [20]. Although enzyme treatment improved the shape of our loaves, they were globally less expanded in the course of baking because of strengthening effect promoted by TG

Table 5 Second-order interactive effects of design factors on consistograph parameters of dough

Parameter	Overall mean	Level[a]	TG/PP	TG/PROT	LAC/PP	LAC/PROT	AMYL/PP	PP/PROT
WA (%)	50.9	00		51.6*	51.6*		50.8*	51.3*
		01		50.6	50.8		50.5	50.1
		10		50.6	49.9		50.6	50.9
		11		50.8	51.4		51.7	51.3
DDT (s)	79	00				88*		
		01				73		
		10				77		
		11				80		
Tol (s)	127	00	126*			139*	122*	
		01	114			114	139	
		10	124			124	129	
		11	144			131	119	
D_{250} (mb)	747	00	787*					
		01	876					
		10	722					
		11	604					
D_{450} (mb)	1199	00						
		01						
		10						
		11						

[a]See Table 2 for levels of design factors

*$p<0.05$ (the effect of the factor is significant with a significance level of 95%)

and the consequent increase of dough tenacity that reduced dough extension during fermentation and oven-spring. Loaf volume probably could be increased by adding additional water.

An opposite effect was observed by adding PROT, since this enzyme increased significantly ($p<0.05$) specific volume and decreased slightly height/width ratio of loaves. The results were in agreement with dough biaxial properties of PROT-supplemented doughs and reflected the weakening action that this enzyme exerts on gluten network. Similar results were obtained by Indrani et al. [58], who stated significant improvements in the specific loaf volume and simultaneous degradation of gluten matrix by PROT. Bombara et al. [60] suggested a limited degree of hydrolysis as responsible of improving product quality. The improvement may be related to flexibility of protein network, without an extensive degradation of glutenins.

The oxidative enzymes GO and LAC also exerted a significant ($p<0.05$) effect on bread quality. The former led to improvements in the shape of loaves whilst the latter affected positively to their specific volume. The strengthening effect of GO on doughs has been widely proved [15, 26, 34, 38, 39, 41, 67] and it would explain a greater loaf height after treatment. Improvements in the wheat and rice bread loaf volume have been obtained by adding GO under different test conditions [26, 39, 42]. However, our tests failed to show an increase in loaf volume, probably because different baking procedures were involved and/or dissimilar GO doses were used. Although LAC action was not confirmed by any change in the rheological properties of dough, the improving effect of this enzyme was probably promoted by two simultaneous mechanisms: the feruloylated arabinoxylans cross-linking [68] and the oxidation of sulphydryl groups [50]. Primo-Martin and Martinez-Anaya [51] also stated improvements in bread volume as consequence of LAC treatment.

PP supplementation caused a significant ($p<0.05$) improvement of loaf specific volume but did not produce changes in its shape. Krishnarau and Hoseney [69] reported how the adverse effects of pentosans addition on the loaf volume were overcome by PP treatment. Indrani et al. [58] also confirmed an important increase in specific volume obtained with xylanase. By means of micrographs of bread doughs with PP, they showed a slight distortion of starch granules accompanied with a thinning of protein film, attributing the observed changes to the breakdown of glycosidic linkages in arabinoxylans.

Similar effect was exerted by AMYL. Although literature emphasizes the use of this enzyme to retard bread staling, additional side effects on bread quality have been also reported. Indrani et al. [58] obtained a high overall quality score in wheat flour breads with a marked increase in loaf volume. Parallel scanning electron microscopy studies revealed the presence of some deformed starch granules due to the action of α-amylase on long starch chains [58] and a slight leakage of amylose [70]. Alpha-amylase also improved rice bread specific volume and crumb firmness but gave very sticky textures [71].

Analysis of second order interactive effects of design factors on bread quality parameters revealed significant ($p<0.05$) interactions between TG and all the other enzymes except LAC (Table 6). TG and GO combined

Table 6 Second-order interactive effects of design factors on bread quality parameters of dough

Parameter	Overall mean	Level[a]	TG/GO	TG/AMYL	TG/PP	TG/PROT	GO/LAC	GO/PP	LAC/AMYL	AMYL/PROT	PP/PROT
Height/width ratio	0.58	00	0.35*	0.48*			0.51*	0.53*			
		01	0.55	0.42			0.51	0.49			
		10	0.68	0.70			0.67	0.63			
		11	0.74	0.72			0.62	0.66			
Specific volume (cm^3 g)	3.70	00			3.86*	3.86*	3.65*	3.36*	3.47*	3.30*	3.35*
		01			4.00	4.01	3.67	3.96	3.69	3.59	3.67
		10			3.16	3.03	3.51	3.66	3.41	3.59	3.54
		11			3.75	3.88	3.94	3.80	4.20	4.30	4.22

[a]See Table 2 for levels of design factors

*$p<0.05$ (the effect of the factor is significant with a significance level of 95%)

exerted a synergist effect on height/width ratio yielding loaves with greater height. This result was supported by significant changes observed previously in dough rheology. The marked decrease in dough extensibility did not allow the correct bi-axial extension of the dough during fermentation. Similar behaviour was showed by samples supplemented with TG and AMYL, although synergistic effect was less marked. The amylases promote yeast action during fermentation by degrading the damaged starch into smaller dextrins, and producing more gas, thus enhancing the TG effect on loaves shape. The binary combination of bacterial alpha-amylase and TG has been reported as an enhancer of sensory and textural bread profile, but significant effect on volume or specific volume was not proved [54], which agrees with our results.

Addition of PP and PROT to doughs treated with TG counteracted partially the negative effects of this latter enzyme on loaf specific volume. As we indicated previously, the release of pentosans associated with proteins improve the quality of gluten network [34], affecting positively to rheological behaviour of doughs. On the other hand, PROT hydrolyse polypeptide chains of different protein fractions, neutralizing partially the excessive increase in dough tenacity promoted by TG. When pentosanases or proteases were used in combination with TG, they allowed a better dough development during fermentation and oven-spring, having positive effects on loaf volume.

GO and LAC combination synergistically led to significant ($p<0.05$) increase in specific volume and height/width ratio of the loaves. The increase in this latter parameter was lower than the one obtained in the presence of GO. Primo-Martin et al. [34] stated an increase of the protein–pentosan interaction by the individual addition of GO and LAC, which would further interfere with the aggregation of the protein network. In addition, they indicated the possible presence of long-chain polysaccharides trapped in the gluten matrix. Both conclusions allowed suggest simultaneous strengthening and softening effects on proteins promoted by the combined use of the enzymes. The gluten network would show a better resistance and extensibility during baking, leading to significant ($p<0.05$) improvements in specific volume and shape of loaves.

Similar significant ($p<0.05$) synergistic effect on bread quality was observed by the combined use of GO and PP. Since gelation of water soluble arabinoxylans promoted by GO could negatively affect bread quality, the generation of small ferulic acid-containing arabinoxylan fragments by xylanase and the subsequent interference action of those in the formation of new arabinoxylan cross-links by GO has been recently proposed as a theory for justifying this synergistic effect [72].

Addition of AMYL to LAC containing doughs significantly ($p<0.05$) increased the specific volume of loaves, whilst their shape stayed practically unaltered, with slight but not significant ($p<0.05$) decreases in height/width ratio. These results were analogous with those obtained by AMYL/PROT and PP/PROT combinations. The positive effect of amylases on yeast action and gas production during fermentation in combination with the softening effect promoted by LAC [34] and PROT [40, 58] on the gluten proteins led to increase in volume of loaves. On the other hand, PP action has been related with the increase of gluten strength and elasticity [34, 55, 62]. In conjunction with weakening effect of PROT, elastic and viscous properties of dough could be improved, suggesting the important increases observed in the quality of final product.

Relationship between rheological properties and bread quality parameters of enzyme-supplemented doughs

Analytical data were subjected to a Pearson correlation analysis in order to establish significant relationships between rheological and bread quality parameters of enzyme-supplemented doughs. A Durbin–Watson (DW) statistic test of the residuals was performed to determine if there

Table 7 Coefficients of significant correlations ($p<0.05$) between rheological and bread quality parameters of dough

Parameter	Height/width ratio	Specific volume ($cm^3\ g^{-1}$)
P (mm H_2O)	0.7447	−0.5828
L (mm H_2O)	−0.7223	0.5155
W ($\times 10^{-4}$ J)	0.6854	
P/L	0.7030	−0.6201
P_{3h} (mm H_2O)	0.7605	−0.5787
L_{3h} (mm H_2O)	−0.8401	
W_{3h} ($\times 10^{-4}$ J)	0.7036	−0.4176
P/L_{3h}	0.6983	−0.5913
D_{250} (mb)	−0.5015	
D_{450} (mb)	−0.6559	0.4183

was any significant correlation based on the order in which they occur in the data file. Significant ($p<0.05$) correlation coefficients (r) are showed in Table 7.

Bread quality parameters showed greater and more significant ($p<0.05$) correlations with alveograph parameters than with consistograph properties of dough. The alveograph test has been described as an empirical method for measuring rheological properties of dough, namely its biaxial extensibility [73]. This test is usually used to elucidate the handling properties of dough, and could represent better its behaviour during baking process. Tenacity and extensibility proved to be acceptable predictors of height/width ratio of loaves. Tenacity was positively correlated with height/width ratio ($r=0.7447$) whereas relationship between extensibility and the mentioned ratio was negative ($r=-0.7223$). Therefore, loaves with better shape corresponded to doughs with higher tenacity and lower extensibility. This relationship increased with dough after 3h resting period, thus the time of the test enhances the prediction of bread quality parameters from rheological properties. Tenacity (P_{3h}) and extensibility (L_{3h}) showed again the best correlation coefficients ($r=0.7605$ and $r=-0.8401$, respectively). Deformation energy (W) and curve configuration ratio (P/L) also showed positive correlations with height/width ratio, being the coefficients of similar magnitude either on rested or non-rested samples (Table 7). Likewise, two parameters in the consitograph test, namely decay at 250 and 450 s (D_{250} and D_{450}) showed negative correlations with the cited ratio. Decay of consistograph curve is related with the loss of dough stability during mixing, thus dough with high mixing stability (lower D_{250} and D_{450}) would lead to high height/width ratio in the loaves. D_{450} showed greater correlation than did D_{250} ($r=-0.6559$ and $r=-0.5015$, respectively).

The relationships between loaf-specific volume and empiric rheological parameters were lower and less significant. For this reason, the results revealed that none of the studied rheological properties could be considered as a good predictor of specific volume of loaves. Correlations that involved specific volume showed the opposite sign to those which involved height/width ratio. Tenacity and curve configuration ratio were negatively correlated with specific volume ($r=-0.5828$ and $r=-0.6201$, respectively), whereas extensibility was positively correlated ($r=0.5155$). In this case, the effect of resting time was not so marked than previously, but the correlation between specific volume and deformation energy (W_{3h}) only became significant ($p<0.05$) after a 3h resting period ($r=-0.4176$). Finally, D_{450} showed a significant ($p<0.05$) positive correlation with specific volume of loaves, although the correlation coefficient was very low ($r=0.4183$). High dough mixing stability corresponded to loaves with less specific volume.

Conclusions

Single addition of gluten cross-linking and gluten degrading enzymes showed more significant and greater effects on rheological properties than polysaccharide degrading enzymes. The most important effect on alveograph parameters were provided by TG and PROT. Resting period accentuated differences between alveograph properties of supplemented and non-supplemented doughs, with more significant effects especially after TG and GO treatments. The analysis of consistograph data revealed a trend similar to alveograph parameters. The simultaneous presence of TG and GO, as well as TG and PROT led to a synergistic effect on deformation energy, improving the rheological behaviour of doughs. Polysaccharide-degrading enzymes exercised a significant effect on rheological properties of dough only when they were used in combination with other enzymes, affecting to consistograph parameters.

Although gluten cross-linking and gluten degrading enzymes had again more significant effects when they were used individually, all enzymes significantly affected the bread quality parameters. Addition of TG led to a significant increase in height/width ratio and a decrease in specific volume. Polysaccharide-degrading enzymes, LAC and PROT, caused a significant improvement of loaf-specific volume but did not produce changes in its shape. Analysis of second order interactive effects of design factors on bread quality parameters revealed significant interactions between TG and all the other enzymes, except LAC. Significant synergistic effect on bread quality was observed by the combined use of GO and LAC, GO and PP, AMYL and LAC, AMYL and PROT, and PP and PROT.

Bread quality parameters showed greater correlations with alveograph parameters than with consistograph properties of dough. As general remark, tenacity (P) and extensibility (L) proved to be acceptable predictors of height/width ratio of loaves. The duration of the alveograph test enhanced the prediction of bread quality parameters. However, none of the studied rheological properties could be considered as a good predictor of specific volume of rolled breads.

Acknowledgements This work was financially supported by Comisión Interministerial de Ciencia y Tecnología Projects (MCYT, AGL2002-04093-C03ALI), Consejo Superior de Investigaciones Científicas (CSIC) and Universidad de Valladolid, Spain. Authors thank Ramiro Martínez (Novo Nordisk, Madrid, Spain) for providing enzyme samples.

References

1. Blumenthal CS, Barlow EWR, Wrigley CW (1993) J Cereal Sci 18:3–21
2. Perrotta C, Treglia AS, Mita G, Giangrande E, Rampino P, Ronga G, Spano G, Marmiroli N (1998) J Cereal Sci 27:127–132
3. Iriki N, Yamauchi H, Takata K, Nishio Z, Ichinose Y, Yoshihira T (2003) Food Sci Technol Res 9:104–109
4. Fisher N, Hutchinson JB, Berry R, Hardy J, Ginocchio AV, Waite Y (1979) Food Cosmet Toxicol 17:33–39
5. Kurokawa Y, Maekawa A, Takahashi M, Hayashi Y (1990) Environ Health Perspect 87:309–335
6. Dupuis B (1997) Cereal Foods World 42:171–183
7. Wolf DC, Crosby LM, George MH, Kilbur SR, Moore TM, Miller RT, DeAngelo AB (1998) Toxicol Pathol 26:724–729
8. Stauffer CE (1990) Functional additives for bakery foods. Van Nostrand Reinhold, New York
9. Gerrard JA (2002) Trends Food Sci Technol 13:389–397
10. Motoki M, Seguro K (1998) Trends Food Sci Technol 9:204–210
11. Gerrard JA, Fayle SE, Brown PA, Sutton KH, Simmons L, Rasiah I (2001) J Food Sci 66:782–786
12. Larré C, Denery PS, Popineau Y, Deshayes G, Desserme C, Lefevre J (2000) Cereal Chem 77:32–38
13. Mujoo R, Ng PKW (2003) Cereal Chem 80(6):703–706
14. Bauer N, Koehler P, Wieser H, Schieberle P (2003) Cereal Chem 80:781–786
15. Rosell CM, Wang J, Aja S, Bean S, Lookhart G (2003) Cereal Chem 80:52–55
16. Gujral HS, Rosell CM (2004) J Cereal Sci 39:225–230
17. Gerrard JA, Fayle SE, Wilson AJ, Newberry MP, Ross M, Kavale S (1998) J Food Sci 63:472–475
18. Basman A, Köksel H, Perry KWN (2002) Eur Food Res Technol 215:419–424
19. Tseng CS, Lai HM (2002) J Food Sci 67:750–755
20. Bauer N, Koehler P, Wieser H, Schieberle P (2003) Cereal Chem 80:787–790
21. Autio K, Kruus K, Knaapila A, Gerber N, Flander L, Buchert J (2005) J Agric Food Chem 53:1039–1045
22. Gerrard JA, Newberry MP, Ross M, Wilson AJ, Fayle SE, Kavale S (2000) J Food Sci 65:312–314
23. Bonet A, Caballero PA, Gomez M, Rosell CM (2005) Cereal Chem 82:425–430
24. Caballero PA, Bonet A, Rosell CM, Gomez M (2000) J Cereal Sci 42:93–100
25. Köksel H, Sivri D, Ng PKW, Steffe JF (2001) Cereal Chem 78:26–30
26. Gujral HS, Rosell CM (2004) Food Res Int 37:75–81
27. Gerrard JA, Sutton KH (2005) Trends Food Sci Technol 16(11):510–512
28. Rakotozafy L, Mackova B, Delcros JF, Boussard A, Davidou S, Potus J, Nicolas J (1999) Cereal Chem 76:213–218
29. Hoseney RC, Faubion JM (1981) Cereal Chem 58:421–424
30. Haarasilta S, Pullinen T, Vaisanen S, Tammersalo-Karsten I (1991) United States Patent 4,990,343
31. Nakai K, Takami K, Yanaka N, Takasaki Y (1995) European Patent Application 0,686,348 A1
32. Vemulapalli V, Hoseney RC (1998) Cereal Chem 75:859–862
33. Aja S, Wang J, Rosell CM (2003) In: Courtin CM, Veraverbeke WS, Delcour JA (eds) Recent advances in enzymes in grain processing. American Association of Cereal Chemists, St. Paul, MN, pp 101–106
34. Primo-Martin C, Valera R, Martínez-Anaya MA (2003) J Agric Food Chem 51:4673–4679
35. Ameille V, Castello P, Garcia R, Rakotozafy L, Potus J, Nicolas J (2000) Sci Aliments 20(4/5):441–455
36. Tilley KA, Benjamin RE, Bagorogoza KE, Moses Okot-Kotber B, Praskash O, Kwen H (2001) J Agric Food Chem 49:2627–2632
37. Rasiah IA, Sutton KH, Low FL, Lin HM, Gerrard JA (2005) Food Chem 89:325–332
38. Martinez-Anaya MA, Jimenez T (1997) J Texture Stud 28:569–583
39. Vemulapalli V, Miller KA, Hoseney RC (1998) Cereal Chem 75:439–442
40. Wikstrom K, Eliasson AC (1998) Cereal Chem 75:331–337
41. Dunnewind B, Van Vliet T, Orsel R (2002) J Cereal Sci 36:357–366
42. Xia P, Jin MG, Liang XY (1999) Food Ind 6:23–24
43. Garcia R, Rakotozafy L, Nicolas J (2004) J Agric Food Chem 52:3946–3953
44. Poulsen C, Hostrup PB (1998) Cereal Chem 75:51–57
45. Si JQ (1994) International Patent Application WO 94/28728
46. Figueroa-Espinoza MC, Morel MH, Rouau X (1998) J Agric Food Chem 46:2583–2589
47. Figueroa-Espinoza MC, Morel MH, Surget A, Asther M, Moukha S, Sigoillot JC, Rouau X (1999) Food Hydrocolloids 13:65–71
48. Labat E, Morel MH, Rouau X (2001) Food Hydrocolloids 15:47–52
49. Oudgenoeg G, Hilhorst R, Piersma SR, Boeriu CG, Gruppen H, Hessing M, Voragen AG, Laane C (2001) J Agric Food Chem 49:2503–2510
50. Labat E, Morel MH, Rouau X (2000) Cereal Chem 77:823–828
51. Primo-Martin C, Martinez-Anaya MA (2003) J Food Sci 68:31–41
52. Gottmann K, Sproessler B (1994) European Patent Application EP 0492406, B1
53. Bollain C, Angioloni A, Collar C (2005) Eur Food Res Technol 220:83–89
54. Collar C, Bollain C, Angioloni A (2005) J Food Eng 70:479–488
55. Collar C, Bollain C (2005) Eur Food Res Technol 221:298–304
56. AACC (2000) Approved methods of the AACC. American Association of Cereal Chemists, St. Paul, MN
57. Rouille J, Della Valle G, Devaux MF, Marion D, Dubreil L (2005) Cereal Chem 82:20–27
58. Indrani D, Prabhasankar P, Rajiv J, Venkateswara-Rao G (2003) J Food Sci 68:2804–2809
59. Pedersen L, Kaack K, Bergsoe MN, Adler-Nissen J (2005) J Food Sci 70:152–158
60. Bombara N, Anon MC, Pilosof AMR (1997) Lebensm Wiss Technol 30:441–447
61. Babiker EE, Fujisawa N, Matsudomi N, Kato A (1996) J Agric Food Chem 44:3746–3750
62. Weegels PL, Hamer RJ (1992) Cereal Foods World 37(5):379–385
63. Pyler EJ (1988) Baking science and technology. Sosland Publising Company, Merriam, KS
64. Armero E, Collar C (1997) Z Lebensm Unters Forsch 204:136–145
65. Duran E, Leon A, Barber B, Benedito de Barber C (2001) Eur Food Res Technol 212:203–207
66. Rouau X, Moreau D (1993) Cereal Chem 70:626–632
67. Collar C, Andreu P, Martinez-Anaya MA (1998) Z Lebensm Unters Forsch 207:133–139
68. Figueroa-Espinoza MC, Rouau X (1998) Cereal Chem 75:259–265
69. Krishnarau L, Hoseney RC (1994) J Food Sci 59:1251–1254
70. Blaszczak W, Sadowska J, Rosell CM, Fornal J (2004) Eur Food Res Technol 219:348–354
71. Gujral HS, Haros M, Rosell CM (2003) Cereal Chem 80:750–754
72. Primo-Martin C, Wang M, Lichtendonk WJ, Plijter JJ, Hamer RJ (2005) J Sci Food Agric 85:1186–1196
73. Dobraszczyk BJ, Morgenstern MP (2003) J Cereal Sci 38:229–245