

2 **BREAD QUALITY AND DOUGH RHEOLOGY OF ENZYME SUPPLEMENTED**  
3 **WHEAT FLOUR**  
4 **EFFECT OF ENZYME COMBINATION ON DOUGH RHEOLOGY AND BREAD**  
5 **QUALITY**

6  
7  
8 **P.A. Caballero<sup>1</sup>, M. Gómez<sup>1</sup>, C.M. Rosell <sup>2</sup>,**

9  
10 <sup>1</sup> Área de Tecnología de Alimentos. Universidad de Valladolid. Avda de Madrid, 44.  
11 34004-Palencia. Spain. E-mail: [pallares@iaf.uva.es](mailto:pallares@iaf.uva.es)

12  
13 <sup>2</sup> Instituto de Agroquímica y Tecnología de Alimentos (CSIC). P.O. Box 73, 46100-  
14 Burjassot. Valencia. Spain.

15  
16 **Running Title:** Enzymes combination for breadmaking

17  
18  
19  
20  
21  
22 Correspondence should be addressed to:  
23 Dr Manuel Gómez  
24 Escuela Técnica Superior de Ingenierías Agrarias  
25 Universidad de Valladolid  
26 Avda Madrid 44, Palencia-34004. Spain  
27 Tel: 34-979 108359  
28 Fax: 34-979 108302  
e-mail: [pallares@iaf.uva.es](mailto:pallares@iaf.uva.es)

2 **Abstract**

4 The enzymatic treatment of wheat flours is an interesting alternative for improving their  
functional properties. Since enzymes with different biochemical activities could induce  
6 synergistic effects on dough behaviour or product quality, the individual and combined  
use of a wide range of enzymes (glucose oxidase, transglutaminase, laccase,  
8 protease, pentosanase,  $\alpha$ -amylase) applied nowadays in bread-making processes  
were investigated. The blend of enzymes allowed to improve the rheological behaviour  
10 of doughs and the quality of final product. The simultaneous presence of  
transglutaminase (TG) and glucose oxidase (GO), as well as TG and protease (PROT)  
12 led to a synergistic effect on alveograph parameters. Polysaccharide-degrading  
enzymes exercised a significant effect on rheology only when they were used in  
14 combination with other enzymes, affecting mainly to consistograph parameters.  
Analysis of breadmaking data revealed significant interactions between TG and all the  
16 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),  
18 amylase (AMYL) and LAC, AMYL and PROT, and PP and PROT. Bread quality  
parameters showed greater correlations with alveograph parameters than with  
20 consistograph properties of dough. Tenacity (P) and extensibility (L) proved to be  
acceptable predictors of height/width ratio of loaves. The duration of the alveograph  
22 test enhanced the prediction of bread quality parameters. On the contrary, none of the  
rheological properties studied showed a high correlation with the specific volume of  
loaves.

24

**Key words:** Enzymes, wheat flour, dough rheology, bread quality.

## 2 Introduction

4 In the last years, the baking industry has undergone very important changes in its  
6 productive processes. The increasing mechanization of its processing unit operations  
8 has been one of the major changes. This fact has contributed to increase the demand  
10 of strong wheat flours, able to generate doughs with high tolerance to handling and  
12 mixing, and stable during fermentation.

14 Functional properties of flours greatly depend on the gluten proteins. On the other  
16 hand, the quality of gluten is dependent on diverse factors such as the wheat variety  
18 and the growth conditions (Blumenthal et al, 1993; Perrotta et al, 1998; Iriki et al,  
20 2003). For this reason, the capacity of some countries to produce high-quality flours is  
22 limited. In this context, the treatment of flours with functional additives must be  
24 considered.

26 Chemical improvers have been used for decades in bread-making as a way to adjust  
28 the variations in flour properties and baking conditions. Nowadays, the baking industry  
30 is deeply involved in research for alternatives to chemical compounds due to their  
32 potential hazards (Fisher et al, 1979, Kurokawa et al, 1990; Dupuis, 1997, Wolf et al,  
34 1998). The enzymatic treatment of wheat flours is an interesting alternative to generate  
36 changes in the structure of the dough and in consequence, for improving functional  
38 properties of the flours. They are generally recognized as safe (GRAS) and do not  
40 remain active in the final product after baking. Therefore enzymes do not have to  
42 appear in the label, which is an additional commercial advantage.

44 The intentional inclusion of enzymes in bread formulas dates back to more than one  
46 century (Stauffer, 1990). Today, a wide range of enzymes produced especially for  
48 bread-making is available for bakers. The aim of enzymes addition can be diverse, for  
50 example to achieve a partial gluten hydrolysis for improving machinability, to obtain  
52 enough sugars for fermentation by means of the starch hydrolysis, to attain a certain  
54 amount of lipid peroxidation for dough strengthening, or to reduce retrogradation and  
56 crumb firming through the hydrolysis of gelatinised starch.

Gluten cross-linking enzymes play an important role in the present baking processes.  
Through different biochemical mechanisms (the oxidative coupling of thiol groups, the  
crosslink 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 bread-making process (Gerrard, 2002).

Transglutaminase (TG) (EC.2.3.2.13) is a transferase able to yield inter- and  
intramolecular  $\epsilon$ -N-( $\gamma$ -glutamyl)lysine crosslinks (Motoki and Seguro, 1998). Its addition  
causes structural changes of gluten proteins, been high molecular weight (HMW)  
glutenin subunits the most affected protein fraction (Gerrard et al., 2001; Larre et al.,  
2000; Mujoo & Ng, 2003; Bauer et al., 2003a; Rosell et al., 2003). TG may also lead to  
the formation of disulfide bridges by oxidation due to the proximity of sulphur containing  
amino acids (Gujral and Rosell, 2004a). Due to this effects, TG have been widely used  
to improve the wheat dough functionality and bread quality (Gerrard et al., 1998; Larre  
et al., 2000; Basman et al., 2002; Tseng and Lai, 2002; Bauer et al., 2003b; Rosell et  
al., 2003; Autio et al., 2005). The possibility of using this enzyme to alleviate some of  
the detrimental effects of frozen storage of the puff pastry and the croissants (Gerrard  
2000), as well as to solve the damage promoted by the insect attack of wheat (Bonet et  
al, 2005; Caballero et al., 2005; Köksel et al., 2001) has been proposed. The results  
obtained with wheat flour have been also extrapolated to other cereals, allowing  
improving the viscoelastic properties of the rice dough and therefore the ability of rice  
flour to retain the carbon dioxide produced during proofing (Gujral and Rosell, 2004b).

2  
4  
6  
8  
10  
12  
14  
16  
18  
20  
22  
24  
26  
28  
30  
32  
34  
36  
38  
40  
42  
44  
46  
48  
50  
52  
54  
56

Glucose oxidase (EC 1.1.3.4) (GO) is an oxidative enzyme that catalyses the oxidation of  $\beta$ -D-glucose to  $\delta$ -D-gluconolactone and hydrogen peroxide (Rakotozafy et al, 1999). 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 (Hoseney and Faubion, 1981; Haarasilta et al., 1991; Nakai et al., 1995; Vemulapalli and Hoseney, 1998; Aja et al, 2003; Primo-Martin et al., 2003; Rosell et al, 2003; Gujral & Rosell, 2004a). Furthermore, it has been related the formation of non-disulfide covalent intermolecular bonds in the gluten proteins by GO treatment, either among glutenins (Ameille et al, 2000; Tilley et al., 2001) or between albumins and globulins (Rasiah et al, 2005). GO modifies the functional properties of dough, increasing its tenacity and elasticity (Martínez-Anaya and Jiménez, 1997; Vemulapalli et al., 1998; Wikstrom and Eliasson, 1998; Dunnewind et al. 2002, Rosell et al., 2003). Gujral and Rosell (2004) revealed even an increase in the elastic and viscous moduli of rice flour dough. As a result of mentioned changes in dough behaviour, GO showed positive effects on bread quality, yielding improved specific volume, bread texture and crumb grain (Vemulapalli et al., 1998; Xia et al., 1999; Gujral and Rosell, 2004).

Through a similar oxidative mechanism, hexose oxidase (EC 1.1.3.5) (HO) has been also suggested as an efficient bread improver (Garcia et al., 2004). 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 (Poulsen and Hostrup, 1998). 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 (1994) 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 (Figueroa-Espinoza and Rouau, 1998). The probable development of a protein-arabinoxylan network by LAC action has been hypothesized. In spite of Figueroa-Espinoza et al. (1999) and Labat et al (2001) have concluded that gluten and arabinoxylans form two distinct networks, Oudgenoeg et al (2001 or 2005 in references) proposed a mechanism by which tyrosine-containing proteins cross-link with arabinoxylans. Due to the simultaneous arabinoxylans gelation and oxidative action, LAC addition significantly improve gluten quality and lead to changes in the rheological properties of dough, diminishing slightly dough extensibility (Primo-Martín et al.; 2003), increasing dough consistency (Labat et al., 2001), reducing time to maximum consistency and accelerating dough breakdown during mixing (Labat et al, 2000). Improvement in the quality of bread elaborated with LAC has been also reported (Primo-Martin and Martinez-Anaya, 2003).

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) according their particular action mechanism. The type of protein being crosslinked appears to be more important than the crosslinking agent or type of crosslink 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) (Gerrard et al., 1998, 2000, 2001), the albumins and globulins play an important role in textural and crumb grain properties (Rasiah et al., 2005). For this reason, association of different gluten modifying enzymes could be an excellent option to improve overall quality of baked products.

2 Besides the gluten network, another secondary crosslinks among minor compounds of  
4 flour such as arabinoxylans and pentosans can be promoted. The combined use the  
6 aforementioned enzymes with non-starch polysaccharide degrading enzymes could  
8 induce synergistic effects on dough behaviour or product quality. Combinations of  
10 hemicellulase/GO/ $\alpha$ -amylase (Haarasilta et al, 1991), TG/amylase/hemicellulase  
(Gottmann and Sproessler, 1994) and TG/pentosanase/ $\alpha$ -amylase (Bollaín et al, 2005;  
Collar et al., 2005; Collar and Bollaín, 2005) have been reported as bread quality  
enhancers. Amylolytic enzymes have been also proposed as a way to contribute

12 actively to fresh bread quality and staling behaviour during storage.  
14 The objective of this study was to analyse the individual and synergistic effects of a  
16 wide enzyme range used nowadays in bread-making processes. In order to improve  
18 the response of some of the most representative enzymes, the effect of combined use  
of gluten cross-linking enzymes, starch and non-starch polisaccharide 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

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

26 Six commercial enzymes were used: a glucose-oxidase [Gluzyme Mono 10000 BG  
(GO)], containing 10000 glucose oxidase units/g, a pentosanase [Pentopan Mono BG  
(PP)] containing 2500 fungal xylanase units/g, a laccase [NZ 27011 (LAC)] containing  
28 10500 phenol oxidase units/g, an amylase [Fungamyl SG (AMYL)] containing 2500  
30 fungal amylase units/g, a protease [Flavourzyme 1000 L (PROT)] containing 1000  
32 aminopeptidase units/g [all of them from Novozymes (Denmark)], and transglutaminase  
[Microbial TGM Activa WM (TG)] containing 100 transglutaminase units/g,  
34 manufactured by Ajinomoto Co. Inc. (Tokyo, Japan). Selected dosages of the enzymes  
were, following the supplier's recommendations, 3 mg, 6 mg, 20  $\mu$ l, 1 mg, 5  $\mu$ l and 500  
36 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)  
38 were mixed during one hour before the tests, using a Rotary Mixer MR 2L (Chopin,  
Tripette et Renaud, France).

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

### Alveograph test.

44 The alveograph test was carried out in an Alveograph MA 82 (Chopin, Tripette et  
Renaud, France) following the AACC Approved Method 54-30 (AACC, 2000). The  
46 parameters determined were tenacity (P, or resistance to extension), dough  
extensibility (L), the deformation energy (W), and the curve configuration ratio (P/L). A  
48 second alveograph test was performed after 3 hours resting period at 25°C in order to  
assess the proteolytic degradation.

### Consistograph test.

52 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  
54 (AACC, 2000). The parameters automatically recorded by the consistograph computer

2 software program were water absorption (WA, water required to yield dough  
consistency equivalent to 1700 mb of pressure in a constant humidity measurement),  
4 dough development time (DDT, time to reach maximum consistency in an adapted  
humidity determination with a maximum pressure of 2200 mb), tolerance (Tol, time  
6 elapsed since dough consistency reaches its maximum until it decreases down to a  
20%), decay at 250s ( $D_{250}$ , consistency difference, in mb units, between height at peak  
8 and to that 250s later), decay at 450s ( $D_{450}$ , consistency difference, in mb units,  
between height at peak and its value 450s later). Decay at 250 s and 450 s are related  
10 with dough mixing stability. Higher stability means lower  $D_{250}$  and  $D_{450}$  values.

### 12 **Breadmaking procedure and evaluation of bread quality.**

Dough formulation, based on 100 g flour, included 57 mL water, 2 g salt, 0.83 g instant  
14 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.  
16 Dough was optimally mixed until dough development, divided into 315 g pieces, hand-  
rounded, mechanically moulded, put on trays, and proofed for 90 min at 30°C and 75%  
18 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  
20 on the surface, which are characteristic of this type of bread (Rouille et al., 2005). The  
pieces were baked into an electric oven for 35 min at 200°C. Loaves were removed  
22 from the trays and cooled for two hours at room temperature.

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

### 26 **Statistical analysis**

28 Experimental design was conducted by means a 2-level half-fractional factorial design  
in order to evaluate all single effects and second order interactions between factors.  
30 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  
32 samples. Fisher's least significant differences (LSD) test was used to describe means  
with 95% confidence.

## 34 **Results and discussion**

### 36 **Rheological properties of enzyme-supplemented doughs.**

Single effects of enzymes on alveograph and consistograph parameters of doughs are  
38 showed in table 3. Gluten cross-linking and gluten degrading enzymes had more  
significant ( $p<0.05$ ) and greater effects on rheological properties than polisaccharide  
40 degrading enzymes, surely due to the implication of gluten network in the rheological  
behaviour of dough. Major effects on alveograph parameters were provided by TG and  
42 PROT. The presence of TG in enzyme-supplemented doughs led to significant  
( $p<0.05$ ) increases in tenacity (P) and deformation energy (W) and decreases in  
44 extensibility (L). As consequence, curve configuration ratio augmented significantly  
( $p<0.05$ ). These results were expected since previous studies have confirmed the  
46 strengthening effect along with dough extensibility reduction by TG addition as a result  
of the promotion of covalent intermolecular cross-links between gluten proteins (Larre  
48 et al., 2000; Tseng and Lai, 2002; Bauer et al., 2003a; Bauer et al., 2003b; Rosell et  
al., 2003; Autio et al., 2005). Conversely, PROT treatment significantly ( $p<0.05$ )  
50 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  
52 obtained by Wikstrom and Eliasson (1998), Indrani et al (2003) and Pedersen et al.  
(2005), who reported increases in the dough relaxation rate, and decreases in dough  
54 resistance to extension and elastic modulus by PROT action. Its weakening action on

2 gluten network seems to be the reason of this behaviour. Proteolytic enzymes  
hydrolyse polypeptide chains of different protein fractions resulting in pronounced  
4 reduction in molecular mass distribution of wheat proteins, especially glutenins  
(Bombara et al., 1997). The micrographs of wheat dough with PROT have revealed a  
6 disruption of gluten matrix with the presence of some small pits (Indrani et al., 2003).

8 Resting period accentuated differences between alveograph properties of  
supplemented and non-supplemented doughs, with more significant ( $p < 0.05$ ) effects  
10 especially after TG and GO treatments. After a three hours period, tenacity (P),  
deformation energy (W) and curve configuration ratio (P/L) of dough containing TG  
12 increased 242%, 68% and 824% respectively, and extensibility decreased 65%. These  
percentages were comparatively more marked than those obtained previously (without  
14 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. (1998) who  
16 suggested a cumulative effect of TG with more protein crosslinks being formed as the  
reaction time increases. The effect of GO was only significant ( $p < 0.05$ ) after incubation  
18 time, and affected to dough extensibility (L). This parameter diminished because of the  
different mechanisms implicated in strengthening action of the enzyme. Although  
20 Rakotozafy et al. (1999) have stated important losses of GO activity during mixing, this  
enzyme maintained a residual activity after this operation. Vemulapalli et al. (1998) has  
22 also established that GO was much more effective at improving bread quality after  
longer fermentation processes, envisaging a direct relation between reaction time and  
24 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  
26 be attributed to the presence of endogenous proteolytic enzymes in the samples  
(deformation energy of non-treated doughs decreased during the resting period) and  
28 the subsequent masking effects on the exogenous proteases action.

The analysis of consistograph data revealed a trend similar to alveograph parameters.  
30 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  
32 drying effect and the decrease in the dough relaxation rate when adding GO  
(Vemulapalli et al., 1998; Wikström and Eliasson, 1998), as well as the modification of  
34 dough consistency and stability during mixing by LAC addition (Labat et al., 2000,  
2001), our consistograph results showed no significant effects neither of GO nor of  
36 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. This  
38 results totally agreed with those obtained in our previous investigations (Bonet et al.,  
2005) but only agreed partially with the findings of Basman et al. (2002) and Gerrard et  
40 al. (1998), who also observed significant changes in flour-water absorption by enzyme  
addition at similar levels. The presence of PROT only showed a significant ( $p < 0.05$ )  
42 effect on decay at 450 s, affecting negatively to dough tolerance to overmixing.  
Mentioned results can be attributed to particular effects of both enzymes on the gluten  
44 network, due to the crosslinking action of TG and the hydrolysing action of PROT.  
Again it was possible to state more marked effects of these enzymes when they had  
46 more time to act, affecting to a greater extent to decay of dough consistence after 450  
s.

48 Statistical design proposed in this study (Table 2) allowed to establish second order  
interactions between enzymes. As can be seen in table 4, TG and GO had significant  
50 ( $p < 0.05$ ) effect on incubated dough rheology when added together, concretely on  
extensibility ( $L_{3h}$ ) and deformation energy ( $W_{3h}$ ). The simultaneous presence of both  
52 enzymes led to a synergistic effect on deformation energy ( $W_{3h}$ ), probably due to both  
enzymes strengthen dough through different mechanisms. Rosell et al. (2003)

2 indicated that wet gluten content slightly increased with the combined addition of TG  
and GO (with respect to individual treatment).

4 Addition of TG to PROT containing samples significantly increased  $P_{3h}$ ,  $W_{3h}$ , and  $P/L_{3h}$ .  
6 The protein polymerisation catalyzed by TG counteracted partially the hydrolytic effect  
of PROT, leading to improvements in rheological behaviour of doughs. The increase in  
8 the mentioned alveograph parameters was lower than the obtained for singly TG  
treated dough except for  $W_{3h}$ , whose values were similar in both cases. Since the  
10 addition of TG and PROT allowed to reduce dough tenacity maintaining deformation  
energy with respect TG treatment, the simultaneous use of both enzymes could be an  
12 interesting alternative for avoiding excessive crosslinking promoted by TG and  
subsequent negative effects. In fact, combination of TG and PROT has been proposed  
as bread improver (Gottmann and Sproessler, 1994).

14 Although TG had no significant ( $p<0.05$ ) effect on dough water absorption (WA), this  
16 consistograph parameter decreased significantly ( $p<0.05$ ) when TG and PROT were  
used jointly (Table 5). Babiker et al. (1996) reported an increase in the hydrophobicity  
18 of protease-treated gluten that would justify the decrease observed in WA after PROT  
treatment. These authors also stated that exposed hydrophobic residues were  
20 incorporated inside polymerized protein molecules by TG addition. This mechanism  
would also explain the dough tightness by TG action observed by Gerrard et al. (1998)  
and Basman et al. (2002) after mixing.

22 Polysaccharide-degrading enzymes exercised a significant ( $p<0.05$ ) effect on  
24 rheological properties of dough only when they were used in combination with other  
enzymes, affecting to consistograph parameters (Table 5). In accordance with the  
26 improvement of dough tolerance (Tol) observed, a synergism between TG and PP  
could be concluded. The significant ( $p<0.05$ ) increase of Tol came accompanied by a  
28 significant decrease of decay at 250 s ( $D_{250}$ ). PP has proved to diminish the amount of  
total pentosans associated with the gluten matrix (Primo-Martin et al., 2003) and  
counteract the over-aggregation of gluten (Weegels and Hamer, 1992).

30 The combined use of LAC and PP allowed overcoming significantly ( $p<0.05$ ) the  
32 individual effects of both enzymes on the water absorption (WA). PP counteracted the  
negative effect of LAC on WA due to their contrary enzymatic action (the first one  
34 release pentosans associated with proteins whereas the latter promotes polymetization  
of the pentosans). The synergistic effect of these enzymes are in accordance with the  
36 findings of Primo-Martin et al (2003), who showed a more marked decrease in total  
pentosans associated with glutenin-macropolymer (GMP) than those obtained by the  
38 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.

40 Dough development time (DDT) and tolerance (Tol) were affected significantly by LAC  
42 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-  
44 treated dough. It can be concluded that simultaneous arabinoxylans gelation and  
oxidative action promoted by LAC counteracted partially the hydrolytic activity of PROT  
46 on dough protein fraction. LAC would also favor the interference of pentosans in the  
aggregation of the glutenins (Primo-Martin et al., 2003) modifying the rheological  
behaviour of dough with respect non-supplemented doughs.

48 AMYL and PP exhibited a significant ( $p<0.05$ ) synergistic effect on dough water  
50 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 were added

2 individually, it was proved an antagonist effect of both enzymes on Tol. Alpha-amylase  
4 has been found to cleave long starch chains producing shorter chains or dextrans that  
6 come accompanied by a rapid loss of dough consistency and water absorption (Pylar,  
8 1988) and an increase of dough stickiness (Armero and Collar, 1998). Dextrans may  
10 interference with interactions between the swollen starch granules and the protein  
12 network (Duran et al., 2001) modifying dough tolerance (Tol). PP brought about a  
14 partial solubilization of water insoluble pentosans (WIP) (Rouau and Moreau, 1993),  
16 reducing also the water absorption capacity of dough by releasing the water bound to  
18 pentosans (Martínez-Anaya and Jimenez, 1997). The progressive liberation of free  
20 water molecules (that aids gluten network development), along with the decrease in  
22 pentosan-protein interaction (Primo-Martin et al., 2003), 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 (Martínez-Anaya  
and Jimenez, 1997), which could explain the behaviour of doughs treated with both  
enzymes.

18 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  
20 present in the samples suggesting a strengthening effect promoted by PP probably due  
22 to the diminution of associations of pentosans with glutenin polymers (Primo-Martin et  
al., 2003) and subsequent improvement of gluten quality.

#### 24 **Bread quality of enzyme-supplemented doughs**

26 Individual effects of enzymes on bread quality parameters of doughs are showed in  
28 Table 3. Although gluten cross-linking and gluten degrading enzymes had again more  
30 significant ( $p < 0.05$ ) effects, all enzymes influenced significantly ( $p < 0.05$ ) the bread  
32 quality parameters.

34 Addition of TG led to a significant ( $p < 0.05$ ) increase in height/width ratio and a  
36 decrease in specific volume. The particular effect of TG on bread quality has been  
38 previously studied with contradictory results, and it seems to be tied with different  
factors such as the quantity of water used (Gerrard et al., 1998, Larre et al., 2000;  
Autio et al, 2005), the dose of TG (Basman et al., 2002), and the baking quality of the  
flour (Bauer et al, 2003b). Although enzyme treatment improved the shape of our  
loaves, they were globally less expanded in the course of baking due to strengthening  
effect promoted by TG 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.

40 An opposite effect was observed by adding PROT, since this enzyme increased  
42 significantly ( $p < 0.05$ ) specific volume and decreased slightly height/width ratio of  
44 loaves. The results were in agreement with dough biaxial properties of PROT-  
46 supplemented doughs and reflected the weakening action that this enzyme exerts on  
gluten network. Similar results were obtained by Indrani et al. (2003) who stated  
significant improvements in the specific loaf volume and simultaneous degradation of  
gluten matrix by PROT. Bombara et al. (1997) 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.

48 The oxidative enzymes GO and LAC also exerted a significant ( $p < 0.05$ ) effect on bread  
50 quality. The first one led to improvements in the shape of the loaves whilst the latter  
52 affected positively to their specific volume. The strengthening effect of GO on doughs  
has been widely proved (Martínez-Anaya and Jimenez, 1997; Collar et al., 1998;  
Vemulapalli et al., 1998; Dunnewind et al.; 2002; Primo-Martin et al., 2003; Rosell et  
al., 2003; Gujral & Rosell, 2004b;) and it would explain a greater loaf height after

2 treatment to the detriment of its width. According with the conclusions of Rasiah et al.  
4 (2005) this enzyme showed a small and selective action on dough proteins, reason  
6 why its macroscopic effects on bread quality would not imply important changes in loaf  
8 volume. Additionally, Primo-Martin et al. (2005) concluded that gelation of pentosans  
10 catalyzed by GO affect negatively to bread quality by interfering protein cross-linking. In  
12 spite of it, improvements in the wheat and rice bread loaf volume have been obtained  
14 by adding GO under different test conditions (Vemulapalli et al., 1998; Xia et al., 1999;  
Gujral & Rosell, 2004b). 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 (Figueroa-Espinoza and Rouau, 1998) and the oxidation of sulphhydryl groups  
(Labat et al, 2000). Primo-Martin and Martinez-Anaya (2003) also stated improvements  
in bread volume as consequence of LAC treatment.

16 PP supplementation caused a significant ( $p < 0.05$ ) improvement of loaf specific volume  
18 but did not produce changes in its shape. Krishnarau and Hosney (1994) reported  
20 how the adverse effects of pentosans addition on the loaf volume were overcome by  
22 PP treatment. Indrani et al (2003) also confirmed an important increase in specific  
24 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. The subsequent release of water and later redistribution to gluten has  
been proposed as a way to improve gluten extensibility and bread quality (Martinez-  
Anaya and Jimenez, 1997).

26 Similar effect was exerted by AMYL. Although literature emphasizes the use of this  
28 enzyme to retard bread staling, additional side effects on bread quality have been also  
30 reported. Indrani et al. (2003) obtained a high overall quality score in wheat flour  
32 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  
 $\alpha$ -amylase on long starch chains (Indrani et al., 2003) and a slight leakage of amylose  
(Blaszczak et al., 2004). Alpha-amylase also improved rice bread specific volume and  
crumb firmness but gave very sticky textures (Gujral et al., 2003).

34 Analysis of second order interactive effects of design factors on bread quality  
36 parameters revealed significant ( $p < 0.05$ ) interactions between TG and all the other  
38 enzymes except LAC (Table 6). TG and GO combined exerted a synergist effect on  
40 height/width ratio yielding loaves with greater height. This result was supported by  
42 significant changes observed previously in dough rheology. The marked decrease in  
44 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 promotes the yeast  
action during fermentation, since they degrade the damaged starch into smaller  
dextrins, being able to produce more gas and accentuate the TG effect on loaves  
shape. The binary combination of bacterial alpha-amylase and TG has been reported  
as enhancer of sensory and textural bread profile, but significant effect on volume or  
specific volume was not proved (Collar et al., 2005), which agrees with our results.

46 Addition of PP and PROT to doughs treated with TG counteracted partially the negative  
48 effects of this latter enzyme on loaf specific volume. As we indicated previously, the  
50 release of pentosans associated with proteins improve the quality of gluten network  
52 (Primo-Martín et al., 2003), 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

2 dough development during fermentation and oven-spring, having positive effects on  
loaf volume.

4 GO and LAC combination synergistically led to significant ( $p < 0.05$ ) increase in specific  
6 volume and height/width ratio of the loaves. The increase in this latter parameter was  
8 lower than the one obtained in the presence of GO. Both enzymes are implicated in  
10 reactions by which take place the oxidation of the free sulfhydryl units from gluten  
12 protein giving disulfide linkages and the gelation of water soluble arabinoxylans.  
14 However, strengthening effect has been attributed fundamentally to GO (Martínez-  
16 Anaya and Jiménez, 1997; Vemulapalli et al., 1998; Dunnewind et al. 2002, Gujral &  
18 Rosell, 2004), which would justify the difference in loaves height obtained by GO and  
LAC individual treatment. Primo-Martin et al. (2003) stated an increase of the protein-  
pentosan interaction by the individual addition of GO and LAC, which would further  
interference 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.

20 Similar significant ( $p < 0.05$ ) synergistic effect on bread quality was observed by the  
22 combined use of GO and PP. Since gelation of water soluble arabinoxylans promoted  
24 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 crosslinks by GO has been recently  
proposed as a theory for justifying this synergistic effect (Primo-Martin et al., 2005).

26 Addition of AMYL to LAC containing doughs significantly ( $p < 0.05$ ) increased the  
28 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  
30 with those obtained by AMYL/PROT and PP/PROT combinations. The positive effect of  
32 amylases on yeast action and gas production during fermentation in combination with  
the softening effect promoted by LAC (Primo-Martin et al., 2003) and PROT (Wikstrom  
34 and Eliasson, 1998; Indrani et al., 2003) on the gluten proteins led to increase in  
36 volume of loaves. On the other hand, PP action has been related with the increase of  
gluten strength and elasticity (Weegels and Hamer, 1992; Primo-Martin et al., 2003;  
Collar and Bollaín, 2005). 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.

38

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

42 Analytical data were undergone to a Pearson correlation analysis in order to establish  
44 significant relationships between rheological and bread quality parameters of enzyme-  
46 supplemented doughs. A Durbin-Watson (DW) statistic test of the residuals was  
performed to determine if there 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 the Table 7.

48 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  
50 properties of dough, namely its biaxial extensibility (Dobraszczyk and Morgenstern,

2 2003). 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  
4 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  
6 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  
8 increased with dough after three hours resting period, thus the time of the test  
enhances the prediction of bread quality parameters from rheological properties.  
10 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  
12 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).  
14 Likewise, two parameters of consistograph test, namely decay at 250 and 450 s ( $D_{250}$   
and  $D_{450}$ ) showed negative correlations with the cited ratio. Decay of consistograph  
16 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.  
18  $D_{450}$  showed greater correlation than  $D_{250}$  ( $r=-0.6559$  and  $r=-0.5015$  respectively).

The relationships between loaf specific volume and empiric rheological parameters  
20 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  
22 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  
24 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  
26 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 three  
28 hours 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  
30 low ( $r=0.4183$ ). High dough mixing stability corresponded to loaves with less specific  
volume.

32

## Conclusions

34 Single addition of gluten cross-linking and gluten degrading enzymes showed more  
significant and greater effects on rheological properties than polysaccharide degrading  
36 enzymes. The most important effect on alveograph parameters were provided by TG  
and PROT. Resting period accentuated differences between alveograph properties of  
38 supplemented and non-supplemented doughs, with more significant effects especially  
after TG and GO treatments. The analysis of consistograph data revealed a trend  
40 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  
42 rheological behaviour of doughs. Polysaccharide-degrading enzymes exercised a  
significant effect on rheological properties of dough only when they were used in  
44 combination with other enzymes, affecting to consistograph parameters.

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

- 2 quality was observed by the combined use of GO and LAC, GO and PP, AMYL and LAC, AMYL and PROT, and PP and PROT.
- 4 Bread quality parameters showed greater correlations with alveograph parameters than  
6 with consistograph properties of dough. As general remark, tenacity (P) and  
8 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.  
On the contrary, none of the studied rheological properties could be considered as a  
good predictor of specific volume of rolled breads.

## 2 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 would like to thank Ramiro Martínez (Novo Nordisk, Madrid, Spain) for providing enzyme samples.

## 8 References.

- AACC. Approved methods of the AACC, 2000. American Association of Cereal Chemists, St. Paul, Minnesota.
- Aja, S., Wang, J., and Rosell, C.M. 2003. Improvement of cereal protein network through enzyme treatment. In: Recent advances in enzymes in grain processing. Ed CM Courtin, WS Veraverbeke, JA Delcour. pp 101-106.
- Ameille, V; Castello, P; Garcia, R; Rakotozafy, L; Potus, J; Nicolas, J. Effects of glucose oxidase or lipase addition on dough consistency and oxygen consumption during mixing of unyeasted flour dough. *Sciences-des-Aliments*. 2000; 20(4/5): 441-455.
- Armero, E.; Collar, C.; 1997. Texture properties of formulated wheat doughs. Relationships with dough and bread technological quality. *Z. Lebensm. Unters. Forsch. A*. 204(2): 136-145.
- Autio, K.; Kruus, K.; Knaapila, A.; Gerber, N.; Flander, L.; Buchert, J., 2005. Kinetics of transglutaminase-induced cross-linking of wheat proteins in dough. *Journal of Agricultural and Food Chemistry* 53(4): 1039-1045.
- Babiker, E.E., Fujisawa, N., Matsudomi, N., Kato, A. 1996. Improvement in the functional properties of gluten by protease digestion or acid hydrolysis followed by microbial transglutaminase treatment. *Journal of Agricultural Food Chemistry*, 44, 3746-3750.
- Basman, A., Köksel H., Perry, K.W.N., 2002. Effects of increasing levels of transglutaminase on the rheological properties and bread quality of two wheat flours *Eur. Food Res. Technol.* 215, 419-424.
- Bauer, N., Koehler, P., Wieser, H., Schieberle, P., 2003a. Studies of the effects of microbial transglutaminase on gluten proteins of wheat I: Biochemical analysis. *Cereal Chem.* 80, 6, 781-786.
- Bauer, N., Koehler, P., Wieser, H., Schieberle, P., 2003b. Studies of the effects of microbial transglutaminase on gluten proteins of wheat II: Rheological properties. *Cereal Chem.* 80, 6, 787-790.
- Blaszczak, W.; Sadowska, J.; Rosell, C.M.; Fornal, J.; 2004. Structural changes in the wheat dough and bread with the addition of alpha-amylases. *European Food Research and Technology* 219(4): 348-354.
- Blumenthal, C.S.; Barlow, E.W.R.; Wrigley, C.W., 1993. Growth environment and wheat quality: the effect of heat stress on dough properties and gluten proteins. *Journal of Cereal Science*, 18(1): 3-21.
- Bollain, C.; Angioloni, A.; Collar, C.; 2005. Bread staling assessment of enzyme-supplemented pan breads by dynamic and static deformation measurements. *European Food Research and Technology* 220(1): 83-89.
- Bombara, N.; Anon, M.C.; Pilosof, A.M.R. 1997. Functional properties of protease modified wheat flours. *Lebensmittel Wissenschaft und Technologie* 30(5): 441-447.
- Bonet, A.; Caballero, P.A.; Gomez, M.; Rosell, C.M.; 2005. Microbial transglutaminase as a tool to restore the functionality of gluten from insect-damaged wheat. *Cereal-Chemistry* 82(4): 425-430.

- 2 Caballero, P.A.; Bonet, A.; Rosell, C.M.; Gomez, M.; 2005. Effect of microbial  
transglutaminase on the rheological and thermal properties of insect damaged  
4 wheat flour. *Journal of Cereal Science* 42(1): 93-100.
- 6 Collar, C.; Andreu, P.; Martinez-Anaya, M.A.; 1998. Interactive effects of flour, starter  
and enzyme on bread dough machinability. *Z. Lebensm. Unters. Forsch. A.*  
207(2): 133-139
- 8 Collar, C.; Bollain, C.; Angioloni, A.; 2005. Significance of microbial transglutaminase  
on the sensory, mechanical and crumb grain pattern of enzyme supplemented  
10 fresh pan breads. *Journal of Food Engineering* 70(4): 479-488.
- 12 Collar, C.; Bollain, C.; 2005. Impact of microbial transglutaminase on the staling  
behaviour of enzyme supplemented pan breads. *Eur. Food Res. Technol.*  
221:298-304.
- 14 Dobraszczyk, B.J.; Morgenstern, M.P.; 2003. Rheology and the breadmaking process.  
*Journal of Cereal Science* 38(3): 229-245.
- 16 Dunnewind, B., Van Vliet, T., and Orsel, R. 2002. Effect of oxidative enzymes on bulk  
rheological properties of wheat flour doughs. *J. Cereal Sci.* 36:357-366.
- 18 Dupuis, B., 1997. The chemistry and toxicology of potassium bromate. *Cereal Foods*  
*World* 42, 171-183.
- 20 Duran, E.; Leon, A.; Barber, B.; Benedito de Barber, C.; 2001. Effect of low molecular  
weight dextrans on gelatinization and retrogradation of starch. *European Food*  
22 *Research and Technology* 212(2): 203-207.
- 24 Figueroa-Espinoza, MC.; Rouau, X. 1998. Oxidative cross-linking of pentosans by a  
fungal laccase and horseradish peroxidase: mechanism of linkage between  
feruloylated arabinoxylans. *Cereal Chemistry* 75(2): 259-265.
- 26 Figueroa-Espinoza, M-C; Morel, M-H; Rouau, X. 1998. Effect of lysine, tyrosine,  
cysteine, and glutathione on the oxidative cross-linking of feruloylated  
28 arabinoxylans by a fungal laccase. *Journal of Agricultural and Food Chemistry*  
46(7): 2583-2589.
- 30 Figueroa-Espinoza, MC; Morel, MH.; Surget, A.; Asther, M.; Moukha, S; Sigoillot, JC.;  
Rouau, X. 1999. Attempt to cross-link feruloylated arabinoxylans and proteins  
32 with a fungal laccase. *Food-Hydrocolloids* 13(1): 65-71.
- 34 Fisher, N.; Hutchinson, J.B.; Berry, R.; Hardy, J.; Ginocchio, A.V.; Waite, Y., 1979.  
Long-term toxicity and carcinogenicity studies of the bread improver potassium  
bromate.1. Studies in rats. *Food Cosmet. Toxicol.* 17, 33-39.
- 36 Garcia, R.; Rakotozafy, L.; Nicolas, J. 2004. Analysis and modeling of the ferulic acid  
oxidation by a glucose oxidase-peroxidase association. Comparison with a  
38 hexose oxidase-peroxidase association. *Journal of Agricultural and Food*  
*Chemistry* 52(12): 3946-3953.
- 40 Gerrard, J.A., Fayle, S.E., Wilson, A.J., Newberry, M.P., Ross, M., Kavale, S., 1998.  
Dough properties and crumb strength of white pan bread as affected by microbial  
42 transglutaminase. *J. Food Sci.* 63, 472-475.
- 44 Gerrard, J.A., Newberry, M.P., Ross, M., Wilson, A.J., Fayle, S.E., Kavale, S., 2000.  
Pastry lift and croissant volume as affected by microbial transglutaminase. *J.*  
*Food Sci.* 65, 312-314.
- 46 Gerrard, J.A., Fayle, S.E., Brown, P.A., Sutton, K.H., Simmons, L., Rasiah, I., 2001.  
Effects of microbial transglutaminase on the wheat proteins of bread and  
48 croissant dough. *J. Food Sci.* 66, 6, 782-786.
- Gerrard, J.A., 2002. Protein-protein crosslinking in food: methods, consequences,  
50 applications. *Trends in Food Science and Technology.* 13:389-397.
- 52 Gottmann, K.; Sproessler, B.; 1994. Baking agent and process for the manufacture of  
doughs and bakery products. *European Patent Application EP 0492406, B1.*
- 54 Gujral, H.S.; Haros, M.; Rosell, C.M.; 2003. Starch hydrolyzing enzymes for retarding  
the staling of rice bread. *Cereal Chemistry* 80(6): 750-754.
- 56 Gujral H.S., and Rosell C.M., 2004b. Improvement of the breadmaking quality of rice  
flour by glucose oxidase. *Food Res. Int.* 37:75-81.

- 2 Gujral, H.S., Rosell, C.M., 2004a. Functionality of rice flour modified with a microbial  
transglutaminase. *J. Cereal Sci.* 39, 225-230.
- 4 Haarasilta, S., Pullinen, T., Vaisanen S., and Tammersalo-Karsten, I. 1991. Enzyme  
product and method of improving the properties of dough and the quality of bread.  
6 United States Patent Nr. 4,990,343.
- 8 Hosoney, R.C., and Faubion J.M. 1981. A mechanism for the oxidative gelation of  
wheat flour water soluble pentosans. *Cereal Chem.* 58:421-424.
- 10 Indrani, D.; Prabhasankar, P.; Rajiv, J.; Venkateswara-Rao, G.; 2003. Scanning  
electron microscopy, rheological characteristics, and bread-baking performance  
12 of wheat-flour dough as affected by enzymes. *Journal of Food Science* 68(9):  
2804-2809.
- 14 Iriki, N.; Yamauchi, H.; Takata, K.; Nishio, Z.; Ichinose, Y.; Yoshihira, T. (2003). Effects  
of genotype and growth conditions on apparent viscosity of heat-treated flour  
paste and their correlation with certain flour properties in wheat produced in  
16 Hokkaido. *Food Science and Technology-Research*, 9(1): 104-109.
- 18 Krishnarau, L.; Hosoney, R.C., 1994. Enzymes increase loaf volume of bread  
supplemented with starch tailings and insoluble pentosans. *Journal of Food  
Science* 59(6): 1251-1254.
- 20 Kurokawa, Y.; Maekawa, A.; Takahashi, M.; Hayashi, Y., 1990. Toxicity and  
carcinogenicity of potassium bromate, a new renal carcinogen. *Environ. Health  
22 Perspect.*, 87, 309-335.
- 24 Labat, E.; Morel, MH.; Rouau, X. 2000. Effects of laccase and ferulic acid on wheat  
flour doughs. *Cereal-Chemistry* 77(6): 823-828.
- 26 Labat, E; Morel, MH.; Rouau, X. 2001. Effect of laccase and manganese peroxidase on  
wheat gluten and pentosans during mixing. *Food-Hydrocolloids* 15(1): 47-52.
- 28 Larré, C., Denery, P.S., Popineau, Y., Deshayes, G., Desserre, C., Lefevre, J., 2000.  
Biochemical analysis and rheological properties of gluten modified by  
transglutaminase. *Cereal Chem.* 77, 32-38.
- 30 Martinez-Anaya, M.A.; Jimenez, T.; 1997. Rheological properties of enzyme  
supplemented doughs. *Journal of Texture Studies* 28(5): 569-583.
- 32 Motoki, M., Seguro, K., 1998. Transglutaminase and its use for food processing.  
*Trends Food Sci. Technol.* 9, 204-210.
- 34 Mujoo, R.; Ng, P.K.W., 2003. Identification of wheat protein components involved in  
polymer formation on incubation with transglutaminase. *Cereal-Chemistry* 80(6):  
36 703-706.
- 38 Nakai, K., Takami, K., Yanaka, N., and Takasaki, Y. (1995). Bread quality-improving  
composition and bread producing process using the same. *European Patent  
Application*. 0,686,348 A1.
- 40 Oudgenoeg, G.; Hilhorst, R.; Piersma, S.R.; Boeriu, C.G; Gruppen, H.; Helsing, M.;  
Voragen, A.G.; Laane, C. 2005. Peroxidase-Mediated Cross-Linking of a  
42 Tyrosine-Containing Peptide with Ferulic Acid. *J. Agric. Food Chem.* 49, 2503-  
2510.
- 44 Pedersen, L.; Kaack, K.; Bergsoe, M.N.; Adler-Nissen, J. 2005. Effects of chemical and  
enzymatic modification on dough rheology and biscuit characteristics. *Journal of  
46 Food Science* 70(2): 152-158.
- 48 Perrotta, C.; Treglia, A.S.; Mita, G.; Giangrande, E.; Rampino, P.; Ronga, G.; Spano,  
G.; Marmiroli, N., 1998. Analysis of mRNAs from ripening wheat seeds: the effect  
of high temperature. *Journal of Cereal Science* 27(2): 127-132.
- 50 Poulsen, C.; Hostrup, PB. 1998. Purification and characterization of a hexose oxidase  
with excellent strengthening effects in bread. *Cereal Chemistry* 75(1): 51-57.
- 52 Primo-Martin, C.; Martinez-Anaya, M.A.; 2003. Influence of pentosanase and oxidases  
on water-extractable pentosans during a straight breadmaking process. *Journal of  
54 Food Science* 68(1): 31-41

- 2 Primo-Martin, C.; Martinez-Anaya, M.A.; Collar, C. 2004. Composition of the glutenin  
 4 macropolymer: effects of flour quality and nonamylolytic enzyme addition.  
 European-Food-Research-and-Technology 218(5): 428-436.
- 6 Primo-Martin, C.; Wang M.; Lichtendonk, W.J.; Plijter, J.J.; Hamer, R.J. 2005. An  
 explanation for the combined effect of xylanase-glucose oxidase in dough  
 systems. *J. Sci Food Agric* 85:1186-1196.
- 8 Primo-Martin, C.; Valera, R., and Martínez-Anaya, M.A. (2003). Effect of pentosanase  
 and oxidases on the characteristics of doughs and the glutenin macropolymer  
 10 (GMP). *J. Agric. Food Chem.* 51:4673-4679.
- 12 Pylar, E.J.; 1988. *Baking Science and Technology*. Sosland Publishing Company.  
 Merriam. Kansas.
- 14 Rakotozafy, L.; Mackova, B.; Delcros, J.F.; Boussard, A.; Davidou, S.; Potus, J.;  
 Nicolas, J. 1999. Effect of adding exogenous oxidative enzymes on the activity of  
 three endogenous oxidoreductases during mixing of wheat flour dough. *Cereal-*  
 16 *Chemistry* 76(2): 213-218.
- 18 Rasiah, I.A., Sutton, K.H., Low, F.L., Lin, H.M. Gerrard, J.A. (2005). Crosslinking of  
 wheat dough proteins by glucose oxidase and the resulting effects on bread and  
 croissants. *Food Chem.* 89:325-332.
- 20 Rosell, C.M., Wang, J.; Aja, S., Bean, S., Lookhart, G., 2003. Wheat flour proteins as  
 affected by transglutaminase and glucose oxidase. *Cereal Chem.* 80, 52-55.
- 22 Rouau, X.; Moreau, D.; 1993. Modification of some physicochemical properties of  
 wheat flour pentosans by an enzyme complex recommended for baking. *Cereal-*  
 24 *Chemistry* 70(6): 626-632.
- 26 Rouille, J.; Della Valle, G.; Devaux, M.F; Marion, D., Dubreil, L.; 2005. French bread  
 loaf volume variations and digital image analysis of crumb grain changes induced  
 by the minor components of wheat flour. *Cereal Chem.* 82 (1):20-27.
- 28 Si, J.Q.; 1994. Use of laccase in baking. International Patent Application WO 94/28728
- 30 Stauffer, C.E., 1990. *Functional additives for bakery foods*. Ed. Van Nostrand Reinhold,  
 New York.
- 32 Tilley KA, Benjamin RE, Bagorogoza KE, Moses Okot-Kotber B, Prakash O, Kwen H.  
 2001. Tyrosine cross-links: molecular basis of gluten structure and function. *J.*  
*Agric. Food Chem.* 49:2627-2632.
- 34 Vemulapalli, V., Hosney, R.C. 1998. Glucose oxidase effects on gluten and water  
 solubles. *Cereal Chem.* 75(6): 859-862.
- 36 Vemulapalli, V; Miller, K.A., and Hosney, R. C. 1998. Glucose oxidase in breadmaking  
 systems. *Cereal Chem.* 75(4): 439-442
- 38 Wikstrom, K; Eliasson, AC. 1998. Effects of enzymes and oxidizing agents on shear  
 stress relaxation of wheat flour dough: additions of protease, glucose oxidase,  
 40 ascorbic acid, and potassium bromate. *Cereal-Chemistry.* 75(3): 331-337.
- 42 Weegels, P.L.; Hamer, R.J.; 1992. Improving the bread-making quality of gluten.  
*Cereal Foods World* 37(5): 379-385.
- 44 Wolf D.C., Crosby L.M., George M.H., Kilbur S.R., Moore T.M., Miller R.T. and  
 DeAngelo A.B., 1998. Time and dose dependent development of potassium  
 bromate induced tumors in male Fischer 344 rats. *Toxicol. Pathol.* 26: 724-729.
- 46 Tseng and Lai (2002)
- 48 Koksel et al (2001)
- Xia et al (1999)
- 50 Armero and Collar (1998)
- Estas citas no aparecen en el listado de referencias.

**Table 1.** Characteristics of wheat flour

	Flour
<i>Chemical composition</i>	
Protein (% d. wt.)	11,00
Ash (% d. wt.)	0,58
Moisture ) (% d. wt.)	12,16
<i>Consistogram</i>	
Water absorption (%)	52,8
<i>Alveogram</i>	
Deformation energy ( $10^{-4}$ J)	146
Curve configuration ratio (P/L)	0,35
<i>Gluten Index</i>	
Gluten Index (%)	94,0
Dry Gluten (%)	9,0
Wet Gluten (%)	26,6
<i>Falling Number</i>	
Time (s)	405

d. wt. : dry weight

**Table 2.** Half fraction factorial design 2<sup>6</sup> for sampling

Sample no.	Factors <sup>a</sup>					
	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

- 4 <sup>a</sup>Levels (0,1) of factors (A to F): A = Transglutaminase (TG): none (0), 500 mg/100g 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).
- 6

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

Parameter	Units	Overall mean	TG <sup>a</sup>		GO		LAC		AMYL		PP		PROT		
			0	1	0	1	0	1	0	1	0	1	0	1	
<i>P</i>	mm H <sub>2</sub> O	46	<b>37</b>	<b>54</b> *	44	47	46	46	46	45	47	44	<b>50</b>	<b>41</b> *	
<i>L</i>	mm H <sub>2</sub> O	119	<b>144</b>	<b>94</b> *	125	113	123	115	120	118	114	124	110	128	
<i>W</i>	x 10 <sup>-4</sup> J	162	<b>142</b>	<b>183</b> *	161	164	164	160	161	164	158	166	<b>173</b>	<b>151</b> *	
<i>P/L</i>		0.44	<b>0.26</b>	<b>0.62</b> *	0.41	0.47	0.42	0.46	0.45	0.43	0.47	0.41	<b>0.52</b>	<b>0.36</b> *	
<i>P<sub>3h</sub></i>	mm H <sub>2</sub> O	69	<b>31</b>	<b>106</b> *	66	71	71	66	72	65	70	67	<b>82</b>	<b>55</b> *	
<i>L<sub>3h</sub></i>	mm H <sub>2</sub> O	69	<b>103</b>	<b>34</b> *	<b>86</b>	<b>51</b> *	69	69	68	70	68	70	61	76	
<i>W<sub>3h</sub></i>	x 10 <sup>-4</sup> J	132	<b>99</b>	<b>166</b> *	131	134	136	129	136	129	130	135	140	125	
<i>P/L<sub>3h</sub></i>		1.89	<b>0.37</b>	<b>3.42</b> *	1.88	1.91	1.97	1.82	1.99	1.80	1.98	1.81	<b>2.54</b>	<b>1.26</b> *	
<i>WA</i>	%	50.9	51.1	50.7	51.0	50.8	51.2	50.6	50.7	51.1	50.7	51.1	51.1	50.7	
<i>DDT</i>	s	79	78	80	79	79	80	78	81	77	80	78	82	76	
<i>Tol</i>	s	127	<b>120</b>	<b>134</b> *	127	127	127	128	130	124	125	129	132	123	
<i>D<sub>250</sub></i>	mb	747	<b>832</b>	<b>663</b> *	751	744	751	744	750	745	755	740	724	771	
<i>D<sub>450</sub></i>	mb	1199	<b>1223</b>	<b>1015</b> *	1120	1119	1118	1121	1101	1138	1117	1122	<b>1084</b>	<b>1155</b> *	
<i>Height/Width ratio</i>		0.58	<b>0.45</b>	<b>0.71</b> *	<b>0.51</b>	<b>0.65</b> *	0.59	0.57	0.59	0.57	0.58	0.57	<b>0.60</b>	<b>0.56</b> *	
<i>Specific volume</i>	(cm <sup>3</sup> /g)	3.70	<b>3.94</b>	<b>3.46</b> *	3.66	3.73	<b>3.58</b>	<b>3.81</b> *	<b>3.44</b>	<b>3.95</b> *	<b>3.51</b>	<b>3.88</b> *	<b>3.44</b>	<b>3.94</b> *	

4 <sup>a</sup>See table 2 for levels of design factors.

\* The effect of the factor is significant with a significance level of 95% (p&lt;0.05)

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

Parameter	Units	Overall mean	Level <sup>a</sup>	TG/GO	TG/LAC	TG/AMYL	TG/PP	TG/PROT	GO/LAC	GO/AMYL	GO/PP	GO/PROT	LAC/AMYL	LAC/PP	LAC/PROT	AMYL/PP	AMYL/PROT	PP/PROT
P	mm H <sub>2</sub> O	46	00	35	37	37	38	42	46	44	46	49	46	47	50	49	50	53
			01	39	37	37	36	32	43	45	43	40	46	45	42	44	43	42
			10	54	55	55	57	60	46	49	49	43	47	48	52	46	52	49
			11	56	55	54	53	50	49	46	46	42	45	44	40	45	39	40
L	mm H <sub>2</sub> O	119	00	156	150	149	140	135	129	129	120	113	130	119	117	115	111	105
			01	132	139	140	148	153	121	121	130	137	117	128	130	125	129	124
			10	94	97	91	89	86	118	111	109	108	110	110	104	114	110	116
			11	94	92	97	100	102	109	115	118	119	120	121	126	123	127	132
W	x 10 <sup>-4</sup> J	162	00	137	141	144	135	152	167	156	156	170	170	161	173	161	167	170
			01	147	143	140	149	132	155	167	166	153	159	167	156	161	155	147
			10	185	188	179	182	195	162	166	161	177	152	156	174	156	180	177
			11	181	179	188	185	171	166	161	167	151	169	166	147	172	148	156
P/L		0.44	00	0.23	0.26	0.26	0.27	0.31	0.42	0.40	0.45	0.48	0.39	0.43	0.47	0.49	0.51	0.56
			01	0.29	0.27	0.27	0.25	0.21	0.41	0.42	0.37	0.34	0.45	0.41	0.37	0.41	0.39	0.38
			10	0.59	0.58	0.64	0.68	0.73	0.42	0.50	0.50	0.56	0.52	0.52	0.57	0.46	0.53	0.48
			11	0.65	0.66	0.60	0.57	0.51	0.52	0.44	0.45	0.38	0.42	0.41	0.36	0.41	0.34	0.34
P <sub>3h</sub>	mmH <sub>2</sub> O	69	00	28	33	33	32	<b>37*</b>	72	68	70	76	72	72	82	77	87	87
			01	35	30	30	31	<b>26</b>	61	65	64	57	71	71	61	67	58	54
			10	105	110	112	109	<b>128</b>	71	76	72	88	72	69	83	64	78	78
			11	107	102	101	104	<b>85</b>	72	66	71	54	60	63	50	67	53	57
L <sub>3h</sub>	mmH <sub>2</sub> O	69	00	<b>140*</b>	104	103	102	95	83	85	88	74	69	70	56	63	60	62
			01	<b>67</b>	102	104	104	112	88	87	84	98	68	67	81	73	75	74
			10	<b>32</b>	33	33	34	28	54	51	48	48	66	65	66	73	61	60
			11	<b>37</b>	36	35	35	41	49	52	56	55	72	72	72	67	78	79
W <sub>3h</sub>	x 10 <sup>-4</sup> J	132	00	<b>107*</b>	102	99	95	<b>112*</b>	134	134	133	135	139	133	140	138	144	142
			01	<b>90</b>	96	99	102	<b>85</b>	128	128	129	127	132	138	131	134	128	118
			10	<b>154</b>	169	172	164	<b>167</b>	137	137	127	145	132	127	139	122	136	138
			11	<b>177</b>	162	159	167	<b>164</b>	130	130	140	122	126	131	119	136	122	131
P/L <sub>3h</sub>		1.89	00	0.22	0.38	0.38	0.40	<b>0.45*</b>	2.14	1.88	1.99	2.37	1.92	2.02	2.44	2.17	2.67	2.73
			01	0.53	0.37	0.36	0.35	<b>0.30</b>	1.62	1.88	1.77	1.39	2.03	1.93	1.51	1.82	1.31	1.23
			10	3.55	3.57	3.60	3.56	<b>4.63</b>	1.81	2.11	1.98	2.7	2.06	1.94	2.64	1.79	2.04	2.34
			11	3.3	3.27	3.24	3.28	<b>2.21</b>	2.02	1.72	1.85	1.13	1.58	1.70	1.00	1.81	1.20	1.28

<sup>a</sup>See table 2 for levels of design factors.

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

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

Parameter	Units	Overall mean	Level <sup>a</sup>	TG/GO	TG/LAC	TG/AMYL	TG/PP	TG/PROT	GO/LAC	GO/AMYL	GO/PP	GO/PROT	LAC/AMYL	LAC/PP	LAC/PROT	AMYL/PP	AMYL/PROT	PP/PROT
WA	%	50.9	00	51,4	51,1	50,7	50,9	<b>51,6*</b>	51,0	50,9	50,9	51,2	50,8	<b>51,6*</b>	51,2	<b>50,8*</b>	50,8	<b>51,3*</b>
			01	50,8	51,0	51,5	51,3	<b>50,6</b>	50,9	51,1	51,1	50,8	51,6	<b>50,8</b>	51,1	<b>50,5</b>	50,5	<b>50,1</b>
			10	50,6	51,2	50,6	50,5	<b>50,6</b>	51,3	50,6	50,5	50,9	50,6	<b>49,9</b>	50,9	<b>50,6</b>	51,4	<b>50,9</b>
			11	50,8	50,2	50,8	50,9	<b>50,8</b>	50,4	51,1	51,1	50,7	50,7	<b>51,4</b>	50,4	<b>51,7</b>	50,9	<b>51,3</b>
DDT	s	79	00	79	80	81	82	83	78	83	73	82	80	84	<b>88*</b>	79	84	82
			01	78	77	76	76	74	79	75	75	76	80	76	<b>73</b>	83	78	78
			10	78	80	81	81	82	82	79	78	83	82	77	<b>77</b>	81	79	82
			11	81	79	79	79	78	77	79	82	76	75	79	<b>80</b>	73	75	74
Tol	s	127	00	120	120	123	<b>126*</b>	127	124	134	129	131	130	131	<b>139*</b>	<b>122*</b>	135	127
			01	120	120	118	<b>114</b>	113	130	120	124	123	123	<b>114</b>	<b>139</b>	126	124	
			10	134	134	138	<b>124</b>	136	130	127	121	133	130	120	<b>124</b>	<b>129</b>	129	136
			11	135	135	131	<b>144</b>	144	124	128	134	122	125	135	<b>131</b>	<b>119</b>	119	122
D <sub>250</sub>	mb	747	00	850	842	840	<b>787*</b>	786	779	731	731	729	747	738	698	768	725	740
			01	813	821	823	<b>876</b>	877	722	771	770	773	756	764	803	731	774	769
			10	652	660	659	<b>722</b>	662	723	768	778	719	752	771	749	742	723	708
			11	675	666	668	<b>604</b>	665	765	720	710	769	735	717	738	749	768	773
D <sub>450</sub>	mb	1199	00	1244	1235	1213	1189	1194	1126	1087	1104	1096	1101	1110	1069	1093	1052	1078
			01	1203	1212	1234	1257	1252	1114	1153	1136	1145	1135	1126	1166	1103	1149	1157
			10	996	1001	988	1048	974	1109	1114	1131	1073	1100	1124	1099	1137	1117	1091
			11	1034	1030	1043	986	1057	1128	1123	1107	1164	1142	1117	1143	1140	1159	1151

<sup>a</sup>See table 2 for levels of design factors.

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

2

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

Parameter	Units	Overall mean	Level <sup>a</sup>	TG/GO	TG/LAC	TG/AMYL	TG/PP	TG/PROT	GO/LAC	GO/AMYL	GO/PP	GO/PROT	LAC/AMYL	LAC/PP	LAC/PROT	AMYL/PP	AMYL/PROT	PP/PROT		
<i>Height/Width ratio</i>		0.58	00	<b>0.35*</b>	0.47	<b>0.48*</b>	0.45	0.47	<b>0.51*</b>	0.51	<b>0.53*</b>	0.54	0.60	0.59	0.60	0.59	0.60	0.60		
			01	<b>0.55</b>	0.43	<b>0.42</b>	0.45	0.43	<b>0.51</b>	0.51	<b>0.49</b>	0.48	0.58	0.59	0.58	0.58	0.58	0.57	0.57	
			10	<b>0.68</b>	0.71	<b>0.70</b>	0.72	0.73	<b>0.67</b>	0.66	<b>0.63</b>	0.65	0.57	0.58	0.60	0.57	0.60	0.57	0.60	0.60
			11	<b>0.74</b>	0.70	<b>0.72</b>	0.70	0.69	<b>0.62</b>	0.63	<b>0.66</b>	0.64	0.56	0.55	0.53	0.57	0.54	0.54	0.54	
<i>Specific volume</i>	(cm <sup>3</sup> /g)	3.70	00	3.88	3.86	3.74	<b>3.86*</b>	<b>3.86*</b>	<b>3.65*</b>	3.47	<b>3.36*</b>	3.40	<b>3.47*</b>	3.42	3.38	3.31	<b>3.30*</b>	<b>3.35*</b>		
			01	3.99	4.00	4.13	<b>4.00</b>	<b>4.01</b>	<b>3.67</b>	3.85	<b>3.96</b>	3.92	<b>3.69</b>	3.74	3.77	3.58	<b>3.59</b>	<b>3.67</b>		
			10	3.44	3.29	3.14	<b>3.16</b>	<b>3.03</b>	<b>3.51</b>	3.41	<b>3.66</b>	3.49	<b>3.41</b>	3.60	3.51	3.72	<b>3.59</b>	<b>3.54</b>		
			11	3.47	3.61	3.77	<b>3.75</b>	<b>3.88</b>	<b>3.94</b>	4.04	<b>3.80</b>	3.96	<b>4.20</b>	4.02	4.11	4.18	<b>4.30</b>	<b>4.22</b>		

<sup>a</sup>See table 2 for levels of design factors.

\* The effect of the factor is significant with a significance level of 95% (p&lt;0.05)

4

2

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

Parameter	P (mm H <sub>2</sub> O)	L (mm H <sub>2</sub> O)	W ( $\times 10^{-4}$ J)	P/L	P <sub>3h</sub> (mm H <sub>2</sub> O)	L <sub>3h</sub> (mm H <sub>2</sub> O)	W <sub>3h</sub> ( $\times 10^{-4}$ J)	P/L <sub>3h</sub>	WA (%)	DDT (s)	Tol (s)	D <sub>250</sub> (mb)	D <sub>450</sub> (mb)
<i>Height/Width ratio</i>	0.7447	-0.7223	0.6854	0.7030	0.7605	-0.8401	0.7036	0.6983				-0.5015	-0.6559
<i>Specific volume (cm<sup>3</sup>/g)</i>	-0.5828	0.5155	-0.3201	-0.6201	-0.5787	0.3130	-0.4176	-0.5913					0.4183