BREAD QUALITY AND DOUGH RHEOLOGY OF ENZYME SUPPLEMENTED WHEAT FLOUR
EFFECT OF ENZYME COMBINATION ON DOUGH RHEOLOGY AND BREAD QUALITY

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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 (glucose oxidase, transglutaminase, laccase, protease, pentosanase, α-amylase) applied nowadays in bread-making processes were investigated. The blend of enzymes allowed to improve the rheological behaviour 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) led to a synergistic effect on alveograph parameters. Polysaccharide-degrading enzymes exercised a significant effect on rheology only when they were used in combination with other enzymes, affecting mainly to consistograph parameters. Analysis of breadmaking 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 height/width ratio of loaves. The duration of the alveograph 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.

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

Introduction

In the last years, the baking industry has undergone very important changes in its productive processes. The increasing mechanization of its processing unit operations has been one of the major changes. This fact has contributed to increase the demand of strong wheat flours, able to generate doughs with high tolerance to handling and mixing, and 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 us the wheat variety and the growth conditions (Blumenthal et al, 1993; Perrotta et al, 1998; Iriki et al, 2003). 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 to adjust the variations in flour properties and baking conditions. Nowadays, the baking industry is deeply involved in research for alternatives to chemical compounds due to their potential hazards (Fisher et al, 1979, Kurokawa et al, 1990; Dupuis, 1997, Wolf et al, 1998). 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 the 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 in the label, which is an additional commercial advantage.

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

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 (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.

Besides the gluten network, another secondary crosslinks 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 (Haarasilta et al, 1991), TG/amylase/hemicellulase (Gottmann and Sproessler, 1994) and TG/pentosanase/α-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 actively to fresh bread quality and staling behaviour during storage.

The objective of this study was to analyse the individual and synergistic effects of a wide enzyme range used nowadays 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 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

A commercial blend of wheat flours provided by Harinera La 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 10000 glucose oxidase units/g, a pentosanase [Pentopan Mono BG (PP)] containing 2500 fungal xylanase units/g, a laccase [NZ 27011 (LAC)] containing 10500 phenol oxidase units/g, an amylase [Fungamyl SG (AMYL)] containing 2500 fungal amylase units/g, a protease [Flavourzyme 1000 L (PROT)] containing 1000 aminopeptidase units/g [all of them from Novozymes (Denmark)], and transglutaminase [Microbial TGM Activa WM (TG)] containing 100 transglutaminase units/g, 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 one hour before the tests, using a Rotary Mixer MR 2L (Chopin, Tripette et Renaud, France).

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.

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 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 3 hours resting period at 25°C in order to assess theproteolytic 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 (AACC, 2000). 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 250s (D_{250}, consistency difference, in mb units, between height at peak 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 with dough mixing stability. Higher stability means lower D_{250} and D_{450} values.

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 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 optimally mixed until dough development, divided into 315 g pieces, hand-rounded, mechanically moulded, put on trays, and proofed for 90 min at 30ºC 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 (Rouille et al., 2005). The pieces were baked into an electric oven for 35 min at 200ºC. Loaves were removed from the trays and cooled for two hours 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 2-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 polisaccharide 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 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 extensibility (L). As consequence, curve configuration ratio augmented significantly ($p<0.05$). These results were expected since previous studies have 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 (Larre 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 ($p<0.05$) 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 (1998), Indrani et al (2003) and Pedersen et al. (2005), 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 (Bombara et al., 1997). The micrographs of wheat dough with PROT have revealed a disruption of gluten matrix with the presence of some small pits (Indrani et al., 2003).

Resting period 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 three hours 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. (1998) who 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 time, and affected to dough extensibility (L). This parameter diminished because of the different mechanisms implicated in strengthening action of the enzyme. Although 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 also established that GO was much more effective at improving bread quality after longer fermentation processes, envisaging 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 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 (Vemulapalli et al., 1998; Wikström and Eliasson, 1998), as well as the modification of 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 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 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 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 ($p<0.05$) 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 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 more time to act, affecting to a greater extent to decay of dough consistence after 450 s.

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 ($p<0,05$) effect on incubated dough rheology when added together, concretely on extensibility (L_{3h}) and deformation energy (W_{3h}). The simultaneous presence of both 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)

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

Addition of TG to PROT containing samples significantly increased P_{3h}, W_{3h}, and P/L_{3h}. The protein polymerisation catalyzed 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 the obtained for singly TG treated dough except for W_{3h}, whose values were similar in both cases. Since the 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 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).

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. (1996) 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 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.

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 concluded. The significant ($p<0.05$) increase of Tol came accompanied by a 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).

The combined use of LAC and PP allowed overcoming significantly ($p<0.05$) the 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 release pentosans associated with proteins whereas the latter promotes polymetization of the pentosans). The synergistic effect of these enzymes are in accordance with the 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 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. 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-suplemented doughs.

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 were 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 (Pyler, 1988) and an increase of dough stickiness (Armero and Collar, 1998). Dextrins may interference with interactions between the swollen starch granules and the protein network (Duran et al., 2001) modifying dough tolerance (Tol). PP brought about a partial solubilization of water insoluble pentosans (WIP) (Rouau and Moreau, 1993), reducing also the water absorption capacity of dough by releasing the water bound to pentosans (Martínez-Anaya and Jimenez, 1997). The progressive liberation of free water molecules (that aids gluten network development), along with the decrease in 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.

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 due to the diminution of associations of pentosans with glutenin polymers (Primo-Martin et al., 2003) 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 (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.

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. (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.

The oxidative enzymes GO and LAC also exerted a significant ($p<0.05$) effect on bread quality. The first one led to improvements in the shape of the loaves whilst the latter 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

treatment to the detriment of its width. According with the conclusions of Rasiah et al. (2005) this enzyme showed a small and selective action on dough proteins, reason why its macroscopic effects on bread quality would not imply important changes in loaf volume. Additionally, Primo-Martin et al. (2005) concluded that gelation of pentosans catalyzed by GO affect negatively to bread quality by interfering protein cross-linking. In spite of it, improvements in the wheat and rice bread loaf volume have been obtained 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 sulphydryl groups (Labat et al, 2000). Primo-Martin and Martinez-Anaya (2003) 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 (1994) reported how the adverse effects of pentosans addition on the loaf volume were overcome by PP treatment. Indrani et al (2003) 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. The subsequent release of water and later redistribution to gluten has been proposed as a way to improve gluten extensibility and bread quality (Martínez-Anaya and Jimenez, 1997).

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. (2003) 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 (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).

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 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 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.

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 (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

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. Both enzymes are implicated in reactions by which take place the oxidation of the free sulfhydryl units from gluten protein giving disulfide linkages and the gelation of water soluble arabinoxylans. However, strengthening effect has been attributed fundamentally to GO (Martínez-Anaya and Jiménez, 1997; Vemulapalli et al., 1998; Dunnewind et al. 2002, Gujral & 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.

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 crosslinks by GO has been recently proposed as a theory for justifying this synergistic effect (Primo-Martin et al., 2005).

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 (Primo-Martin et al., 2003) and PROT (Wikstrom and Eliasson, 1998; Indrani et al., 2003) 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 (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.

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

Analytical data were undergone 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 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.

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 (Dobraszczyk and Morgenstern,

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 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 increasedwith dough after three hours 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 of 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 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 three 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 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. On the contrary, none of the studied rheological properties could be considered as a good predictor of specific volume of rolled breads.

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Table 1. Characteristics of wheat flour

	Flour
Chemical composition	
Protein (% d. wt.)	11,00
Ash (% d. wt.)	0,58
Moisture) (% d. wt.)	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

d. wt. : dry weight

2

Table 2. Half fraction factorial design 2^6 for sampling

Sample no.	Factors [a]					
	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 [a]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 =
6 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).

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

Parameter	Units	Overall mean	TG[a]			GO			LAC			AMYL			PP			PROT		
			0	1		0	1		0	1		0	1		0	1		0	1	
P	mm H_20	46	**37**	**54**	*	44	47		46	46		46	45		47	44		**50**	**41**	*
L	mm H_20	119	**144**	**94**	*	125	113		123	115		120	118		114	124		110	128	
W	x 10^{-4} J	162	**142**	**183**	*	161	164		164	160		161	164		158	166		**173**	**151**	*
P/L		0.44	**0.26**	**0.62**	*	0.41	0.47		0.42	0.46		0.45	0.43		0.47	0.41		**0.52**	**0.36**	*
P_{3h}	mm H_20	69	**31**	**106**	*	66	71		71	66		72	65		70	67		**82**	**55**	*
L_{3h}	mm H_20	69	**103**	**34**	*	**86**	**51**	*	69	69		68	70		68	70		61	76	
W_{3h}	x 10^{-4} J	132	**99**	**166**	*	131	134		136	129		136	129		130	135		140	125	
P/L_{3h}		1.89	**0.37**	**3.42**	*	1.88	1.91		1.97	1.82		1.99	1.80		1.98	1.81		**2.54**	**1.26**	*
WA	%	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	
DDT	s	79	78	80		79	79		80	78		81	77		80	78		82	76	
Tol	s	127	**120**	**134**	*	127	127		127	128		130	124		125	129		132	123	
D_{250}	mb	747	**832**	**663**	*	751	744		751	744		750	745		755	740		724	771	
D_{450}	mb	1199	**1223**	**1015**	*	1120	1119		1118	1121		1101	1138		1117	1122		**1084**	**1155**	*
Height/Width ratio		0.58	**0.45**	**0.71**	*	**0.51**	**0.65**	*	0.59	0.57		0.59	0.57		0.58	0.57		**0.60**	**0.56**	*
Specific volume	(cm^3/g)	3.70	**3.94**	**3.46**	*	3.66	3.73		**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.
* The effect of the factor is significant with a significance level of 95% ($p<0.05$)

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

Parameter	Units	Overall mean	Level[a]	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_20	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_20	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^{-4} 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_{3h}	mmH_20	69	00	28	33	33	32	**37***	72	68	70	76	72	72	82	77	87	87
			01	35	30	30	31	**26**	61	65	64	57	71	71	61	67	58	54
			10	105	110	112	109	**128**	71	76	72	88	72	69	83	64	78	78
			11	107	102	101	104	**85**	72	66	71	54	60	63	50	67	53	57
L_{3h}	mmH_20	69	00	**140***	104	103	102	95	83	85	88	74	69	70	56	63	60	62
			01	**67**	102	104	104	112	88	87	84	98	68	67	81	73	75	74
			10	**32**	33	33	34	28	54	51	48	48	66	65	66	73	61	60
			11	**37**	36	35	35	41	49	52	56	55	72	72	72	67	78	79
W_{3h}	x 10^{-4} J	132	00	**107***	102	99	95	**112***	134	134	133	135	139	133	140	138	144	142
			01	**90**	96	99	102	**85**	128	128	129	127	132	138	131	134	128	118
			10	**154**	169	172	164	**167**	137	137	127	145	132	127	139	122	136	138
			11	**177**	162	159	167	**164**	130	130	140	122	126	131	119	136	122	131
P/L_{3h}		1.89	00	0.22	0.38	0.38	0.40	**0.45***	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	**0.30**	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	**4.63**	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	**2.21**	2.02	1.72	1.85	1.13	1.58	1.70	1.00	1.81	1.20	1.28

[a]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[a]	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	**51,6***	51,0	50,9	50,9	51,2	50,8	**51,6***	51,2	**50,8***	50,8	**51,3***
			01	50,8	51,0	51,5	51,3	**50,6**	50,9	51,1	51,1	50,8	51,6	**50,8**	51,1	**50,5**	50,5	**50,1**
			10	50,6	51,2	50,6	50,5	**50,6**	51,3	50,6	50,5	50,9	50,6	**49,9**	50,9	**50,6**	51,4	**50,9**
			11	50,8	50,2	50,8	50,9	**50,8**	50,4	51,1	51,1	50,7	50,7	**51,4**	50,4	**51,7**	50,9	**51,3**
DDT	s	79	00	79	80	81	82	83	78	83	73	82	80	84	**88***	79	84	82
			01	78	77	76	76	74	79	75	75	76	80	76	**73**	83	78	78
			10	78	80	81	81	82	82	79	78	83	82	77	**77**	81	79	82
			11	81	79	79	79	78	77	79	82	76	75	79	**80**	73	75	74
Tol	s	127	00	120	120	123	**126***	127	124	134	129	131	130	131	**139 ***	**122 ***	135	127
			01	120	120	118	**114**	113	130	120	124	123	123	123	**114**	**139**	126	124
			10	134	134	138	**124**	136	130	127	121	133	130	120	**124**	**129**	129	136
			11	135	135	131	**144**	144	124	128	134	122	125	135	**131**	**119**	119	122
D_{250}	mb	747	00	850	842	840	**787***	786	779	731	731	729	747	738	698	768	725	740
			01	813	821	823	**876**	877	722	771	770	773	756	764	803	731	774	769
			10	652	660	659	**722**	662	723	768	778	719	752	771	749	742	723	708
			11	675	666	668	**604**	665	765	720	710	769	735	717	738	749	768	773
D_{450}	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

[a]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 6.- Second-order interactive effects of design factors on bread quality parameters of dough

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

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

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

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

Parameter	P (mm H_20)	L (mm H_20)	W (x 10^{-4} J)	P/L	P_{3h} (mm H_20)	L_{3h} (mm H_20)	W_{3h} (x 10^{-4} J)	P/L_{3h}	WA (%)	DDT (s)	Tol (s)	D_{250} (mb)	D_{450} (mb)
Height/Width ratio	0.7447	-0.7223	0.6854	0.7030	0.7605	-0.8401	0.7036	0.6983				-0.5015	-0.6559
Specific volume (cm^3/g)	-0.5828	0.5155	-0.3201	-0.6201	-0.5787	0.3130	-0.4176	-0.5913					0.4183