EFFECT OF HIGH PRESSURE PROCESSING ON WHEAT DOUGH AND BREAD CHARACTERISTICS

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ABSTRACT

Microbial, physical and structural changes in high pressured wheat dough were studied as a function of pressure level (50-250 MPa) and holding time (1-4 min). Thereafter, selected conditions of high hydrostatic processing (HPP) were applied to bread dough and the technological quality of the obtained breads was studied. The effect of HPP on wheat dough was investigated by determining microbial population (total aerobic mesophilic bacteria, moulds and yeasts), color and mechanical and texture surface related dough parameters (cohesiveness, adhesiveness, hardness and stickiness). HPP reduced the endogenous microbial population of wheat dough from 10^4 colony forming units/g (CFU) to levels of 10^2 CFU. HPP treatment significantly ($P<0.05$) increased dough hardness and adhesiveness, whereas treatment time reduced its stickiness. Scanning electron micrographs suggested that proteins were affected when subjected to pressure levels higher than 50 MPa, but starch modification required higher pressure levels. HPP treated yeasted doughs led to wheat breads with different appearance and technological characteristics; crumb acquired brownish color and heterogeneous cell gas distribution with increased hardness due to new crumb structure. This study suggests that high hydrostatic processing in the range 50-200 MPa could be an alternative technique for obtaining novel textured cereal based products.

Keywords: wheat dough, high pressure processing, microbiology, dough texture, bread.

INTRODUCTION

In the last decades, the development of non conventional methods for food processing, like high pressure processing (HPP), has attracted much attention. This technology consists in submitting foods to high hydrostatic pressure (usually among 100 and 1000 MPa) with the purpose of inhibiting both pathogen and spoiled microorganisms and of inactivating enzymes that cause undesirable changes (Farr, 1990; Hoover, Metrick, Papineau, Farkas & Knorr, 1989). The application of this technology in food storage has been growing, even at industrial level, due to its effect on microorganisms and enzymes leading to high quality food products (Mertens & Knorr, 1992; Norton & Sun, 2007). Additionally, this technology is being applied to food and raw material processing for obtaining innovative sensorial and functional properties (Welti-Chanes, López-Malo, Palou, Bermúdez, Guerrero-Beltrán & Barbosa-Canovas, 2005; Norton et al., 2007). High hydrostatic pressure has been successfully applied to different food matrices. Currently, it is possible to find fruit juices, oysters, sliced jam, avocado puree, and so on in the market of different countries (San Martín-González, Welti-Chanes & Barbosa-Canovas, 2006; Norton et al., 2007). Nevertheless, there are scarce studies about the use of high hydrostatic pressure in the discipline of cereals and cereal based products. In the Japanese market it can be found HPP treated sake and rice sake (Cheftel, 1995). Different scientific reports described the effect of HPP on specific cereal components properties or model systems, namely starch and gluten (Gomes, Clark & Ledward, 1998; Apichartsrangkoon, Ledward, Bell & Brennan, 1998; Kieffer, Schurer, Köhler & Wieser, 2007). HPP induces starch gelatinization, following different mechanism than thermally-induced gelatinization (Gomes et al., 1998). HPP treatment provokes swelling of starch but keeping granule integrity; as a consequence HPP treated starches modify their microstructure and rheological properties in a different way than thermally treated ones

(Gomes et al., 1998; Stolt, Oinonen & Autio, 2000), and the extend of swelling highly depends on the type of starch, pressure level and time of treatment (Stute, Heilbronn, Klingler, Boguslawski, Eshtiaghi & Knorr, 1996; Stolt et al., 2000). In addition, thermal properties of pressure-treated starches show decrease in both gelatinization temperature and enthalpy; besides starch granules loose crystallinity and they are prone to aggregate (Wang, Li, Wang, Chiu, Chen & Mao, 2008). Simultaneously, some studies carried out on gluten showed that HPP produces a weakening effect on gluten when low pressure levels (200 MPa) were applied, but an increase in pressure and temperature (800 MPa, 60°C) induces the opposite effect, the strengthening of gluten losing its cohesiveness (Kieffer et al., 2007), because HPP brings about the formation of disulphide bonds (Apichartsrangkoon et al., 1998; Kieffer et al., 2007). Nonetheless, there is no study about the effect of HPP on the complete matrix of wheat dough and the potential use of HPP for leading to HPP new cereal based product development with novel texture.

The aim of this study was firstly to determine the effect of diverse hydrostatic pressure levels applied for different periods of time on the microbiological, physical and structural characteristics of wheat dough and secondly, to explore the possible use of HPP for obtaining wheat breads with novel texture characteristics.

MATERIALS AND METHODS

Materials

A commercial blend of wheat flours (14.21 g/100g moisture content, 11.44 g/100g protein content, 0.61 g/100g ash content, 34.5 g/100g wet gluten) was used in this study. Salt and compressed baker's yeast were acquired in the market.

Wheat dough preparation and HPP treatment

Wheat flour was mixed with salt (1.5 g/100g , flour basis), and the amount of water required for reaching 500 Brabender units dough consistency. Mixing was carried out in a 300 g bowl Brabender Farinograph (Brabender, Duisburg, Germany) for 10 minutes. Then dough was divided in 30 g pieces, hand moulded and packed in polyethylene bags (11x12 cm), that were thermosealed under vacuum (Murtivac, España). For HPP, the packed doughs were placed into the pressurization cabinet of the HPP device (Engineering Pressure Systems SO 12644, Belgium), and then subjected to different pressures (50, 100, 150, 200 or 250 MPa) for various holding times (1, 2, 3 or 4 minutes). Control dough without HPP treatment was used as reference. Samples after each treatment of pressure and time were evaluated.

Microbiological analysis

For determining the endogenous amount of total aerobic mesophilic bacteria (TAMB) in the wheat dough, 10^{-1} and 10^{-2} dilutions of the sample were prepared in water peptone solutions (15g/100 mL), and then 0.1 mL aliquot was inoculated in agar plates (PCA, Scharlau). Colony forming units (CFU) were counted after incubation at 38°C for 24 h.. A sample of each dough (1g) was homogenized with 9 mL peptone solutions, serially diluted and plating on potato-dextrose Agar (PDA, Scharlau Chemie, Barcelona, Spain) for yeast and mould counts. Microbial counts were determined after aerobic incubation at 25°C, for five days. Determinations were carried out in triplicate.

Dough machinability and surface related profile

Mechanical and surface related properties were determined in the reference (non HPP treated) and in HPP treated doughs. Dough machinability was determined by assessing the texture profile analysis (TPA) and dough stickiness in a TA-XT2i texturometer

(Stable Micro Systems, Godalming, UK) as described by Armero and Collar (1997) using the Chen and Hoseney cell. Primary textural properties were measured in absence of dough adhesiveness by using a plastic film on the dough surface to avoid the distortion induced by the negative peak of adhesiveness (Collar & Bollaín, 2005). The adhesiveness was measured without the plastic film. Three and ten repetitions for the TPA parameters and stickiness were done, respectively. Compression test was performed with a 25mm diameter cylindrical aluminium probe (SMSP/25), 60% compression rate followed of 75s interval. TPA profile recorded the following parameters: hardness (N), adhesiveness (Ns) and cohesiveness. The Chen and Hoseney cell with a cylindrical probe of 25mm diameter was used for dough stickiness (N) determination (Armero et al., 1997).

Wet gluten and gluten index determination

The amount of wet gluten and the gluten index were determined to assess the effect of HPP on the gluten characteristics. Gluten was extracted from 10 g of wheat dough using a gluten washer (Glutomatic, Stockholm, Sweden). Gluten index was determined according to the approved method (ICC, 2004). Values are the average of three replicates.

Scanning electron microscopy

The microstructure of the reference and the treated dough samples was analysed by scanning electron microscopy (SEM). Freeze dried samples were mounted on metal stubs using double tape active carbon and sputter-coated with 100-200Å thick layer of gold and palladium by Ion Sputter (Bio-Rad SC-500). Sample analysis was performed at an accelerating voltage of 10kV with a SEM Hitachi 4100 from the SCSIE Department of the University of Valencia.

Breadmaking procedure

Wheat bread doughs or yeasted doughs were obtained following the same procedure as described above for dough preparation but including compressed yeast (4%, flour basis) in the recipe. After HPP treatment, bread dough was hand rounded and put it into pans. Proofing was made in a fermentation cabinet (National MF6.C0, US) at 28ºC and 80% relative humidity during the required time to reach three times the initial bread dough volume. Pan breads were baked in an electric oven (Eurofours, France) at 210ºC for 11 min, and then they were cooled down for one hour before running the further analysis.

Dough and bread color

Color was determined with a Color Guard System Colorimeter (Hunter Laboratory, Reston, VA). Color was determined by reflectance mode and expressed by *L* (luminosity), *a* (green-red) and *b* (blue-yellow) Hunter parameters. The colorimeter was calibrated by utilizing the black tile and the white standard (L=92.89, a=-1.05, b=0.82). The net difference of color (ΔE) was calculated by equation 1:

$$\Delta E = \sqrt{(L_c - L_m)^2 + (b_c - b_m)^2 + (a_c - a_m)^2} \qquad \text{(Eq.1)}$$

Where the subscript *m* corresponded to HPP treated dough or bread obtained from treated bread dough, and subscript *c* indicated the values of the reference dough or bread (without HPP treatment).

Bread quality assessment

In order to determine the bread quality, the volume (rapeseed displacement), weight, height/width ratio of the slices, and moisture content were measured. Moisture content was determined following the ICC Method (2004). Besides, a texture profile analysis (TPA) of the breadcrumbs was performed by a Texture Analyzer TA-XT2i texturometer. A bread slice of 2-cm-thickness was compressed up to 50% of its original height at a crosshead speed of 1 mm/s with a cylindrical stainless steel probe (diameter 25 mm). Values were the mean of four replicates. The cross-section of bread slices was visually observed. Sensory perception was performed by ten trained panellists (5 women and 5 men), whose age ranged from 24 to 50 years old. The trained panel scored the overall acceptability of the breads using a semi-structured scale (0: extremely dislike, 10: extremely like).

Statistical analysis

Experimental data from wheat dough characterization were submitted to multifactor analysis of variance (MANOVA) using Statgraphics Plus, versión 5.1 (Statistical Graphics Corp., 1994-2001). Experimental data from bread quality assessment were submitted to one-way analysis of variance (ANOVA). All data were presented as mean values of at least three replicates ± standard error (SE). When analysis of variance indicated significant *F* values, multiple sample comparison was also performed by Tukey HSD test in order to detect significant differences ($P<0.05$).

RESULTS AND DISCUSSION

Effect of high pressure processing on the microbial population of the wheat dough

A preliminary study of the effect of HPP on wheat dough was carried out to select the most adequate HPP conditions considering the endogenous microbial dough population and dough mechanical and structural properties. The effect of different HPP levels, applied for different duration, on the colony forming units (CFU) of total aerobic mesophilic bacteria (TAMB) of wheat dough can be observed in Figure 1. An important reduction of microorganisms was observed after one minute of exposure at HPP, and no further significant decrease was obtained by increasing the duration of the treatment, with the exception of samples treated at hydrostatic pressure of 50 MPa, which required prolonged treatment (two minutes). Generally, an increase in pressure has been related to high microbial inactivation, but that relationship has not been found with the time of treatment (Palou, López-Malo, Barbosa-Canovas, Welti-Chanes, Davidson & Swanson, 1998). In fact, it is necessary to determine the threshold HPP required for each microorganism inactivation and above that an increase in the time of exposition does not promote a significant reduction in the microbial counts, always having in mind the essential role of the environment on the microorganism resistance to HPP (Palou et al., 1998). Nonetheless, HPP below 200 MPa can induce spore germination of certain bacterial strains (Gould & Sale, 1970), increasing the amount of cells in vegetative state and thus the colony forming units, although the present results did not suggest any germination.

Similarly, it was determined the total moulds and yeasts counts presented in wheat dough treated at different HPP during different time (Figure 1). Regardless the lowest HPP tested, one minute of HPP was enough for reducing the moulds and yeast endogenous population of the wheat dough. Similarly, with the exception of 50 MPa, no further moulds and yeast counts decrease was observed by increasing treatment time. In opposition, when doughs were HPP treated at 50 MPa, moulds and yeasts counts showed additional decrease by extending the treatment time. Moulds and yeast are very sensitive to HPP affecting the external shape of the cells and very high HPP treatment (500 MPa) induces disruption and damage of the cell walls (Ogawa, Fukuhisa, Kubo & Fukumoto, 1990; Shimada, Andou, Naito, Yamada, Osumi & Hayashi, 1993).

The pairs HPP-time used in this study were enough to promote a significant reduction of the TAMB and moulds and yeast counts, and after one or two minutes of treatment the final microbial populations of the doughs were approximated 10^2 CFU/g. Lately, the effect of HPP on the microorganism has been widely studied showing good efficiency for microorganism inactivation (Carlez, Rosec, Richard, & Cheftel, 1993; Patterson, Quinn, Simpson & Gilmour, 1995; Palou, López-Malo & Welti-Chanes, 2002), but higher pressure and time than the ones used in the present study are usually applied. Very mild HPP level and treatment time were tested in this study to ensure treatment effectiveness with retention of dough functionality.

Effect of HPP on wheat dough color

Experimental color data were submitted to statistical analysis to determine the level of significance of HPP level and the treatment duration on the color parameters (*L, a, b*) and the total color change (ΔE) (Table 1). The color of wheat dough was affected by HPP treatment; nevertheless absolute differences among experimental data were rather small. Luminosity (L) of the samples varied with the HPP treatment and the holding time at constant pressure, but no significant differences were observed between the control (untreated dough) and the treated doughs due to the pressure intensity. Pressure levels of 100 and 150 MPa produced the greatest effect on the *a* and *b* color parameters, showing a tendency to red and yellow, respectively. Concerning the period of HPP treatment, increasing duration of HPP resulted in decreased values of *a* parameter, whereas no significant differences of *b* parameter were observed between the untreated sample (time 0) and the HPP treated samples. The total change of color (ΔE) only was significantly ($P<0.05$) increased when the highest HPP level (250 MPa) was applied, and regarding the duration of HPP, all the times tested affected significantly ($P<0.05$) this parameter. There is a general assumption that HPP is a preservative technique that protects food color but only once circumspect treatment is applied (Norton et al., 2007). Hydrostatic pressure applied to wheat dough modified the color parameters but only at the highest pressure tested (250 MPa) was observed a significant change of the total color.

Effect of HPP on gluten

The amount of wet gluten and gluten index was determined in wheat dough samples subjected to different levels of HPP (Table 2). Only the pressure level applied during HPP induced significant effect on the amount of wet gluten and its quality assessed as gluten index. However, no significant differences were observed between the untreated dough and the HPP treated doughs. The duration of the HPP or holding time at constant pressure did not have any significant effect on the characteristics of the gluten. Some studies carried out on wheat gluten stated that at 20ºC, only hydrostatic pressure higher than 200 MPa modifies the gluten structure and the effect was dependent on the holding time (20 or 50 min) and temperature (20-60ºC) (Apichartsrangkoon et al., 1998; Apichartsrangkoon, Bell, Ledward & Schofield, 1999). Conversely, Kieffer et al. (2007) observed a decrease in the gluten strength when treated at low pressure (200 MPa). Therefore, slightly contradictory results have been obtained when gluten was treated at low pressure, but a consensus has been reached concerning that changes in gluten structure associated to disulfide cross-linking only became significant at extreme conditions (400-800MPa, 60ºC or lower temperature but prolonging the exposure) (Apichartsrangkoon et al., 1998; Kieffer et al., 2007). HPP conditions applied in the present study did not reveal substantial changes on gluten characteristics, either wheat dough provided a shielding effect on gluten, or HPP conditions were too mild to induce gluten physical changes.

Effect of HPP on the texture parameters of wheat dough

Data of mechanical and texture surface related parameters are included in Table 2. The pressure level had a significant effect on the hardness, cohesiveness and adhesiveness, whereas the holding time at constant pressure had only significant effect on the stickiness. Increasing values of hardness and adhesiveness were obtained when raising the hydrostatic pressure, besides a decrease in stickiness when increasing the time of treatment (Table 2). No differences were detected on the cohesiveness of untreated dough and HPP treated wheat doughs. Results agree with Apichartsrangkoon et al. (1998) findings that described an increase in the hardness of high pressure treated wheat gluten when applying hydrostatic pressure within the range 200-800 MPa, and that effect was markedly dependent on the pressure, temperature and holding time of HPP. It is advisable for proper breadmaking to keep dough stickiness at low levels, because it seriously constrains dough machinability and that is even more important in automated breadmaking processes (Armero et al., 1997). Considering that dough cohesiveness has been reported as a good predictive parameter of fresh bread quality and keepability, and maximized dough cohesiveness and minimized dough stickiness are recommended trends for providing good bread-making performance (Armero et al., 1997), the range of HPP conditions applied in this study would provide wheat dough with satisfactory breadmaking performance.

Microstructure of HPP treated wheat dough

Scanning electron microscopy was used to determine the effect of the HPP on dough microstructure. Scanning electron micrographs of wheat doughs treated at 50, 150 and 250 MPa for four minutes are showed in Figure 2. Untreated wheat dough (0 MPa) was characterized by having a continuous structure with the intact starch granules embedded in the matrix structure of proteins and soluble solutes. Two distinct populations of starch granule sizes could be envisaged, ones with lenticular shape and the others smaller and with spherical shape, which agree with previous reported two populations of A and B-type of starch granules (Angold, 1975). In the present study the reticular structure of the wheat dough, previously reported (Rojas, Rosell, Benedito, Pérez-Munuera & Lluch, 2000), was not evident because samples were not subjected to sublimation, which is necessary in cryo-scanning electron microscopy. After HPP treatment the continuous matrix appeared disaggregated and the starch granules were clearly identified as individual structures, however their structure became more distorted as the pressure level increase. Dough treated at pressure of 50 and 150 MPa showed well defined starch granules with diverse size, and the surrounding structures (mainly of protein nature) were progressively reduced, being confined in the case of 150 MPa to agglomerates of starch granules. Drastic changes were observed in dough treated at 250 MPa where starch granules as individual structures disappeared adopting a discontinuous film like organization similar to what happened after swelling and gelatinization. The effect of high hydrostatic pressure on different types of starch has been widely studied (Stolt et al., 2000; Stute et al., 1996; Gomes et al., 1998; Katopo, Song & Jane, 2002). High pressure induces a gelatinization process keeping intact the starch morphology (Stute et al., 1996; Katopo et al., 2002). The effect of HPP on starch granules led to a limited swelling that is highly dependent on the level of pressure, water moisture content, time of exposure, and starch type (Stute et al., 1996). Studies focused on the effect of HPP on diverse proteins described that pressure levels equal or higher than 1000-2000 MPa do not have a significant effect on covalent bonds (Mozhaev, Heremans, Frank, Masson & Balny, 1994), hydrogen bonds are induced by HPP treatment and the hydrophobic linkages tend to be stabilized at pressure levels higher than 100 MPa (Hoover et al., 1989). Following previously reported explanations, gluten structure should not be severely affected by the HPP carried out in the present study, but considering that HPP also favors unfolding and dissociation of oligomeric proteins (Masson, 1992), some gluten modification could be expected. Apichartsrangkoon et al. (1998) and Kieffer et al. (2007) found that gluten treatment with HPP (ranged from 200-800 MPa for 20-50 minutes or combining high temperature) yielded the formation of additional disulphide bonds that besides the hydrogen and hydrophobic bonds, formed or altered by the HPP, originated changes in the protein matrix of gluten, which differed from the heat induced ones. Therefore, microscopy studies revealed that HPP treatment up to 150 MPa for four minutes induced microstructural changes on wheat dough related to matrix disorganization, likely due to protein unfolding, but drastic starch modification required higher pressure levels (250 MPa).

Effect of HPP on technological quality of wheat bread

Studies carried out on wheat dough allowed selecting an intermediate holding time (2 min) and hydrostatic pressures ranged from 50-200 MPa for inducing modifications on bread dough (yeasted dough) without loosing its breadmaking performance. Those HPP conditions were applied to bread dough, which were then submitted to the conventional breadmaking process for obtaining wheat breads. Cross-section of the bread slice obtained from HPP treated yeasted doughs is shown in Figure 3. Bread pictures show that HPP greatly affected the crumb microstructure with uneven distribution of the gas cells, and increased size of the alveoli. Even the lowest HPP level (50 MPa) induced the formation of bigger gas cells compared to the control. Large gas cells were observed in some places of the treated crumbs, besides a brownish color, and that appearance was more noticeable when increasing pressure levels. Despite high hydrostatic processing reduced the microbial population in wheat dough, the HPP treated yeasted doughs had enough microbes' survival (around 10^4 CFU/g in all HPP yeasted samples) for ensuring dough fermentations.

The sensory evaluation of the treated breads revealed that, with the exception of the 200MPa treated sample, HPP gave acceptable products, with the same overall acceptance as the non-treated bread (score 7). Judges emphasized the original layered structure of the treated bread crumb that was very soft and attractive, reminding the croissant structure.

Therefore the use of HPP on the yeasted dough might lead to the development of new bakery products with novel crumb characteristics, resembling croissant structure.

Regarding bread technological quality (Table 3), HPP significantly ($P<0.05$) reduced the volume and specific volume of the loaves, obtaining more compact and flat breads at higher hydrostatic pressure treatment, as indicated the width/height ratio. Presumably, the effect of HPP either on gluten network structure or on yeast cells fermentation ability led to reduced dough expansion during proofing and baking, and in consequence lower specific volume. Crumb hardness was also modified obtaining higher crumb hardness with breads from treated doughs, which was expected due to the protein network modification induced by HPP. Hardness increase has been always described in breads that have undergone gluten network damage, for instance due to ice crystal formation and growing like in frozen doughs or even from frozen partially baked breads (Bhattacharya, Langstaff & Berzonsky, 2003; Bárcenas & Rosell, 2006).

The moisture content of the bread showed a steady increase as function of pressure levels. Likely, crumb structure was holding higher amount of water molecules partly due to chemical pressure-induced changes in proteins (Apichartsrangkoon et al., 1998; Kieffer et al., 2007) and the pressure-induced gelatinization of starch that allows completing starch gelatinization at lower temperatures (Stute et al., 1996; Katopo et al., 2002).

Color of crumb and crust of the bread was modified due to the HPP treatment of the bread doughs (Table 4). Luminosity, *a* and *b* color parameters of crust were reduced with the pressure levels, showing a significant ($P<0.05$) decrease at the higher pressure levels tested. Those results agree with studies carried out by Tamaoka, Itoh and Hayashi (1991), who showed that HPP in the range 50 and 500 MPa at 50° C on model systems formed by glyceraldehyde, glycolaldehyde or xylose and aminoacids did not have any effect on the condensation reaction but suppressed the browning process in the total course of the Maillard reaction. The overall HPP effect considered as the total change of color (ΔE) showed a significant increase.

Concerning the crumb color, significant decrease in luminosity together with a large increase in the total change of color were observed, in agreement with the visual observation of the cross-section of the slices (Figure 3). No general trend could be established regarding the *a* and *b* color parameters. Although color changes induced by HPP in wheat dough were rather small, the temperature applied during baking resulted in significant crumb color changes.

Treatment of wheat dough with high hydrostatic pressure induced rapid reduction of the microbial population but sufficient mold and yeast survival, for ensuring bread dough fermentation, can be obtained using mild pressure conditions (50-250 MPa, for two minutes at 20ºC). Regarding dough properties, HPP treatment significantly (P<0.05) increased hardness and adhesiveness, whereas stickiness was reduced by increasing the time of exposure to HPP. SEM micrographs suggested that proteins were affected at pressure levels ranged from 50 to 150 MPa, but starch modification required higher pressure levels. HPP treated yeasted doughs allowed obtaining wheat breads with different appearance and technological characteristics; crumb acquired brownish color and uneven cell gas distribution with increased hardness due to new crumb structure. This study suggests that high hydrostatic processing in the range 50-200 MPa could be an alternative technique for obtaining novel textured cereal based products.

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Table 1. Effect of high pressure processing on color parameters of wheat dough. Experimental data were submitted to multifactor analysis of variance (MANOVA).

	L			*a*			*b*			Δ*E*		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE	
Grand mean	80.30			1.88			17.04			3.58		
Pressure level (MPa)												
50	80.64	0.32	ab	1.72	0.15	ab	16.18	0.46	a	3.27	0.23	a
100	79.44	0.39	a	2.54	0.18	c	18.67	0.56	c	3.19	0.29	a
150	79.84	0.39	a	2.07	0.18	bc	18.29	0.56	bc	3.26	0.29	a
200	79.90	0.39	a	1.75	0.18	ab	16.39	0.56	ab	3.55	0.29	a
250	81.70	0.39	b	1.33	0.18	a	15.68	0.56	a	4.64	0.29	b
Treatment time (min)												
0	77.33	0.79	a	2.95	0.37	c	17.55	1.12	ab	1.01	0.23	a
1	81.06	0.32	b	1.74	0.15	b	17.36	0.46	ab	3.98	0.23	b
2	81.26	0.32	b	2.03	0.15	bc	17.98	0.46	b	4.41	0.23	b
3	80.55	0.32	b	1.57	0.15	ab	16.19	0.46	ab	3.99	0.23	b
4	81.31	0.32	b	1.10	0.15	a	16.14	0.46	a	4.53	0.23	b

Means of three replicates followed by different letters within rows and groups were significantly different at $P<0.05$.

SE: Standard error

Table 2. Effect of high pressure processing on gluten and mechanical and texture surface related parameters of wheat dough. Experimental data were submitted to multifactor analysis of variance (MANOVA).

	Wet gluten (g)			Gluten index (%)			Hardness (N)			Cohesiveness			Adhesiveness (Ns)			Stickiness (N)		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
Grand mean	16.9			51.1			4.61			0.65			4.34			0.44		
Pressure level (MPa)																		
50	17.3	0.3	ab	53.1	2.9	ab	3.36	0.37	a	0.63	0.02	ab	2.75	0.51	a	0.41	0.03	
100	16.5	0.4	ab	49.0	3.5	ab	3.81	0.45	ab	0.70	0.02	b	2.44	0.62	a	0.41	0.03	
150	16.1	0.4	a	42.3	3.5	a	5.09	0.45	bc	0.65	0.02	ab	6.79	0.62	b	0.46	0.03	
200	17.0	0.4	ab	56.4	3.5	b	5.48	0.45	c	0.67	0.02	ab	5.09	0.62	b	0.46	0.03	
250	17.6	0.4	b	54.9	3.5	ab	5.43	0.45	c	0.60	0.02	a	4.95	0.62	b	0.43	0.03	
Treatment time (min)																		
0	15.9	0.7		50.5	7.0		4.06	0.90		0.63	0.05		4.95	0.52		0.60	0.07	b
1	17.5	0.3		50.6	2.9		4.50	0.37		0.65	0.02		4.70	0.51		0.52	0.03	b
2	16.8	0.3		51.2	2.9		5.63	0.37		0.65	0.02		4.20	0.51		0.36	0.03	a
3	16.7	0.3		48.9	2.9		4.26	0.37		0.68	0.02		3.56	0.51		0.40	0.03	a
4	17.8	0.3		54.4	2.9		4.62	0.37		0.64	0.02		4.30	0.51		0.41	0.03	a

Means of three replicates (ten in the case of stickiness) followed by different letters within rows and groups were significantly different at $P<0.05$.

SE: Standard error

Values were not followed by letters, when ANOVA indicated no significant *F* values

Table 3. Effect of different levels of high pressure processing and holding time of two minutes on fresh bread quality parameters. Experimental data were submitted to one-way analysis of variance (ANOVA).

Pressure level (MPa)	Moisture content (g/100g)			Volume (cm$_3$)			Specific volume (cm$_3$/g)			Width/Height ratio			Hardness (N)		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
0	29.6	0.0	a	126	7	d	4.2	0.0	c	1.3	0.0	a	2.48	0.10	a
50	30.2	0.0	b	84	2	c	2.8	0.0	b	1.3	0.0	a	5.39	0.10	b
100	34.9	0.0	c	80	1	b	2.7	0.0	b	1.6	0.0	b	6.26	0.10	c
200	35.0	0.0	d	59	2	a	2.0	0.0	a	1.5	0.0	b	15.87	0.10	d

Means of three replicates followed by different letters within rows were significantly different at $P<0.05$.
SE: Standard error

Table 4. Effect of different levels of high pressure processing and holding time of two minutes on crust and crumb color parameter of fresh bread. Experimental data were submitted to one-way analysis of variance (ANOVA).

	Crust												Crumb											
Pressure level (MPa)	*L*			*a*			*B*			Δ*E*			*L*			*a*			*b*			Δ*E*		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
0	49.87	0.33	b	10.03	0.20	bc	21.54	0.27	b	0.38	0.28	a	60.58	0.99	d	-0.72	0.02	bc	8.61	0.11	b	2.08	0.65	a
50	52.03	0.33	c	9.59	0.20	b	22.76	0.27	b	2.54	0.28	b	53.66	0.99	c	-0.94	0.02	a	10.59	0.11	c	7.22	0.65	b
100	47.24	0.33	a	10.50	0.20	c	17.79	0.27	a	4.69	0.28	c	44.96	0.99	b	-0.65	0.02	c	7.42	0.11	a	15.67	0.65	c
200	45.96	0.33	a	8.48	0.20	a	18.46	0.27	a	5.23	0.28	c	39.62	0.99	a	-0.78	0.02	b	7.38	0.11	a	21.02	0.65	d

Means of three replicates followed by different letters within rows were significantly different at $P<0.05$.

SE: Standard error

FIGURE CAPTIONS

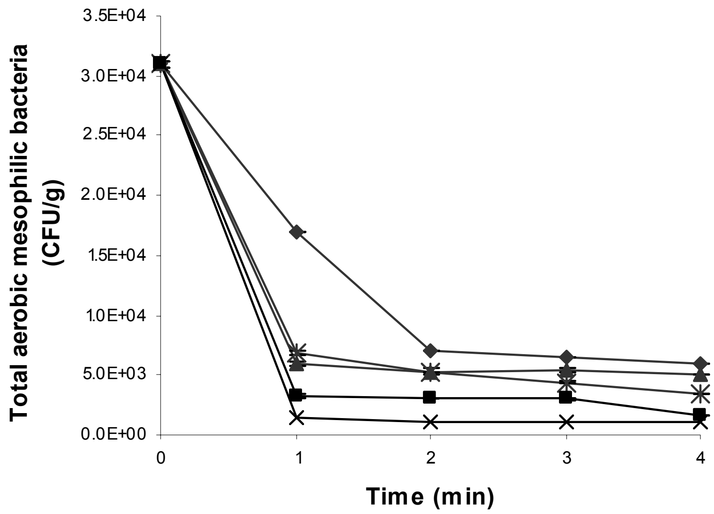
Figure 1. Effect of HPP treatment on total aerobic mesophilic bacteria (A) and on molds and yeasts (B) of wheat dough. Error bars indicate standard deviation (n=3). Symbols: ♦: 50MPa; ▲: 100MPa; ∗: 150MPa; ■: 200MPa; ×: 250MPa.

Figure 2. Scanning electron micrographs (SEM) of wheat dough (2500X) exposed to different levels of high pressure processing (0, 50, 150, 250 MPa) for 4 minutes.

Figure 3. Cross section of the different bread slices obtained from bread doughs treated at different high pressure processing levels and two minutes of exposure. Values indicate the pressure level used for HPP.

Figure 1

A.


3.5E+04
3.0E+04
2.5E+04
2.0E+04
1.5E+04
1.0E+04
5.0E+03
0.0E+00
Total aerobic mesophilic bacteria (CFU/g)
0
1
2
3
4
Time (min)

B

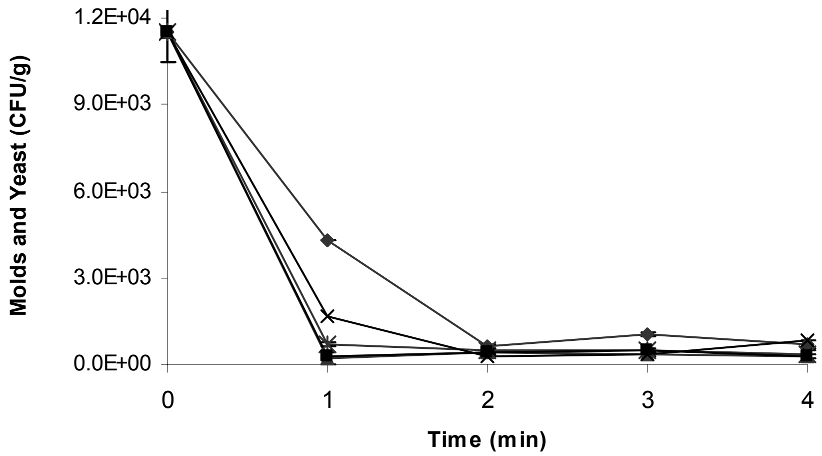
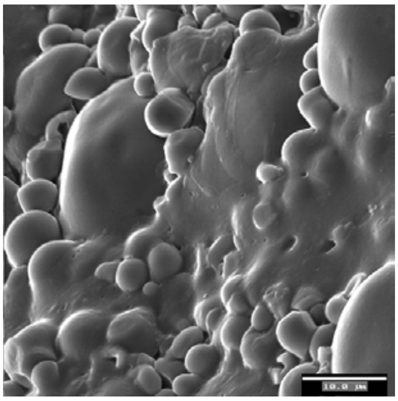
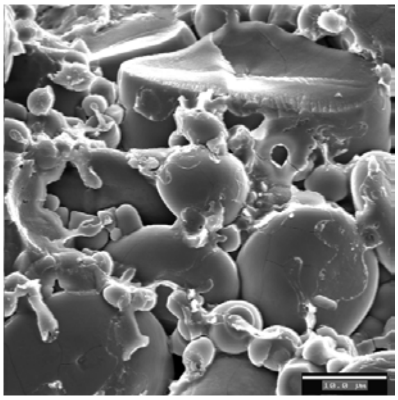

1.2E+04
9.0E+03
6.0E+03
3.0E+03
0.0E+00
Molds and Yeast (CFU/g)
0
1
2
3
4
Time (min)

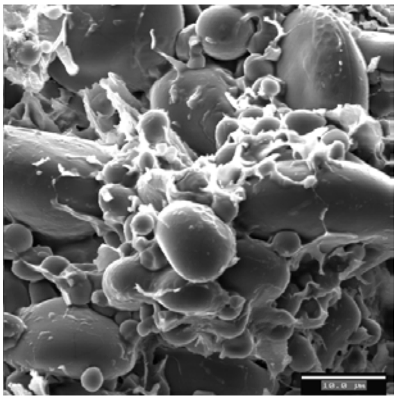
Figure 2



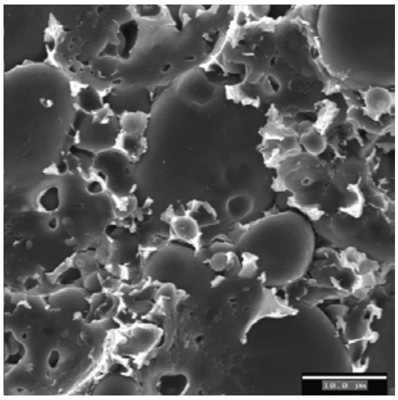
0 MPa



50 MPa

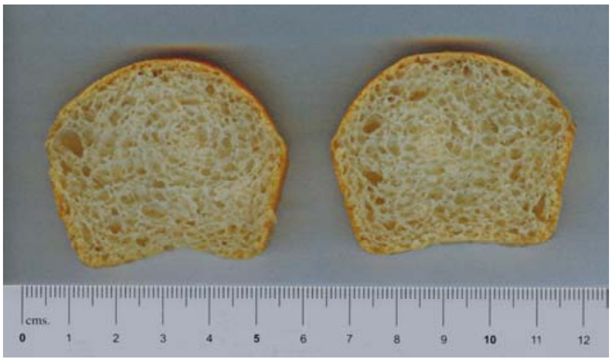


150 MPa

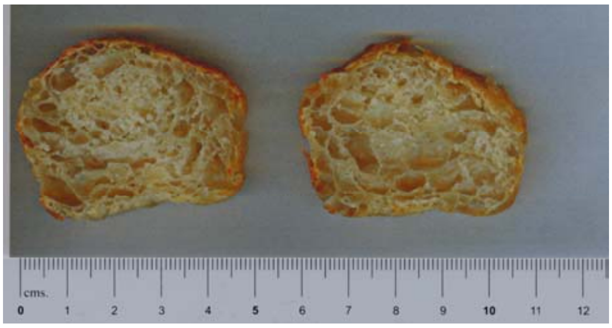


250 MPa

Figure 3.



Control



50 MPa

100 MPa

200 MPa