WHOLEMEAL WHEAT BREAD: A COMPARISON OF DIFFERENT BREADMAKING PROCESSES AND FUNGAL PHYTASE ADDITION

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ABSTRACT

The effect of different breadmaking processes (conventional, frozen dough, frozen partially baked bread) and the effect of the storage periodon the technological quality of the fresh wholemeal wheat breads are investigated. In addition, the impact of the exogenous fungal phytase on the phytate content was also determined. Results showed that breadmaking technology significantly affected the quality parameters of wholemeal breads (specific volume, moisture content, crumb and crust colour, crumb texture profile analysis and crust flaking) and frozen storage affected in different extent the quality of the loaves obtained from partially baked breads and those obtained from frozen dough, particularly crust flaking. Freezing and frozen storage of wholemeal bread in the presence of fungal phytase decreased significantly the phytate content in whole wheat breads. The combination of fungal phytase addition, breadmaking process and frozen storage could be advisable for overcoming the detrimental effect of bran on the mineral bioavailability. Key words: wholemeal, breadmaking, quality, phytates.

INTRODUCTION

Until recently, the nutrient value of the wheat bran, although long understood, has been ignored and the bran discarded and used as animal feed. However, its rich nutrient composition (Antoine et al., 2004), and its dietary fibre content has motivated numerous campaigns for increasing the consumption of whole wheat products. Whole grain products are perceived as more nutritionally balanced, healthy and natural, being bread the most consumed product (Claupein et al., 2007). Nevertheless, recommendations for the increase of wholemeal cereal consumption have raised questions about the increase intake of antinutritive compounds such as phytates (Sanz Penella et al., 2008). Whole wheat flours contain phytate or myo-inositol hexaphosphate that decreases the bio-availability of multivalent cations due to the formation of insoluble complexes in the gastrointestinal tract (Lopez et al., 2002). Significant reduction in the content of phytates in whole wheat bread have been obtained by adding exogenous phytase (Haros et al., 2001a,b), and also using different lactic acid bacteria with phytase activity (Palacios et al., 2008).

Despite the beneficial effect of consuming whole wheat bread, public's acceptance of this product is limited due to its lower volume, coarser texture and faster staling compared to refined wheat bread (Gan et al., 1992; Zhang and Moore, 1999). Therefore, some technological efforts are needed in the performance of whole wheat breads to meet consumer's needs and demands. Convenience bakery products are solving the constraints on food preparation and shopping imposed by the accelerated consumer lifestyles. Concerning bakery products, freezing dough, partially baked or fully baked bread become in many cases necessary to face the present demands (Rosell and Gómez, 2007). The use of frozen dough is very attractive because the low volume of the unfermented dough, which is very convenient when frozen storage is involved in the process, having satisfying quality characteristics even after nine months of storage (Giannou and Tzia, 2007). The partially baked bread (part-baked, par baked bread or pre-baked bread), also called bake off technology (BOT), consists in partial baking till the dough structure is fixed, giving a product with aerated crumb but without a crunchy crust that is formed along the second baking (Bárcenas and Rosell, 2006a,b). Numerous studies have been focussed on the sensory and technological quality of refined wheat loaves obtained from frozen dough or partially baked breads (; Bárcenas and Rosell, 2006a, b; Fik and Surowka, 2002; Poinot et al., 2008). Those revealed that breads with qualities close to the ones obtained from conventional breadmaking process are obtained. Nevertheless, scarce information exists about the impact of those breadmaking processes on whole wheat breads loaves, where studies have been addressed to the improvement of formulation for counteracting the negative effects of the bran particle size on the breadmaking performance and bread quality (Collar et al., 2006; Rosell et al., 2006; Shogren et al., 1981; Zhang and Moore, 1999).

The aim of this research was to determine the effect of different breadmaking processes (conventional, frozen dough, frozen partially baked bread) and diverse storage time on the technological quality (specific volume, texture, crumb structure and crust flaking) of the resulting fresh wholemeal wheat breads and to assess the impact of the exogenous fungal phytase on the phytates profile of the different breads.

MATERIALS AND METHODS

Commercial wholemeal wheat flour for breadmaking was used in this study. The characteristics of the flour were: 14.20% moisture content (ICC 110/1), 12.61% protein content (ICC 105/2), 1.82% fat content (ICC 136) and 1.46 % ash content (ICC 104/1). Commercial phytase (3.13.8) from *Aspergillus niger* (11.4 U ml^{-1}, Ronozyme Phytase) provided by Novozymes (Madrid, Spain) was used. One unit of phytase activity was defined as 1.0 mg of Pi liberated per minute at pH 5.0 and 30ºC (Haros et al., 2001a). The bread improver was provided by Puracor (Groot-Bijgaarden, Belgium). The rest of the ingredients were acquired in the food market.

Breadmaking process

Three different breadmaking processes were followed: conventional, frozen dough (FD) and partially baked frozen (PBF). Basic wholemeal wheat dough formula on 100 g flour basis consisted of 3% (w/w) compressed yeast, 1.8% (w/w) salt and 1% (w/w) bread improver. When required, fungal phytase (200 μL/100 g flour) was added. In conventional and partially baked process the amount of water necessary to give 500 Brabender Units (BU) of dough consistency was used, whereas dough consistency of

600 BU was used in the case of frozen dough. Bread doughs were prepared by mixing ingredients in a spiral mixer (AV18/2, Vimar Industries 1900, S.L., Spain) for six minutes, after 10 min resting, dough was divided into 70 g pieces and hand moulded. Fermentation was carried out in a proofing cabinet at 35°C and 95% relative humidity for 45 min. In conventional process, complete baking was carried out in an electric oven at 185°C for 15 min with steam injection at the beginning of the baking. Partial baking was carried at 170°C for 16 minutes, and then loaves were cooled down at room temperature. Partially baked bread and doughs were placed directly in a blaster freezer at -30°C till the bread core reached -18°C. Loaves and frozen doughs were taken out of the freezer and thawed at room temperature for 60 minutes. Full baking of partially baked breads was carried out at 185°C for 8 minutes. Frozen-thawed doughs were fermented in a proofing cabinet at 35°C and 95% relative humidity for one hour and then baked in an electric oven at 185°C for 15 min, as has been previously described for conventional process.

For storage studies, partially baked frozen loaves and frozen dough were packed in polypropylene bags and stored at -18°C for three months. Technological characteristics were evaluated along the storage period taking samples after 1, 2, and 3 months. Two different sets of breads were made for each breadmaking process comprising at least 20 loaves for performing further analysis.

Bread quality parameters

Technological parameters of bread quality included: volume, specific volume (rapeseed displacement, AACC Standard 10-05), moisture content (AACC Standard 44-15A), crumb texture profile analysis (TPA), crust and crumb colour, crust flaking, and crumb image analysis. TPA was measured in a Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, UK) using two bread slices of 1-cm-thickness, which underwent two double compression test up to 50% penetration of its original height at a crosshead speed of 1 mm/s and a 30 s gap between compressions, with a cylindrical stainless steel probe (diameter 25 mm) (Collar and Bollaín, 2005).

Bread crust and crumb coloration were measured in four different locations by using a Minolta colorimeter (Chroma Meter CR-400/410, Konica Minolta, Japan) after standardization with a white calibration plate (L = 96.9, a = -0.04, b = 1.84). The colour was recorded as L^*, C and h colour parameters, where L^* is the lightness or clearness, C the chroma and h the shade or hue angle.

For crumb to crust ratio determination, crust was separated from the crumb using the razor blade. Crumb to crust ratio was expressed as weight ratio and as volume ratio on a wet basis.

Crust flaking test was carried out in specific crushing system developed by Le Bail et al. (2005). Bread was crushed on its flanks and on its base by 30 % of its diameter and height in crushing system. Crust pieces were collected and weighed and then a digital picture of crust pieces was taken. Using an UTHSCSA Image Tool 3.0 Software, average crust flakes size was measured.

Crumb cell analysis was performed by scanning longitudinal and cross sections of bread sample, 10 mm thick. Images were analyzed by Image J software according to Gonzales-Barron and Butler (2006). Number of cells, average cells area, average diameter and cell circularity were calculated. Values were the mean of four replicates.

Determination of *myo*-inositol phosphates

Myo-inositol hexaphosphate (Ins*P*6) concentration in flour and the remained concentration of InsP6 and the lower inositol phosphates contained in bread were measured by HPLC method following the method described by Türk and Sandberg (1992) and lately modified by Sanz Penella et al. (2008).

Statistical analysis

Experimental data were subjected to analysis of variance (ANOVA) using Statgraphics V.7.1 program (Bitstream, Cambridge, MN), to determine significant differences among the factors combination. When ANOVA indicated significant *F* values, multiple sample comparison was also performed and Fisher's least significant difference (LSD) procedure was used to discriminate among the means.

RESULTS AND DISCUSSION

Effect of breadmaking processes and phytase addition on bread technological quality

The type of process significantly affected the specific volume of the bread ($P<0.001$), and also the moisture content ($P<0.01$) (Table 1), and phytase addition did not promote any significant effect on those parameters. The combination process x phytase affected the specific volume of the loaves (P<0.05). In the absence of phytase (-), bread from partially baked showed the lowest specific volume (Table 2), whereas no significant differences were observed between the ones obtained from conventional process or frozen dough. The same trend was observed in the moisture content, although in this case bread from PB had the highest value. Taking into account that FD and PB, in the absence of phytase, were subjected to freezing during breadmaking, it seems that freezing itself did not affect the specific volume or it could affect in different manner to frozen dough and partially baked bread. Poinot et al. (2008) found that breadmaking processes (conventional, frozen dough and partially baked bread), when running with the same formulation, does not produce any effect on the density of white wheat breads. The presence of phytase decreased the specific volume of the bread when it was obtained following conventional process. This result disagrees with previous findings of Haros et al (2001a). Likely wholemeal flour composition might be responsible of this divergence, since the action of the phytase affects the endogenous alfa-amylase activity through the inhibitory role of phytates. No significant effect on the bread specific volume was observed when phytase was added in the other breadmaking processes.

Haros et al. (2001b) did not find any significant effect when fungal phytase was added to wholemeal bread. Neither breadmaking processes, nor the presence of phytase induced any significant effect on the bread shape (width/height ratio), in agreement with previous results when similar amount of phytase was added to wholemeal conventional breadmaking (Haros et al., 2001a). Freezing and thawing processes exert some stress on the refined wheat dough that cause a deterioration in the quality of the baked product, mainly affecting the protein fraction of the wheat flour and the shelf-life of the baker's yeasts. In consequence, extended proofing times are needed and reduced loaf volumes are obtained from frozen dough (Phimolsiripol et al., 2008). However, freezing process without frozen storage seems to have less detrimental effect on the whole wheat dough as revealed results of the present study.

Process significantly (P<0.001) affected texture profile parameters of the crumb, whereas phytase addition did not showed any significant effect on those parameters (Table 1). Regarding breadmaking process, similar effect has been observed on white wheat breads obtained by different breadmaking processes (Poinot et al., 2008). The combined effect of process and enzyme had significant ($P<0.01$) effect on hardness, chewiness and ($P<0.05$) resilience. Regarding the individual effect induced by each breadmaking process (Table 2), partially baked bread gave the softest crumb followed by the conventional process and frozen dough. Only in the frozen dough process was observed a significant softening effect derived from phytase addition, likely derived from the relationship existing between phytase and alfa-amylase activities, previously described. In the present study likely the presence of bran could modify that behaviour in whole meal bread, since the disruption of the structure induced by the bran particles will be enhanced with the formation of ice crystals. However, it seems that in the case of PB, where crumb was already formed when freezing, ice crystal formation could induce breaking of the gluten fibrils that form the skeletal framework of coarse pore walls, as has been previously described for frozen dough (Naito et al., 2004). As a consequence a disrupted crumb structure might be obtained, which is manifested as softer crumb. Breads from PB also showed significantly lower springiness, cohesiveness, chewiness and resilience, which again could be attributed to crumb rupture.

When crumb cells were analyzed deeply (number of alveoli, average area, average diameter and circularity), no significant difference could be attributed to the breadmaking process, neither to the presence of phytase (Table 1). However, although they were not significant (Table 3), some differences were induced by phytase in the number of alveoli, which could explain differences observed in the crumb texture. Rapid freezing and the absence of frozen storage might be responsible of those results, since the main effect on crumb microstructure occurs during prolonged frozen storage (Bárcenas et al., 2004; Ribotta et al., 2001).

Colour of crumb and crust changes due to process and phytase addition were estimated by *L*C*h* colour space (Table 3). Crust colour was significantly affected by type of process but no by the presence of enzyme, neither it was observed any interaction process x enzyme (Table 1). Luminosity and hue angle were significantly ($P<0.05$) higher in the case of bread from PB, whereas crust of conventional breads showed significantly higher chroma values (*C*) than FD(-) and PB(-), indicating more vivid colouration (Table 3). The two-stages baking that occur in the bread obtained from partially baked significantly modified the crust luminosity, likely the lower baking temperatures or shorter baking times are responsible of reduced Maillard reactions, yielding increased lightness. In fact, the aromatic profile observed in loaves from partially baked breads shows significantly lower amount of volatile compounds compared to frozen dough, mainly due to reduced amount of volatile compounds from fermentative process (Poinot et al., 2008). Despite differences observed in the crust colour, no significant differences were observed on the crust section or crust wideness (Table 3). Crumb colour parameters L^* and C were significantly affected by process and only L^* was significantly (P<0.001) modified by the enzyme, also the combined action of process x enzyme did modify significantly this parameter (Table 1). Again, crumb of bread from PB had significantly higher L^* than the ones obtained from the other processes. Phytase presence significantly affected L^* in bread from conventional and FD, but no change was observed in breads from PB (Table 3).

Crust properties have been considered an important factor for bread quality assessment. Crust flaking resulting from the detachment of some part of the crust constitutes an important drawback, which has been related to excessive drying of the bread surface at the end of the post-baking chilling and freezing process (Hamdami et al., 2007; Lucas et al., 2005). Breadmaking processes only affected significantly ($P<0.05$) the crust flaking size and the crumb to crust weight ratio, whereas the enzyme did not influence crust properties (Table 1, Table 3). In the absence of phytase, bread from FD gave significantly smaller crust flakes than the bread obtained from conventional process, whereas breads from PB showed an intermediate behaviour (Table 3). Crust flaking has been mainly investigated on partially baked bread stored under frozen conditions (Le Bail et al., 2005). This phenomenon has been ascribed to two different processes, first, the concentration of water as ice under the crust due to the presence of the freezing front. Second process is the interfacial differences between the crust and crumb associated with the tensile forces and stresses induced by the thermo-mechanical shock (Lucas et al., 2005). However, the similar values of crust flakes amount (CF_M) obtained with the different breadmaking processes stressed the role of the frozen storage on this phenomenon, because freezing itself did not induce significant differences. In addition, temperature fluctuations between crust and crumb produced during baking and cooling could be responsible of the observed effect on the size of the crust flakes. Le Bail et al.

(2005) reported that chilling conditions after partial baking are the most determinant parameter on the crust flaking followed by the proofing conditions, being advisable high air humidity during those processes for reducing crust flaking.

Effect of frozen storage and phytase addition on bread technological quality

Frozen dough and partially baked bread were stored at sub-zero temperatures during three months to determine the effect of frozen storage on the technological quality of wholemeal breads. Storage and breadmaking processes induced significant effects on the specific volume ($P<0.001$), crumb hardness ($P<0.001$) and crust flaking ($P<0.001$) (Figure 1), whereas phytase addition did not modify those parameters. Breads obtained from FD showed increasing specific volume when extending the storage period (Figure 1). Taking into consideration that the bran particles contained in the wholemeal dough causes disruption of the dough structure, structural changes derived from frozen storage and the redistribution of water associated to them might affect positively further wholemeal dough expansion leading a slight increase during storage. No trend was observed with the wholemeal breads obtained from PB. Bárcenas et al. (2004) observed that although the specific volume of white partially baked bread was not significantly affected by the duration of the frozen storage after 42 days, breads obtained from those PB showed a slight hardness increase. Conversely, previous findings regarding the quality of white breads obtained from stored frozen dough revealed that dough weight loss and bread crumb firmness increase with increasing storage time, although temperature fluctuations during storage could explain this divergence (Phimolsiripol et al. 2008; Ribotta et al., 2001).

Breadmaking process promoted significant effect ($P<0.001$) on the crumb hardness of bread, being loaves obtained from PB significantly ($P<0.001$) softer than those obtained from FD during all the frozen storage period tested (Figure 1). Results obtained during frozen storage confirmed that ice crystals formation and growing do not affect in the same way to FD and PB. Crumb hardness of the breads obtained from PB was kept almost constant during the whole storage (three months) and the presence of phytase did not induce any effect on this parameter. Breads from FD showed irregular values of crumb hardness, although an increasing trend could be envisaged, and no effect was clearly observed due to the presence of phytase. Ice crystals initially formed during freezing at the gas pore interface (Esselink et al., 2003) grow during frozen storage since the amount of freezable water in frozen doughs increases with frozen storage (Lu and Grant, 1999), yielding baked breads with harder crumbs and coarse texture (Sharadanant and Khan, 2003).

The amount of crust flakes underwent the greatest variation related to frozen storage (Figure 1). Along frozen storage, breadmaking process significantly ($P<0.001$) affected the amount of crust flakes (CF_M). Although freezing did not significantly affect the CF_M, frozen storage dramatically augmented this parameter. Principally, in breads obtained from FD crust flaking significantly ($P<0.001$) increased after one month of storage, and further storage only induced a smooth increase in CF_M. In breads obtained from PB, the effect of frozen storage on CF_M was only significant ($P<0.05$) after prolonged storage (three months).

Effect of different breadmaking technologies on the phytate content

During fermentation the cereal phytate-degrading enzyme degraded the total InsP_6 initially present in the wheat flour and generated lower *myo*-inositol phosphates as released hydrolysis products (Table 4). With the exception of breads obtained from PB, the residual content of phytates was significantly and positively affected by the addition of fungal phytase, which increased the percentage of InsP_6 hydrolysis from 22.9-34.3% to 54.9-60.9%. The *myo*-inositol phosphate profile was also significantly affected by the fungal phytase addition, particularly the lower phosphate compounds (InsP_4 and InsP_3) and that effect was independent on the breadmaking process followed (Table 4). In the case of higher phosphate compounds, fungal phytase significantly reduced the amount of InsP_6 and InsP_5 in breads obtained from conventional process and frozen dough.

Breadmaking process and the addition of fungal phytase significantly ($P<0.05$) affected the phytates hydrolysis during frozen storage (Figure 2). Initially, the InsP_6 amount showed a slight increase during frozen storage compared to the samples without storage, which was significant ($P<0.05$) in the case of PB samples. Presumably, the effect of freezing and frozen storage on dough microstructure could favor both the substrate liberation and the accessibility of the fungal phytase to the phytate compounds, as has been observed at dough level (Sanz Penella et al., 2007), being the overall result an increase in the level of InsP_6. A reverse tendency was observed with longer storage, the InsP_6 amount significantly (P<0.05) decreased, thus enzymes were not inactivated. On the other hand, in frozen systems although the low temperatures decrease reactions rate, the increment of solute concentration in the unfrozen phase could increase the rates. Another factor that may be involved is a possible catalytic effect of ice crystals, greater proton mobility in ice than in water, a favorable substrate catalyst orientation caused by freezing or a greater dielectric constant for water than ice (Fennema et al., 1973).

Regarding the type of process, in general the InsP_6 level was significantly (P<0.05) higher in breads from PB samples than those from FD samples (Figure 2). Partial baking could induce partial inactivation of the fungal phytases, whereas in FD samples the enzyme might remain active during the storage. Therefore, although phytase did not induce a significant effect on bread specific volume and crust flaking, significantly softer crumbs were obtained in breads from FD containing phytase.

A comparison of different breadmaking processes for obtaining wholemeal breads showed that the type of process significantly affects technological quality parameters like specific volume, crust and crumb color, crumb texture and moisture content, whereas the addition of fungal phytase only induced significant effect on the crumb lightness. However, there was significant interaction between breadmaking process and enzyme addition concerning specific volume, crumb lightness and crumb texture. Freezing and frozen storage influenced in diverse way the quality of wholemeal breads obtained from frozen dough or partially baked breads.

Freezing and frozen storage of wholemeal bread in the presence of fungal phytase decreased significantly the phytate content, independently of the breadmaking process followed, thus the combination of both variables could be a good approach to increase the mineral bioavailability in whole wheat breads.

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It does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

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FIGURE CAPTIONS

Figure 1. Effect of breadmaking process and frozen storage on some technological quality parameters of breads containing phytase (+) or in the absence of phytase (-). FD: frozen dough, PB: partially baked.

Figure 2. Effect of fungal phytase addition, breadmaking process and frozen storage on residual InsP6 content in whole wheat bread. Breads containing phytase (+) or in the absence of phytase (-). FD: frozen dough, PB: partially baked.

Table 1. Significant effects of the breadmaking process and the presence of fungal phytase on the technological quality parameters of wholemeal breads.

Parameters	Process	Enzyme	Process x Enzyme
Specific volume	***	ns	*
Crust colour			
*L**	***	ns	ns
C	*	ns	ns
h	**	ns	ns
Crumb colour			
*L**	*	***	*
C	**	ns	ns
h	ns	ns	ns
Crumb texture, TPA			
Hardness	***	ns	**
Springiness	***	ns	ns
Cohesiveness	***	ns	ns
Chewiness	***	ns	**
Resilience	***	ns	*
Moisture content	**	ns	ns
Crust flaking			
CF_M	ns	ns	ns
CF_S	*	ns	ns
Crust section	ns	ns	ns
Crumb to crust ratio			
Volume	ns	ns	ns
Weight	*	ns	ns
Crumb cell analysis			
Number of alveoli	ns	ns	ns
Average area	ns	ns	ns
Average diameter	ns	ns	ns
Circularity	ns	ns	ns

CF_M: g crust/100g bread; CF_S: average crust flaking size
ns: no significant effect; * significant effect at $P<0.05$; ** significant effect at $P<0.01$; *** significant effect at $P<0.001$.

Table 2. Effect of different breadmaking process and fungal phytase addition on technological quality parameters of the fresh loaves.

Process	Phytase	Specific Volume (g/cm3)	Moisture content (%)	width/height ratio	Crumb texture fresh bread				
					Hardness, g	Springiness	Cohesiveness	Chewiness, g	Resilience
Conventional	-	3.6 b	35.4 a	1.43 a	340 b	0.990 d	0.839 b	281 b	0.432 c
	+	3.3 a	34.9 a	1.43 a	337 b	0.982 cd	0.829 b	274 b	0.423 c
FD	-	3.7 b	35.1 a	1.65 a	418 c	0.960 bc	0.834 b	334 c	0.420 c
	+	3.9 b	35.3 a	1.69 a	344 b	0.981 cd	0.837 b	282 b	0.427 c
PB	-	3.2 a	38.5 b	1.66 a	256 a	0.922 a	0.794 a	188 a	0.345 a
	+	3.2 a	38.4 b	1.60 a	278 a	0.947 b	0.805 a	212 a	0.366 b

Means followed by the same letter within a column were not significantly different (P<0.05) (n=4).
Process: FD: bread obtained from frozen dough; PB: bread obtained from frozen partially baked bread
Phytase: -: in the absence of phytase; +: in the presence of phytase

Table 3. Effect of different breadmaking process and fungal phytase addition on the characteristics of crust and crumb of the crust and crumb of fresh loaves.

Process	Phytase	Crust flaking		Crust section, mm	Crumb to crust ratio		Crust Colour			Crumb Colour		
		CF_M	CF_S, mm^2		volume	weight	L^*	C^*	h	L^*	C^*	h
Conventional	-	0.10 a	0.77 b	2.7 a	1.19 ab	1.38 abc	56.5 ab	34.0 b	70.6 a	61.7 ab	18.1 a	78.7 b
	+	0.10 a	0.58 ab	2.5 a	0.94 a	1.17 abc	58.4 b	34.3 b	71.2 a	64.3 c	18.6 abc	78.6 ab
FD	-	0.08 a	0.37 a	2.9 a	1.48 ab	1.60 c	56.2 ab	31.4 a	71.9 a	59.9 a	19.3 c	77.4 a
	+	0.07 a	0.53 ab	2.7 a	1.73 b	1.54 bc	55.7 a	32.5 ab	71.6 a	62.2 b	19.4 c	78.6 ab
PB	-	0.13 a	0.67 ab	2.9 a	1.36 ab	1.07 a	63.5 c	31.4 a	75.8 b	66.4 d	19.1 bc	78.7 ab
	+	0.13 a	0.83 b	2.9 a	1.31 ab	1.11 ab	61.5 c	33.1 ab	74.7 b	65.3 cd	18.2 ab	78.9 b

Phytase: -: in the absence of phytase; +: in the presence of phytase

CF_M: g crust/100g bread; CF_S: average crust flakes size

Table 4. Residual amount of *myo*-inositol phosphates in whole wheat bread[ab]

Process	**Phytase**	**Hydrolysis**	***Myo*-inositol phosphates, μmol/ g d.m.**			
		%	**InsP_6**	**InsP_5**	**InsP_4**	**InsP_3**
Conventional	-	22.9 ± 13.5 a	5.88 ± 1.03 a	1.00 ± 0.27 a	0.47 ± 0.08 a	0.13 ± 0.06 a
	+	60.9 ± 7.8 b	2.99 ± 0.60 b	0.42 ± 0.06 b	0.15 ± 0.04 b	n.d.
FD	-	34.3 ± 14.7 a	5.01 ± 0.71 a	0.98 ± 0.32 a	0.58 ± 0.26 a	0.18 ± 0.01 a
	+	54.9 ± 5.4 b	3.44 ± 0.49 b	0.53 ± 0.10 b	0.20 ± 0.04 b	n.d.
PB	-	21.7 ± 11.1 a	5.97 ± 0.91 a	0.88 ± 0.12 a	0.45 ± 0.05 a	0.11 ± 0.03 a
	+	35.0 ± 14.4 a	4.96 ± 0.55 a	0.58 ± 0.09 b	0.13 ± 0.07 b	n.d.

[a]Means ± standard deviation followed by the same letter in the same column are not significantly different at 95% confidence level.
[b]**d.m.**: dry matter; **n.d.**: not detected; **InsP_3- InsP_6**: *myo*-inositol phosphate containing 3-6 phosphates per inositol residues.

Figure 1.

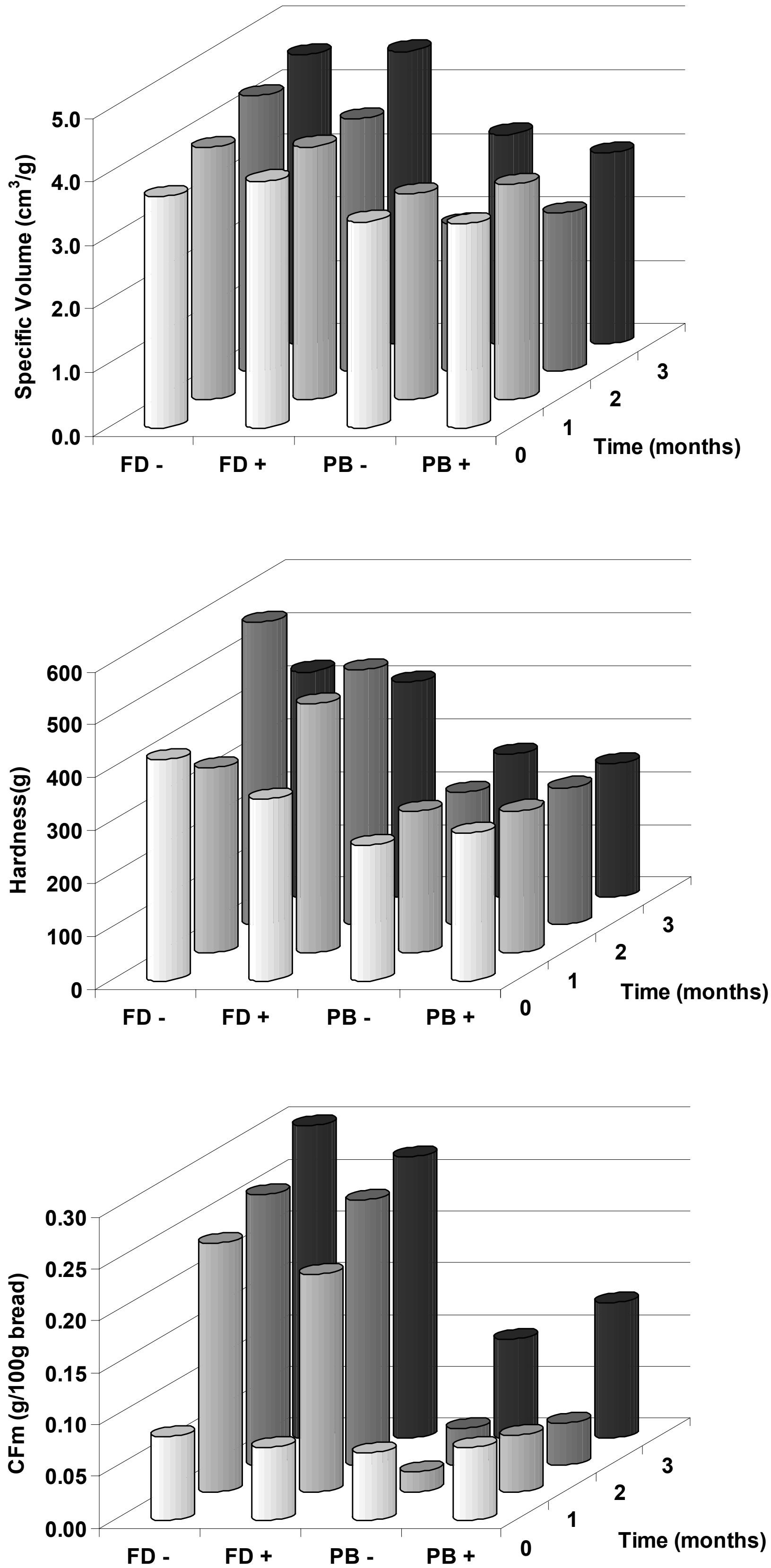
Specific Volume (cm3/g)
0.0
1.0
2.0
3.0
4.0
5.0
FD -
FD +
PB -
PB +
0
1
2
3
Time (months)
Hardness(g)
0
100
200
300
400
500
600
FD -
FD +
PB -
PB +
0
1
2
3
Time (months)
CFm (g/100g bread)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
FD -
FD +
PB -
PB +
0
1
2
3
Time (months)

Figure 2.