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# The effect of different enzymes on the quality of high-fibre enriched brewer's spent grain breads

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ABSTRACT

The brewing industry produces large quantities of waste co-products. There is increasing pressure to ensure total utilisation of such products to address economic and environmental concerns. Brewer's spent grain (BSG) the main by-product of the brewing industry is rich in dietary fibre and has a strong potential to be recycled. The overall objective of this study was to incorporate BSG into wheat flour breads together with a range of different enzymes (Maxlife 85, Lipopan Extra, Pentopan Mono BG and Celluclast) and evaluate the bread quality. A number of nutritional and textural properties of the finished product were studied. The incorporation of BSG significantly ($P < 0.0001$) improved the dietary fibre but the major difficulty encounted was to achieve a good structure and high loaf volume. Increasing the level of dietary fibre significantly ($P < 0.001$) increased dough development time, dough stability and crumb firmness but decreased the degree of softening and loaf volume. It was found that addition of Lipopan Extra (LE), Pentopan Mono (PE) and a mixture of Pentopan Mono and Celluclast (PCE) enzymes improved the texture, loaf volume and shelf life while Maxlife 85 enzyme (ME) was not significantly different from control samples (wheat flour breads containing 0%, 10%, 20% and 30% BSG). Image analysis of the bread structure obtained from the C-cell analyzer showed that the most significantly ($P < 0.001$) open network was obtained using LE, followed by PE and PCE.



## 1. Introduction

Consumer awareness of using healthy high-fibre foods is increasing, which is resulting in the need to develop a variety of foods with higher levels of dietary fibre. Brewer's spent grain (BSG), the residue left after separation of the wort during the brewing process (Santos, Jimenez, Bartolome, Gomez-Cordoves, & Nozal, 2003) is rich in cellulose (17%) and non-cellulosic polysaccharides mainly arabinoxylans (Mandalari et al., 2005). Around 3.4 million tonnes of BSG from the brewing industry are produced in the EU every year (Faulds et al., 2006), of which UK brewers contribute over 0.5 million tonnes of waste annually. The big advantage using BSG is that the brewing process uses material approved for human consumption, so there is a real potential for developing new products that can meet full regulatory approval. It has a strong potential for being recycled and used as a cheap source of fibre that may provide a number of benefits when incorporated into human diets such as a prevention of certain diseases including cancer, gastrointestinal disorders, diabetics and coronary heart disease (Aman, Zhang, Hallmans, & Lundin, 1994; Jacobs, Meyer, Kushi, & Folsom, 1998).

BSG was incorporated and significantly improved the dietary fibre breads (Dreese & Hoseney, 1982; Finley & Hanamoto, 1980; Plessas et al., 2007; Prentice & D'Appolonia, 1977; Stojceska, Ainsworth, & Plunkett, 2007). One of the main problems associated was to achieve a good structure and high loaf volume probably as a result of arabinoxylans, the mayor polymers in BSG (Kabel, Schols, & Voragen, 2002; Mandalari et al., 2005) and the most important dietary fibre compound. Arabinoxylans have significant influence on bread quality parameters as loaf volume, crumb texture and bread staling (Biliaderis, Izydorczyk, & Rattan, 1995; Wang, vanVliet, & Hamer, 2004). Arabinoxylan-fortified breads retain more water and exhibit a greater rate of starch retrogradation. The beneficial effects of arabinoxylans rich-fibre, prepared as a by-product of wheat flour processing, on lowering of postprandial glucose and insulin responses was reported in a study of Lu, Walker, Muir, Mascara, and Dea (2000). The other constituents of BSG that might affect rheological properties of dough and bread quality are cellulose, lipids and β-glucans.

Therefore, the objective of this study was to add BSG (without creating additional by-products) into bread formulations and investigate the potential of a number of different enzymes to improve the structure and loaf volume of baked breads. A number of authors have improved the structure and quality of high-fibre enriched breads by adding enzymes or yeast and lactic acid

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bacteria into the bread making process (Caballero, Gomez, & Rosell, 2007; Katina, Salmenkallio-Marttila, Partanen, Forssell, & Autio, 2006; Laurikainen, Harkonen, Autio, & Poutanen, 1998). Polysaccharide-degrading enzymes such as amylases and pentosanases have been usually used for these trials. A number of different enzyme mixtures such as α-amylase, xylanase and lipase have been used in rye bran (Laurikainen et al., 1998) and wheat bran (Katina et al., 2006) breads. Xylanases are hydrolytic enzymes that solubilized arabinoxylans and consequently change their physicochemical and functional properties, making dough slacker, softer and more viscous. Caballero et al. (2007) used a number of cross-linking enzymes along with polysaccharide and gluten degrading enzymes in bread making. Recently, Plessas et al. (2007) employed *Lactobacillus casei* that was immobilized on BSG using straight-dough and sourdough methods. To the author's knowledge there is no published work on the impact of enzymes on the quality of BSG breads and for that reason the data from this study are compared with results from studies involving different sources of fibre.

## 2. Materials and methods

The ingredients used for the bread making were: wheat flour with composition (w/w) of 12.6% protein, 1.3% fat, 0.9% sugar, 77.3% starch and 0.9% fibre (Smiths Flours Mills, Worksop, UK), yeast (Fermipan, Gist-Bracades, Holland), shortening (Cardowan Creameres Ltd., Glasgow), sugar and salt (purchased from a local supermarket in Manchester).

A commercial sample of brewer's spent grain with composition of 75% moisture, 1% ash, 4.8% protein, 16% fibre, 2.1% fat and 1.1% carbohydrates content was supplied by the Joseph Holts Brewery (Manchester, UK).

Four commercial enzymes were used: α-amylase Grindamyl Max-Life 85 (100–300 ppm), containing 100 Fungal Amylase Units/gram (Danisco Ingredients, Denmark); Lipopan Xtra BG enzyme (20–30 ppm), containing 7.2 Kilolipase Units/gram; Pentopan Mono BG (20–120 ppm), containing 2500 Fungal Xylanase Units(wheat)/grams and Celluclast L (10–60 ppm), containing 700 Endo-Glucanase Units/gram (Novozymes, Denmark).

### 2.1. Preparation of dry BSG

The source of BSG was based on brewing of barley hops and is the remains after wort mashing and straining, before fermentation of the beer. The BSG was refrigerated immediately after collection and then dried the following day at 150 °C for 4 h in a Teknotronic reel oven (Teknigas Ltd., Sussex, England) to a moisture content of 5–7%. The dried sample was milled using 0.5 mesh screens, packed and then sealed in polyethylene bags and kept at room temperature until use. The composition of dried BSG was 20.30% protein, 53.39% fibre, 8.32% fat and 10.76% carbohydrates.

### 2.2. Flour characteristics measurements

Farinograph measurements on the flours were carried out using a Barbender Farinograph (mixer bowl 300 g, Barbender OHG, Duisberg, Germany; ICC Standard method No. 115/1 (ICC, 1992)). Measurements obtained from the Farinograph torque curve were flour water absorption, dough development time, dough stability and degree of softening. Three repeats of the Farinograph measurements were carried and the results were averaged.

### 2.3. Bread making

The ingredients added were based on a % flour weight as follows: added water as per water absorption value (Barbander Farinograph) (range 58–61%), 2.3% salt, 6% sugar, 4% shortening and 3% yeast. Mixing of the ingredients was carried out in a Hobart NCM mixer (Process Plant and Machinery Ltd., UK) at 1st speed. The resulting dough was divided into three 300 g pieces, moulded by hand and placed into pre-greased 454 g tins. The dough was proofed for 55 min at 30 °C and 85% humidity, and baked in a Teknitronic reel oven (Teknigas, Ltd., Sussex, England) for 20 min at 230 °C.

The following bread types were studied:

- Four control samples were made: white wheat bread (WB) with the substitution levels of BSG at 0%, 10%, 20% and 30%.
- Four samples using Maxlife 85 enzyme (ME) in white wheat bread with the substitution levels of BSG at 0%, 10%, 20% and 30%. ME was added in flour with the dosage of 200 ppm which corresponds to 20 g/100 kg flour.
- Four samples using Lipopan Extra enzyme (LE) in white wheat bread with the substitution levels of BSG at 0%, 10%, 20% and 30%. LE was added in flour with the dosage of 25 ppm.
- Four samples using Pentopan enzyme (PE) in white wheat bread with the substitutional levels of BSG at 0%, 10%, 20% and 30%. PE was added in flour with dosage of 70 ppm.
- Four samples using a mixture of Pentopan + Celluclast enzymes (PCE) in white wheat bread with the substitutional levels of BSG at 0%, 10%, 20% and 30%. PE was added in flour with dosage of 70 ppm and Celluclast with 35 ppm.

ME, LE and PE were in powdered form and added to the flour while Celluclast was liquid and was added to water. Selected dosages of the enzymes were added following the suppliers recommendations.

### 2.4. Protein content (PC)

PC was estimated from the crude nitrogen content of the sample determined by the Kjedahl method (Nx 6.25), (AOAC, 1984).

### 2.5. Total dietary fibre content (TFC)

The total dietary fibre content of extrudates was determined using a combination of enzymatic and gravimetric methods (Sigma–Aldrich, Inc., Saint Louis, Missouri, US) (AOAC, 1997). Milled and dried samples were gelatinized with heat stable α-amylase and then enzymatically digested with protease and amyloglucosidase to remove protein and starch present in the sample. Ethanol was added to precipitate the soluble dietary fibre. The residue was then filtered and washed with ethanol and acetone. After drying, the residue was weighed. Half of the samples were analyzed for protein and the others ashed. Total dietary fibre is the weight of the residue less the weight of the protein and ash.

### 2.6. Colour

The colour of the bread crumb and crust was assessed using a Hunter-Lab colorimeter (Optical Sensor, Hunter Associates Laboratory Inc., Reston Virginia, USA). In the Hunter-Lab colorimeter, the colour of a sample is denoted by the three dimensions, $L^*$, $a^*$ and $b^*$. The $L^*$, $a^*$ and $b^*$ readings were then taken from a digital display unit (Model D 25-2). The $L^*$ value gives a measure of the lightness of the product colour from 100 for perfect white to zero for black, as the eye would evaluate it. The redness/greenness and yellowness/blueness are denoted by the $a^*$ and $b^*$ values, respectively. The colour of the samples was measured after grinding the samples to fine flour (particle size < 200 μm) using a type ZM100 ultra centrifugal mill (Retsch GmbH & Co, Haan, Germany).

### 2.7. Evaluation of bread quality

Quality analysis of fresh bread samples was carried out by measuring weight, volume (determined by seed displacement in a loaf volume meter) and specific loaf volume.

Crumb firmness was measured using a texture analyzer (TA-XT2i, Stable Micro Systems, Surrey, UK) at days 1, 2, 5 and 8 days to assess potential shelf life of the breads. Bread crumb firmness during storage was determined as maximum compression force according to AACC 1998, modified method 74-09. The loaves were sliced by hand into equal slice thickness of 25 mm thick. The crust slices of the loaves were discarded. Nine bread slices originating from three loaves were measured and results were expressed as mean values.

Image analyses were carried our using a C-Cell analyzer (Calibre Control International Ltd., Appleton, UK). Slices were selected from near the centre of each loaf and measurements of fineness (cell/cm$^2$), total cell area and wall thickness were analysed.

### 2.8. Reproducibility

All the experiments were replicated, so that the data in the paper are all mean values of three tests. The coefficient of variation (CV) of all the tests was lower than 10%.

### 2.9. Statistical analysis

Statistical analyses were conducted using a multiple comparison test using SPSS 12.0.1 (SPSS Inc., Chicago, Illinois, US).

## 3. Results

### 3.1. The addition of BSG into bread making

#### 3.1.1. Nutritional analysis and colour of breads

The fibre, protein and fat content together with the colour of breads containing BSG are given in Table 1. As a result of adding up to 30% BSG a wide variation in fibre content was observed with values ranging between 2.3% and 11.5%. BSG addition significantly improved the fibre level of breads ($r^2 = 0.99^{***}$) which is in agreement with the other studies where BSG has been used as a fibre source (Finley & Hanamoto, 1980; Kawka, Gorecka, & Gasiorowski, 1999; Prentice & D'Appolonia, 1977). Variation in protein content of breads was between 10.7% and 11.0% and was not related to the addition of BSG. Fat content varied between 3.4% and 4.4% and was significantly correlated ($r = 0.9^{***}$) to the level of BSG. The colour values recorded for the breads containing different levels of BSG (0–30%) showed that there was a significant difference ($P < 0.001$) between control samples and samples containing 10% and 30% BSG, and samples containing 20% BSG with 10% and 30% BSG. The redness and yellowness of breads were significantly different as BSG levels increased ($r = -0.9^{***}$).

**Table 1**
Some nutritional analyses, colour, water absorption and Farinograph characteristics of BSG breads

| BSG (%) | 0 | 10 | 20 | 30 |
|---|---|---|---|---|
| Fibre (%) | 2.3$^a$ | 6.3$^b$ | 9.7$^c$ | 11.5$^d$ |
| Protein (%) | 11.0$^a$ | 10.7$^c$ | 11.4$^d$ | 10.9$^a$ |
| Fat (%) | 3.4$^a$ | 3.4$^a$ | 3.8$^b$ | 4.4$^c$ |
| Colour | | | | |
| $L^*$ | 43.4$^a$ | 41.9$^b$ | 43.4$^a$ | 41.7$^b$ |
| $a^*$ | 0.7$^a$ | −0.3$^b$ | −1.1$^c$ | −1.6$^d$ |
| $b^*$ | 17.6$^a$ | 16.4$^b$ | 16.0$^b$ | 14.4$^c$ |
| Water absorption (%) | 58.0$^a$ | 61.0$^b$ | 60.5$^{b,c}$ | 60.0$^c$ |
| Dough development time (min) | 3.5$^a$ | 7.0$^b$ | 13.0$^c$ | 18.0$^d$ |
| Stability (min) | 6.5$^a$ | 9.5$^b$ | 10.0$^b$ | 18.0$^c$ |
| Degree of softening (BU) | 25.0$^a$ | 15.0$^b$ | 20.0$^c$ | 5.0$^d$ |

Different letters in the same row shows statistically significant values (0.05).

#### 3.1.2. The effect of fibre on Farinograph parameters

Farinograph information of the dough mixing behaviour of the bread mixture with different levels of BSG (0–30%) is shown in Table 1. Water absorption increased with fibre addition with a variation between 58% and 61% at a fixed dough consistency of 700 BU. Dough development time (3.5–18 min) and dough stability (6.5–18 min) increased while degree of softening (5–25 BU) decreased as the level of fibre increased. Increasing the water absorption as a result of dietary fibre addition using different sources as rye bran, rye pentosans and arabinoxylans were reported by a number of workers (Billiaderis et al., 1995; Laurikainen et al., 1998; Michniewicz, Biliaderis, & Bushuk, 1991), probably as a result of the presence of hydroxyl groups in the fibre structure that allow more water interactions through hydrogen bonding (Rosell, Rojas, & Benedito de Barber, 2001). In a study of Sudha, Vetrimani, & Leelavathi (2007) barley, wheat, oat and rice brans were used as a source of dietary fibre and the highest increase in water absorption was obtained by the addition of barley bran, followed by oat and rice bran blends while dough development time was higher with wheat and rice bran blends and dough stability increased significantly with increased oat and barley blends.

### 3.2. The addition of enzymes into high-fibre breads

In order to improve the loaf volume, shelf life and the structure of high-fibre enriched breads, a number of different enzymes were employed such as: Maxlife 85 (ME), Lipopan Extra (LE), Pentopan mono (PE), combination of Pentopan mono and Celluclast (PCE).

Grindamyl Max-Life 85 as an amylolytic enzyme complex was suggested by the manufacturer to improve the loaf volume of high-fibre enriched breads (Danisco Ingredients, Denmark).

Lipopan Xtra BG enzyme was suggested since it has good dough-strengthening properties and unlocks the dough-strengthening potential of the natural lipids in flour (Novozymes, Denmark).

Pentopan mono BG was recommended because of its ability to improve dough properties such as extensibility and stability which leads to increased loaf volume and improved crumb structure (Novozymes, Denmark).

Celluclast L was suggested for its special dough-conditioning application in combination with Pentopan mono BG (Novozymes, Denmark).

#### 3.2.1. The effect of fibre and enzymes on loaf volume

The specific loaf volume of sixteen samples of bread containing ME, LE, PE and PCE were studied while samples with the level of BSG ranging between 0% and 30% were used as control (Fig. 1).

Specific loaf volume of breads containing different levels of fibre varied between 2.06 and 3.22 ml/g with significant correlation between fibre content and resulting loaf volume $r = -0.8^{***}$. The greatest loaf volume reduction was detected at 30% BSG addition. These results were comparable to those previously reported (Finley & Hanamoto, 1980; Kawka et al., 1999; Prentice & D'Appolonia, 1977) where addition of fibre content from BSG significantly decreased the loaf volume of baked breads. Arabinoxylans the most important dietary fibre compound in BSG seems the main contributor for decreasing loaf volume. Some of the investigators increased loaf volume and improved the crumb structure of BSG breads by adding shortening or sodium stearoyl lactylate (Dreese & Hoseney, 1982). Plessas et al. (2007) employed a sourdough

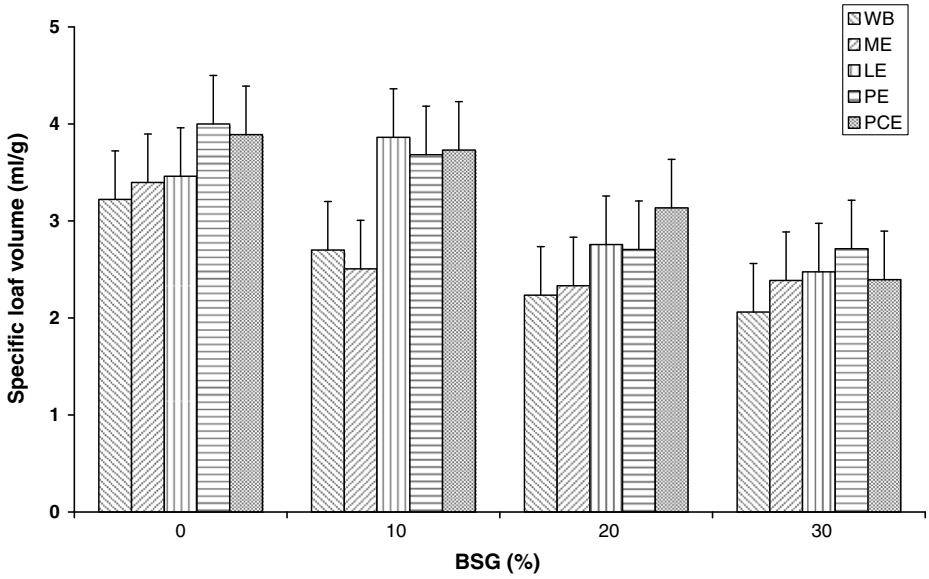


**Fig. 1.** Specific loaf volume obtained using different levels of BSG and different enzymes.

method with a higher moisture retention during baking, which caused acidification of the dough, proteolysis of gluten and moderate hydrolysis of starch that affected some physicochemical changes (Corsetti et al., 1998).

Addition of ME into the bread formulations with specific loaf volume ranged between 2.4 and 3.4 ml/g resulting in a significant ($P < 0.001$) decrease as the level of fibre increased. There was no significant difference when compared to the control samples containing different fibre levels.

Specific loaf volume of samples containing enzymes LE (2.4–3.9 ml/g), PE (2.7–4 ml/g) and PCE (2.4–3.9 ml/g) behaved in a similar way with significantly ($P < 0.0001$) lower specific loaf volume at 20% and 30% of BSG. No significant difference in specific loaf volume was found among these samples at different fibre levels except at 10% BSG where LE was significantly ($P < 0.001$) higher than PE and PCE samples. Comparing these samples (LE, PE and PCE) with their equivalent of control samples and ME samples, all of them showed significantly ($P < 0.0001$) higher specific loaf volume.

### 3.2.2. The effect of fibre and enzymes on shelf life

The shelf life of the breads containing different levels (0–30%) of BSG and different enzymes was tested at 1 day, 2 day, 5 day and 8 day (Fig. 2). Fibre addition significantly ($P < 0.001$) increased crumb firmness in samples containing 20% and 30% BSG while no significant difference was found with 10% addition. This significant difference was observed at each day of storage with the 20% and 30% BSG samples. Biliaderis et al. (1995) reported that the molecular weight of arabinoxylans significantly increased the firmness of crumb but the level of arabinoxylans significantly decreased in wheat flour breads over 7 days storage. This could be explained by the fact that cereal brans consist of different tissues and thus the actual fine structures of isolated arabinoxylans are very diverse (Mandalari et al., 2005).

ME breads showed a significant ($P < 0.001$) increase in hardness as fibre increased while no significant difference was found compared with their equivalent control sample. The same trend was observed during each day of storage. LE showed no significant difference in crumb firmness at 0% and 10% BSG while a significant ($P < 0.0001$) difference was observed at 20% and 30% BSG. Compared with the equivalent control samples, LE gave significantly ($P < 0.0001$) lower crumb firmness at all levels of BSG (0–30%) for each day of storage. PE showed a significant ($P < 0.0001$) increase of crumb firmness as level of BSG increased up to 20% but decreased at 30% BSG. Compared with the equivalent control samples the only significant ($P < 0.001$) decrease of crumb firmness was detected at 30% BSG. Again this was apparent during each day of storage. PCE showed a significant difference in hardness ($P < 0.0001$) at all levels of dietary fibre. Compared with its equivalent control samples the only difference was found at 20% and 30% BSG. Bread containing LE, PE and PCE showed a clear tendency towards a softer crumb and a reduced rate of staling compared with the control samples and samples containing ME. PE showed significantly ($P < 0.0001$) increased hardness during storage compared with LE and PE at 20% BSG.

In this study, the best results in terms of crumb firmness were obtained with LE with a significant ($P < 0.001$) delay in staling compared with their equivalent control samples. It was observed that PE and PCE extended the shelf life at higher levels of dietary fibre. The positive influence of enzymes on the shelf life of breads have also been observed by a few investigators (Caballero et al., 2007; Katina et al., 2006; Laurikainen et al., 1998), where a combination of bran, sourdough and enzyme mixture was used. However, it has been reported that pentopans have slight transgutaminase site activity and may caused hardening by cross-linking gluten proteins (Autio et al., 2005).

### 3.2.3. Image C-cell analysis

Figs. 3–5 present the image analysis made on slices of breads containing different levels of BSG and different enzymes. A number of characteristics was obtained from the C-cell analyzer such as: density of the cells, area of cells and wall thickness. Increasing the level of fibre did not affect these parameters. There was no significant difference between ME samples and the control samples in terms of mean cell area at all levels of BSG. It was expected that ME would open the network structure as reported previously in the study of Caballero et al. (2007), where amylose increased mean cell area. The most significant ($P < 0.0001$) open network was detected using LE with samples containing 10% and 30% BSG, followed by PE at 30% BSG ($P < 0.05$) (Fig. 4). Breads containing LE showed a significant decrease in cell density with 10%, 20% and 30% BSG ($P < 0.0001$) while ME breads showed a significant ($P < 0.0001$) increase in cell density at 10% BSG. PE significantly ($P < 0.05$) decrease cell density at 30% BSG (Fig. 3). Consequently, the wall

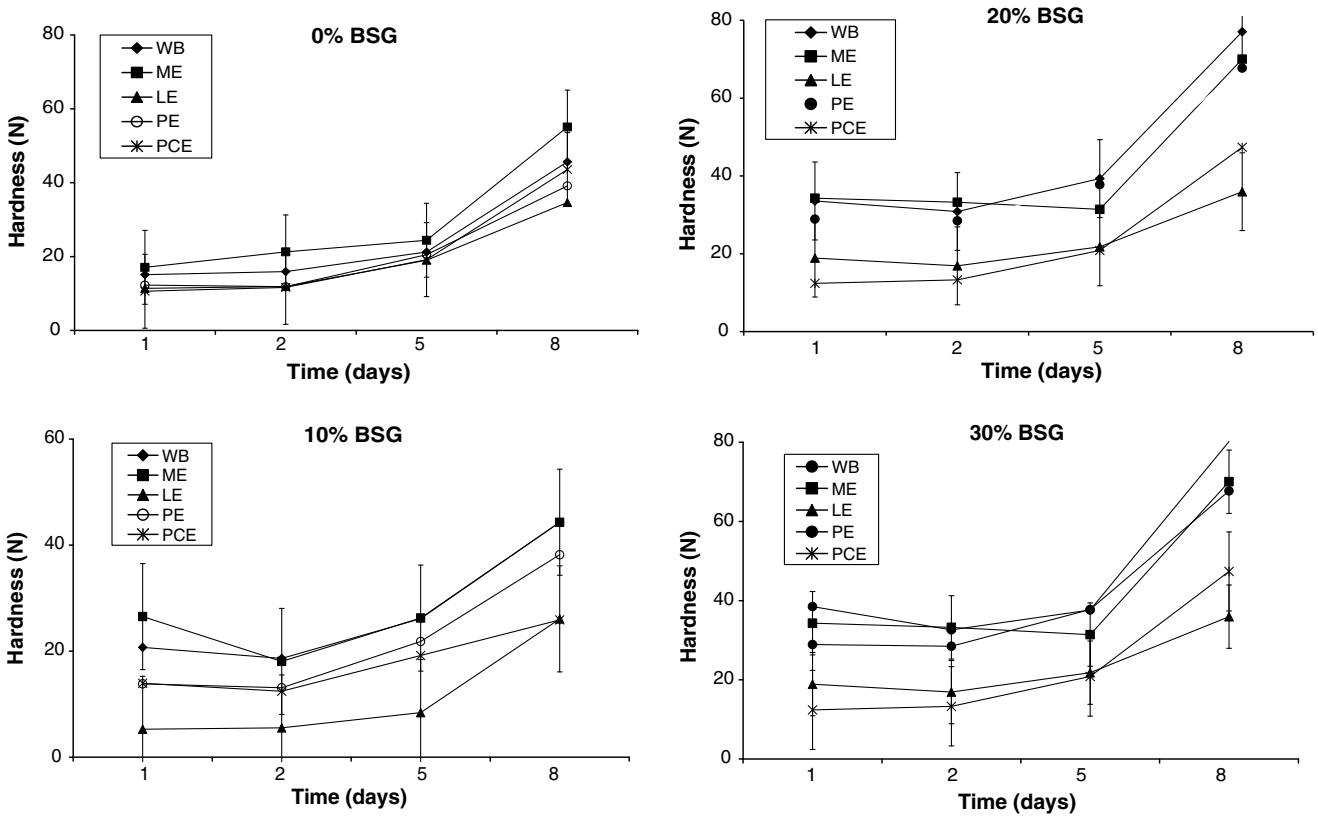


**Fig. 2.** Maximum hardness of breads made with different levels of BSG and different enzymes during storage for 8 days.

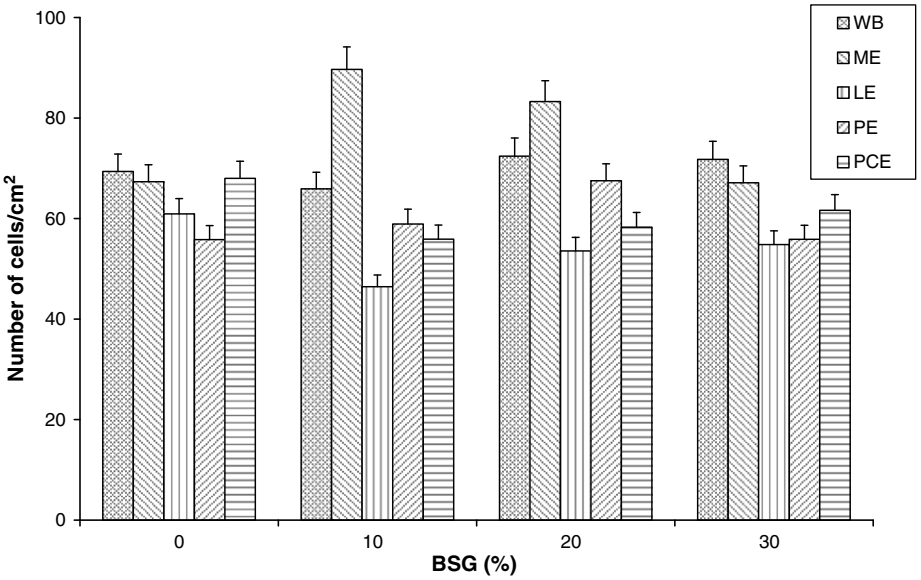


**Fig. 3.** Density of cells in breads made with different levels of BSG and different enzymes.

thickness of breads with LE was significantly ($P < 0.0001$) increased with 10% ($P < 0.001$) and 20% ($P < 0.0001$) BSG. ME significantly ($P < 0.001$) decreased wall thickness with 10% BSG whereas PCE increased with 20% BSG (Fig. 5). Similar findings were reported by Lurikainen et al. (1998), when as a result of the addition of different enzymes, the crumb pore size in wheat breads increased slightly with the greatest effect achieved using xylanase.

## 4. Discussion

The incorporation of dietary fibre in food from new sources is a challenging task for cereal technologists. Cereal co-products such as brewer's spent grain represent untapped resources for obtaining industrially important hydrocolloids, such as arabinoxylan and protein (Mandalari et al., 2005). Pentose content (sum of xylose

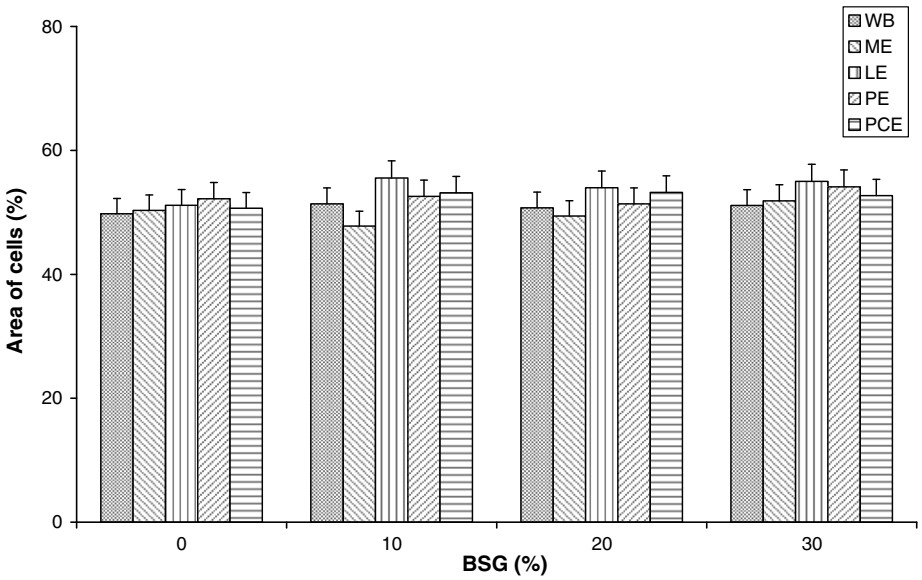


**Fig. 4.** Total area of cells in breads made with different levels of BSG and different enzymes.

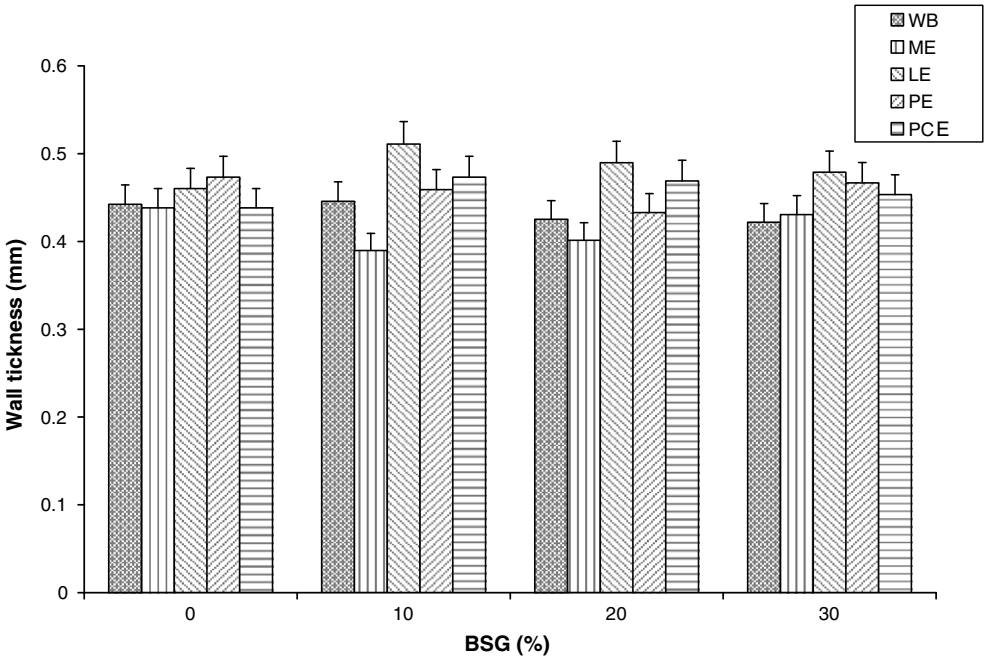


**Fig. 5.** Wall thickness in breads made with different levels of BSG and different enzymes.

and arabinose) of BSG has been found to vary between 21.0% and 27.3% dry weight for oven dried samples (Santos et al., 2003). The addition of BSG into dough formulations significantly improved the dietary fibre content in baked breads (Table 1). It was apparent that increased level of pentosans mainly arabinoxylans in BSG significantly affected Farinograph water absorption, dough development time, dough stability, degree of softening, loaf volume of bread, moisture content and bread staling rate (Table 1). Similar results were reported by Sudha et al. (2007) where barley, wheat, oat and rice bran blends were incorporated into the bread making process and Farinograph parameters were affected in the same manner. Machniewicz et al. (1991) and Billiaderis et al. (1995) added various levels of wheat and rye pentosans and different arabinoxylans into wheat flours and they found that dietary fibre significantly increased dough development time and dough stability while decreasing the degree of softening. However, there are contradictory reports obtain from Farinograph analyses for high-fibre enriched breads supplemented with different sources of bran: rye bran increased water absorption and dough development time with little effect on stability and softening of dough (Laurikainen et al., 1998). It was found that the addition of arabinoxylans above 0.7% (w/w) into bread formulations decreased loaf volume (Biliaderis et al., 1995). Fibre supplementation restricts the amount of water available for gluten hydration (Lai, Hoseney, & Davis, 1998), disrupts the starch–gluten matrix and affects the gas-holding capacity (Gan, Ellis, & Schofield, 1995) that contributes significantly to the low specific volume. The deleterious effects of the addition of fibre on dough structure have been suggested to be due to the dilution of the gluten network, which in turn impairs gas retention rather than gas production (Autio & Laurikainen,

1997). Therefore, it could be concluded that crumb firming during storage mainly depends on initial crumb firmness (Armero & Collar, 1998).

In this study, the quality of BSG breads was improved by addition of enzymes in the bread making process. Apart from ME, the other enzymes used LE, PE and PCE promoted a similar significant ($P < 0.001$) increase in loaf volume (Fig. 1), improved the shelf life (Fig. 2) and bread structure (Figs. 3–5).

ME is an amylolytic enzyme complex produced by fermentation with selected fungal and bacterial strains. α-Amylase enzymes are widely used in the food industry to hydrolyze starch to glucose. In this study, ME did not improve loaf volume, shelf life and had no effect of mean cell area which is not in agreement with the other researchers (Caballero et al., 2007; Katina et al., 2006). Katina et al. (2006) found that ME combined with other enzymes such as xylanase and lipase significantly improved loaf volume, texture and shelf life of breads supplemented with wheat bran. The delay in staling and increasing mean cell area due to ME activity has been found in wheat flour breads (Caballero et al., 2007). These authors reported that the effectiveness of amylases in high-fibre baking to retard bread staling is due to the changes in cell wall polysaccharides of wheat flour. In a combination of a bran, sourdough and enzyme mixture, the reported beneficial effect of enzymes staling is most likely due to an improved loaf volume. This significantly reduces starch retrogradation and possibly alerts the distribution of water between the starch and protein phases during storage confirming the effectiveness of amylases in retarding bread staling in high-fibre bread. In our study, ME was used as a single enzyme and there was no significant difference in terms of loaf volume, texture characteristics and shelf life compared to the control samples. It appears that this enzyme would be better used in combination with other enzymes because the changes involving the other dough constituents such as gluten, non-starch polysaccharides, lipids and water are probably as important as well. However, differences have been shown to result when different sources of arabinoxylans are need, e.g. Katina et al. (2006) and Caballero et al. (2007) who used wheat flour and wheat bran. It has been suggested that barley arabinoxylan has a higher arabinose: xylose ratio than wheat endospermic arabinoxylan but in the case of by-products, the opposite appears to be true (Mandalari et al., 2005). This may be due to the solubility of highly substituted arabinoxylan from BSG being removed during the brewing process. Wheat bran has not been through such a processing route. BSG is also representative of the whole grain, not just the bran. Furthermore, BSG had undergone enzymatic processing as part of malting during the brewing process, which could influence the secondary extraction of feruloylated arabinoxylans (Mandalari et al., 2005).

The effect of LE showed an opposite trend to that observed for ME. LE produced beneficial effects during bread making, positively affecting loaf volume, staling rate and crumb structure. It is well-known that flour lipids strongly correlate with loaf volume (Gan et al., 1995). The additional amount of lipids derived from BSG into a dough mix is about 9% (Prentice & Refsguard, 1978). Lipases hydrolyse the ester bonds of triglycerides, yielding mono- and diglycerides and free fatty acids (Olesen, Qi Si, & Donelyan, 1994) that lead to improved loaf volume and dough rheological properties as well as the quality of the baked products. Furthermore, it has been postulated that lipases modify the interaction between the flour lipids and gluten (Olesen et al., 1994). The interaction of pentosans and starch (Jankiewicz & Michniewicz, 1987) and the increased level of monoglyceride that forms amylase-lipid complexes (Johnson & Welsh, 1968) play an important role in retarding the starch retrogradation process and staling of breads.

PE and PCE showed a similar trend to that observed with LE, positively affecting the loaf volume, staling rate and crumb structure. Similar effects have been already reported, where xylanase significantly increased the stickiness of wheat and rye bran dough, slightly increased of crumb pore size, reduced staling rate and softened the bread crumb (Laurikainen et al., 1998). Xylanases are endo-active enzymes that solubilize arabinoxylanases and consequently change their physicochemical and functional properties while cellulases should contribute to enzymatic hydrolysis of cellulose to glucose (Tomme, Warren, & Gilkes, 1995). They profoundly impact on gluten agglomeration behaviour during gluten–starch separation and indirectly provide insight into the role of arabinoxylans in gluten aggregates (Frederix, Courtin, & Delcour, 2004). In a study of Caballero et al. (2007), Pentopan Mono BG exerted a softening effect on the crumb of transglutaminase-supplemented pan breads, leading to significant decreases in hardness, gumminess and chewiness. However, the interaction between xylanase and the other enzymes and different sources of fibre should be taken into consideration as well. The enzyme mixtures used in a study by Laurikainen et al. (1998), were more efficient than individual xylanase in softening the bread crumb, giving larger volume and reducing the staling rate of wheat breads and rye bran breads. The same authors found that added enzymes reduced the total dietary fibre content of the breads but doubled the amount of soluble pentosans.

Altogether, these results indicate that BSG could be added up to 30% level to a bread formulation and together with appropriate enzymes improve the loaf volume, texture and shelf life. LE in breads gave the most open network, increased wall thickness and decreased cell density, which resulted in a higher loaf volume and extended shelf life. PE and PCE were more effective in increasing loaf volume and extending shelf life of breads containing higher levels of fibre probably because the levels of arabinoxylans and celluloses derived from BSG were higher. This work is still continuing by developing wholemeal BSG breads by forming a sourdough with the addition of a range of enzymes in order to improve texture and sensory quality.

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