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Oxidative and proteolytic enzyme preparations as promising improvers for oat bread formulations: Rheological, biochemical and microstructural background

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ABSTRACT

Commercial preparations of laccase (LAC) and glucose oxidase (GO) (0.01% and 0.1% addition levels), as well as of a protease (PR) (0.001% and 0.01% addition levels) were tested for their impact on the breadmaking performance of gluten-free oat flour. LAC 0.1%, PR 0.001% and PR 0.01% additions significantly improved oat bread quality, as they increased specific volume and decreased crumb hardness and chewiness. In contrast, GO 0.1% addition revealed detrimental effects, as it resulted in the hardest bread crumb. The improved breadmaking performances of oat breads with LAC and PR addition was explained by the increase in batter softness, deformability and elasticity which were achieved upon addition of these enzyme preparations, both containing discernible levels of endo-β-glucanase side activity. With LAC, the effect is due to prevalence of β-glucan depolymerisation over protein polymerisation while, with PR, it is due to the combined effect of protein and β-glucan degradation. Extensive protein hydrolysis during baking may have increased functionality of the soluble protein fraction. In contrast, extensive protein polymerisation was detrimental, as indicated by GO addition.

1. Introduction

Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible persons (Catassi & Fasano, 2008). At present, the only safe and effective treatment for CD sufferers is the avoidance of gluten-containing foods, such as wheat, rye and barley products (Ciclitira, Johnson, Dewar, & Ellis, 2005). A general growing awareness of CD has increased the interest of the food industry in marketing gluten-free cereal products.

Oats are rich in fibres, i.e. β-D-glucan, and contribute significant levels of dietary minerals and vitamins (Welch, 1995) as well as of essential amino acids. Therefore, gluten-free (GF) oat breads may well contribute to a nutritionally improved and diversified GF diet for CD sufferers. However, the development of such breads poses a major technological hurdle, as oat proteins do not have the viscoelastic properties of wheat gluten. Recently, enzymatic processing of GF flours has been investigated as a tool to improve the breadmaking performance of the flours by modifying the structure–functionality of its proteins (Gujral & Rosell, 2004a, 2004b; Marco & Rosell, 2008; Renzetti, Behr, Vogel, & Arendt, 2008; Renzetti, Dal Bello, & Arendt, 2008).

Oxidative enzymes, such as glucose oxidase (GO) and laccase (LAC), are of particular interest in the breadmaking industry as bread improvers. GO (EC 1.1.3.4) catalyses the oxidation of glucose to form gluconic acid and hydrogen peroxide. In wheat flour breadmaking, the hydrogen peroxide promotes intermolecular and intramolecular disulphide linkages in the gluten network and the gelation of water-soluble arabinoxylan (Primo-Martin, Valera, & Martinez-Anaya, 2003; Vemulapalli & Hoseney, 1998). LAC (EC 1.10.3.2) catalyses the oxidation of various aromatic compounds, particularly *o*-diphenols, producing semiquinones with the concomitant reduction of molecular oxygen to water. The free radical may lead to polymerisation of the semiquinones. In wheat flour, LAC catalyses the polymerisation of feruloylated arabinoxylans by dimerisation of their ferulic esters (Figueroa-Espinoza & Rouau, 1998). Protein cross-linking may also result from oxidation of sulfhydryl groups, resulting in disulphide bonds (Labat, Morel, & Rouau, 2000a). In wheat flour-based breadmaking applications, GO and LAC treatments increase dough strength and stability, increase loaf bread volume and improve crumb structure and softness (Goesaert

Abbreviations: CD, celiac disease; CLSM, confocal laser scanning microscopy; CON, control; dm, dry matter; G^*, complex modulus; GO, glucose oxidase; GF, gluten-free; LAC, laccase; LMW, low molecular weight; δ, phase angle; PR, protease; RVA, rapid visco-analysis; TPA, texture profile analysis; WEAX, water-extractable arabinoxylans.

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et al., 2005; Labat, Morel, & Rouau, 2000b; Si, 1994; Vemulapalli & Hoseney, 1998). Therefore, GO and LAC may well promote the formation of a protein and/or non-starch polysaccharide network in oat batters, resulting in improved breadmaking performances.

GF systems are quite liquid and resemble cake batter more than dough, leading to a need for water binders and surface-active substances. In sponge cake systems, protein-foaming properties are fundamental in determining the overall textural quality of the product (Çelic, Yilmaz, Işik, & Üstün, 2007). Limited enzymatic hydrolysis of wheat gluten significantly increases its foaming and emulsifying properties (Drago & González, 2001; Kong, Zhou, & Qian, 2007), improving cake volume and moistness (Bombara, Añón, & Pilosof, 1997). In GF systems, limited hydrolysis may also improve protein functionality in the batters and, therefore, the breadmaking performance. We have recently reported that protein degradation improves the breadmaking performance of brown rice flour by increasing batter deformability and paste stability during proofing and in the early stages of baking (Renzetti & Arendt, 2009). Therefore, protease (PR) treatment of oat flour could well improve its breadmaking performance.

The aim of the present study was to investigate the impact of two oxidative (GO and LAC) and one proteolytic (PR) enzyme preparation on the breadmaking performance of oat flour. Standard baking tests and texture profile analysis were used as tools to evaluate the breadmaking performance, while batter properties were assessed using batter development tests and fundamental rheology measurements. Furthermore, the physicochemical and microstructural impact of the enzymatic treatments was investigated by means of capillary gel electrophoresis, rapid visco-analysis (RVA) and confocal laser scanning microscopy (CLSM).

2. Materials and methods

2.1. Materials

Oat flour (9.4% protein, ash 1.2%, 10.5% moisture; Flavahans Co., Waterford, Ireland), was used, in conjunction with instant dried yeast (Pante, Puratos, Groot-Bijgaarden, Belgium), salt, sugar and tap water.

Three types of enzymes, in the form of commercial preparations, were used: a glucose oxidase (GO, Gluzyme Mono 10000 BG, Novozymes, Baegsvaard, Denmark) containing 10,000 GO units/g, a laccase (LAC, NS26021, Novozymes, Baegsvaard, Denmark) containing 1000 LAC units/g, and a protease (PR, Neutrase 1.5 MG, Novozymes, Baegsvaard, Denmark) containing 1.5 AU-NH/g [equivalent to 169 and 288 endo-protease units/g at pH 7 and 40 °C, based on *Bacillus licheniformis* and *Bacillus subtilis* proteases, respectively (azo-casein kit, Megazyme)]. According to supplier specifications, GO (from *Aspergillus oryzae*) had pH and temperature activity ranges of ca. 3.3–7 and 15–50 °C, respectively (optima: pH 5.3, 35 °C); LAC (from *Myceliophthora thermophila*) of ca. 4.5–6.5 and 15–65 °C (optima: not provided), respectively; and PR (from *Bacillus amyloliquefaciens*) of ca. 5–8 and 25–70 °C, respectively (optima: pH 6.5, 45 °C). Following the supplier's recommendations, the selected enzyme dosages on flour weight were: 0.01% and 0.1% for GO and LAC, and 0.001% and 0.01% for PR.

LAC did not contain significant endo-protease (azo-casein kit, Megazyme International Ireland Ltd., Bray, Ireland) or xylanase activities (Xylazyme AX tablets, Megazyme International Ireland Ltd., Bray, Ireland).

No α-amylase activity was present in the LAC and GO preparations (Alpha-amylase Assay Procedure – Ceralpha method, Megazyme International Ireland Ltd., Bray, Ireland). Instead, the commercial PR showed an α-amylase activity of 75 International Units (IU)/g of preparation, which corresponds to a maximum of 0.008 IU/g of flour at the addition levels of this study. It has been recently demonstrated, by RVA experiments with pure corn starch, that this level of activity has no significant effects on starch pasting properties (Renzetti & Arendt, 2009).

All enzyme preparations had some β-glucanase side activities, which were 11, 2.6 and 127 U/g of LAC, for GO and PR preparations, respectively (Malt and Bacterial β-glucanase and cellulose Assay Procedure, Megazyme International Ireland Ltd., Bray, Ireland). At the highest levels of enzyme used, the activities corresponded to 11, 2.6 and 12.7 mU/g of flour for LAC 0.1, GO 0.1 and PR 0.01 treatments, respectively.

2.2. Breadmaking

The formulation used consisted of 100 parts of oat flour (relative mass), 89 parts of water, two parts of salt, two parts of sugar and three parts of instant dry yeast and, where applicable, an enzyme preparation. All dry ingredients were placed in the bowl of a Kenwood Major mixer (Chef Major KM250, Kenwood, Hampshire, UK), with the exception of the LAC preparation which was added as a solution to the water. Batter preparation, fermentation, and baking were performed as previously described (Renzetti et al., 2008). Mixing was performed for 2 min with a paddle tool (K beater) at slow/medium speed (level 2 of 6). The batters were scaled to 400 g into lubricated (Bakels Sprink Spray fat, Bakel, Oxon, UK) baking tins (930 ml volume; 7.3 cm height; 9.5 × 15.2 cm top; 7.5 × 13.2 cm bottom) and proofed at 30 °C and 85% RH for 30 min (Koma popular, Koma, Roermond, Netherlands). Baking was performed at 190 °C, top and bottom heat, for 35 min in a deck oven (MIWE, Arnstein, Germany). The oven was pre-injected with steam (0.3 l of water) and, after loading, the oven was steamed again with 0.7 l of water. After baking, the loaves were depanned and cooled for 90 min on cooling racks at room temperature. Three independent batches (three loaves per batch) were prepared with either GO 0.01% and 0.1% (GO 0.01 and GO 0.1), LAC 0.01% and 0.1% (LAC 0.01 and LAC 0.1) or PR 0.001% and 0.01% (PR 0.001 and PR 0.01) additions. Bread without enzymes was used as control (CON).

Bread crumbs were frozen, freeze-dried and ground into powder for further analysis.

2.3. Bread evaluation

Standard baking tests and texture profile analysis (TPA) were conducted on three loaves ($n = 3$) from each of the seven bread types, as previously described (Renzetti et al., 2008). TPA was performed 2 h after baking with a universal testing machine, TA-XT2I (Stable Microsystems, Surrey, UK), equipped with a 25-kg load cell and a 35-mm aluminium cylindrical probe, as previously described (Renzetti et al., 2008). All measurements obtained with the three loaves from one batch were averaged into one value (one replicate). Three replications were performed for each bread type.

2.4. Rheofermentometer test

The gas release and development characteristics of the batters were measured using a rheofermentometer (Chopin, Villeneuve-La-Garenne, France). Batters were prepared in the same manner as for breadmaking. The test was conducted for 90 min at 30 °C on 300 g of batter. A number of characteristics were determined from the batter development and gas release curves produced, the details of which have been previously described (Gobbetti, Corsetti, & Rossi, 1995). All results are averages of three independent replicates.

2.5. Batter fundamental rheology

Rheological measurements were performed on a controlled stress and strain rheometer (Anton Paar MCR 301, Ostfildern, Germany), using a parallel plate geometry (50-mm diameter) with a gap between the two plates of 1 mm. Yeastless samples were prepared as described for breadmaking and the resultant batters were also incubated for 30 min at 30 °C. Frequency sweeps were performed immediately afterwards, as described elsewhere (Renzetti et al., 2008). After loading, the batter was rested for 5 min to allow relaxation of residual stresses. Frequency sweeps from 0.1 to 10 Hz was performed with a target strain of 10^{-3} (0.1%). Preliminary tests indicated that the strain was well within the linear visco-elastic region. Ten measuring points were recorded. Temperature was kept constant at 30 °C. Creep-recovery tests were performed by applying, for 90 s, a constant shear stress of 35 Pa, which was outside the linear visco-elastic region of batters. The resultant compliance (stress/strain) was monitored as a function of time. Afterwards, the stress was removed and the compliance monitored for 210 s. All results are the average of at least two independent replicates.

2.6. Chemical analysis

Biochemical analyses were performed on the batter samples with the highest enzyme levels, as well as on the control (CON, LAC 0.1, GO 0.1, and PR 0.01). Yeastless batters were prepared as described above. After "proofing", samples were immediately frozen and subsequently freeze-dried. The freeze-dried samples were reduced to powder using a friabilimeter (Pfeuffer, Kitzingen, Germany). Two independent replicates, for each batter sample, were produced for biochemical analysis.

2.7. Analysis of extractable α-amino nitrogen

For α-amino nitrogen analysis, 1.25 g of freeze-dried batter samples were suspended in 25 ml of 1.0 M NaCl and shaken for 2 h. The suspension was then centrifuged at 18,000*g* for 30 min at 4 °C. The supernatant was collected and analysed for α-amino nitrogen by the ninhydrin method (Analytica, European Brewery Convention, method 8.10).

2.8. Analysis of free sulfhydryl groups of batters

Accessible sulfhydryl groups of the untreated and enzyme-treated oat flour proteins were measured as described by Elkhalifa et al. (2006) directly on suspensions of the freeze-dried batters in 0.05 M potassium phosphate buffer (pH 7.6) containing 0.2 mM 5,5′-dithiobis-(2-nitrobenzoate), 6.0 M urea and 0.15 M NaCl.

2.9. Capillary electrophoresis analysis of proteins from batters and bread crumbs

Proteins were extracted from batter and bread samples under non-reducing conditions by suspending 50 mg of freeze-dried material in 1.0 ml of extraction buffer solution, containing 0.05 M potassium phosphate buffer (pH 7.6), 6.0 M urea and 0.15 M NaCl. For protein extraction under reducing conditions, 0.1 M dithiothreitol was added to the above mentioned buffer. Samples were shaken for 2 h and then centrifuged at 18,000*g* for 30 min. The supernatant was collected and proteins in the range 6.5–80 kDa were separated using the Protein 80+ LabChip in the Agilent 2100 Bioanalyser (Agilent Technologies, Palo Alto, CA, USA), as previously described (Renzetti et al., 2008). For each sample, the relative concentration of the polypeptides separated by molecular size was calculated against the internal standard present in the Agilent sample buffer. Five extractions per sample were used. Detailed description of the Bioanalyser system and principle of analysis have been recently reported (Klose, Schehl, & Arendt, 2009).

For analysis of protein from bread crumbs, analyses were performed, in duplicate, with five extractions per sample.

2.10. Total analysable carbohydrate contents

Total analysable carbohydrate contents of untreated and enzymatically treated oat batters, and aqueous extracts thereof were determined in duplicate, by gas-chromatography, as described by Courtin, Van den Broeck, and Delcour (2000). Water-extractable arabinoxylans (WEAX) content was calculated as 0.88 times the sum of xylose and arabinose contents. Because the galactose content of the extracts from batters was comparable to or higher than the arabinose content and the structure or content of arabinogalactan in oat has not been studied, no correction of the arabinose present in arabinogalactan was made. We also used the water-extractable xylose content as a measure of arabinoxylans content.

2.11. Flour pasting properties

A Rapid Visco Analyser (Newport Scientific Pty Ltd., Warriewood, Australia) was used to determine the pasting properties of untreated and enzyme-treated oat batters with 14.0% (w/w) suspensions of freeze-dried batters (3.5 g, 14.0% moisture basis) in water. Analyses were performed in duplicate according to Ragaee and Abdel-Aal (2006). The temperature profile involved holding at 50 °C for 1 min, then heating to 95 °C over 7.3 min and holding at 95 °C for 5 min, and finally cooling to 50 °C over 7.7 min. The paddle speed was 960 rpm for the first 7 s and was then adjusted to 160 rpm. Sample moisture (for 14.0% moisture basis correction) was determined using the air-oven method (Approved method 44-15A, AACC International, 2000).

2.12. Confocal laser scanning microscopy of bread crumbs

Bread samples for microscopy were prepared as previously described (Renzetti et al., 2008). Fuchsin acid (0.02% in 1.0% acetic acid) was used to stain protein (red) and Calcofluor (0.01%) to stain cell walls (blue).

A FV300 confocal laser scanning system (Olympus, Hamburg, Germany) mounted on an Olympus IX80 inverted microscope with a 40× dry objective, was used. Fluorescence images of a number of optical sections were acquired by scanning the sample along the optical axis using a 405 nm excitation line for cell walls and a 543 nm excitation line for proteins. A micrograph was taken of the projection of the layers. To obtain 3D images, Volocity 3.1.0 (Improvision Limited, Coventry, England) was used.

2.13. Statistical analysis

Multiple sample comparison was performed with the Statgraphics Plus 5.0 software (Statpoint Technologies Inc., Warrenton, VA, USA). Fisher's least significant differences test was used to describe means at the 5% significance level.

3. Results

3.1. Breadmaking performances

Table 1 shows the effects of the different enzymatic treatments on the breadmaking performances of oat flour. All enzymatic treatments increased the specific volume of oat bread. However, only

Table 1
Effect of enzymatic treatments on oat bread quality and batter development.

Bread type	Bread quality parameters					Batters
	Specific volume (ml/g)	Crumb hardness (N)	Crumb chewiness (N)	Crumb springiness	Crumb cohesiveness	Maximum height (mm)
CON	1.97 ± 0.08^{ab}	11.37 ± 0.33^{a}	10.45 ± 0.33^{a}	1.05 ± 0.02^{a}	0.87 ± 0.00^{a}	36.5 ± 0.1^{a}
LAC 0.01	2.08 ± 0.09^{bc}	10.33 ± 0.11^{b}	10.19 ± 1.99^{ab}	1.14 ± 0.22^{a}	0.87 ± 0.01^{a}	39.6 ± 0.5^{c}
LAC 0.1	2.15 ± 0.06^{cd}	9.71 ± 0.24^{c}	8.67 ± 0.42^{bc}	1.03 ± 0.03^{a}	0.87 ± 0.00^{ab}	40.1 ± 0.8^{c}
GO 0.01	2.06 ± 0.02^{bc}	11.51 ± 0.34^{a}	10.31 ± 0.58^{ab}	1.04 ± 0.06^{a}	0.87 ± 0.00^{ab}	37.2 ± 0.6^{ab}
GO 0.1	2.04 ± 0.08^{bc}	12.21 ± 0.35^{e}	11.05 ± 1.07^{a}	1.05 ± 0.08^{a}	0.86 ± 0.00^{bc}	38.1 ± 0.6^{b}
PR 0.001	2.25 ± 0.05^{d}	9.54 ± 0.16^{c}	8.27 ± 0.31^{c}	1.01 ± 0.14^{a}	0.86 ± 0.00^{c}	39.5 ± 0.3^{c}
PR 0.01	2.21 ± 0.10^{d}	8.61 ± 0.35^{d}	8.52 ± 0.66^{c}	1.18 ± 0.14^{a}	0.85 ± 0.00^{d}	43.3 ± 1.0^{d}

All data represent the mean values ± standard errors, of three replicates. Mean values followed by the same superscript in each column are not significantly different ($p < 0.05$).

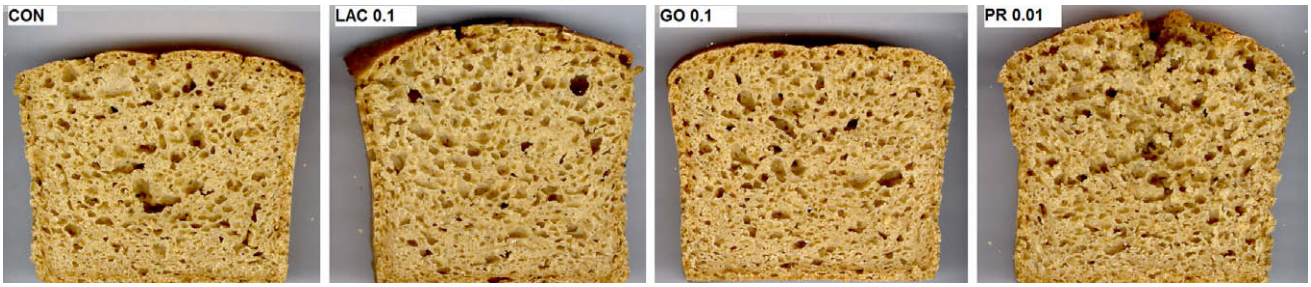

Fig. 1. Bread slices from untreated and enzyme-treated oat flour: untreated (CON), LAC 0.1% (LAC 0.1), glucose oxidase 0.1% (GO 0.1) and protease 0.01% (PR 0.01).

LAC 0.1, PR 0.001 and PR 0.01 showed a significant improvement over the control ($p < 0.05$). PR treatment showed the highest increments in specific volume (14% and 12% for PR 0.001 and PR 0.01 additions, respectively). The significant increase in specific volume evidently resulted in a significant decrease in crumb hardness with increasing levels of LAC and PR addition ($p < 0.05$, Table 1). In contrast, GO 0.1 addition yielded a significant increase in crumb hardness ($p < 0.05$). LAC 0.1, PR 0.001 and PR 0.01 additions also significantly decreased crumb chewiness ($p < 0.05$). A slight decrease in crumb cohesiveness was detected with GO 0.1, PR 0.001 and PR 0.01 additions ($p < 0.05$). The enzyme treatments did not produce any significant changes in bake loss, whose values varied between 10.5% and 11.2% for the control and enzyme-treated breads. The effect of the enzymatic treatments was clearly reflected in the appearance of the bread slices (Fig. 1). LAC 0.1 and PR 0.01 additions yielded increased height and a round-shaped top when compared to CON and GO 0.1 addition breads. A coarser crumb structure was observed with PR 0.01 addition than with the control (Fig. 1).

Batter development during proofing was significantly affected by the enzymatic treatments (Table 1). The maximum height of the batter was increased, irrespective of the LAC concentration added ($p < 0.05$). GO 0.1 addition also induced a significant increase in maximum height, but the effect was lower than that of the different LAC treatments. PR 0.001 addition increased maximum height to a similar extent as did either LAC 0.01 or LAC 0.1 addition, while PR 0.01 addition led to the highest increase among all enzymatic treatments ($p < 0.05$). All enzymatic treatments, except GO 0.01, showed a slight increase in the carbon dioxide retention coefficient which was highest with PR 0.01 supplementation (data not shown). However, results were not statistically significant ($p < 0.05$).

3.2. Frequency sweeps

Batters were proofed for 30 min at 30 °C and frequency sweeps were performed immediately afterwards. Independent of the type and level of enzyme added, all batters showed that the elastic modulus (G') was higher than the viscous modulus (G''), indicating that the batters had a solid, elastic-like behaviour (data not shown). Table 2 shows the effects of the enzymatic treatments on the complex modulus (G^*) and phase angle (δ). LAC and PR treatments significantly decreased G^* over the whole frequency range tested (0.1–10 Hz, $p < 0.05$), indicating a decrease in the resistance to

Table 2
Effect of enzymatic treatments on oat batter rheology.

Batter type	Frequency sweeps						Creep-recovery	
	G^* (Pa)			δ			J_{max} (10^3/Pa)	Relative recovery (%)
	0.1 Hz	1.29 Hz	10 Hz	0.1 Hz	1.29 Hz	10 Hz		
CON	7015^{a}	12,300^{a}	16,850^{a}	16.90^{a}	13.60^{a}	14.55^{ac}	0.491^{a}	47.3^{a}
LAC 0.01	4270^{c}	6140^{d}	8425^{d}	16.15^{ab}	13.80^{a}	14.80^{ab}	–	–
LAC 0.1	3260^{d}	4355^{e}	5815^{e}	10.75^{c}	11.95^{b}	15.55^{b}	0.930^{b}	64.9^{b}
GO 0.01	6605^{a}	10,440^{b}	13,900^{b}	15.20^{ab}	12.60^{ab}	13.85^{c}	–	–
GO 0.1	6385^{a}	9845^{b}	13,100^{bc}	14.65^{ab}	12.80^{ab}	14.25^{ac}	0.514^{a}	37.1^{a}
PR 0.001	5335^{b}	8040^{c}	10,850^{c}	14.75^{ab}	13.10^{ab}	15.60^{b}	–	–
PR 0.01	4295^{c}	6020^{d}	7780^{cd}	12.95^{bc}	12.15^{b}	14.45^{ac}	0.964^{b}	62.2^{b}

All data represent the mean values of two replicates. Mean values followed by the same superscript in each column are not significantly different ($p < 0.05$).

Table 3
Protein size distribution of untreated and enzymatic treated oat batters.

Non-reducing conditions					Reducing conditions				
Size (kDa)	CON (ng/μl)	LAC 0.1 (ng/μl)	GO 0.1 (ng/μl)	PR 0.01 (ng/μl)	Size (kDa)	CON (ng/μl)	LAC 0.1 (ng/μl)	GO 0.1 (ng/μl)	PR 0.01 (ng/μl)
14	26[a]	47[b]	37[ab]	39[ab]	13–19	296[a]	311[a]	336[a]	356[b]
21–27	702[a]	535[b]	339[c]	572[b]	25–30	1693[a]	1731[a]	1428[b]	2063[c]
45–51	397[a]	317[b]	190[c]	386[a]	39–50	1747[a]	1659[a]	1435[b]	1810[a]
70	1792[a]	1354[b]	1221[b]	1740[a]	70	67[a]	68[a]	68[a]	76[a]

All data represent the mean values of two replicates. Mean values followed by the same superscript in each row are not significantly different ($p < 0.05$).

deformation of batters. The decrease was higher with increasing levels of enzyme (Table 2). Any GO addition revealed a significant decrease in G^* only in the medium–high frequency range (1.29–10 Hz, $p < 0.05$) and to a far less extend than in LAC and PR samples. LAC 0.1 and PR 0.01 additions also decreased δ at low-medium frequencies (0.1–1.29 Hz, $p < 0.05$), indicating an increased degree of elasticity of the batters. Both samples showed a significant increase in δ at 10 Hz ($p < 0.05$).

3.3. Creep-recovery tests

Creep-recovery tests were carried out to study the effects of enzyme treatments on batter rheology at large deformations, i.e. in deformation ranges similar to those experienced by the batters during processing. Based on the results of the frequency sweep tests, CON, LAC 0.1, GO 0.1 and PR 0.01 samples were chosen as representative of each enzyme treatment for the creep-recovery tests. Maximum compliance and relative recovery were calculated from the creep-recovery curves. Maximum compliance was calculated as the ratio of the strain at the end of creep time to the stress applied (35 Pa), while relative recovery was calculated as the ratio of absolute recovery to compliance. The results for the two parameters are displayed in Table 2. LAC 0.1 and PR 0.01 samples showed significant increase in maximum compliance (89% and 96%, respectively), indicating that the batter was softened ($p < 0.05$). LAC 0.1 and PR 0.01 samples also revealed a significant increase in relative recovery ($p < 0.05$), indicating increased elasticity of the batters. No significant differences were detected with GO 0.1 addition ($p < 0.05$).

3.4. Chemical analysis

3.4.1. Determination of extractable α-amino nitrogen and free sulfhydryl groups in batters

LAC 0.1 and GO 0.1 samples showed levels of extractable α-amino nitrogen of 165 and 148 μg/mg of dry matter (dm), respectively, which were significantly lower than the 177 μg/mg dm of the control sample ($p < 0.05$). The decrease was significantly higher for the GO 0.1 than for the LAC 0.1 sample ($p < 0.05$). The PR 0.01 samples had an extractable α-amino nitrogen content of 182 μg/mg of dm, which was significantly higher than the control, as a result of the proteolytic activity ($p < 0.05$). The oxidative treatments induced a (statistically insignificant) decrease in the levels of free sulfhydryl groups, which was 1.17 μmol-SH/g of dm for the control, 0.86 and 0.97 μmol-SH/g of dm for LAC 0.1 and GO 0.1 samples, respectively.

3.4.2. Capillary gel electrophoresis analysis of proteins from batters

LAC 0.1 and GO 0.1 samples had a significantly lower relative concentration (Table 3) of bands from 21 to 70 kDa ($p < 0.05$), the decrease being higher for the GO 0.1 than for the LAC 0.1 sample in the 21–50 kDa range ($p < 0.05$). The PR 0.01 sample revealed a significant decrease in the relative concentration of low molecular weight proteins at 21–27 kDa.

Under reducing conditions, the GO 0.1 sample produced a significant decrease in the relative concentration of the two major bands at 25–30 and 39–50 kDa ($p < 0.05$), while no significant effects were detected with the LAC 0.1 sample. PR 0.01 addition produced a significant increase in the relative concentration of proteins in the 13–30 kDa range ($p < 0.05$).

3.4.3. Total analysable carbohydrate content of batters

The enzymatic treatments did not produce any significant changes in the WEAX levels of the batter samples which, for the control and enzyme-treated samples, varied between 0.114% and 0.119% of dm only. Significant increases in the glucose content of the water extract were found for LAC 0.1 and PR 0.01 samples, namely 1.15% and 1.33% of dm, respectively, compared to the control with 0.83% of dm ($p < 0.05$).

3.5. Flour pasting properties

The freeze-dried batters were analysed by RVA to detect the influence of the enzymatic treatments on the flour pasting properties (Table 4). All parameters (namely pasting temperature, peak viscosity, breakdown, final viscosity and setback) were significantly affected by the enzymatic treatments ($p < 0.05$). Pasting temperature increased with all of the enzymatic treatments, while viscosity at peak and breakdown were significantly reduced ($p < 0.05$). Final viscosity during the cooling stage was reduced with LAC 0.1 and GO 0.1 additions ($p < 0.05$), while no effects were detected for PR 0.01 addition. Setback significantly increased with PR 0.01 supplementation, while LAC 0.1 and GO 0.1 addition produced a significant decrease ($p < 0.05$).

3.6. Capillary electrophoresis analysis of proteins from bread crumbs

Under non-reducing conditions, both LAC 0.1 and GO 0.1 breads showed a significant decrease in the relative concentration of bands at 21–27 and 45–51 kDa ($p < 0.05$) (data not shown). Drastic changes were detected for the PR 0.01 sample, as an increase in the level of proteins of low molecular weight was observed (Fig. 2). The relative concentration of protein bands at 70 and 45–51 kDa was significantly decreased ($p < 0.05$) (data not shown), while new protein bands at 30 and 37 kDa were detected (Fig. 2).

Under reducing conditions, LAC 0.1 and GO 0.1 treatments induced a significant decrease in the relative concentration of the

Table 4
Pasting properties of untreated and enzyme-treated oat batters.

Batter type	Pasting T (°C)	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)
CON	84.7[a]	3258[a]	1315[a]	3697[a]	1754[a]
LAC 0.1	86.6[bc]	2583[b]	795[b]	3456[b]	1668[b]
GO 0.1	87.0[b]	2827[c]	1075[c]	3400[b]	1649[c]
PR 0.01	85.9[c]	2336[d]	718[d]	3655[a]	2037[d]

All data represent the mean values of two replicates. Mean values followed by the same superscript in each column are not significantly different ($p < 0.05$).

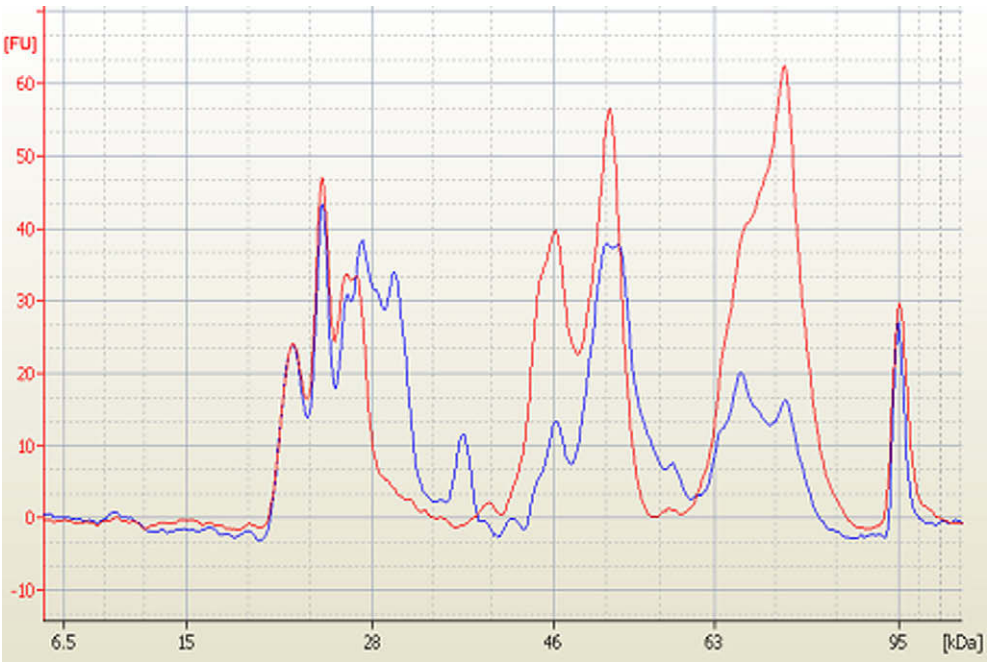

Fig. 2. Electrophoretic pattern of proteins from CON (—) and PR 0.01 (—) bread crumbs extracted under non-reducing conditions. Proteins were separated in the 6.5–80 kDa range. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

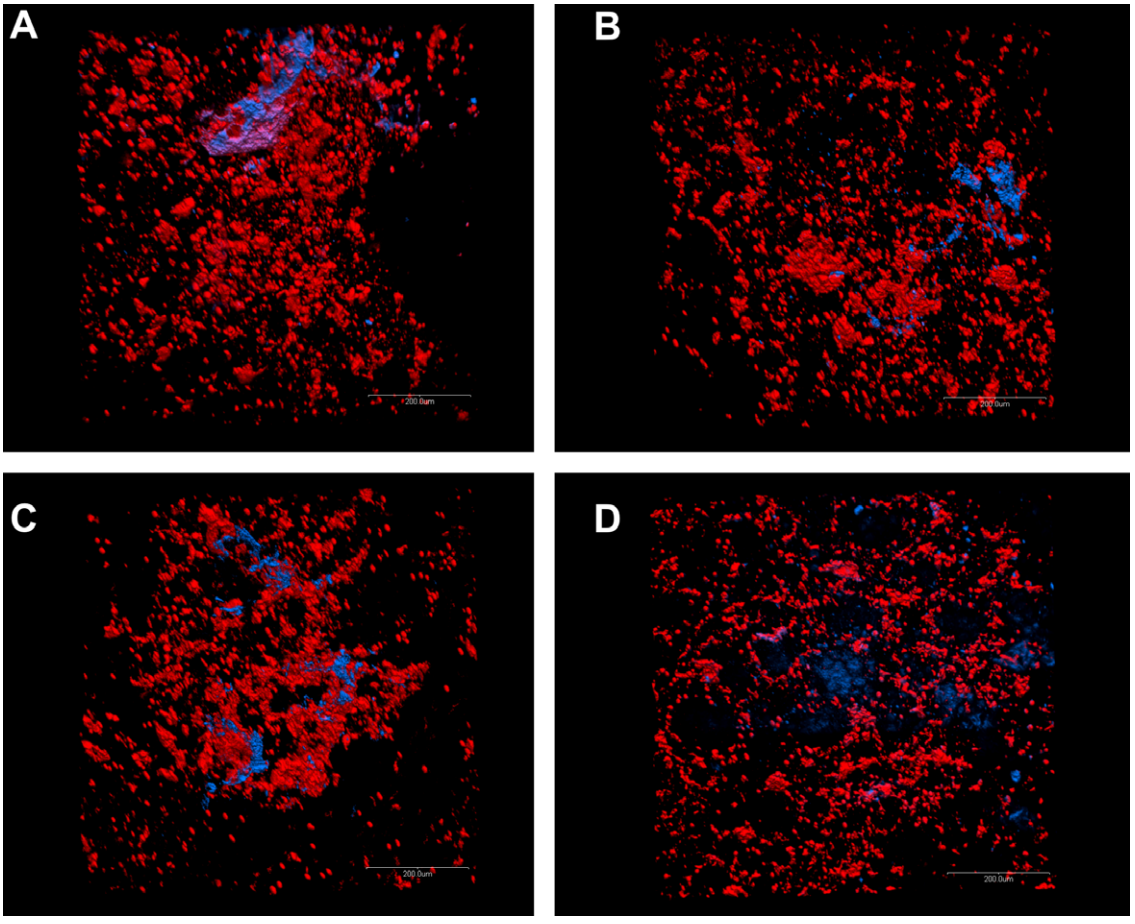

Fig. 3. 3D elaboration of CLSM images of untreated and enzyme-treated oat bread crumbs (40× magnification): CON (A), LAC 0.1 (B), GO 0.1 (C) and PR 0.01 (D). Proteins are stained red, together with yeast cells, which appear round-shaped, while cell walls appear in light blue. Scale bar is 200 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

two major bands at 25–30 and 39–50 kDa ($p < 0.05$) (data not shown). The decrease was significantly higher for the GO 0.1 than for the LAC 0.1 sample ($p < 0.05$). PR 0.01 addition induced a significant increase in the level of low molecular weight proteins, i.e. 24–37 kDa, with a concomitant decrease in the relative concentration of proteins in the 39–51 kDa range ($p < 0.05$) (data not shown).

3.7. Confocal laser scanning microscopy of bread crumbs

The microscopy of CON bread crumbs revealed a microstructure of small protein clusters and high protein density (Fig. 3A). With LAC 0.1 treatment, some of these clusters formed small aggregates (Fig. 3B), while the GO 0.1 sample revealed the presence of large and globular protein aggregates (Fig. 3C). In contrast, PR 0.01 showed the presence of seemingly smaller protein clusters dispersed in the system (Fig. 3D). No clear differences were observed in the cell wall structures with any of the enzymatic treatments.

4. Discussion

The development of good quality GF bread is a challenging task for cereal technologists and enzymatic processing of GF flour is a promising tool to improve their breadmaking potentials. This study shows that treatment of oat flour with LAC and PR preparations can significantly improve the textural quality of oat bread, by increasing loaf specific volume and lowering crumb hardness and chewiness (Table 1). The improvements could be related to improved batter development (Table 1), which is a marker for baking quality. LAC and PR batters showed increased deformability and elasticity at both small and large deformation scale. Two depolymerisation mechanisms, namely β-glucan and protein hydrolysis, were responsible for the changes in the rheological properties. In contrast, GO treatment is detrimental for the textural quality of oat bread, as extensive protein polymerisation resulted in a significant increase in crumb hardness (Table 1), while specific volume was unaffected.

The results of this study are in contrast with those of Flander et al. (2008) who reported detrimental effects of LAC on oat bread quality in terms of decreased specific volume and increased crumb hardness. The effects were related to polymerisation of WEAX into water-unextractable arabinoxylans and protein cross-linking by disulphide bonds. Lower arabinoxylan and protein content in the present oat batter, different microbial origin of LAC and presence of β-glucanase side activity might explain the contradictory results. On the contrary, improvements in specific volume and crumb softness with PR treatments of oat flour (Table 1) are in agreement with the results recently reported on PR treatment of brown rice flour (Renzetti & Arendt, 2009). These improvements were related to increased batter deformability and paste stability during proofing and in the early stages of baking, which resulted from the release of low molecular weight (LMW) proteins and the opening up of macromolecular protein complexes (Renzetti & Arendt, 2009).

LAC and PR treatments significantly affected the rheological properties of batters (Table 2). Frequency sweep tests showed decreases in G^* over the whole frequency range, with any LAC and PR addition (Table 2). Significant reduction in G^* with LAC and PR treatments has been reported in wheat doughs, together with a decrease in dough elasticity (i.e. higher tan δ) (Caballero, Gómez, & Rosell, 2007). Surprisingly, the increased deformability was accompanied by an increased degree of elasticity of the batters with LAC 0.1 and PR 0.01 addition (Table 2). The effects of LAC and PR on batter deformability and elasticity were confirmed at larger deformation by creep-recovery tests. The increased deformability could be one explanation for the improved breadmaking performance of oat batters with LAC and PR addition. Additionally, improved elasticity results in increased stability of the batter films during expansion of the gas cells. The improved batter film stability would prevent premature gas cell rupture and collapsing of dough during proofing and oven spring, explaining the increases in maximum height obtained with LAC 0.1 and PR 0.01 supplementation. High correlation between maximum recovery and loaf volume were reported for wheat flour breadmaking (Wang & Sun, 2002). It can be suggested that the increase in softness, deformability and elasticity of LAC- and PR-treated batters are responsible for the improved breadmaking performances of the oat flour. These effects were not detected with GO samples, explaining the lack of improvements.

Chemical analyses of untreated and enzymatic treated batters were conducted in order to relate rheological properties to chemical modifications. Oat proteins mainly consist of globulins, which account for up to 75% of total seed proteins (Luthe, 1987). The main storage globulin in oats endosperm is the 12S globulin, a hexameric protein consisting of α (ca. 35 kDa) and β (ca. 22 kDa) subunits, linked together by disulphide bonds (Peterson, 1978). Under non-reducing conditions, the oat protein profile was dominated by the 12S subunits, i.e. 70 kDa, and by two minor bands at 21–27 and 45–51 kDa (Table 4), most probably prolamins and albumins, respectively (Klose et al., 2009). The significant decrease in the relative concentration of these protein bands with LAC 0.1 and GO 0.1 suggests their polymerisation through either tyrosine cross-links (Gujral & Rosell, 2004b) or disulphide bridges (Labat et al., 2000a; Vemulapalli, Miller, & Hoseney, 1998) (Table 3). Similar results with LAC treatment of oat dough were recently reported (Flander et al., 2008). This hypothesis is confirmed by the significant decrease in the amount of α-amino nitrogen in the LAC 0.1 and GO 0.1 samples ($p < 0.05$) and by the lower levels of free sulfhydryl groups, despite the fact that the effects were not significant. Under both non-reducing and reducing conditions, the significant differences between GO 0.1 and LAC 0.1 samples in the relative concentration of protein bands ($p < 0.05$) suggests extensive polymerisation in high molecular mass structures with GO 0.1 addition (Table 3). On the other hand, the proteolytic activity in the PR 0.01 sample resulted in a significant decrease in the relative concentration of the 21–27 kDa proteins under non-reducing conditions (Table 3). Most probably, these proteins were degraded to products below the 6.5 kDa detection limit of the capillary gel electrophoresis system. This was confirmed by a significant increase in the total protein content of the supernatant (data not shown) and by the significant increase in the amount of α-amino nitrogen ($p < 0.05$). Under reducing conditions, the substantial increase in the relative concentration of the 25–30 kDa bands for PR 0.01 could be related to the hydrolysis of protein subunits participating in inter/intra molecular disulphide bridges (Table 3). Overall, PR activity in batters prevalently resulted in opening up of the macromolecular structures, while partially releasing LMW proteins. These structural changes increase batter deformability by lowering the water-holding capacity and the rigidity of the protein complexes, and by modifying protein–protein and protein–starch interactions (Renzetti & Arendt, 2009).

The similarities in the rheological properties of LAC- and PR-treated batters suggest the prevalence of a depolymerisation mechanism in LAC samples. Oxidative treatments did not affect WEAX extractability, indicating that the oxidative treatments did not promote polymerisation of polysaccharides. Instead, the significant increase in the glucose content of the water extract with LAC and PR addition suggests depolymerisation of insoluble glucose-based oligomers ($p < 0.05$). The LAC preparation did not contain any detectable α-amylase side activity, while α-amylase side activity at the levels of PR addition was too low to significantly affect starch pasting properties (Renzetti & Arendt, 2009). Considering the endo-β-glucanase activity present in both preparations, it can

be suggested that the increase in glucose material comes mainly from the degradation of insoluble β-glucan. Evidence that the LAC preparation used in this study did not decrease the consistency of batters from other GF flours, with irrelevant β-glucan content (unpublished data), confirms the hypothesis of β-glucan depolymerisation in the oat system. Therefore, it can be suggested that the new rheological properties of batters with LAC and PR addition are the result of (i) the prevailing effect of β-glucan degradation over protein polymerisation in LAC samples and (ii) protein and β-glucan degradation in PR samples. Instead, a high degree of protein polymerisation and low endo-β-glucanase activity in the GO supplemented batters results in zero net effect on the rheological properties.

The enzymatic treatments significantly affected the pasting properties of oat flour (Table 4). In general, pasting temperature was increased, while peak viscosity and breakdown were decreased. High starch pasting temperatures have been related to high specific volume and good crumb texture (Kusunose, Fujii, & Matsumoto, 1999), which might have contributed to the increases in specific volume with the enzymatic treatments. Changes in the pasting profiles can be related to modifications in protein–starch interactions (Ragaee & Abdel-Aal, 2006), as well as β-glucan degradation. Lower paste viscosities of flours after PR addition have been reported by several authors (Derycke et al., 2005; Hamaker & Griffin, 1993; Xie, Chen, Duan, Zhu, & Liao, 2008). In concentrated regime conditions in the RVA, starch granules cannot swell to their maximum because of space restrictions (Derycke et al., 2005). Under such conditions, protein structures surrounding the starch granules confer rigidity to the paste, and the rheology of the system is dictated by the rigidity of the suspended particles (Steeneken, 1989). By disrupting the paste rigidity, protein hydrolysis decreases RVA viscosity. Additionally, β-glucan degradation also contributes to the reduced paste viscosity. On the other hand, reduction of peak viscosity with LAC and GO treatments can be explained by protein aggregation strengthening of the protein barrier surrounding starch granules, thus reducing starch swelling (Hamaker & Griffin, 1993). Additionally, β-glucan degradation in the LAC sample may also be responsible for reduced paste viscosity, explaining why the GO sample had higher peak viscosity than LAC, despite the higher degree of protein polymerisation. The strengthened protein structure makes the swollen granules less susceptible to breakdown during shear, either by conferring strength to the granules or by reducing the degree of swelling (Hamaker & Griffin, 1993). As recently reported (Renzetti & Arendt, 2009), the decreased peak viscosity, coupled to the reduction in paste breakdown, contributed to improve the breadmaking potentials of LAC- and PR-treated batters, limiting detrimental effects of protein hydrolysis to a slightly coarser crumb structure (Fig. 1). The protein barrier can also explain the differences in final viscosity and setback between PR and LAC or GO treatments. Reduced swelling and/or increased granules strength would lower starch re-aggregation, thus decreasing setback. Instead, the disruption of the protein barrier by PR addition would favour starch re-aggregation during cooling, explaining the increase in setback compared to the control batter.

The protein profile of bread crumbs was analysed to gain insight in the structural changes promoted by the enzymatic treatments after baking. LAC and GO treatments confirmed the results obtained in the batter systems (data not shown). The greater decrease in the relative concentration of protein bands with GO 0.1 addition compared to the LAC 0.1 sample confirms the higher degree of protein polymerisation with GO treatment. On the other hand, the extensive hydrolysis of the 12S subunit, i.e. 70 kDa band, coupled with the significant increase in the relative concentration of low molecular weight proteins ($p < 0.05$) and the presence of new protein bands (Fig. 2) suggests that the enzyme was active during the early stages of baking when temperatures rose to the optimum, i.e. 45 °C. Due to their globular structure, globulins are unable to stabilise the gas/liquid interface of the expanding gas cells during baking; instead, oat albumins have functional properties comparable to that of egg white and soy isolate (Ma & Harwalkar, 1984). The low molecular weight protein hydrolysates produced by PR during baking may have increased the functional properties of the soluble fraction, stabilizing the gas/liquid interface by creating a layer of unfolded/soluble proteins (Gan, Ellis, & Schofield, 1995) and thus contributing to the improvements in oat bread quality. The microstructure of PR 0.01 bread crumbs, where small protein clusters were detected, seems to confirm such a conclusion (Fig. 3D). Instead, the large and globular protein aggregates present in the GO-treated breads (Fig. 3C) might have resulted in detrimental effects on the breadmaking performance, resulting in harder crumbs. Small protein aggregates were detected with LAC 0.1, which may have resulted in better breadmaking performance than in the GO 0.1 bread (Fig. 3B).

5. Conclusion

LAC and PR preparations significantly improved the breadmaking performances of oat flour and the textural quality of oat bread, by increasing specific volume and lowering crumb hardness and chewiness. On the contrary, GO preparation was detrimental for the textural quality of oat bread as a significant increase in crumb hardness was observed. The improved breadmaking performances could be related to the increased softness, deformability and elasticity of oat batters with LAC and PR supplementation. Both LAC and GO promoted protein cross-linking, which was more evident with addition of GO, resulting in larger protein aggregates in the bread crumbs than in the breads with LAC. It can be suggested that these globular aggregates, which were observable in the CLSM images, negatively affected the breadmaking performances of oat bread. Instead, endo-β-glucanase side activity in the LAC preparation promoted β-glucan depolymerisation. The effects of this hydrolysis prevailed over protein polymerisation, resulting in the observed rheological properties. Endo-β-glucanase and protease activities in the PR preparation resulted in oat breads of the best textural quality. Most probably, extensive protein hydrolysis during baking increased the functionality of the soluble fraction, stabilizing the gas/liquid interface by creating a layer of unfolded/soluble proteins.

Protein modifications and β-glucan degradation produced by the enzymatic treatments significantly affected flour pasting properties. The decreased paste viscosity and improved paste stability with LAC and PR treatments positively contributed to the improved breadmaking performance.

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References

AACC International (2000). *Approved methods of the American Association of Cereal Chemists* (10th ed.) Methods 44-15A and 46-30. The Association: St. Paul, MN, USA.

Analytica, European Brewery Convention (2004). Fachverlag Hans Carl GmbH & Co., Nürnberg, Germany.

Bombara, N., Añón, M. C., & Pilosof, A. M. R. (1997). Functional properties of protease modified wheat flour. *LWT – Food Science and Technology, 30*, 441–447.

Caballero, P. A., Gómez, M., & Rosell, C. M. (2007). Improvement of dough rheology, bread quality and bread shelf-life by enzyme combination. *Journal of Food Engineering, 81*, 42–53.

Catassi, C., & Fasano, A. (2008). Celiac disease. In E. Arendt & F. Dal Bello (Eds.), *Gluten-free cereal products and beverages* (pp. 1–27). London, UK: Elsevier.

Çelic, I., Yilmaz, Y., Işik, F., & Üstün, Ö. (2007). Effect of soapwort extract on physical and sensory properties of sponge cakes and rheological properties of sponge cake batters. *Food Chemistry, 101*, 907–911.

Ciclitira, P. J., Johnson, M. W., Dewar, D. H., & Ellis, H. J. (2005). The pathogenesis of celiac disease. *Molecular Aspects of Medicine, 26*, 421–458.

Courtin, C. M., Van den Broeck, H., & Delcour, J. A. (2000). Determination of reducing end sugar residues in oligo- and polysaccharides by gas liquid chromatography. *Journal of Chromatography A, 866*, 97–104.

Derycke, V., Veraverbeke, W. S., Vandeputte, G. E., De Man, W., Hoseney, R. C., & Delcour, J. A. (2005). Impact of proteins on pasting and cooking properties of nonparboiled and parboiled rice. *Cereal Chemistry, 82*, 468–474.

Drago, S. R., & González, R. J. (2001). Foaming properties of enzymatically hydrolysed wheat gluten. *Innovative Food Science and Emerging Technologies, 1*, 269–273.

Elkhalifa, A. E. O., Bernhardt, R., Bonomi, F., Iametti, S., Pagani, M. A., & Zardi, M. (2006). Fermentation modifies protein/protein and protein/starch interactions in sorghum dough. *European Food Research International, 222*, 559–564.

Figueroa-Espinoza, M. C., & Rouau, X. (1998). Oxidative cross-linking of pentosans by a fungal laccase and horseradish peroxidise. Mechanism of linkage between feruloylated arabinoxylans. *Cereal Chemistry, 75*, 259–265.

Flander, L., Rouau, X., Morel, M. H., Autio, K., Seppänen-Laakso, T., Kruus, K., et al. (2008). Effects of laccase and xylanase on the chemical and rheological properties of oat and wheat doughs. *Journal of Agricultural and Food Chemistry, 56*, 5732–5742.

Gan, Z., Ellis, P. R., & Schofield, J. D. (1995). Gas cell stabilisation and gas retention in wheat bread dough. *Journal of Cereal Science, 21*, 215–230.

Gobbetti, M., Corsetti, A., & Rossi, J. (1995). Interaction between lactic acid bacteria and yeasts in sour-dough using a rheofermentometer. *World Journal of Microbiology and Biotechnology, 11*, 625–630.

Goesaert, H., Brijs, K., Veraverbeke, W. S., Courtin, C. M., Gebruers, K., & Delcour, J. A. (2005). Wheat flour constituents: How they impact bread quality and how to impact their functionality. *Trends in Food Science and Technology, 16*, 12–30.

Gujral, H. S., & Rosell, C. M. (2004a). Functionality of rice flour modified with a microbial transglutaminase. *Journal of Cereal Science, 39*, 225–230.

Gujral, S. H., & Rosell, M. C. (2004b). Improvement of the breadmaking quality of rice flour by glucose oxidase. *Food Research International, 37*, 75–81.

Hamaker, B. R., & Griffin, V. K. (1993). Effect of disulphide bond containing protein on rice starch gelatinization and pasting. *Cereal Chemistry, 70*, 377–380.

Klose, C., Schehl, B. D., & Arendt, E. K. (2009). Fundamental study on protein changes taking place during malting of oats. *Journal of Cereal Science, 49*, 83–91.

Kong, X., Zhou, H., & Qian, H. (2007). Enzymatic preparation and functional properties of wheat gluten hydrolysates. *Food Chemistry, 101*, 615–620.

Kusunose, C., Fujii, T., & Matsumoto, H. (1999). Role of starch granules in controlling expansion of dough during baking. *Cereal Chemistry, 76*, 920–924.

Labat, E., Morel, M. H., & Rouau, X. (2000a). Effects of laccase and ferulic acid on wheat flour doughs. *Cereal Chemistry, 77*, 823–828.

Labat, E., Morel, M. H., & Rouau, X. (2000b). Effect of laccase and manganese peroxidise on wheat gluten and pentosans during mixing. *Food Hydrocolloids, 15*, 47–52.

Luthe, D. S. (1987). Storage protein synthesis during oat (*Avena sativa* L.) seed development. *Plant Physiology, 84*, 337–340.

Ma, C. Y., & Harwalkar, V. R. (1984). Chemical characterization and functionality assessment of oat protein fractions. *Journal of Agricultural and Food Chemistry, 32*, 144–149.

Marco, C., & Rosell, C. M. (2008). Effect of different protein isolates and transglutaminase on rice flour properties. *Journal of Food Engineering, 84*, 132–139.

Peterson, D. M. (1978). Subunit structure and composition of oat seed globulin. *Plant Physiology, 62*, 506–509.

Primo-Martin, C., Valera, R., & Martinez-Anaya, M. A. (2003). Effect of pentosanase and oxidases on the characteristics of doughs and the glutenin macropolymer. *Journal of Agricultural and Food Chemistry, 51*, 4673–4679.

Ragaee, S., & Abdel-Aal, E.-S. M. (2006). Pasting properties of starch and protein in selected cereals and quality of their food products. *Food Chemistry, 95*, 9–18.

Renzetti, S., & Arendt, E. K. (2009). Effect of protease treatment on the baking quality of brown rice bread: From textural and rheological properties to biochemistry and microstructure. *Journal of Cereal Science, 50*, 22–28.

Renzetti, S., Behr, J., Vogel, R. F., & Arendt, E. K. (2008). Transglutaminase polymerization of Buckwheat (Fagopyrum esculentum Moench) proteins. *Journal of Cereal Science, 48*, 756–763.

Renzetti, S., Dal Bello, F., & Arendt, E. K. (2008). Microstructure, fundamental rheology and baking characteristics of batters and breads from different gluten-free flours treated with a microbial transglutaminase. *Journal of Cereal Science, 48*, 33–45.

Si, J. Q. (1994). *Use of laccase in industry*. International patent application PCT/DK94/00232.

Steeneken, P. A. M. (1989). Rheological properties of aqueous suspensions of swollen starch granules. *Carbohydrate Polymers, 11*, 23–42.

Vemulapalli, V., & Hoseney, R. C. (1998). Glucose oxidase effects on gluten and water solubles. *Cereal Chemistry, 75*, 859–862.

Vemulapalli, V., Miller, K. A., & Hoseney, R. C. (1998). Glucose oxidase in breadmaking systems. *Cereal Chemistry, 75*, 439–442.

Wang, F. C., & Sun, X. S. (2002). Creep-recovery of wheat flour doughs and relationship to other physical dough tests and breadmaking performance. *Cereal Chemistry, 79*, 567–571.

Welch, R. W. (1995). Oats in human nutrition and health. In R. W. Welch (Ed.), *The oat crop production and utilisation* (pp. 279–320). London: Chapman and Hall.

Xie, L., Chen, N., Duan, B., Zhu, Z., & Liao, X. (2008). Impact of proteins on pasting and cooking properties of waxy and non-waxy rice. *Journal of Cereal Science, 47*, 372–379.