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# Effect of protease treatment on the baking quality of brown rice bread: From textural and rheological properties to biochemistry and microstructure

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ABSTRACT

In this study, protease treatment of brown rice (BR) batters was investigated in order to evaluate its impact on the textural and baking properties of BR bread. The enzymatic treatment improved bread quality by significantly increasing specific volume ($p < 0.05$), while decreasing crumb hardness and chewiness ($p < 0.05$). Fundamental rheology and viscometry of batters revealed that protein hydrolysis induced lower complex modulus and initial viscosity, while phase angle was unaffected. Flour pasting properties were also affected, with a significant decrease in paste viscosity and breakdown ($p < 0.05$). Protein analysis of batters revealed that the enzymatic treatment induced the release of low molecular weight proteins from macromolecular protein complexes. In conclusion, a lower resistance to deformation of batters during proofing and in the early stages of baking as well as the preserved batter elasticity and the increased paste stability positively affected the breadmaking performance.



## 1. Introduction

Celiac Disease (CD) is one of the most common lifelong disorders on a worldwide basis; an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible persons (Catassi and Fasano, 2008). Despite the advances been made in the understanding of CD pathogenesis and the potential development of novel therapies, at present the only safe and effective treatment for CD sufferers is the avoidance of gluten-containing foods such as wheat, rye and barley (Ciclitira et al., 2005). Recently, a general growing awareness in the diffusion of CD has increased the interest of the food industry in the market of gluten-free cereal products.

Rice (*Oryza sativa* L.) is one of the most important cereal foodstuffs in the world and is naturally gluten-free, highly digestible and hypoallergenic. Additionally, brown rice (BR) contributes good nutritional properties as it contains considerably higher amounts of proteins and minerals than rice (Itani et al., 2002; Lamberts et al., 2007). Therefore, BR flour would be an ideal raw material for the production of gluten-free products, i.e. breads. However, rice proteins do not possess the visco-elastic properties typically found in gluten, thus making rice flour unsuitable for the production of yeast-leavened products. For this reason, hydrocolloids and gums are generally used as gluten replacements to confer structure and gas-retaining capacity to rice batters (Nishita et al., 1976; Sivaramakrishnan et al., 2004). Recently, the use of enzymatic processing of rice flour with and without addition of hydrocolloids has been investigated in order to further improve its breadmaking performances by promoting protein cross-linking and thus increasing the elastic and viscous behaviour of batters (Gujral and Rosell, 2004a,b; Renzetti et al., 2008a).

Rice batters are quite liquid and resemble more a cake batter rather than a dough. Limited protein hydrolysis might be beneficial to improve the foaming properties of the batter and consequently its breadmaking performances. In sponge cake systems, protein foaming properties are fundamental in determining the overall textural quality of the product (Çelic et al., 2007), and limited protein hydrolysis is beneficial to improve cake qualities such as volume and moisture (Bombara et al., 1997). In gluten-free breads, improvements in the textural quality with sourdough fermentation of gluten-free flours were recently reported (Moore et al., 2007; Schober et al., 2007), and related to protein hydrolysis by the bacterial proteases. Improvements were mainly related to a better appearance of the crumb structure and a slight retardation of staling, while no significant effects were reported for the specific volume. Furthermore, lactic acid fermentation of the total

*Abbreviations:* BR, brown rice; G*, complex modulus; CLSM, confocal laser scanning microscopy; LMW, low molecular weight; $\delta$, phase angle; PR, protease; RVA, rapid visco analysis.

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gluten-free flour in the formulation and complex recipes which included hydrocolloids were used in these studies.

Modifications in protein profile can affect the behaviour of the other flour components, i.e. starch. The importance of protein–starch interactions in the pasting properties of rice flour has been previously reported (Hamaker and Griffin, 1993). In sorghum batters, protein hydrolysis significantly influenced flour pasting properties by increasing paste viscosity, positively affecting the breadmaking performances (Schober et al., 2007).

The aim of the present study was to investigate the effects of protease treatment on the breadmaking performances of BR flour and relate them to the modifications in the rheological and biochemical characteristics of batters. Rice bread formulations were developed following a previous methodological approach without addition of any hydrocolloids in order to avoid synergistic effects (Renzetti et al., 2008a). Standard baking tests, texture profile analysis, and fundamental rheology were performed to evaluate the breadmaking performances. 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

BR flour (9.5% protein, 13% moisture, ash 1.4%; Doves Farm Foods Ltd., Berkshire, UK), was used in conjunction with instant dried yeast (Pante, Puratos, Groot-Bijgaarden, Belgium), salt, sugar and tap water.

A commercial protease was used (Neutrase 1.5 MG, Novozymes, Bagsvaerd, Denmark), containing 1.5 AU-NH/g protease activity [equivalent to 169 and 288 endo-protease units/g based on *Bacillus licheniformis* and *Bacillus subtilis* proteases respectively (azo-casein kit, Megazyme International Ireland Ltd., Bray, Ireland)]. The protease (from *Bacillus amyloliquefaciens*) had pH and temperature ranges of ca. 5–8 and 25–70 °C, respectively (optima: pH 6.5, temperature 45 °C). Following supplier's recommendations, the selected enzyme dosages on flour weight were 0.001 and 0.01% (w/w).

### 2.2. Breadmaking

The formulation used consisted of 100 parts BR flour (relative mass), 93 parts of water, 2 parts of salt, 2 parts of sugar and 3 parts of dried yeast. The amount of flour was interpreted as flour weight basis. All dry ingredients were placed in the bowl of a Kenwood Major mixer (Kenwood, Hampshire, UK). Batter preparation and baking were performed as previously described (Renzetti et al., 2008a). Mixing was performed for 2 min with a paddle tool (K beater) at slow/medium speed (level 2 out of 6). The batters were scaled to 400 g into 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. 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. Two breads containing different levels of protease addition were tested: 0.001% and 0.01% (PR0.001 and PR0.01). A bread without enzymes was used as control (CON).

### 2.3. Bread evaluation

Standard baking tests were conducted on three loaves ($n = 3$) from each bread type as previously described (Renzetti et al., 2008a). Texture profile analysis (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., 2008a). Pre-test speed, test speed and post test speed were 2 mm/s, trigger force was 20 g, distance was 10 mm (40% compression) and wait time between first and second compression cycle was 5 s. 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. Batter fundamental rheology

Rheological measurements were performed on a controlled stress and strain rheometer (Anton Paar MCR 301, Germany), using a parallel plate geometry (50 mm diameter) with a gap between the two plates of 1 mm. Samples were prepared as previously described but without yeast. The batters were incubated for 30 min at 30 °C as described for breadmaking. Immediately after, samples were loaded on the rheometer and the batters were allowed to rest for 5 min in order to allow relaxation of residual stresses. Frequency sweeps from 0.1 to 10 Hz were performed as previously described (Renzetti et al., 2008a) with a target strain of $10^{-3}$ (0.1%). Preliminary tests indicated that the strain was well within the linear viscoelastic region. Ten measuring points were recorded. Temperature was kept constant at 30 °C. All results are the average of at least two measurements.

### 2.5. Flow behaviour

Shear measurements were performed to evaluate the flow behaviour of batters. Apparent viscosity was measured as a function of shear rate over the range 0.06–5.0 $s^{-1}$ using the viscometry function of a controlled stress and strain rheometer (Anton Paar MCR 301, Ostfildern, Germany). The batters were incubated for 30 min at 30 °C as described for breadmaking but without yeast and viscometry measurements performed immediately after. Temperature was kept constant at 30 °C. Apparent viscosity is reported as the mean of three independent replicates for each batter type.

### 2.6. Capillary electrophoresis analysis of proteins from batters

Batters were prepared as described for breadmaking but without yeast. After proofing, samples were immediately frozen and subsequently freeze-dried. The freeze-dried samples were ground and sieved. Proteins were extracted from batter samples under non-reducing conditions by dissolving 50 mg of freeze-dried material in 1 ml of extraction buffer solution containing 50 mM potassium phosphate buffer, pH 7.6, 6 M urea and 0.15 M NaCl. N-ethylmaleimide (20 μl, 0.1%) was added in order to stop any further enzymatic activity. For protein extraction under reducing conditions 0.1 M dithiothreitol (DTT) was added in the 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 from 5 to 80 kDa were separated using the Protein 80 + LabChip in the Agilent 2100 Bioanalyser (Agilent Technologies, Palo Alto, CA) as previously described (Renzetti et al., 2008b). For each sample, the relative concentration of the polypeptides separating on molecular size was calculated against the internal standard present in the Agilent sample buffer. Analyses were performed in