



Dietary fibre enzymatic treatment: a way to improve the rheological properties of high-fibre enriched dough

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Complete List of Authors:	Marti, Alessandra; University of Milan, DeFENS, Department of Food, Environmental and Nutritional Sciences Bottega, Gabriella; University of Milan, DeFENS, Department of Food, Environmental and Nutritional Sciences Casiraghi, Maria; University of Milan, DeFENS, Department of Food, Environmental and Nutritional Sciences Faoro, Franco; University of Milan, DiSAA, Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy Iametti, Stefania; University of Milan, DeFENS, Department of Food, Environmental and Nutritional Sciences Pagani, M. Ambrogina; University of Milan, DeFENS, Department of Food, Environmental and Nutritional Sciences
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11 4 Alessandra Marti ¹, Gabriella Bottega ¹, Maria Cristina Casiraghi ¹, Franco Faoro ², Stefania Iametti ¹,
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13 Maria Ambrogina Pagani ^{1*}
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17 6 ¹ Department of Food, Environmental and Nutritional Sciences - Università degli Studi di Milano, via
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19 7 Giovanni Celoria 2, 20133 Milan, Italy
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22 8 ² Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy -
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24 Università degli Studi di Milano, via Giovanni Celoria 2, 20133 Milan, Italy
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30 11 *Corresponding author:

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32 12 Prof. Maria Ambrogina Pagani

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34 13 2, Via G. Celoria

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36 14 20133 Milan, Italy

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38 15 E-mail: ambrogina.pagani@unimi.it

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3 18 The demand for functional products is increasing, driven largely by the market interest in foods suitable
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5 19 for improving the health and well-being of consumers (Charalampopoulos *et al.*, 2002). In this regard,
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8 20 diets rich in dietary fibre (DF) are more and more recommended, since it is widely accepted that Non-
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10 21 Starch Polysaccharides (NSP) are associated with the prevention of cardiovascular diseases, the
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12 22 regulation of intestinal function, the promotion of gut health, and the protection against colon cancer
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15 23 (Kumar *et al.*, 2012). Bakery products, particularly bread, may be a convenient tool for increasing DF
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17 24 content in habitual western diet, since they are consumed daily in high amounts.

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20 25 However, the long chains in NSP molecules interfere with the development of a regular and strong
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22 26 gluten network, resulting in a worsening of the rheological properties and handling of bread dough,
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24 27 giving loaf with low volume, hard crumb, bitter flavour, and dark colour (Ktenioudaki & Gallagher,
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27 28 2012). The negative effect of fibre on bread texture and consumer acceptability is greatly dependant on
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29 29 the type and components, level of substitution, and particle size of the fibre (Ktenioudaki & Gallagher,
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31 30 2012). Many approaches have been employed to improve the quality of fibre-enriched bread, mainly
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34 31 based on the use of bioprocessing such as fermentation (Salmenkallio-Marttila *et al.*, 2001; Katina *et*
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36 32 *al.*, 2006) and/or on the addition of enzymes or additives directly in the bread formulation (Laurikainen
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38 33 *et al.*, 1998; Haros *et al.*, 2001; Katina *et al.*, 2006; Shah *et al.*, 2006). Consequently, in all these
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40 34 studies the enzymatic action is simultaneous to the bread-making process. None of the enzymatic
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42 35 treatments already proposed has been carried out as a pre-treatment of the fibre. Only Napolitano *et al.*
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44 36 (2006) highlighted that the conversion of the highly polymerised insoluble dietary fibre of durum wheat
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46 37 into soluble feruloyl oligosaccharides can be achieved by using hydrolytic enzymes. However, no
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48 38 information is available on the effect of fibre enzymatic treatment on the rheological properties of
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50 39 wheat dough when total fibre content is higher than 6%. The aim of the present work was to investigate
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52 40 the modifications induced by enzymatic treatment of fibre on the rheological properties of fibre-
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54 41 enriched bread dough.

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3 42 Oat bran (cv. Raisio, Finland) containing 15% beta-glucans (BG15; total starch = 26 g/100 g; protein =
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5 43 19 g/100 g; lipids = 10 g/100 g;) was used in this study. The enzymatic hydrolysis of the samples was
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8 44 performed with a commercial food grade cellulolytic and glycosidic enzyme mixture (Cytolase M102,
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10 45 Genencor International, New York, USA, with a cellulase activity of 2400 viscosimetric units,
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13 46 measured as detailed by Caldini *et al.* (1994)), obtaining BG15+E sample. An aliquot of 50 µl of the
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15 47 enzyme solution was added to one gram of sample suspended in 4 ml of deionised water. The
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18 48 suspension was placed in agitation for 6 hours at 25 °C, then freeze dried, according to Caldini *et al.*
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20 49 (1994). The amount of total (DF), soluble (SDF) and insoluble dietary fibre (IDF) was determined
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22 50 according to the gravimetric enzymatic method proposed by Prosky *et al.* (1988). The soluble residue
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25 51 in the supernatant was measured after centrifugation of the mixture at 4000 x g, for 15 min at 25°C, and
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27 52 overnight drying at 105°C. Sugar content was determined according to Zygmunt *et al.* (1982).
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29 53 Microscopy images were obtained by means of an Olympus BX50 microscope, using Lugol (I₂KI) as
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32 54 staining.

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34 55 Untreated and treated fibre samples were added to a common wheat flour of good baking properties
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36 56 (protein = 13.8 g/100 g; fiber content 2.95g/100g; alveographic W = 358 *10⁻⁴ J; alveographic P/L =
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39 57 0.55) in order to prepare a bread dough enriched with 20 g BG15/80 g flour or 20 g BG15+E/80 g
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41 58 flour. Dough mixing properties were evaluated with the farinographic test (Brabender OHG, Duisburg,
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44 59 Germany). Dough development and CO₂ produced from the yeast activity during the leavening phase
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46 60 were measured by means of a Rheofermentometer F3 (Chopin, Tripette & Renaud, Villeneuve La
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49 61 Garenne, France).

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51 62 The data were processed by Statgraphic Plus for Windows v. 5.1. (StatPoint Inc., Warrenton, VA,
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53 63 USA). A one-way analysis of variance (Anova) was performed using the Least Significant Differences
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55 64 (LSD) test to compare the sample means; differences were considered significant at P < 0.05.

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3 65 The enzymatic treatment of fibre was associated with a significant decrease in the total fibre content
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5 66 (Table 1). In particular, the SDF/IDF ratio greatly changed; this trend could be related to the formation
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8 67 of disaccharides and/or other soluble oligosaccharides having size and molecular weight too low to be
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10 68 classified as SDF by using the Prosky method (Prosky *et al.*, 1988). This finding was confirmed by a
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13 69 significant increase of the soluble residue and by the higher sugar content measured after enzymatic
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15 70 treatment (Table 1). The relevant breakdown of the macrostructure of NSP into smaller species was
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17 71 also made evident by microscopy (Fig. 1). Before the enzymatic treatment, BG15 sample appears
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20 72 characterized by fibre particles between 500 and 650 μm in size (Fig. 1a). The enzymatic action caused
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22 73 a disintegration of the fibre particles, broken down to fractions in the 20-100 μm range (Fig. 1b).

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25 74 The effects of the enrichment in fibre on mixing properties, as evaluated by the farinographic test, are
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27 75 reported in Fig. 2. As expected, the water absorption and the development time of the flour increased,
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30 76 whereas the stability decreased. The high water affinity of the NSP and the weakening effect of fibre on
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32 77 dough have been already described (Sudha *et al.*, 2007). On the contrary, adding high level of untreated
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34 78 fibre resulted in an increase of both water absorption of the dough and development time, accounting
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37 79 for a difficult workability. Adding enzymatically treated fibre promoted a decrease of the amount of
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39 80 water absorption and a more relevant impact on the macromolecule hydration and, consequently, on the
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41 81 development time (see insert in Fig. 2).

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44 82 The dough development during leavening and the amount of gas produced and retained by the dough
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46 83 system of the dough enriched with fibre was investigated by the rheofermentographic test. The addition
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48 84 of untreated fibre compromised a good dough development (Fig. 3). This phenomenon had been
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51 85 attributed to impaired protein hydration in presence of NSP (Sudha *et al.*, 2007). The addition of pre-
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53 86 treated fibre allowed a fast and great dough development, likely due to the sugar formation promoted
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56 87 by the enzymatic treatment. With regard to the ability of the dough to produce CO_2 , the incorporation
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3 88 of BG15+E to flour resulted in a product with high gas production and retention, very similar to that
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6 89 obtained for the common wheat flour (see insert in Fig. 3).

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8 90 These results suggest that the enzymatic treatment allows to add high level of soluble fibre (>5%) to
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10 91 the flour without worsening the dough properties during both mixing and leavening. The enzymatic
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12 92 pre-treatment proposed in this study may be easily applied by milling companies both for the
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14 93 enhancement of wheat milling by-products and for increasing their range of products. From the point of
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16 94 view of the final user, in a production reality as the Italian one, characterized by numerous small bread-
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18 95 making companies where very often experience and tradition matter more than a scientific approach,
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20 96 the use of pre-treated flours/ingredients permits to minimize some of the problems associated with the
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22 97 direct use of enzymes in bread-making.

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27 98 Further studies are currently being carried out to investigate the effect of treated fibre on bread
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29 99 characteristics.

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3 137 Table 1. Fibre and sugars content (g/100g db) of untreated and enzymatically treated high fibre
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6 138 samples.

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8 139 BG15 = oat bran (15% beta-glucans); BG15+E = enzymatically treated oat bran (15% beta-glucans).
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11 140 Figure 1. Microscope images of untreated (a) and enzymatically treated (b) high fibre samples.
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14 141 BG15 = oat bran (15% beta-glucans); BG15+E = enzymatically treated oat bran (15% beta-glucans).
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17 142 Figure 2. Mixing properties of untreated and treated fibres.
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20 143 BG15 = oat bran (15% beta-glucans); BG15+E = enzymatically treated oat bran (15% beta-glucans).
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23 144 Figure 3. Leavening properties of fibre-enriched wheat dough.
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26 145 BG15 = oat bran (15% beta-glucans); BG15+E = enzymatically treated oat bran (15% beta-glucans).
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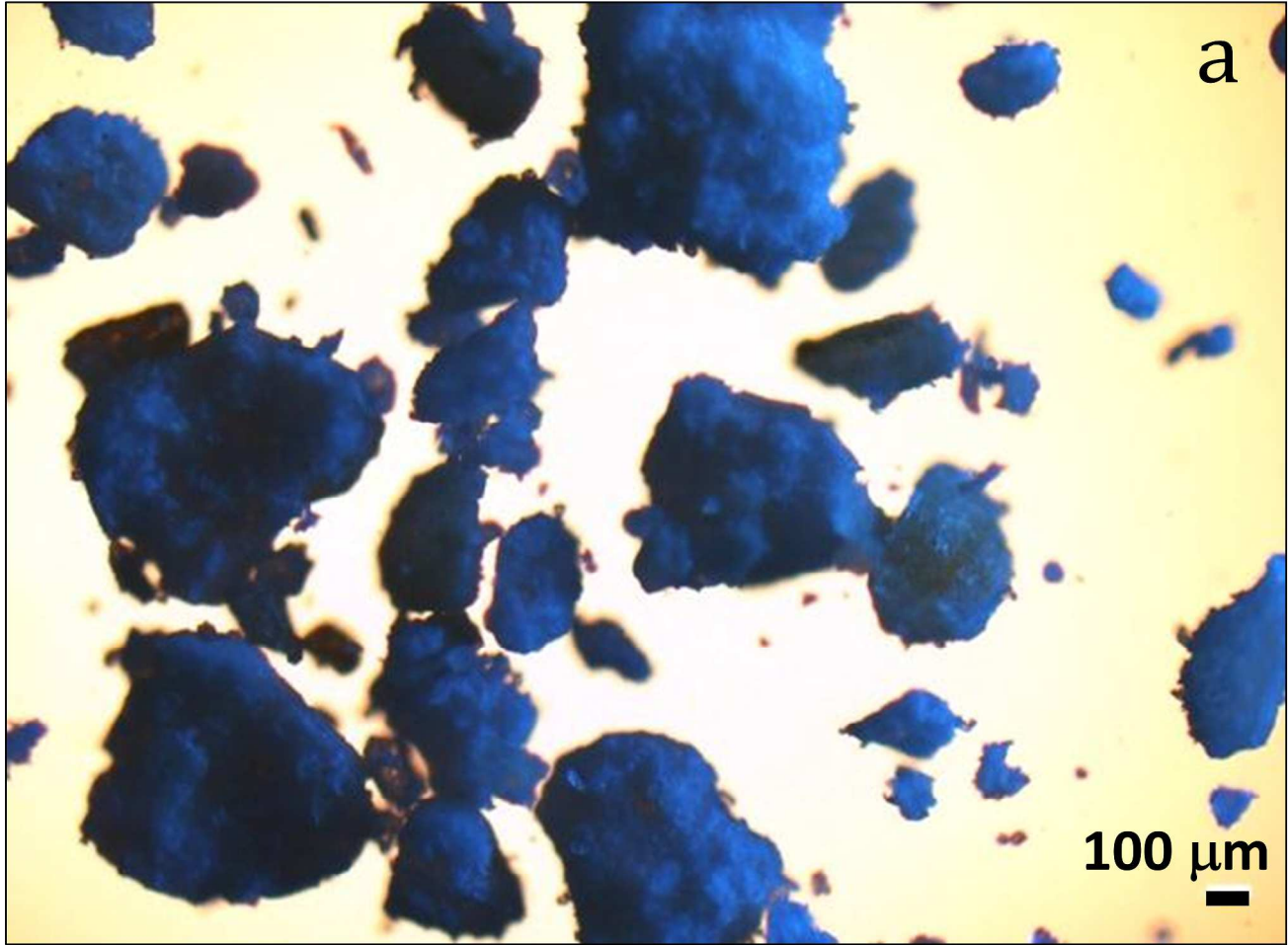
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Table 1. Fibre and sugars content of untreated and enzymatically treated high fibre samples.

BG15 = oat bran (15% beta-glucans); BG15+E = enzymatically treated oat bran (15% beta-glucans).

Parameters	BG15	BG15 + E
	(g/100g db)	
Total Fibre	31.10 ± 0.93b	24.10 ± 0.72a
Soluble Fibre	15.36 ± 0.46a	20.23 ± 0.61b
Insoluble Fibre	15.69 ± 0.47b	3.89 ± 0.12a
Soluble/Insoluble Fibre ratio	0.98	5.2
Soluble residue	11.8 ± 0.35b	23.5 ± 0.71a
Sugars	3.16a	5.19b
Glucose	0.03 ± 0.001a	3.22 ± 0.04b
Fructose	0.07 ± 0.002a	1.10 ± 0.005b
Sucrose	2.34 ± 0.01b	0.18 ± 0.02a
Maltose	0.40 ± 0.05b	0.27 ± 0.04a

Different letters in the same line are significantly different (**Least Significant Differences**; p<0.05).



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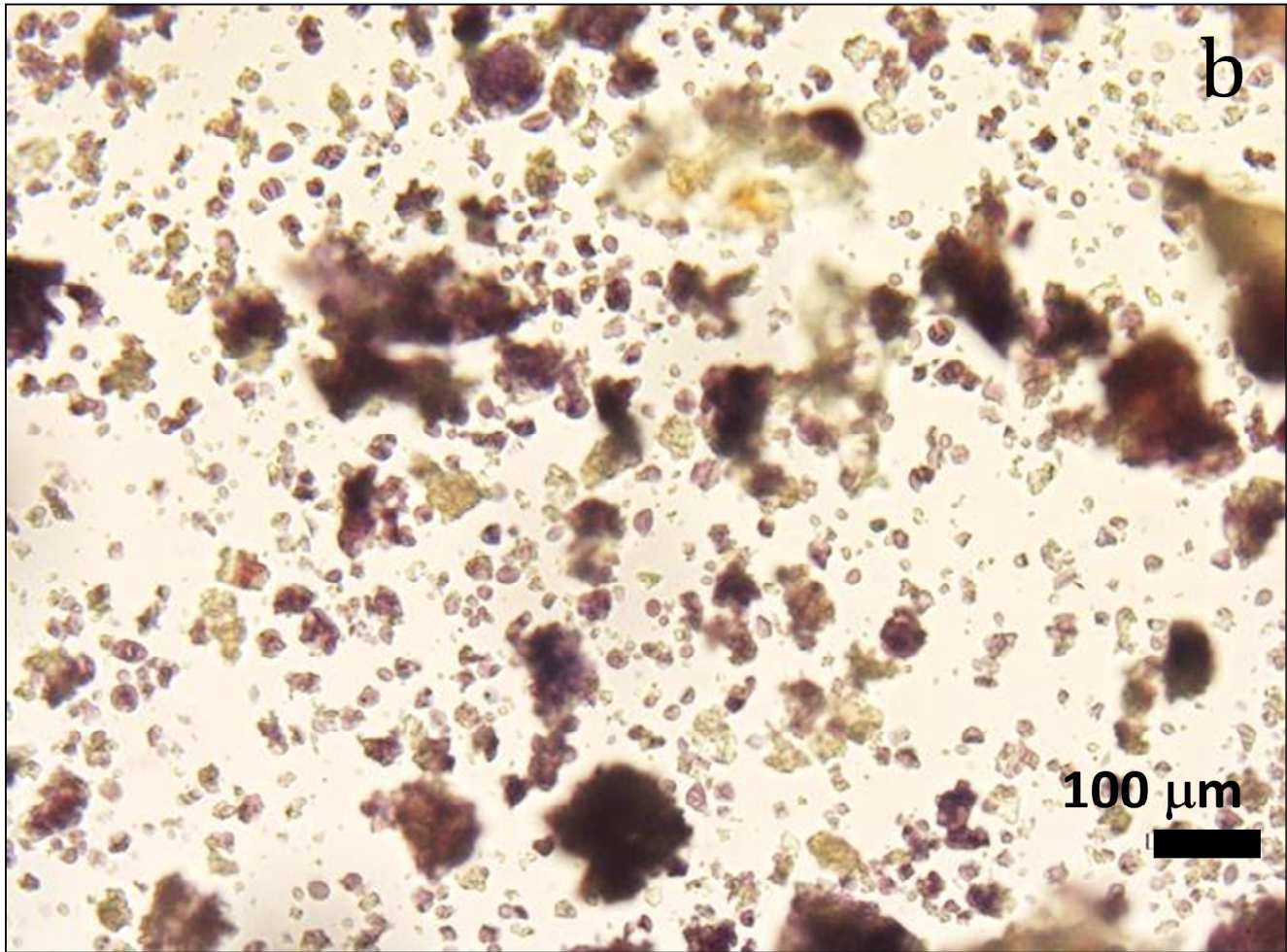


Figure 1. Microscope images of untreated (a) and enzymatically treated (b) high fibre samples.

BG15 = oat bran (15% beta-glucans); BG15+E = enzymatically treated oat bran (15% beta-glucans).

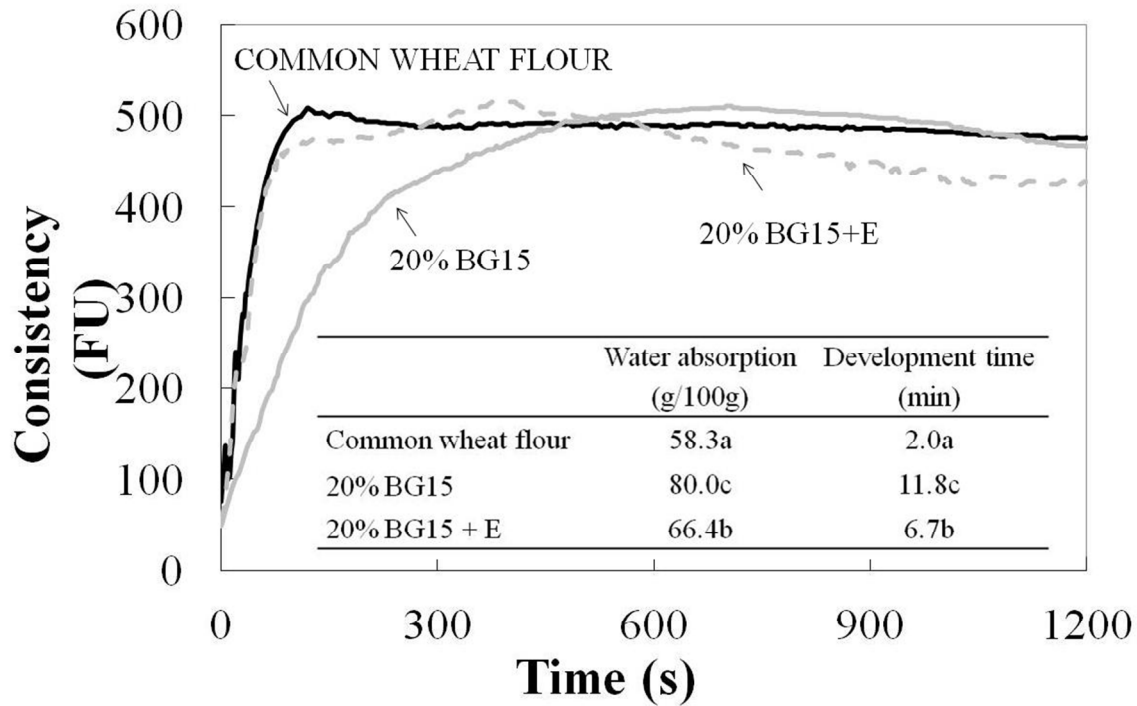
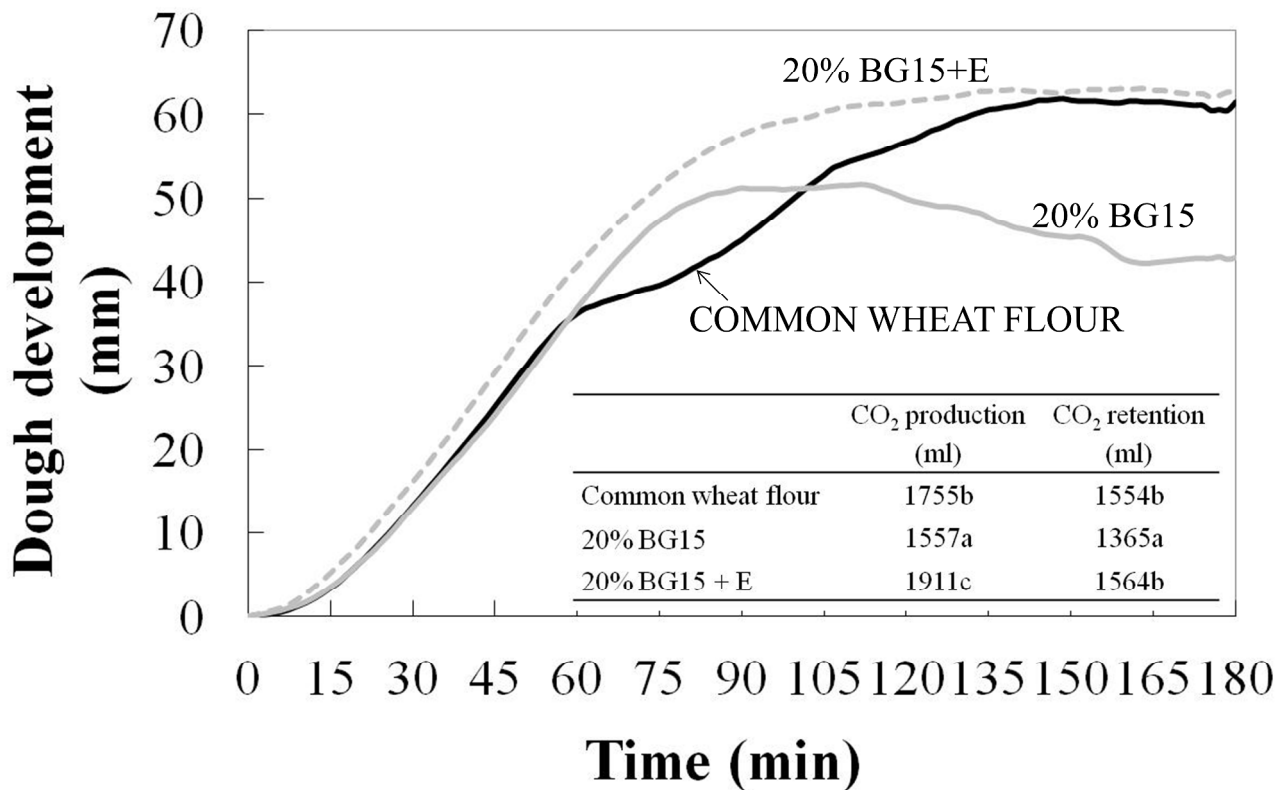


Figure 2. Mixing properties of untreated and treated fibres.

Different letters in the same column are significantly different (Least Significant Differences; $p < 0.05$).

BG15 = oat bran (15% beta-glucans); BG15+E = enzymatically treated oat bran (15% beta-glucans).



183
184 Figure 3. Leavening properties of fibre-enriched wheat dough. Different letters in the same parameter
185 are significantly different (Least Significant Differences; $p < 0.05$).

186 BG15 = oat bran (15% beta-glucans); BG15+E = enzymatically treated oat bran (15% beta-glucans).