

1                    **Effect of high pressure processing on the features of wheat milling by-products**

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3                    Alessandra Marti, Alberto Barbiroli, Francesco Bonomi, Andrea Brutti, Stefania Iametti, Mauro

4    Marengo, Matteo Miriani, and Maria Ambrogina Pagani

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6                    <sup>1</sup> Università degli Studi di Milano - Department of Food, Environmental and Nutritional Sciences – 2,

7    via Giovanni Celoria, 20133 Milan, Italy

8                    <sup>2</sup> Experimental Station for the Food Preserving Industry – SSICA - Viale Tanara, 31/a, 43100 Parma,

9    Italy

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11                    \*Corresponding author: Prof. Maria Ambrogina Pagani

12                    2, Via G. Celoria

13                    20133 Milan, Italy

14                    E-mail: ambrogina.pagani@unimi.it

15                    Phone: +39 0250316658

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**ABSTRACT**

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18 The ability of high hydrostatic pressure processing to promoting changes in both the structural  
19 properties of fiber and of the interaction of fiber with water were addressed. Both coarse and fine bran  
20 from milling of common wheat were considered. Treatment-induced morphological changes were most  
21 pronounced in fine bran, whereas treatment of coarse bran resulted in the largest change of water  
22 holding capacity. The significance of the process-induced changes is discussed as for their practical  
23 relevance in the production of fiber-enriched foods.

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26 High hydrostatic pressure (HHP) processing uses high hydrostatic pressure (usually between  
27 100 and 1000 MPa) to improve food shelf-life and, in some cases, to modify food properties (Hayashi  
28 1991). HHP modifies the structure of biopolymers (protein, starch, and DNA), thus producing foods  
29 with novel texture and providing satisfying effects (Estrada-Giron et al 2005). HHP processing has  
30 been shown to significantly affect the amorphous and ordered starch structure and, thus, to cause starch  
31 gelatinisation as a function of applied pressure, treatment time and temperature, concentration and type  
32 of starch (Pei-Ling et al, 2010). Recent studies demonstrated that the HHP treatment of gluten-free  
33 cereal raw materials may improve baking performance of gluten-free cereals, mostly as a consequence  
34 of changes in their protein fractions that may occur concomitantly with modifications in other  
35 biopolymers in the system (Huttner et al 2009; Huttner et al 2010; Vallons et al 2011).

36 The application of HHP processing and its effect on wheat milling by-products has not been  
37 much studied until now. The objective of this study was to investigate the effects of HHP treatment on  
38 microstructure and hydration properties of by-products from wheat milling. This could promote  
39 changes in overall structural properties of fiber resulting in improved quality and easier handling of  
40 fiber-enriched dough, and promote further intervention on fiber structure (e.g., enzymatic treatments  
41 aimed at altering the soluble/insoluble ratio).

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## MATERIALS AND METHOD

44 Coarse and fine bran from common wheat were supplied by Molino Quaglia S.p.A. (Vighizzolo  
45 D'Este, Padova, Italy). The particle size distribution of both samples are reported in Table 1.

46 HHP processing was performed on both coarse and fine bran. For each HHP treatment, 8 g of  
47 sample were added to 80 ml of deionised water and sealed in a plastic bag after 15 minutes of resting.  
48 Treatment was carried out at SSICA in a QFP 35L-600, (Avure Technologies, Franklin, TN, USA), at

49 36 °C and 600 MPa for 5 or 15 minutes. The treated mixture was freeze-dried within 4 h from  
50 treatment and kept at 0–4 °C until further analysis.

51 Water-holding capacity (WHC), swelling capacity (SC), and water binding capacity (WBC)  
52 were determined, in triplicate, as reported by Lebesi and Tzia (2012) with few modifications. WHC  
53 (g/g) was expressed as the residue hydrated weight and the original sample weight ratio. SC (ml/g) is  
54 defined as the ratio of the volume occupied when the sample is immersed in excess of water after  
55 equilibration to the actual weight. WBC (g/g) was defined as the quantity of water that remains bound  
56 to the hydrated fiber following the application of an external force (centrifugation).

57 Microscopy images were obtained by means of an Olympus BX50 microscope, using Lugol  
58 (I<sub>2</sub>KI) as staining. Coarse and fine bran, before and after HHP processing, were incorporated at 20%  
59 replacement level to a wheat flour of good bread-making properties (protein = 14.5 g/100 g;  
60 alveographic W = 430 \*10<sup>-4</sup> J; alveographic P/L = 0.80).

61 The effect of treatment on gluten aggregation properties of bran-enriched flour was measured  
62 using the GlutoPeak (Brabender GmbH and Co KG, Duisburg, Germany), that allows to measure  
63 rheological parameters of highly hydrated flours under conditions of high shear stress. An aliquot of 9  
64 g of blend was dispersed in 10 ml of distilled water. Sample temperature was maintained at 35 °C by  
65 circulating water through the jacketed sample cup. The paddle was set to rotate at 3000 rpm and each  
66 test ran for 5 min. The time required to achieve maximum torque development (Peak Maximum Time,  
67 PMT; s) and the area under the peak (equivalent to energy, and expressed in arbitrary units) were  
68 considered. Measurements were performed at least in triplicate.

69 Data were processed by Statgraphic Plus for Windows v. 5.1. (StatPoint Inc., Warrenton, VA,  
70 USA). One-way analysis of variance (Anova) was performed using the Least Significant Differences  
71 (LSD) test to compare the sample means; differences were considered significant at P < 0.05.

72

## RESULTS AND DISCUSSION

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### 74 Hydration properties

75 The hydration properties of samples before and after HHP treatment are presented in Figure 1.  
76 Water Holding Capacity (WHC) is the ability of the hydrated fiber matrix to retain water, in the form  
77 of linked water, hydrodynamic water, and physically trapped water, the latter contributing most to this  
78 property (Alfredo et al 2009). A fiber WHC is highly indicative of its physiological role in intestinal  
79 function and in blood sugar level control (Wolever 1990). Coarse bran retained more water than fine  
80 bran (Figure 1). HHP processing significantly ( $p < 0.05$ ) affected WHC, almost regardless of treatment  
81 time ( $p > 0.05$ ). Since the structural characteristics and chemical composition of fiber play important  
82 roles in water uptake and consequently in swelling (Figuerola et al 2005), the increase in WHC after  
83 HHP treatment could be explained by a modified organization of some fiber component.

84 Swelling Capacity (SC) indicates to what extent the fiber matrix swells as water is absorbed  
85 (Femenia et al 2009). The SC of coarse bran did not change after HHP processing (Figure 1). In the  
86 case of fine bran, HHP treatment increased the swelling ability by 25%.

87 Water Binding Capacity (WBC) is related to the sample ability to strongly bind water.  
88 Untreated coarse bran showed a slightly higher WBC than fine bran (Figure 1), confirming that water  
89 binding to fiber decreases as particle size decreases (Auffret et al 1994). Particle size and shape affect  
90 the centrifuge separation performance of the material, as coarse wheat bran is less firmly packed than  
91 smaller particle bran after centrifugation (Zhang and Moore 1997). The HHP treatment promoted a  
92 greater increase in WBC in the case of fine bran (24%) than in coarse bran (10%), confirming the  
93 greater effect of HHP processing on the hydration properties of fine bran. Again, no significant  
94 ( $p > 0.05$ ) differences were measured according to the time of treatment. For this reason, further  
95 investigation was carried out on samples treated just for 5 minutes.

96

## 97 **Microstructural features**

98           Microscope images of coarse and fine bran before and after HHP treatment are shown in Figure  
99 2. Coarse bran has many particles greater than 1000  $\mu\text{m}$ ; the largest one also reached 1500  $\div$  2000  $\mu\text{m}$   
100 (Figure 2a). Fine bran ranged from 500  $\mu\text{m}$  to 1000  $\mu\text{m}$  (Figure 2b). HHP-treated samples have a size  
101 smaller than the corresponding untreated samples (Figure 2c, d). In both cases, particles of 100 $\div$ 200  
102  $\mu\text{m}$  with a less compact structure appeared after HHP processing, suggesting that the treatment  
103 promoted a weakening of the structure and its fragmentation, thus accounting for the change in  
104 hydration properties (Figure 1).

105

## 106 **Gluten aggregation behaviour**

107           The GlutoPeak Test (GPT) is a new approach for testing gluten quality, that uses shear force to  
108 uniformly mix flour and water and measures the torque associated with high-speed mixing. The GPT  
109 profiles of flour and bran-enriched flours are shown in Figure 3. As the gluten aggregation develops,  
110 the shear force increases as does the energy required for mixing.

111           Peak Maximum Time (PMT) relates to the time required for gluten to aggregate and to exhibit  
112 maximum spindle torque. The addition of any type of bran significantly ( $p<0.05$ ) decreased PMT  
113 indicating a weakening of the gluten network, as observed in presence of cellulosic fiber (Goldstein et  
114 al 2010).

115           The area under the peak is an indicator of the amount of work required for mixing and gluten  
116 formation. Bran significantly decreased this parameter ( $p<0.05$ ), again suggesting a worsening of  
117 gluten aggregation properties. HHP processing gave a significant ( $p<0.05$ ) increase in PMT (only for  
118 coarse bran) and a significant ( $p<0.05$ ) increase in energy for both coarse and fine bran. These effects  
119 could be related to changes in both bran hydration properties (Figure 1) and particle size (Figure 2).

120 Indeed, if tightly bound water is only available, it will take more time and energy to get the water-  
121 dependent structural changes involved in gluten development (Huschka et al 2011).

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## CONCLUSIONS

124 This work indicates that HHP processing alters the physical and structural characteristics of  
125 milling by-products, and – as a consequence – their ability to interact with water. HHP-induced  
126 morphological modifications were greater in the case of fine bran and resulted in large changes in the  
127 bran hydration properties. The hydration properties of bran are relevant in many cereal-based systems  
128 where water availability at various process stages (e.g, during mixing in bread or pasta making) affects  
129 the handling of mixtures, and the physical and sensory properties of the resulting product. Furthermore,  
130 the highly solvated bran that forms as a consequence of HHP treatment could be a convenient substrate  
131 for bioconversions aimed either at modifying the structural/nutritional features of the product (for  
132 example, by altering the ratio between soluble and insoluble bioactive poly- and oligosaccharides), or  
133 for improving the recovery of other bioactive components, such as polyphenols.

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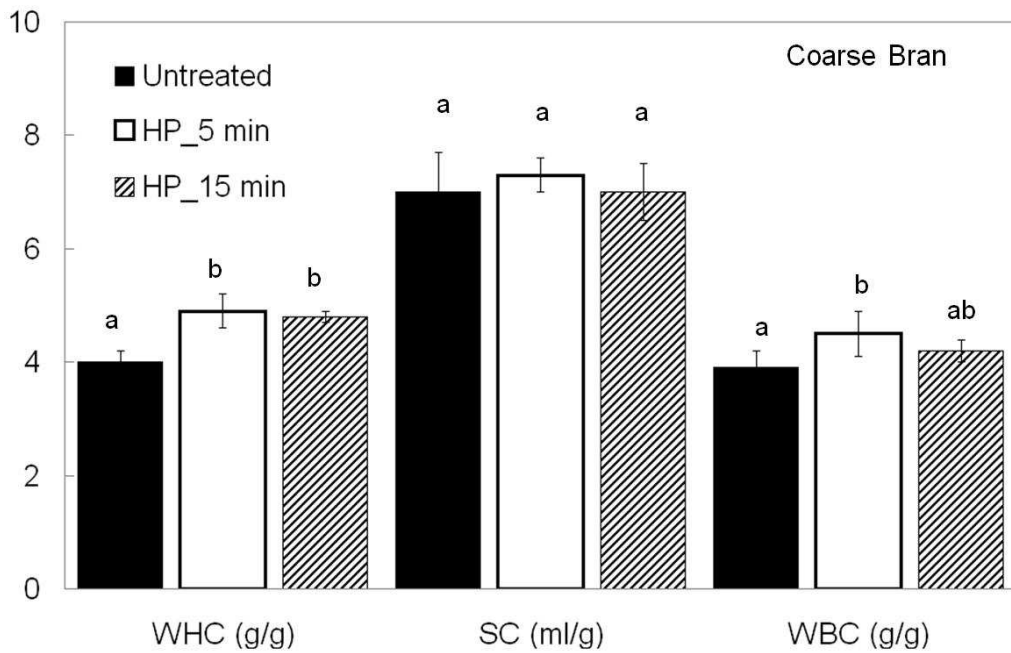
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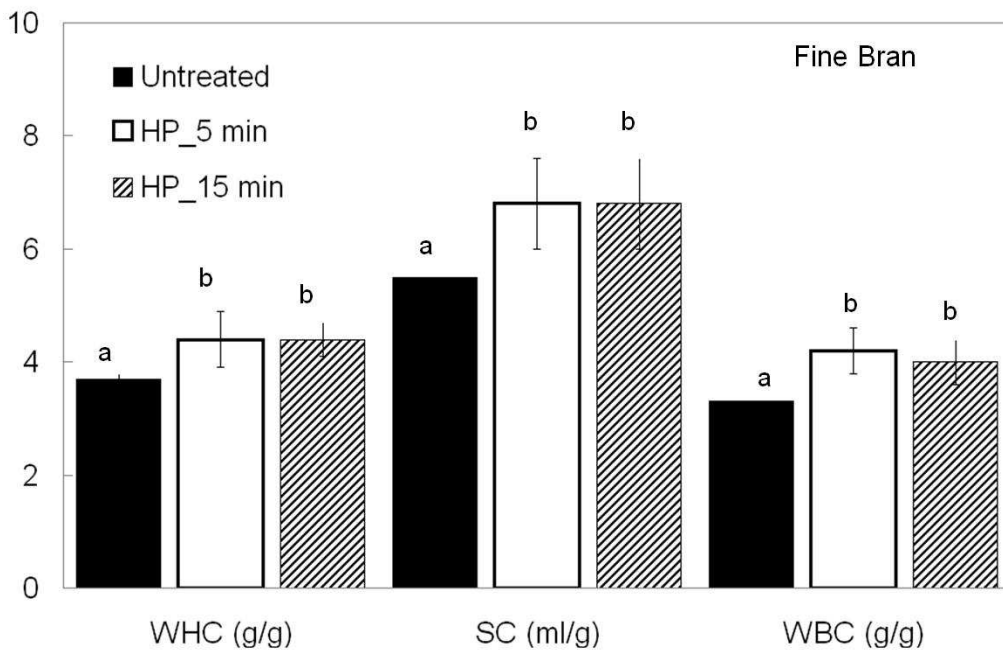
168 **Table 1**  
169 **Coarse and fine bran particle size distribution (expressed in g/100g).**

	<b>Coarse bran</b>	<b>Fine bran</b>	
170			
171			
172			
173			
174	> 800 $\mu\text{m}$	32	1
175			
176	500 $\div$ 800 $\mu\text{m}$	35.1	35.1
177			
178	350 $\div$ 500 $\mu\text{m}$	27.5	40.2
179			
180	180 $\div$ 350 $\mu\text{m}$	5.4	20.2
181			
182	50 $\div$ 180 $\mu\text{m}$	0	3.4
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184	< 50 $\mu\text{m}$	0	0.1
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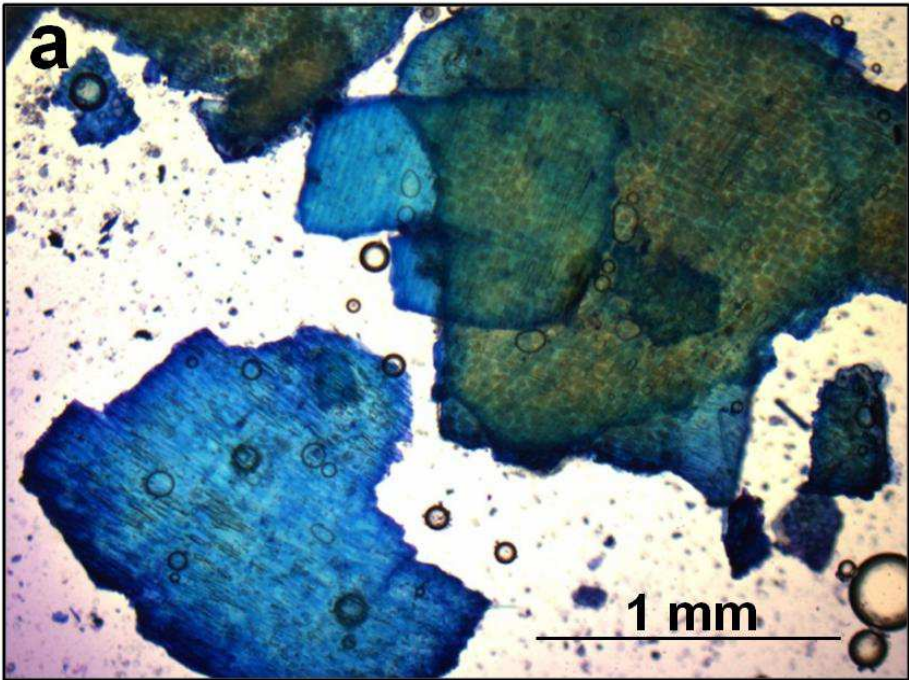


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191 **Fig. 1.** Hydration properties of coarse and fine bran, before and after HHP processing.

192 HP\_5 min = sample after HHP processing for 5 minutes; HP\_15 min = sample after HP processing for  
 193 15 minutes; WHC = water holding capacity; SC = swelling capacity; WBC = water binding capacity.

194 Different letters for each parameter are significantly different ( $p < 0.05$ ).

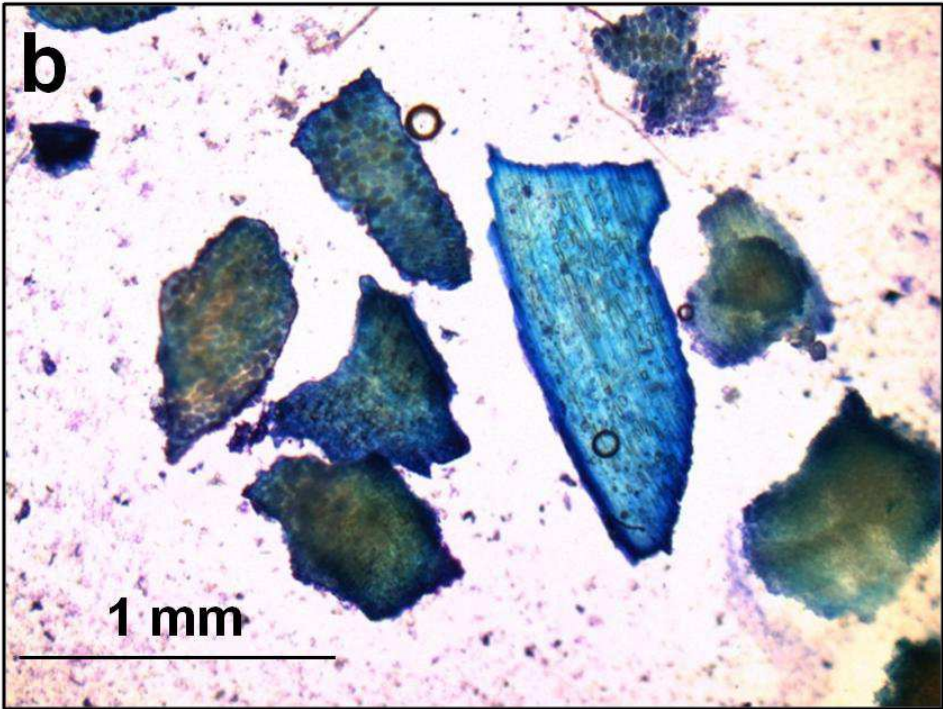


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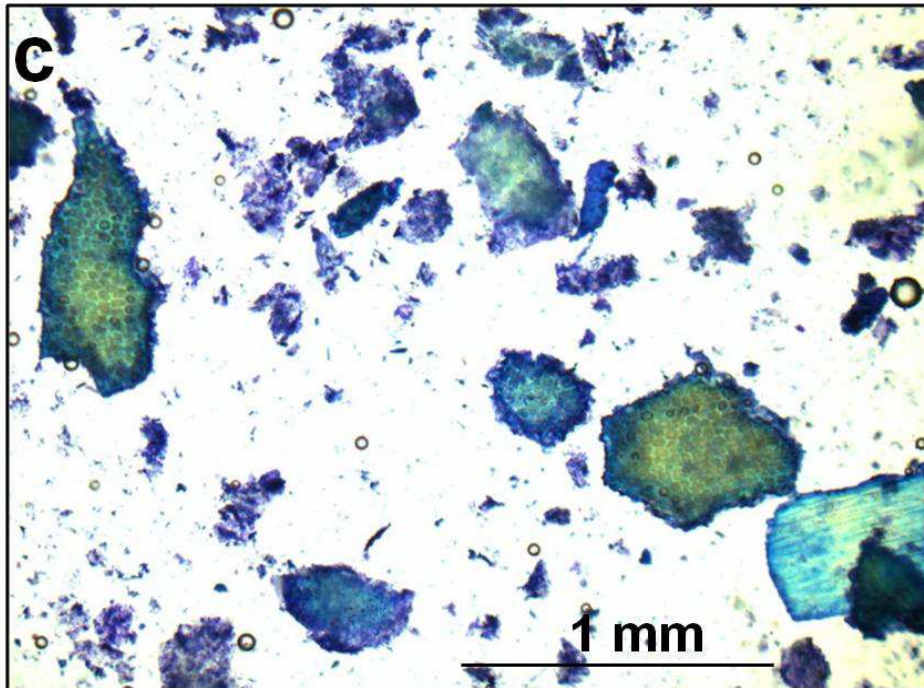
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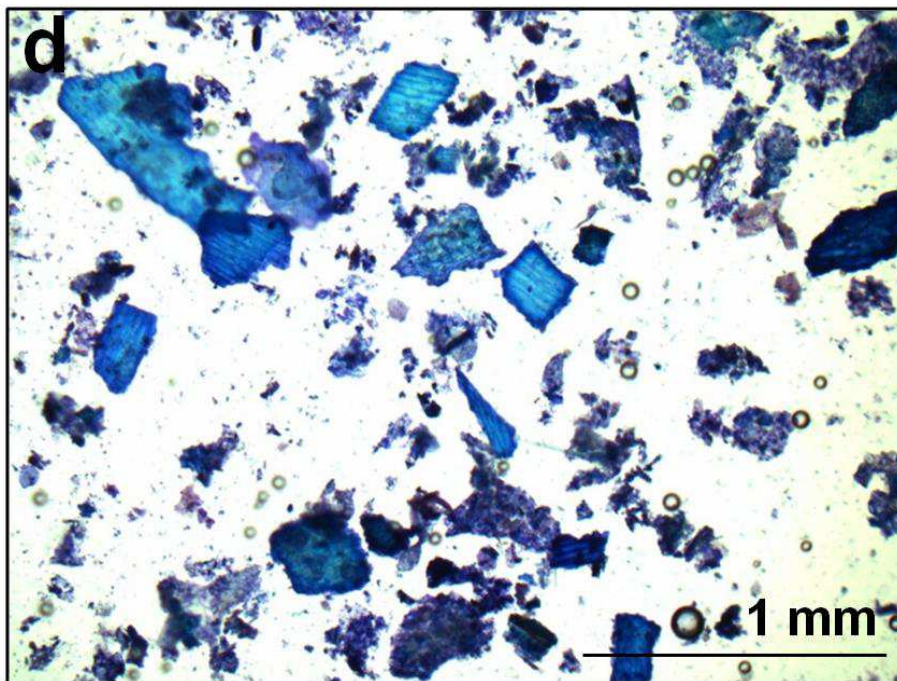


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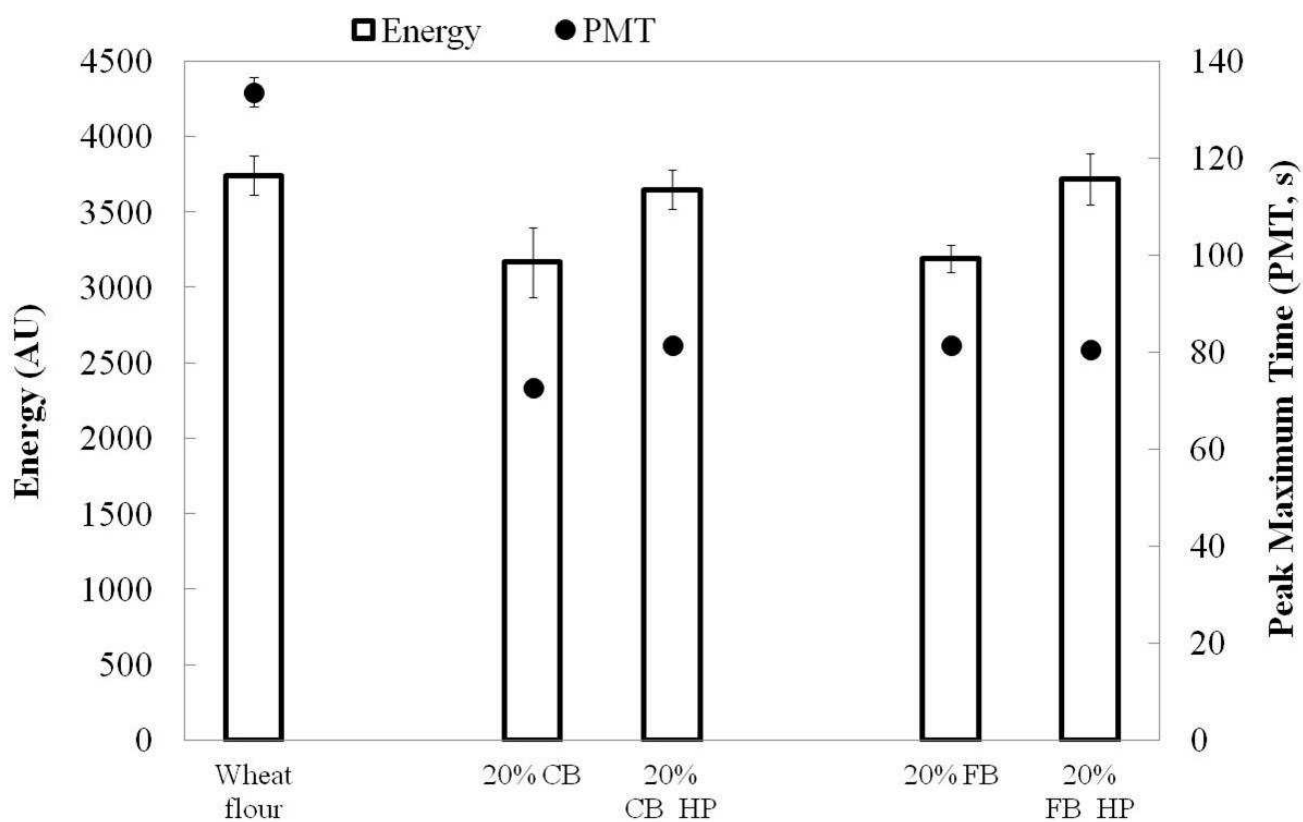


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203 **Fig. 2.** Microscope images of coarse (a) and fine (b) bran and effects of HHP processing on coarse (c)

204 and fine (d) bran.





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207 **Fig. 3.** Gluten aggregation properties of bran-enriched flour. PMT = Peak Maximum Time, is indicated

208 by dots. Column height refers to energy, measured by considering the area underneath the torque peak,

209 and given in arbitrary units. CB = coarse bran; HPCB\_5 = coarse bran after HHP processing for 5

210 minutes; FB = fine bran; HPFB\_5 = fine bran after HHP processing for 5 minutes.