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Effect of (bio-)technological approaches on bran to improve the quality of cereal products

[Scientific field AGR/15]

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Abstract

The design of foods enriched in dietary fiber has recently received great attention among academia and food industry, since in many European countries the daily intake of dietary fiber is much lower than the recommended levels (25 g; EFSA, 2010). Being a staple food category, cereal products may represent a valid resource to satisfy the optimal amount of soluble and insoluble fiber and other bioactive components. Despite the nutritional benefits, the incorporation of fiber into flour negatively affects the textural and sensory properties of bread and pasta products.

This PhD project dealt with the application of physical and/or biotechnological strategies to modify the structural properties of peculiar macromolecules (such as non-starch-polysaccharides or proteins) to enhance the quality of fiber-enriched foods. Samples from the outermost layers of cereals and pseudocereals particularly rich in bioactive compounds (e.g. purple wheat and buckwheat) were included in pasta or bread after treatment and evaluated in terms of technological behavior.

The first part of the thesis regarded the application of a two-step debranning process to purple wheat that allowed the selective recovery of bioactive compounds, such as fiber and phenolic compounds. Debranned fractions from the first and the second step were used separately to produce fiber-enriched pasta. Bran from conventional milling was also used as a control. Both fractions had higher or comparable content in total and soluble fiber than bran. Moreover, both of them exhibited a higher ferric reducing-antioxidant power (FRAP) than bran, whereas the highest amount of anthocyanins was found in the first fraction. When compared with pasta enriched in bran, the samples enriched either in the first or second fraction had similar FRAP values and higher amount of anthocyanins, while retaining a fair cooking quality.

In the second part of the thesis micronization was applied to buckwheat bran to evaluate the effect of granulometry on dough/bread quality. The role of coarse and fine buckwheat bran enrichment on wheat dough was studied at increasing levels (5, 10, 20%). Dough and bread properties were negatively affected by the interplay of bran addition level and particle size. The thermo-mechanical behavior of dough was found to be mainly related to starch phase transitions during heating with no regards of particle size. Front-face fluorescence studies revealed differences in gluten structural arrangement and solvation with a distinct effect of particle size. Despite the better or similar dough rheological properties (e.g. elasticity, resistance to extension, extensibility), bread enriched in fine bran had lower baking quality in comparison with samples enriched in coarse bran, in terms of loaf specific volume and crumb softness. Concerning the texture, the variations in the perceived crumb hardness were mainly related to changes in crumb density. The application of an adapted Ashby-Gibson model to correct hardness values for the variation in density revealed that the mechanical properties of the solid crumb matrix were controlled by the differences in moisture and in bran volume fraction.

Buckwheat bran was used also to evaluate the effects of superheated steam (SS) on bran properties. SS is an innovative technology that has allowed drying of many food matrices while limiting collateral effect on the product. After screening the process conditions, the selected treated bran samples (120 °C, 140 °C, or 160 °C;

40 min; 0.7 a_w) were incorporated into high-enriched dough (20%) and their influence on the rheological properties and on the baking quality were considered. The treatment deeply affected the chemical/physical properties of buckwheat bran. In particular, changes in water affinity were progressively observed with increasing temperature, also due to the formation of protein aggregates. These modifications influenced the thermo-mechanical properties of high-bran enriched doughs that reflected baking behavior. When appropriate setting was chosen (temperature: 160 °C) SS treatment increased buckwheat bran performances in terms of specific volume and crumb softness in comparison with untreated bran.

The last part of the thesis regarded the use of specific enzymatic treatment (i.e. pectinases and cellulases) to decrease the size of non-starch-polysaccharide chains, aiming at mitigating the worsening effects of fiber on enriched products. Either coarse buckwheat bran or SS bran (treated at 140 °C; 40 min; 0.7 a_w) were used. From a technological standpoint, the preliminary bran treatment with cellulases could be a feasible solution to produce bread enriched in buckwheat bran. Besides this, no synergistic effect was observed between SS treatment and enzymatic treatment.

Future studies will include the assessment of the effects of bran-treatments on the macromolecular structure of fiber, to define not only its rheological performance but also its nutritional functionality.

Riassunto

La produzione di alimenti arricchiti in fibra è attualmente oggetto di grande attenzione sia per il mondo accademico che per l'industria alimentare, in quanto in molti paesi europei l'assunzione giornaliera di questo nutriente non sempre raggiunge la quantità raccomandata (25 g; EFSA, 2010).

I prodotti derivati dai cereali, essendo alimenti di base, rappresentano una valida risorsa per soddisfare i livelli ottimali di fibra solubile e insolubile, nonché di altri componenti bioattivi. Malgrado i benefici nutrizionali, l'integrazione della farina raffinata con materiale ricco in fibra influenza negativamente le proprietà strutturali e sensoriali dei semilavorati (impasti) e dei prodotti finiti.

Questo progetto di dottorato ha riguardato il trattamento di materiale cruscale con approcci fisici e/o biotecnologici, allo scopo di modificare le proprietà funzionali di particolari macromolecole (polisaccaridi non amido o proteine) e, conseguentemente, migliorare la qualità tecnologica degli alimenti arricchiti in fibra. Le frazioni derivate dai tessuti più esterni di cereali e di pseudocereali particolarmente ricchi in composti bioattivi (frumento pigmentato e grano saraceno) sono state perciò incorporate, dopo trattamento, in prodotti come pasta e pane.

La prima parte della tesi ha previsto l'utilizzo di un processo di decorticazione in due step per il recupero selettivo di componenti bioattivi quali fibra e composti fenolici. Le frazioni di decorticazione ottenute dal primo e dal secondo passaggio sono state utilizzate separatamente per produrre una pasta arricchita in fibra. La crusca ottenuta dalla macinazione convenzionale è stata invece utilizzata come controllo. Entrambe le frazioni di decorticazione hanno presentato una quantità maggiore o equivalente di fibra totale e di fibra solubile rispetto alla crusca. Inoltre, entrambi i campioni hanno mostrato valori superiori di attività antiossidante, valutata tramite saggio FRAP ("Ferric reducing-antioxidant power"), mentre il contenuto maggiore di antocianine è stato ritrovato nella prima frazione di decorticazione. L'arricchimento della pasta con le frazioni di decorticazione ha permesso di ottenere un prodotto con valori simili di FRAP e superiori di antocianine rispetto al controllo, pur mantenendo una buona qualità in cottura.

Nella seconda parte della tesi è stata utilizzato un altro sottoprodotto di macinazione ricco in composti bioattivi, la crusca (o tritello) di grano saraceno. Dopo trattamento di micronizzazione, il tritello è stato incorporato in impasti di frumento in percentuali crescenti (5, 10 e 20%) per la valutazione della qualità reologica e panificatoria. Le proprietà dell'impasto e del prodotto finito sono risultate essere influenzate negativamente dall'interazione tra il livello di arricchimento e la dimensione della crusca. Il comportamento termomeccanico dell'impasto è risultato essere invece strettamente legato alle transizioni di fase dell'amido durante il riscaldamento, indipendentemente dalla dimensione delle particelle cruscale. Le misurazioni di fluorescenza superficiale delle proteine hanno rilevato una differente organizzazione strutturale del glutine e della sua capacità di solvatazione negli impasti arricchiti in tritello, con un effetto distintivo della granulometria. Nonostante il pane arricchito in crusca micronizzata abbia mostrato proprietà reologiche (elasticità dell'impasto, resistenza all'estensione ed estensibilità) migliori o equivalenti rispetto alla crusca tal quale, il

comportamento in panificazione è risultato peggiore (in termini di volume specifico e di sofficità della mollica). Considerando in particolare la texture, le variazioni di consistenza, sono state imputabili principalmente alle differenze nella densità della mollica. L'adattamento di un modello della teoria di Ashby-Gibson per la correzione dei valori di consistenza per la densità ha mostrato come le proprietà meccaniche della matrice solida sono controllate principalmente dalle differenze di umidità e dalla frazione di volume della crusca.

Il tritello di grano saraceno è stato utilizzato anche per valutare gli effetti del trattamento con vapore surriscaldato ("superheated steam" – SS) sulle proprietà della crusca. Il vapore surriscaldato è una tecnologia di disidratazione innovativa che limita gli effetti collaterali sul prodotto finito. Dopo uno studio preliminare delle condizioni operative, i campioni trattati alle condizioni selezionate (120 °C, 140 °C o 140 °C; 40 min; 0.7 a_w) sono stati incorporati in impasti al 20%, ed è stata valutata la loro influenza sulle proprietà reologiche e sulla qualità panificatoria. Il trattamento SS ha modificato profondamente le proprietà chimico-fisiche del tritello di grano saraceno. In particolare, è stato osservato, un cambiamento progressivo nell'affinità con l'acqua con l'incremento della temperatura di trattamento, dovuto anche alla formazione di aggregati proteici. Queste modificazioni hanno influenzato le proprietà termomeccaniche degli impasti arricchiti e dei prodotti finiti, migliorandone le performance in particolari condizioni di trattamento (temperatura: 160 °C).

L'ultima parte della tesi ha riguardato l'uso di specifici trattamenti enzimatici (pectinasi e cellulasi) allo scopo di diminuire la dimensione delle catene di polisaccaridi non amido, per mitigarne l'effetto negativo nei prodotti finiti. Il trattamento enzimatico è stato condotto sia sul tritello di grano saraceno tal quale, sia sul tritello trattato con SS (140 °C, 40 min; 0.7 a_w). Il trattamento preliminare del tritello con cellulasi rappresenta dal punto di vista tecnologico una soluzione interessante per la produzione di pane arricchito in crusca. Non è stata invece osservata alcuna sinergia tra il trattamento SS e quello enzimatico.

Studi futuri prevedono la valutazione degli effetti dei trattamenti del tritello sulla struttura macromolecolare della fibra, in modo da definirne non solo le proprietà reologiche, ma anche la funzionalità nutrizionale.

I. Preface

In the last decades, the demand for healthy foods has grown thanks to a more awareness of the role of nutrition in preventing or lowering the risk of developing chronic diseases as cardiovascular disease, cancer, or type 2 diabetes (Who and Consultation, 2003).

Cereal and pseudo-cereal products, being a staple food category, may represent a valid resource to provide the adequate amount of nutrients such as non-digestible cell wall polymers (dietary fiber, DF) and related compounds with relevant bio-activity (Vitaglione et al., 2008). DF is concentrated in bran and represents between 18.1% and 86.7% of the outermost fractions in kernel. Its absolute amount depends on species and variety, seed morphology and on the different bran layers considered (Vitaglione et al., 2008).

Despite the positive result of fiber on the nutritional quality of enriched products, it should be noted that cereal fiber incorporation generally leads to negative effect on sensorial and technological properties of final products.

Several studies report on the quality of pasta enriched in DF derived by cereal, indicating these products of poorer textural quality and darker color in respect to pasta made from refined semolina (Manthey and Schorno, 2002). Main problems associated with the fiber presence regard higher cooking losses, generally related to the dilution and weakening of the gluten network (Kunerth and Youngs, 1984). Increased water absorption may be promoted by the disruption of gluten matrix and facilitating of starch granule swelling and rupture (Manthey et al., 2004). Enriched pasta is reported to have lower cooked firmness (Edwards et al., 1995) and lower elasticity (Tudorica et al., 2002) and higher surface stickiness (Sozer et al., 2007) than semolina pasta. Fiber-rich pasta has more bitter and branny flavor than pasta from refined wheat flour (West et al., 2013); these traits can be attributed especially to the presence of phenolic compounds in the outer layers of grain (Heiniö et al., 2008). Bran inclusion is also responsible for decreasing the brightness and yellowness and increasing the redness of uncooked pasta (Mercier et al., 2016). The consumers generally perceive these attributes as negative (Debbouz et al., 1995).

The use of bran has also deleterious effects on bread making, starting from an “altered” rheology in respect to the same products obtained by refined products. Fiber incorporation into dough increases the flour water absorption, decreases the stability and results in a moist and shorter dough (Poutanen et al., 2014). Dough extensibility, development and gas retention are decreased, whereas dough stiffness and stickiness are increased. Bread from high-fiber dough has consequently reduced loaf volume, denser and less aerated structure and harder crumb (Ktenioudaki and Gallagher, 2012), resulting in a product not fully accepted by consumers due to unattractive appearance, altered texture, and unpleasant flavor and taste (Foschia et al., 2013). The detrimental effects of fiber on dough/bread properties are related to dilution of gluten forming proteins (Pomeranz et al., 1977), restriction of the available water for gluten development (Autio, 2006), physical disruption of the matrix and piercing of the gas cells (Gan et al., 1992).

In the last years, many studies have addressed and attempted to minimize the detrimental effects of fiber in enriched products. As regard cereal bran modification, these approaches are mainly based on the use of dry fractionation technologies to collect fractions that are highly “concentrated” in bioactive compounds. Alternately, the application of different strategies to modify the native bran structure have been proposed.

The most common processes used for wheat bran treatment are summarized in Fig. 1.1 and briefly described here below.

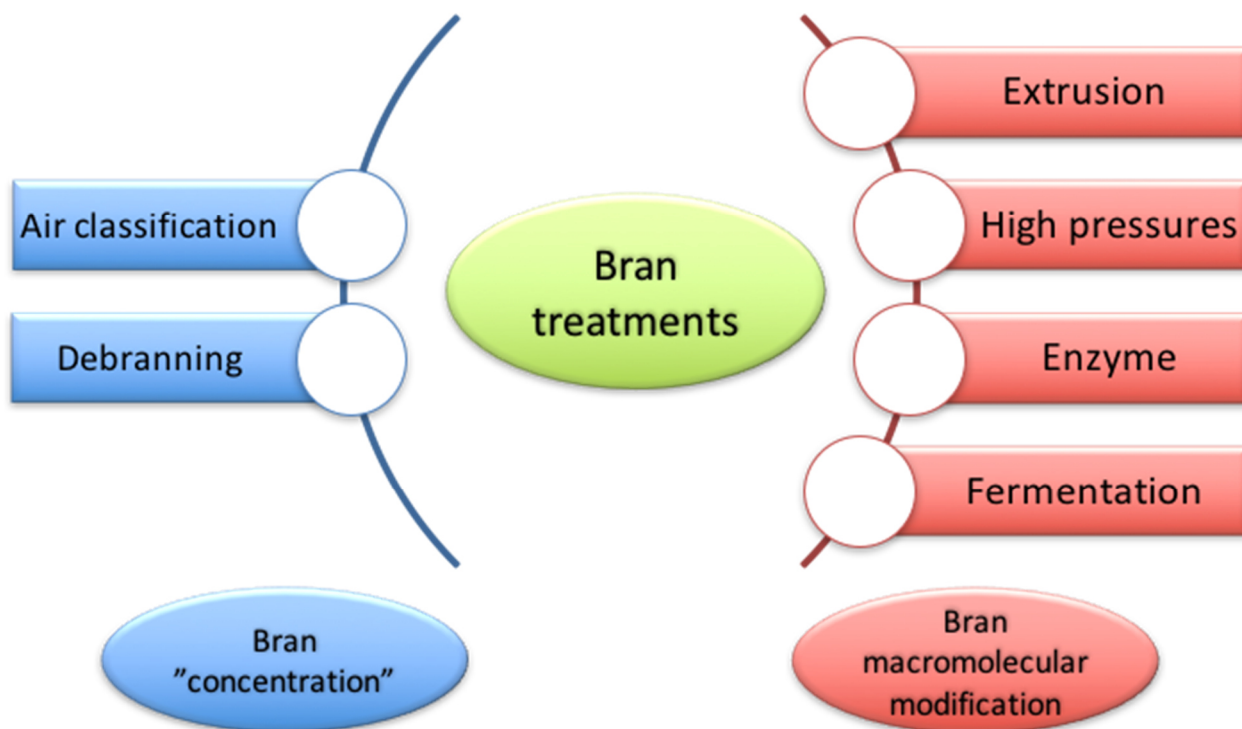


Fig. 1.1. Most common technologies used to modify cereal bran for enhancing the technological quality of enriched products.

Air classification permits the separation of bran into fractions with different sizes, properties and compositional traits (Andersson et al., 2000). The process often involves a preliminary severe grinding of incoming material (Ferrari et al., 2009; Ranhotra et al., 1994; Wu and Doehlert, 2002) aimed at fractionating tissues and at isolating their sub-cellular constituents (Hemery et al., 2007). Air classification has been applied to barley (Ferrari et al., 2009; Knuckles and Chiu, 1995; Verardo et al., 2011) and oat (Wu and Doehlert, 2002) to obtain fractions enriched in β -glucans. Only few studies regarded the application to wheat bran (Antoine et al., 2004; Ranhotra et al., 1994). Fractions separated by air-classification were successful incorporated into pasta (Verardo et al., 2011).

Debranning is based on sequential and controlled removal of grain layers by abrasion, pearling and rubbing the kernels against an abrasive stone (Hemery et al., 2007). It has demonstrated to be an effective strategy to recover the bioactive compounds in the external layers of barley or other grain kernels (Beta et al., 2005). This pre-milling process applies abrading forces to separate the outer region from the inner part of the kernel, and

results in a gentler and effective fractionation of the wheat kernel layers with respect to the conventional roller milling (Bottega et al., 2009; Delcour et al., 2012).

Bran treatment by extrusion-cooking has been observed to increase the solubilization of chemical compounds, especially dietary fiber (Ralet et al., 1990; Rashid et al., 2015). During extrusion, the material is subjected to a combination of moisture, pressure, temperature, and mechanical shear (Gómez et al., 2011) but the main resulting effects are likely related to the mechanical transformations rather than the thermal effect (Ralet et al., 1990). Extrusion has been observed to improve the functional properties (in terms of gelatinization temperature, solubility, apparent viscosity and consistency coefficient) of Soluble Dietary Fiber (SDF) from oat bran (Zhang et al., 2011) and the baking quality (in terms of volume and crumb softness) of enriched bread (Gómez et al., 2011).

Differently from what happens in extrusion treatment, during High Hydrostatic Pressure (HHP) process, fluid or properly packaged product is only subjected to compression and decompression cycles without any change in system temperature. Pressures ranges between 150 and 900 MPa (Betoret et al., 2015). HPP treatment of wheat bran produced a modification of its physical/structural properties in relation to the ability in interacting with water, suggesting a positive effect on the handling of mixtures and on the physical properties of the final product (Marti et al., 2014).

The use of bioprocess to pre-treat bran by partial hydrolysis of cell walls is a suitable strategy to improve its technological behavior. These approaches are mainly based on the action of specific microorganisms, such as lactic acid bacteria or yeast or on the use of enzymes isolated from different sources (Delcour et al., 2012).

Sourdough fermentation is one of the oldest food biotechnologies used to produce leavened products with high sensory, nutritional, structural and storage qualities (Gobbetti et al., 2014). It has been traditionally used for rye bread production and it may be used as pre-treatment to modify ingredients rich in fiber, as bran and germ (Katina and Poutanen, 2013). Main positive effects on dough and bread quality are linked to the acidification, the gluten proteolysis and the starch hydrolyses by specific yeast and lactic acid employed as starters (Rizzello et al., 2010). The use of tailored enzymes to pre-treat bran before dough production are more and more used to induce remarkable modification in the structure and functionality of fiber-rich material (Poutanen, 1997).

However, when selecting enzymatic mixtures, it is important to consider the physio-chemical characteristics of the bran and to note that added enzymes can keep their activity during the bread making. Finally, sourdough in combination with enzyme mixture was observed to produce better effects on bread quality in respect to separate treatment (Katina et al., 2007, 2006).

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II Rationale and aims

This PhD project dealt with the application of several physical and/or biotechnological approaches to modify the structural properties of peculiar macromolecules (such as non-starch-polysaccharides or proteins) to enhance the quality of fiber-enriched foods.

These approaches were carried out on the outermost layers from: i) a pigmented (purple) variety of common wheat; and from ii) buckwheat. These materials were considered since they are among the cereals and pseudocereals richest in bioactive compounds. Additionally, the effects of similar physical and/or biotechnological processes on the overall structure of non-starch polysaccharides from purple wheat and from buckwheat have poorly been addressed so far.

Samples from the outermost layers of either purple wheat or buckwheat – treated by various physical and biotechnological approaches – were characterized by physico-chemical measurements and included into staple food as pasta or bread, followed by the evaluation of their effects on the technological performance of the final product.

In Chapter 1, a debranning process was applied on purple wheat, aimed at collecting the external coats and the aleurone layer of the kernel, particularly rich in bioactive compounds, i.e. fiber and phenolic acids. These activities were performed in collaboration with the CRA-QCE of Rome.

In Chapter 2, the effects of micronization on the physico-chemical properties of buckwheat bran were addressed to pave the way to the following activity. In this frame, although bran micronization is well known to increase the nutritional potential of food products, its effect on dough/bread quality are not univocally defined yet.

The third part of the project (Chapter 3) aimed at evaluating the physical mechanisms that describe the influence of buckwheat bran of different granulometry on the thermo-mechanical behavior and bread-making performance of wheat dough. These experimental activities were carried out in collaboration with the laboratories of TNO (Functional Ingredients Group, Zeist, The Netherlands) and with the Section of Chemical and Biomolecular Sciences of the Department of Food, Environmental and Nutritional Sciences (DeFENS).

In Chapter 4, we addressed the effects of superheated steam (SS) on buckwheat bran properties. SS is an innovative drying technology that has allowed drying of a number of food matrices while limiting collateral effect on the product. After screening the process conditions, the selected treated bran samples were incorporated into high-enriched dough and their influence on the rheological properties and on the baking quality was considered. These experimental activities were carried out in collaboration with the laboratories of TNO and with the Section of Chemical and Biomolecular Sciences of the DeFENS.

In the last part of this project (Chapter 5), specific enzymatic treatments were carried out at a low bran:water ratio, aiming at decreasing the size of non-starch-polysaccharide chains and, consequently, at mitigating the worsening effects of fiber on dough technological performance. Either coarse buckwheat bran or SS-treated

bran was used. The consequences on dough properties and on enriched-bread quality were evaluated. These research activities were performed in collaboration with TNO.

1. Debranning of purple wheat: recovery of anthocyanin-rich fractions and their use in pasta production

The results presented here below are published in Zanoletti et al., 2017.

1.1 Introduction

Whole wheat grain is a good source of dietary fiber and antioxidants which can promote health benefits towards several chronic diseases usually associated with oxidative stress (Yu, 2008). Although most of the cultivated cultivars are white- or red- grained, some varieties – such as purple and blue wheat grains - have drawn the attention of researchers and food industry due to their high content in anthocyanin pigments and to their antioxidant properties (Abdel-Aal et al., 2006; Escribano-Bailón et al., 2004; Zeven, 1991).

Anthocyanins are the largest group of water-soluble natural pigments that provide many fruits, vegetables, and cereal grains with red, violet, and blue color (Escribano-Bailón et al., 2004; Mazza and Miniati, 1993). These bioactive compounds not only scavenge free radicals, they also have a detoxifying effect towards heavy metals (Jan et al., 2015). Various fruits and vegetables are good sources of anthocyanins (Mazza and Miniati, 1993). However, all of these foods are less frequently consumed in comparison with cereal products. Consequently, blue and purple grains would be potential candidates for the development of bioactive food ingredients. At present, these grains are underutilized, and their contribution to the human diet is very little. For this reason, only limited data are available about their functional characteristics.

Currently, blue and/or purple corns are used for the production of naturally colored blue tortillas. As for wheat-based products, anthocyanins-rich biscuits (using whole purple wheat; Pasqualone et al., 2015), muffins (using bran from purple wheat; Li et al., 2007) and pasta (from purple durum wheat; Ficco et al., 2016) have been recently studied, focusing on the effects of processing conditions on the antioxidant properties.

In the case of pasta from durum purple wheat, the technological process led to a dramatic decrease in nutritionally-relevant antioxidant compounds (Ficco et al., 2016), suggesting that greater attention needs to be paid to optimize either the extrusion or drying conditions and to ensure their preservation. Furthermore, it can be considered the effect of the partial leaching of bioactive compounds into the cooking water.

Since the purple pigments are likely to be located in the outer layers of the pericarp (Zeven, 1991), most of the anthocyanins are removed along with fiber during milling in the case of refined semolina (Ficco et al., 2016). On the other hand, whole-grain products appear less palatable than those that are refined, due to the high bran content, which affects their technological and sensory properties (Heiniö et al., 2016). Pearling or debranning has demonstrated to be an effective strategy to recover the bioactive compounds in the external layers of barley or other grain kernels (Beta et al., 2005). This pre-milling process applies abrading forces to separate the outer region from the inner part of the kernel, and results in a gentler and effective fractionation of the wheat kernel layers with respect to the conventional roller milling (Bottega et al., 2009; Delcour et al., 2012).

In view of developing functional, grain-based ingredients, such as anthocyanin-rich flours, and foods (i.e. pasta), the present work aims at: (i) thoroughly evaluating the effect of debranning pre-treatment on dietary fiber and anthocyanins content, and on the antioxidant capacity of the various milling fractions of purple wheat in comparison with the conventional roll-milling process; (ii) producing pasta with high fiber and anthocyanins content along with retaining a good cooking quality.

1.2 Materials and methods

1.2.1 Materials

Commercial purple common wheat was provided by Molino Quaglia S.p.A. (Vighizzolo D'Este, Italy) and processed as shown in Fig. 1.1. A part of it was milled in a lab-scale mill (Labormill, BONA, Monza, Italy) to produce refined flour, middlings, and bran. Another part of purple wheat underwent two debranning steps prior to conventional milling. Kernels were first hydrated (adding 2 g of water to 100 g of wheat) to make the seed coats less brittle and prevent kernel breakage (Bottega et al., 2009). Debranning was carried out by using two sequential steps in a laboratory debranning machine equipped with an abrasive stone element (NAMAD Impianti, Roma, Italy). The first step allowed the collection of the fraction F1, corresponding to a debranning level of 3.7% of whole grain; the second passage removed the fraction F2, corresponding to a debranning level of 6%. Both fractions were stored at 4 °C until analysis. Debranned grains were then milled in a lab-scale mill (Labormill, BONA, Monza, Italy) to obtain refined flour, middlings, and bran.

Particle size distribution of bran and debranning fractions was determined by mechanical sieving 50 g of sample on Sieve Shaker (EFL 300, Endecotts Ltd, London, UK), equipped with six sieves with sieve aperture sizes of 2 mm, 1 mm, 500 µm, 250 µm, 125 µm, and 40 µm.

1.2.2 Methods

1.2.2.1 Microstructure approaches

Microscopy images of wheat kernels were obtained by using a stereo microscope Zeiss Axio Zoom V16 (Carl Zeiss AG; Oberkochen, Germany). Debranning fractions F1 and F2 and milling by-products CB and DB were observed by using a light Olympus BX50 microscope (Olympus, Tokyo, Japan) after staining with 1 g/L Toluidine blue in water, which is a generic dye for plant tissues. Samples were layered on the glass slide, covered with a coverslip and a small drop of staining was left to permeate in between. For each sample, at least ten observations (magnification: 4x) were made in order to obtain a semi-quantitative analysis of particle size.

1.2.2.2 Chemical analysis

Ash (AOAC 942.05), protein (AOAC 960.52), total starch (AOAC 996.11), and total (TDF), and insoluble (IDF) dietary fiber (AOAC 991.43) were determined according to official methods (AOAC, 2005). Soluble (SDF) dietary fiber was determined as the difference between TDF and IDF.

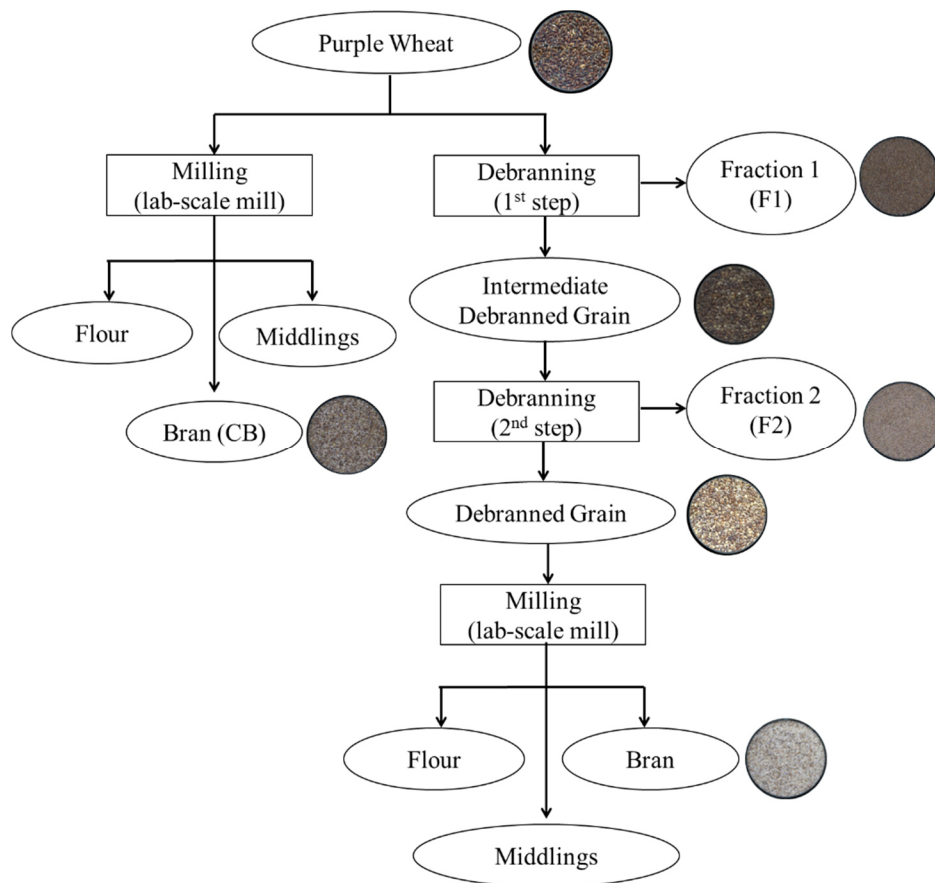


Fig. 1.1. Flow-chart of purple wheat milling and debranning process.

1.2.2.3 Anthocyanins Content

About 2 g of milling fractions, debranning fractions, or cooked pasta - after freeze drying - were defatted by overnight soaking in 30 mL petroleum ether, which contributed to improving the efficiency of anthocyanins extraction. Fractions were extracted with 15 mL of solvent solution for 16 h at room temperature with continuous stirring. The solvent solution was prepared with ethanol (65 mL), water (35 mL) and HCl (0.1 mL). The mixture was centrifuged at $10000 \times g$ for 10 min, the supernatant recovered and the solid residue was twice re-extracted using 15 mL of the same solvent. A further extraction step did not increase anthocyanin recovery. Hence, the three supernatants of the first, second and third extraction steps were eventually pooled together. All extractions were performed in duplicate.

Total monomeric anthocyanins were measured according to the pH differential method (Lee et al., 2005). Samples were diluted 10 times to a final volume of 2 mL, respectively with 0.03 mol/L potassium chloride buffer, pH 1.0; or with 0.4 mol/L sodium acetate buffer, pH 4.5, respectively. The absorbance of each sample was measured at 520 nm against distilled water as a blank. Correction at 700 nm was carried out to eventually correct haze. The concentration of each anthocyanin was calculated and expressed as micrograms of cyanidin 3-O-glucoside equivalents per gram of dry product.

1.2.2.4 Ferric reducing-antioxidant power (FRAP)

The FRAP assay was performed on milling and debranning fractions and on pasta extracts (see previous paragraph), according to a previously-described procedure (Benzie and Strain, 1996).

Briefly, FRAP reagent was prepared by adding: 25 mL of 0.3 mol/L acetate buffer, pH 3.6; 2.5 mL of 0.01 mol/L 2,4,6-tripyridyl-s-triazine in 0.04 mol/L HCl; and 2.5 mL of 0.02 mol/L FeCl₃. The reaction mixture contained 0.4 mL of each extract prepared as described above, diluted with the same solvent solution used for anthocyanins content determination, and 3 mL of FRAP reagent. The increase in absorbance at 593 nm was evaluated after 4 min of incubation at 37 °C against a blank, where extract was not added. For each extract, 2 to 4 different dilutions were assessed in duplicate. A solution of FeSO₄·7H₂O was used for calibration. Results were expressed as micromoles of Fe(II) sulfate equivalents per gram of dry product.

1.2.2.5 Dough rheology

Taking into account the antioxidant capacity and the anthocyanins content, the CB, F1, and F2 fractions were respectively used for producing wheat mixtures enriched in bioactive components. These fractions were added to commercial flour from common wheat (sample C; Molino Quaglia, Vighizzolo d'Este, Italy; protein: 13.5 g/100 g; alveographic W: 380 * 10⁻⁴ J; alveographic P/L: 0.65) in such amounts to reach the same total fiber content (8.5 g/100 g) in each mixture, a quantity generally higher than that found in commercial wholegrain pasta (6-6.5 g/100 g). Consequently, CB, F1 and F2 were added at 20.4, 14.2, and 21.1 g/100 g, respectively. The related mixtures were labeled as M-CB, M-F1, and M-F2.

Gluten aggregation properties of mixtures were measured by using the GlutoPeak (Brabender GmbH and Co KG, Duisburg, Germany), according to Marti et al. (2014). An aliquot of sample (9 g) was dispersed in 10 mL of distilled water, while keeping water temperature at 35 °C. The paddle was set to rotate at 3000 rpm and each test was run for 5 min. Maximum Torque (BE – Brabender Equivalent) and Peak Maximum Time (s) – which is the time required to achieve maximum torque development - were considered. Measurements were performed at least in triplicate.

1.2.2.6 Pasta-making

Distilled water was added to the flour mixtures containing 20.4 g/100 g CB, 14.2 g/100 g F1, and 21.1 g/100 g F2 in order to obtain dough with 32 g/100 g final moisture. Samples were hand-mixed at room temperature for 10 min. After mixing, the dough was covered with plastic stretch-film and kept at 4 °C for 30 min. A home dough-sheeter (SP50, Imperia 1932, Moncalieri, Italy) was used to prepare pasta. The thickness of the sheet was gradually reduced by five consecutive steps through decreasing roll gaps (respectively of 6 mm, 4 mm, 2 mm -twice, folding the sheet - and 1 mm). Pasta dough was shaped into “tagliatelle” (length: 300 mm; width: 13.5 mm; thickness: 1.0 mm) and dried in a cell (M710 Thermostatic oven, Fratelli Galli, Milano, Italy) at 40 °C for 18 hours and stored at 4 °C until analysis.

Pasta containing CB, F1, and F2 fractions was labeled as P-CB, P-F1, and P-F2, respectively. Pasta prepared from refined wheat flour (P-C) was produced in the same conditions described above and used as a control.

1.2.2.7 Pasta Quality

Pasta was cooked in distilled water (pasta:water ratio = 1:25) at the Optimum Cooking Time, according to the AACC method 16-50 (1995). Cooking losses (g of solid loss/100 g of dry pasta) were evaluated according to the AACC standard method (16-50, 1995). After cooking, pasta was drained for 1 min, and the weight increase was evaluated gravimetrically. Then, the cooking water was collected, brought back to the initial volume, and an aliquot (50 mL) was dried to constant weight at 105 °C. Cooking loss and water absorption values were replicated five times and the average values were used. Cooked pasta was freeze-dried, ground to a particle size lower than 500 µm with a lab-scale mill (IKA Universalmuhle M20, Janke and Kunkel GmbH & Co KG, IKA Labortechnik, Staufen, Germany) and stored at 4 °C until analysis.

1.2.2.8 Statistical Analysis

Analysis of variance (one-way ANOVA) was performed by using Statgraphic Plus v. 5.1 (StatPoint Inc., Warrenton, VA, USA). Different milling fraction or pasta samples were considered as factors for ANOVA. When a factor effect was found to be significant ($p \leq 0.05$) significant differences among the respective means were determined using Fischer's Least Significant Difference (LSD) test.

1.3 Results and discussion

1.3.1 Milling and debranning material

The debranning effects on purple wheat kernels are shown in Fig. 1.2. Both the first and second abrasion steps promoted a heterogeneous removal of the external layers. Indeed, after the first debranning, few grains appeared intact (Fig. 1.2B), as in the case of wheat before debranning (Fig. 1.2A), whereas the purple bran layers were mostly removed from some grains, thus leaving exposed the tissues below the bran layers exposed. Even after two debranning steps (Fig. 1.2C), which removed about 10% of material, the mechanical abrasion of the kernel surface was non-homogeneous. Nevertheless, the ventral-side of caryopsis seems to be more prone to abrasion, probably due to its flat surface.

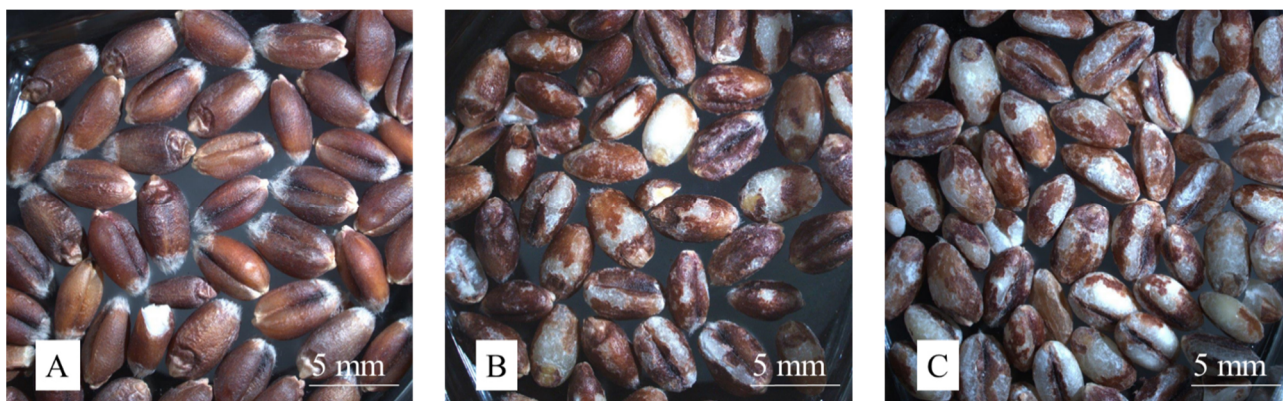


Fig. 1.2. Stereo-microscope images of purple wheat kernel before A) and after the first B) or second C) debranning step.

Microscopic features of bran from intact grain milling (CB), bran from debranned grain milling (DB), F1 and F2 fractions are shown in Fig. 1.3. In CB, many particles were larger than 1000 µm (Fig. 1.3A). Milling of

debranned grains resulted in a dramatic decrease in bran particle size, down to about 600 μm in average (Fig. 1.3B).

Debranning operations led to the recovery of fractions with particles smaller than those for CB (Fig. 1.3C, 1.3D). The amount of debranning fractions is usually expressed as debranning level and considered as a marker of the debranning intensity. The higher the debranning level, the lower the particle size of the removed material and the higher the starch amount, as shown in Table 1.1. In particular, the average size of F1 was about 500-700 μm , while F2 particle size was in the 300-400 μm range. Furthermore, in F2 sample, fragments of aleurone layer and many starch granules were recognizable (Fig. 1.3D).

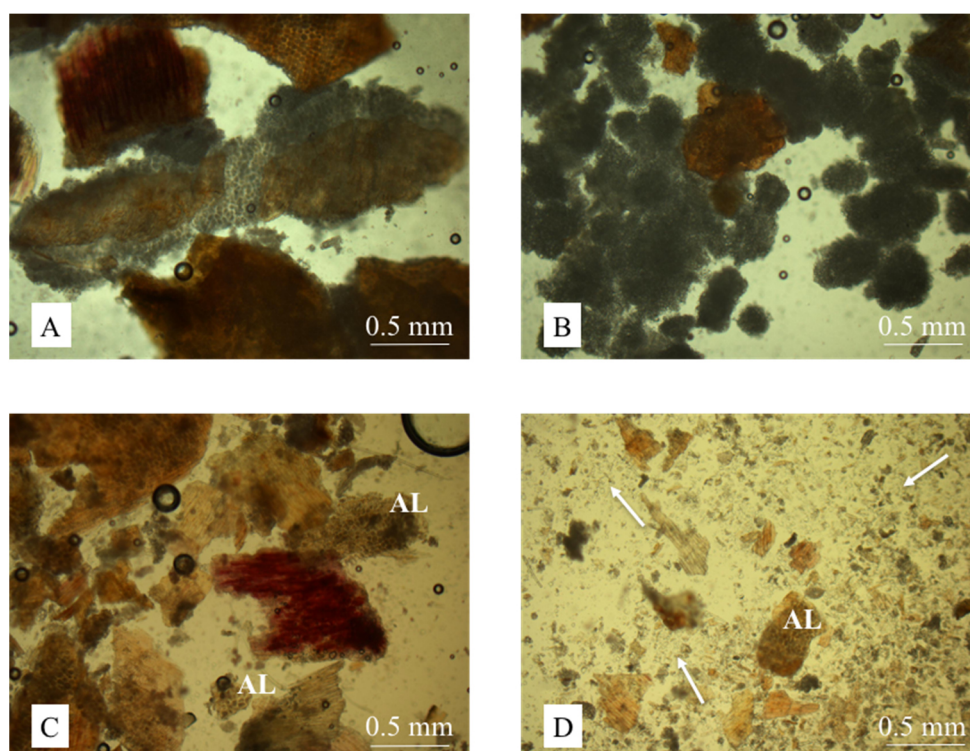


Fig. 1.3. Light-microscope images of milling and debranning fractions. A) bran from conventional milling; B) bran from debranned grain; C) fraction from the first debranning step; D) fraction from the second debranning step. AL: aleurone layer; arrows: starch granules.

The compositional features of purple wheat, debranning fractions and milling products are shown in Table 1.1. Lab-scale milling provided three fractions (flour, bran and middlings, see Fig.1.1) characterized by a recovery yield, and by contents in starch, protein, ash, and fiber comparable to those reported for common wheat (Lai and Lin, 2006). As expected, F1 and F2 showed different compositions: F1 (debranning level = 3.7%) contained a lower amount of starch and protein than F2 (debranning level = 6.0%). On the other hand, the ash and fiber content progressively decreased as debranning level increased from the first to the second debranning step, as evident in previous findings (Bottega et al., 2009). In particular, the total starch content relates to the progressive removal of the kernel layers that included the starchy endosperm (Bottega et al., 2009).

Table 1.1 Yield and chemical composition of milling and debranning fractions of purple wheat.

		Yield	Starch	Protein	Ash	Dietary Fiber		
						Total (TDF)	Insoluble (IDF)	Soluble (SDF)
Whole Purple Kernel		100	59.9 ± 0.8	14.38 ± 0.09	1.86 ± 0.02	14.51 ± 0.09	12.5 ± 0.4 (86)	2.0 (14)
Conventional milling	Flour	50.0	76.17 ± 0.07 ^a	12.92 ± 0.02 ^c	0.50 ± 0.02 ^g	3.69 ± 0.08 ^g	1.05 ± 0.04 ^f (29)	2.6 (71)
	Bran	22.2	23.7 ± 0.2 ^c	19.18 ± 0.03 ^a	5.59 ± 0.01 ^b	43.5 ± 0.4 ^b	41.86 ± 0.02 ^b (96)	1.7 (4)
	Middlings	27.7	69 ± 1 ^b	13.8 ± 0.2 ^d	1.02 ± 0.03 ^f	7.58 ± 0.03 ^f	5.48 ± 0.03 ^e (72)	2.1 (28)
Debranning	Fraction 1	3.7	12 ± 2 ^f	12.60 ± 0.04 ^f	5.80 ± 0.02 ^a	62.6 ± 0.3 ^a	60.1 ± 0.4 ^a (96)	2.5 (4)
	Fraction 2	6.0	25.9 ± 0.3 ^c	16.06 ± 0.06 ^b	4.67 ± 0.01 ^c	42.1 ± 0.8 ^c	39.6 ± 0.8 ^c (94)	2.5 (6)
Milling after debranning	Flour	49.2	77 ± 2 ^a	11.91 ± 0.04 ^g	0.51 ± 0.01 ^g	2.70 ± 0.06 ^b	0.89 ± 0.06 ^f (33)	1.8 (67)
	Bran	23.3	45 ± 2 ^d	19.4 ± 0.2 ^a	3.99 ± 0.01 ^d	33.9 ± 0.2 ^d	27.4 ± 0.2 ^d (81)	6.5 (19)
	Middlings	27.5	66 ± 2 ^c	14.8 ± 0.1 ^c	1.38 ± 0.01 ^e	9.51 ± 0.07 ^e	4.93 ± 0.02 ^e (52)	4.6 (48)

Yield is expressed as g/100 g kernel, whereas compositional data are expressed as g/100 g sample (d.b.). IDF (or SDF) percentage on TDF are reported in bracket. All data are expressed as the mean ± standard deviation (n = 2). Different letters in the same column indicate statistically significant differences (LSD; p ≤ 0.05).

Interestingly, F1 contained only 12 ± 2 g/100 g of starch; the amount of this component doubled in F2, up to the level measured in the bran produced in the conventional milling process. The higher protein amount in F2 compared to F1 (16.06 ± 0.06 vs 12.60 ± 0.04 g/100 g) confirmed the presence of some aleuronic cells, as highlighted by microscopic observations (Fig. 1.3D). However, some aleurone fragments were still present in F1 (Fig. 1.3C), although F1 (debranning level = 3.6%) is most likely formed by the outer pericarp. Indeed, Shetlar et al. (1947) stated that the outer pericarp and the aleurone layer represented 3.9 and 9.0% of the kernel weight, respectively. However, a certain variability in wheat grain structure should be considered (Pomeranz, 1988; Kent, 1983).

As for the total dietary fiber content, whole purple wheat exhibited comparable values to those reported for other varieties (TDF: 11.6-17.0 g/100 g; Vitaglione et al. (2008)). As expected, bran showed a high TDF content (43.5 ± 0.4 g/100 g), composed of almost insoluble macromolecules (96 g/100 g of TDF). Both F1 and F2 showed a remarkable amount of TDF, confirming the data reported by Blandino et al. (2013) and Sovrani et al. (2012). The latter found a TDF content of 58.0-61.5 g/100 g in the outermost layer (corresponding to 5% of debranning level) of various common wheat varieties, whereas the amount of TDF in the second pearling fraction (up to 10% of debranning level) ranged from 36.4 to 40.9 g/100 g. In our study, F1 exhibited a TDF content more than 40% higher than bran, but a similar IDF/SDF ratio. On the contrary, sample F2 showed a comparable TDF content, but a slight higher SDF as compared with bran, likely related to the high SDF content in the aleurone layer (Hemery et al., 2007). Differences in both the composition and the particle size (Table S1, Fig. 1.3) of the various bran fractions may strongly affect their hydration properties and technological behavior (Marti et al., 2014).

As regards the composition of flour and middling before and after kernel debranning, the former did not exhibit any relevant modification, whereas the middle fraction obtained from debranned grain showed an unexpected enrichment in the soluble fiber, likely due to part of the aleurone cells that could have flown into this milling fraction, contributing to a higher amount of protein and total fiber amount.

The anthocyanins content and FRAP of purple wheat and its milling and debranning fractions are reported in Table 1.2. In the whole purple kernel the anthocyanins content was 52.2 ± 0.4 $\mu\text{g/g}$, values in accordance with the data reported by Abdel-Aal et al. (2006) for purple wheat (38-96 $\mu\text{g/g}$). Conventional milling promoted the recovery of anthocyanins pigments in the bran fraction (Table 1.2), allowing to obtain 3-8 times more pigment than that collected in other pigmented cereals (e.g. black rice, red rice, blue wheat, black, brown, and red sorghum bran fractions) (Abdel-Aal et al., 2006). A single step of debranning, associated with the removal of 3.7% of the grains, was a useful strategy to concentrate anthocyanins, as in F1 sample the anthocyanin content increased significantly ($p \leq 0.05$) to 695 ± 64 $\mu\text{g/g}$. The anthocyanins collected in the F2 were 295 ± 7 $\mu\text{g/g}$, the same amount determined in bran. Consequently, F1 contained more than 50% of the total anthocyanins content of whole wheat grains. Studies on purple barley showed that the bran-rich fraction, corresponding to a pearling level of 10%, contained up to 75% of the anthocyanins in the kernel (Bellido and Beta, 2009).

Table 1.2 Anthocyanins content and antioxidant capacity (expressed as FRAP) of milling and debranning fractions from purple wheat.

		Anthocyanins ($\mu\text{g/g}$)	FRAP ($\mu\text{mol Fe(II)/g}$)
Whole Purple Kernel		$52.2 \pm 0.4^{\text{d}}$	$4.2 \pm 0.1^{\text{c}}$
Conventional milling	Flour	n.d.	$0.45 \pm 0.05^{\text{f}}$
	Bran	$295 \pm 7^{\text{b}}$	$12.7 \pm 0.2^{\text{b}}$
	Middlings	$9.1 \pm 0.3^{\text{c}}$	$2.09 \pm 0.02^{\text{cd}}$
Debranning	Fraction 1	$695 \pm 64^{\text{a}}$	$25 \pm 1^{\text{a}}$
	Fraction 2	$295 \pm 21^{\text{b}}$	$26 \pm 3^{\text{a}}$
Milling after debranning	Flour	n.d.	$0.25 \pm 0.01^{\text{f}}$
	Bran	$93 \pm 3^{\text{c}}$	$13 \pm 3^{\text{b}}$
	Middlings	$11.9 \pm 0.4^{\text{c}}$	$2.5 \pm 0.2^{\text{cd}}$

All data are expressed as the mean \pm standard deviation ($n = 4$ from two independent extractions). Different letters in the same column indicate statistically significant differences (LSD; $p \leq 0.05$). n.d. = not determined.

F1 and F2 samples also exhibited the highest FRAP values, about $25 \mu\text{mol Fe(II) eq/g}$ (Table 1.2). In CB and DB samples, the antioxidant capacity was halved. These results suggest that the debranning of purple wheat is a more efficacious approach than conventional milling to separate and collect anthocyanin-rich fractions with higher antioxidant activity, thus maximizing the potential health benefits of wheat-based products. The lack of differences in FRAP values between F1 and F2 (Table 1.2) might be due to compensation between the removal of the outer layers - where anthocyanins are accumulated - and a simultaneous passage of part of aleurone and endosperm, regions richer than the pericarp in phenolic acids (Martini et al., 2015) and other compounds with antioxidant activity.

Besides antioxidant content, physical parameters of the wheat bran, such as the particle sizes affecting the total surface area, could influence antioxidant solubility and, in turn, antioxidant capacity in solid-liquid system. However, no remarkable effect of particle sizes on antioxidant capacity is observed, when the measuring conditions resulted in plateau values to be reached (Serpen et al., 2008). For this purpose, in the present study, a long 3-step antioxidant capacity was performed.

Interestingly, the antioxidant capacity of wheat bran increased when the medium particle size decreased, due to the breakdown of the aleurone cell-wall (Rosa et al., 2013; Zhou et al., 2004). As shown in Fig. 1.3D, F2 contained disrupted aleurone cells, which can account for its high antioxidant activity.

1.3.2 Dough Rheology

Based on the anthocyanins content in bran fractions of purple wheat, we focused on bran, F1 and F2 fractions for preparing bioactive compounds-enriched products. Each fraction was added to commercial flour at different levels (20.4, 14.2, and 21.2 g/100 g, respectively) in order to have a fiber content of 8.5 g/100 g in

the final product. This percentage is higher than that one usually present in commercial whole pasta from durum wheat semolina (Casiraghi et al., 2013), thus allowing us to obtain a pasta with greater nutritional value.

The GlutoPeak Test is a new approach for testing gluten quality in common (Marti et al., 2015) and durum (Marti et al., 2013) wheat. The GlutoPeak indices of bran-enriched flours are shown in Table 1.3. Dough enrichment in fiber generally increased the maximum torque, likely due to the high fiber content and its water absorption capacity.

Peak maximum time relates to the time required for gluten to aggregate and to exhibit the maximum spindle torque. The addition of any type of bran significantly ($p \leq 0.05$) decreased peak maximum time, thus weakening the gluten network, as shown in previous studies (Marti et al., 2014). However, M-F1 exhibited a significantly longer aggregation time, most likely due to the lower replacement level, than either M-CB or M-F2. Indeed, no differences in the gluten aggregation profile were found in flour enriched with either bran or aleurone fraction (Adams, 2015).

Table 1.3 Gluten aggregation properties of bran-enriched mixtures. CF; control flour; M-CB, CB-enriched wheat mixture; M-F1, F1-enriched wheat mixture; M-F2, F2-enriched wheat mixture.

	CF	M-CB	M-F1	M-F2
Maximum torque (BE)	63 ± 2 ^b	67.7 ± 0.6 ^a	69 ± 3 ^a	70 ± 2 ^a
Peak maximum time	105 ± 4 ^a	52 ± 3 ^c	58 ± 2 ^b	50 ± 3 ^c

All data are expressed as the mean ± standard deviation (n = 3). Different letters in the same row indicate statistically significant differences (LSD; $p \leq 0.05$).

1.3.3 Enriched pasta

The quality characteristics of pasta are summarized in Table 1.4. Despite the addition of fiber, which has been demonstrated to decrease the cooking time (Aravind et al., 2012), no differences in optimal cooking time were observed between the samples (4 min), probably because of the low thickness (1 mm) and the high surface of the tagliatella-shape which ensured a fast water diffusion and absorption.

During cooking, P-CB and P-F1 absorbed a similar amount of water which was significant lower than that for P-CF and P-F2 (Table 1.4). The enrichment in IDF generally decreased the water absorption because of the competition for water between bran and starch, resulting in a low starch swelling capacity (Aravind et al., 2012).

Measurement of cooking loss is an important parameter in assessing overall pasta quality. During pasta cooking soluble components, including starch fractions, proteins and non-starch polysaccharides, leached into the cooking water, which became cloudy and thick. For good-quality pasta, the cooking loss should be lower than 4-5 g/100 g (Marti et al., 2013).

Table 1.4 Pasta cooking properties: optimal cooking time, water absorption, cooking loss, antioxidant capacity (expressed as FRAP) and anthocyanins content of cooked pasta. P-C, control pasta from refined wheat flour; P-CB, CB-enriched wheat pasta; P-F1, F1-enriched wheat pasta; P-F2, F2-enriched wheat pasta.

	P-C	P-CB	P-F1	P-F2
Optimal cooking time (min)	4	4	4	4
Water absorption (g/100 g)	128.6 ± 0.8 ^a	105 ± 11 ^b	110 ± 2 ^b	127 ± 1 ^a
Cooking loss (g/100 g)	2.5 ± 0.4 ^b	2.8 ± 0.1 ^{ab}	3.2 ± 0.3 ^a	3.18 ± 0.03 ^a
FRAP (μmol Fe(II)/g)	n.d.	2.41 ± 0.06 ^{ab}	2.6 ± 0.1 ^a	2.3 ± 0.2 ^b
Anthocyanins (μg/g)	n.d.	28 ± 2 ^b	67.9 ± 0.9 ^a	60 ± 1 ^a

Mean ± standard deviation (Water absorption and cooking loss: n = 5; FRAP and Anthocyanins: n = 4 from two independent extractions). Different letters in the same row indicate statistically significant differences (LSD; p ≤ 0.05). n.d. = not determined.

Regardless of the number of debranning steps, both debranned fraction-enriched samples P-F1 and P-F2 showed higher cooking losses than the control pasta (Table 1.4). This may be due to changes in the elasticity of the gluten network because of the interference of the dietary fiber content (Table 1.1). Indeed, several studies have shown that the addition of non-gluten flours in the production of pasta decreases the gluten strength, and weakens the overall structure of pasta (Tudorica et al., 2002). Our results agree with those reported in literature regarding fiber-enriched pasta (Marti et al., 2011) and might be explained by the use of a mild forming process (i.e. roll-sheeting) which does not impart extreme pressure and stress to dough. On the contrary, conventional extrusion usually leads to damage or breakage of the gluten structure in products characterized by a less tenacious protein network (i.e. common wheat and wholegrain flours) (Marti et al., 2011; Pagani et al., 1989).

In the case of pasta samples, anthocyanins content and FRAP values were considered only for the cooked products, which are more important for the consumers' standpoint. Enriched wheat pasta showed a relatively high antioxidant capacity, with P-F1 exhibiting no significant differences in FRAP values from P-CB, despite the amount added was lower for F1 than for CB (Table 1.4). Comparison between the antioxidant activity values of the samples and those referred to in the literature is difficult, due to the various approaches used both for product preparation (sheeting vs extrusion) and for the evaluation of the antioxidant capacity. The FRAP assay was chosen among the electron-transfer based reactions (e.g. Folin-Ciocalteu assay, Trolox equivalence antioxidant capacity, TEAC) because it is carried out at low pH, that allows the highest anthocyanin stability (Mazza and Miniati, 1993).

The anthocyanin content values ranged from 28 ± 2 to 67.9 ± 0.9 μg/g for P-CB and P-F1, with no significant differences between P-F1 and P-F2. The highest anthocyanins found in cooked P-F1 is in accordance with the FRAP values (Table 1.4). In addition, P-F1 and P-F2 enriched samples showed a higher anthocyanins content (67.9 ± 0.9 and 60 ± 1 μg/g, respectively) than values found by Ficco et al. (2016) in pasta prepared from purple wholemeal semolina and ranging from 16.89 and 37.27 μg/g for cooked dried pasta and fresh pasta, respectively. During cooking, only P-CB and P-F1 showed a loss of anthocyanin content, whereas P-F2 was

able to retain the anthocyanins during cooking. The effect of particle size of bran and debranning fractions (Table 1.5) on cooking behavior can not be neglected. Indeed, superior cooking quality has been observed in pasta from wheat with finer particle size (Hatcher et al., 2002).

Table 1.5 Particle size distribution of bran from conventional milling (CB), fraction from the first debranning step (F1), fraction from the second debranning step (F2).

Particle size interval	CB	F1	F2
<40 μm	0	0	0
40-125 μm	0.6	15.4	22.6
125-250 μm	2.2	26.3	31.6
250-500 μm	3.6	37.0	24.9
500-1000 μm	22.7	17.4	15.3
1000-2000 μm	66.8	3.7	5.4
>2000 μm	4.0	0.1	0.1

1.4. Conclusions

Debranning can be considered a more efficacious processing than conventional milling to mechanically concentrate the bioactive compounds – mainly fiber and anthocyanins- present in the pericarp layers of purple wheat. The selective recovery of these fractions and their use in pasta could represent an interesting approach to encourage the diffusion and the use of wheat pericarp layers in making foods with functional properties. However, the presence of natural and synthetic contaminants in the most external layers pose a risk for consumer safety and need to be taken into serious consideration.

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2. Effect of micronization on the physical and chemical properties of buckwheat bran

2.1 Introduction

The decrease in bran particle size, associated with severe grinding as micronization, has been observed in wheat samples to improve, in only few cases (Lai et al., 1989; Moder et al., 1984; Pomeranz et al., 1977) the technological performance of bran.

However, the impact of bran particle size on dough and bread features remains unclear, mostly due to the different methods used for size reduction or dough and bread preparation (Hemdane et al., 2016). Despite this not univocal trend, it is well known that bran micronization is a tool to increase the nutritional potential of food products (Delcour et al., 2012) both in wheat (Hemery et al., 2010) and in buckwheat (Zhu et al., 2014).

This study aimed at evaluating the effect of micronization on the physical and chemical properties of buckwheat bran.

2.2 Materials and methods

2.2.1 Materials

Common buckwheat (*Fagopyrum esculentum*) bran was provided by Filippini s.p.a. (Teglio, Italy) as coarse bran (CB). Part of the supplied bran was processed in a micronizer system (KMX-300i; Separ Microsystem, Brescia, Italy) in order to reduce the particle size and to obtain fine bran (FB).

2.2.2 Methods

2.2.2.1 Color evaluation

The color evaluation of bran samples was carried out with a hand-held tristimulus colorimeter (Minolta Chroma Mether CR-210, Minolta, Osaka, Japan). Before each measurement, the apparatus was calibrated on the Hunterlab color space system using a white ceramic tile (Minolta calibration plate, $Y = 94.1$, $x = 0.3158$, $y = 0.3333$). The color was expressed in the CIE LAB system. At least five replicates were carried out for each sample.

2.2.2.2 Particle size distribution

Particle size distribution of coarse and fine bran was determined by mechanical sieving 20 g of sample for 30 min on Retch Vibratory Sieve Shaker (Aartselaar, Belgium) equipped with sieves with mesh sizes of 710, 500, 355, 180, 150 and 63 μm .

The average diameter (d_{av}) was calculated as follows:

$$d_{av} = \sum d_i \cdot m_i$$

with d_i the average particle size of particles on sieve i and m_i the mass percentage of the fraction retrieved on sieve i . Particle size distribution analyses was made in duplicate.

2.2.2.3 Dynamic water vapor sorption behavior

Dynamic water vapor sorption experiments were performed using an automatic multi-sample moisture sorption analyzer SPSx-111 (Projekt Messtechnik, Ulm, Germany). In this instrument, the relative humidity (RH) inside the climatic chamber was conditioned by mixing a dry nitrogen gas flow with a gas flow saturated with water. The instrument is equipped with a dew point analyzer and a microbalance (WXS206SDU Mettler-Toledo, Greifensee, Switzerland) for accurate measurements of RH and weight, respectively. Water sorption was determined by measuring the variation in weight of a sample caused by changes in environmental RH. Weight changes were measured at time intervals of at least of seven minutes. Samples were dried above P₂O₅ for at least three days before the start of the dynamic water vapor sorption experiments. The sorption measurement procedure involved an initial drying at 30°C for 500 min followed by increases from 0% up to 90% RH at 10% RH increments at 25°C, with 50 h equilibration times at each stage. The mass at the maximum residence time was assumed to correspond to that of the moisture sorption isotherms. All analyses were performed in duplicate.

2.2.2.4 Compositional traits

Proximate analyses of buckwheat bran fractions were performed according to AOAC (2005) for ash (942.05), protein (960.52 conversion factor: 6.25), total starch (996.11), Soluble Dietary Fiber (SDF) and Insoluble Dietary Fiber (IDF) (991.43). Total Dietary Fiber (TDF) was determined as sum between SDF and IDF. Damaged starch (76-31.01) was measured according to AACC (1999).

Ash, protein, SDF and IDF analyses were made in duplicate. Total starch and damage starch analyses were made at least in triplicate.

2.2.2.5 Water Binding Capacity (WBC)

Bran (1.5 g) was soaked in 45 mL of Milli Q water in a falcon tube and shaken for 16 h at room temperature. Therefore, sample was centrifuged for 60 min at 10000 x g, supernatant (SN) was discarded from the pellet and collected for further analysis. WBC was expressed as the ratio between water absorbed by gel and initial bran weight. At least 3 replicates were carried out for each sample.

2.2.2.6 Water solid extraction: charge level and supernatant sugar composition

The charge level (ion content) of the supernatant was measured using a ion meter expressed in µS/cm.

The residual supernatant was freeze-dried and total amount of solid extracted (Solid SN) by water (mg/mL) was calculated as the weight of freeze-dried supernatant and the supernatant volume before freeze-drying. Charge level and Solid SN analysis were performed in triplicate, deriving from three different water extractions.

Monosaccharide composition of supernatant was evaluated by hydrolyzing with 2M HCl at 100 °C for 1 h about 10 to 20 mg of freeze-dried material. Samples were then diluted 50 times, filtered through a 0.25 µm membrane filter and injected in a HPLC (Dionex)-Pad. Sugar standards were used. Sample preparation

included α -amylase treatment followed by precipitation with 80 % ethanol. Monosaccharide composition was performed in duplicate.

2.2.2.7 Microstructure features

Coarse and fine bran samples were observed by using a light Olympus BX50 microscope (Olympus, Tokyo, Japan) after staining with 1 g/L Sudan Orange dye in water, which is a generic dye for lipids tissues. Samples were layered on the glass slide, covered with a coverslip and a small drop of staining was left to permeate in between.

2.2.2.8 Statistical Analysis

A two-sample t-test was used for analyzing color indices, compositional and particle size data. Test was performed by using XLSTAT Version 2016.02 (Addinsoft, Paris, France).

2.3 Results and discussion

2.3.1 Color evaluation

Micronization promoted a significant change in all color indices considered, especially in lightness (L^*) (Fig. 2.1). As observed in wheat flour by Hidalgo et al. (2014), the particle size determines color perception, and in particular, L^* was found to increase with flour size decrease. Color of buckwheat bran used in this study was quite different from the result reported by Bonafaccia et al. (2003) who found lower lightness (L^*) (≈ 30) and higher yellowness (≈ 30) values. Differences were likely due to the buckwheat variety considered, bran extraction rate and granulometry.

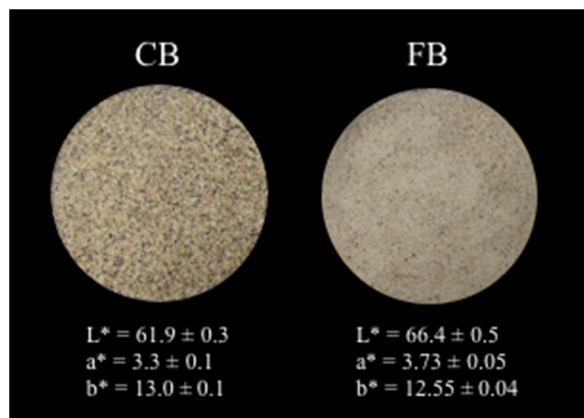


Fig. 2.1. Images and color indices of coarse and fine buckwheat bran. All data are expressed as the mean \pm standard deviation ($n = 5$). All color indices were statistically significant different at 95.0% confidence level (t-test).

2.3.2 Particle size, moisture sorption behavior and chemical-physical characterization

The particle size of coarse buckwheat bran (CB) was mostly distributed in a wide range, between 180 and 710 μm (Table 2.1), with an average diameter of 359 μm . After micronization the average diameter was 110 μm with most of the particles of micronized bran (FB) having a diameter in the range of 63-150 μm . However, a considerable fraction (28%) still presented a relatively high particle size ($>180 \mu\text{m}$).

Table 2.1. Particle size distribution of coarse (CB) and fine (FB) buckwheat bran expressed as percentage.

	CB	FB
<63 μm	0.4 ± 0.3	$2 \pm 1^*$
63-150 μm	1.3 ± 0.2	$63.9 \pm 0.3^*$
150-180 μm	1.3 ± 0.6	$6.5 \pm 0.4^*$
180-355 μm	20 ± 1	22 ± 2 ns
355-500 μm	46 ± 1	$5.21 \pm 0.07^*$
500-710 μm	30.011 ± 0.007	$1.09 \pm 0.02^*$
>710 μm	0.9 ± 0.3	0
Average diameter (μm)	358.5 ± 0.7	$113 \pm 3^*$

* indicates statistically significant differences (t-test; $p \leq 0.05$); ns indicates lack of significant differences ($p \leq 0.05$).

Coarse and fined bran displayed the characteristic shape of a type II isotherm (Fig. 2.2), as defined by the Brunauer classification scheme (Brunauer et al., 1940). The sorption isotherms were similar, thus indicating that micronization did not alter the surface sorption properties of the bran. On the contrary, the decrease in particle size resulted in differences in WBC and supernatant characteristics (Table 2.2). Since hydration properties are related to the water held by polysaccharide chains through hydrogen bonds, the alteration of the matrix structure by grinding could be responsible for the lower WBC in FB (Zhu et al., 2010). Beside particle size, hydration properties can depend on IDF/SDF ratio (Cadden, 1987). The apparent contrast in sorption isotherms and WBC values could be ascribable in their own mechanism in terms of water uptake: in fact, the first method is based on the uptake of water vapor, the second one combines the effect of fiber soaking, swelling and application of centrifugal force (Chen, Piva, & Labuza, 1984).

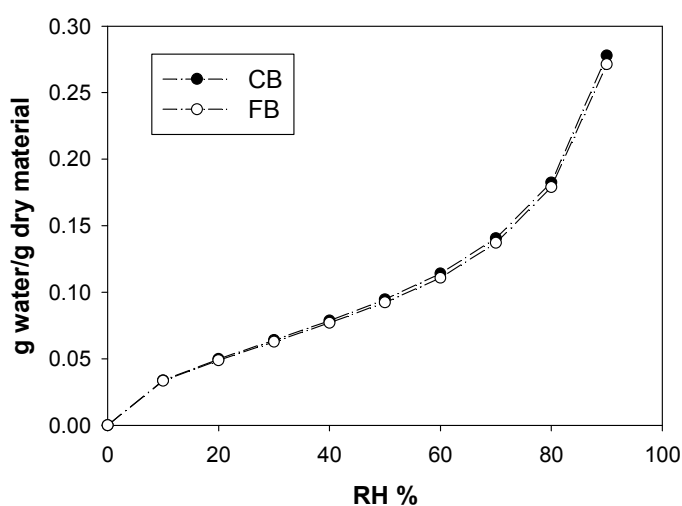


Fig. 2.2. Water vapor sorption of coarse (CB) and fine (FB) buckwheat bran.

The micronization had also a significant effect on the composition of the water extracted solids (Table 2.2). The total amount of solids (Solid SN) increased particularly due to high glucose content in the supernatant phase, while the charge level (i.e. the ion content in the water phase) increased likely due to the more extraction after grinding. The composition changes could be an indication of the alteration of the polysaccharide structure induced by mechanical treatment. However, this aspect needs further investigation.

Table 2.2. Chemical composition of coarse (CB) and fine (FB) buckwheat bran.

	CB	FB
WBC (g water/g sample)	2.8 ± 0.2	2.18 ± 0.09*
Solid SN (mg/mL)	5.36 ± 0.03	6.5 ± 0.1*
Charge level (µS/cm)	1151 ± 22	1251 ± 38*
Sugar total (mg/g)	12.5 ± 0.4	14.0 ± 0.2*
Fucose (mg/g)	0.04 ± 0.01	0.023 ± 0.003 ns
Rhamnose (mg/g)	0.65 ± 0.06	0.7 ± 0.1 ns
Arabinose (mg/g)	1.9 ± 0.3	2.41 ± 0.07 ns
Galactose (mg/g)	5.1 ± 0.1	4.7 ± 0.1*
Glucose (mg/g)	4.0 ± 0.2	5.5 ± 0.3*
Xylose (mg/g)	0.55 ± 0.02	0.52 ± 0.02 ns
Mannose (mg/g)	0.26 ± 0.03	0.24 ± 0.04 ns

WBC, supernatant characteristics and solid extracted sugar composition of coarse and fine buckwheat bran. All data are expressed as mean ± standard deviation (WBC: n = 3; Solid SN and Charge level: n = 3 deriving from 3 independent water extractions; Sugar composition: n = 2). * indicates statistically significant differences (t-test; p ≤ 0.05); ns indicates lack of significant differences (p ≤ 0.05).

2.3.3 Compositional traits

Buckwheat bran was composed, before micronization, approximately by one third of fiber and one third of proteins, whereas starch, lipid and ash contents accounted respectively for 21.5, 7.6 and 6% (Table 2.3).

The high content in fiber and proteins confers indeed a high nutritional value to this fraction. Our sample contains a higher protein and fiber content than what is reported in literature (Bonafaccia et al., 2003), likely due to differences in variety, crop yield and seed weight. Interestingly, SDF content in our sample was 3-folds of than one reported in the cited study. This aspect is of great interest from a nutritional standpoint, since SDF has been associated with a number of health benefits, including the maintenance of physiological blood cholesterol level, due to its ability to form viscous solutions in the intestine (Kumar et al., 2012). Steadman et al. (2001) found in bran obtained from the milling of dehulled groats, about the same content in protein and starch but lower TDF which was composed mainly by insoluble fibers.

Table 2.3. Chemical composition of coarse (CB) and fine (FB) buckwheat bran.

	CB	FB
Protein	31.74 ± 0.08	29.6 ± 0.2*
Ash	5.954 ± 0.005	5.57 ± 0.01*
Lipids	7.6 ± 0.3	7.3 ± 0.4 ns
Total starch	21.5 ± 0.3	20.2 ± 0.5*
Damaged starch	0.84 ± 0.02 (3.9%)	1.26 ± 0.02* (6.3%)
TDF	36 ± 2	32.7 ± 0.2 ns
SDF	3.48 ± 0.03 (9.7%)	4.2 ± 0.2* (12.9%)
IDF	33 ± 2	28.5 ± 0.5 ns

Compositional data are expressed as g/100 g sample (d.b.). Damage starch percentage on Total starch content is reported in Damage starch row in bracket. SDF percentage on TDF is reported in Soluble Fiber row in bracket. * indicates statistically significant differences (t-test; $p \leq 0.05$); ns indicates lack of significant differences ($p \leq 0.05$).

The micronization had small effects on the chemical composition of buckwheat bran: protein, ash, total starch content slightly decreased ($p \leq 0.05$), while lipid content did not change after the mechanical treatment as shown in Table 2.3. The small differences observed could be due to the discard of non-homogenous material during the grinding (yield of micronization process: 94.8%).

Nevertheless, the overall impact of micronization has been reported to promote large changes in chemical composition, as reported by Coda et al. (2014) who found a starch and protein content increase in wheat bran progressively ground. As expected, the severe mechanical action applied largely increased the fraction of damaged starch. The grinding caused physical damage to a certain amount of starch granules, facilitating a rapid hydration and hydrolysis by α - and β -amylases (Gibson et al., 1992).

The grinding promoted also a significantly increase in the SDF from 9.7% to 12.9% without decrease the TDF and IDF. Zhu et al. (2014) observed an increase in SDF after micronization of buckwheat hulls, likely due to the fiber degradation into small molecular substances and to a redistribution of fiber components caused by ultrafine grinding, as reported also in Table 2.2.

2.3.4 Microstructure features

Although no difference was observed in lipid content before (CB) and after grinding (FB), mechanical treatment promoted a redistribution of fat droplets, as shown by light microscopy pictures (Fig. 2.3). In coarse bran (Fig 2.3A) it was clearly possible to recognize single husk particles not colored by Sudan Orange dye, likely due to the absence of lipids on bran surface.

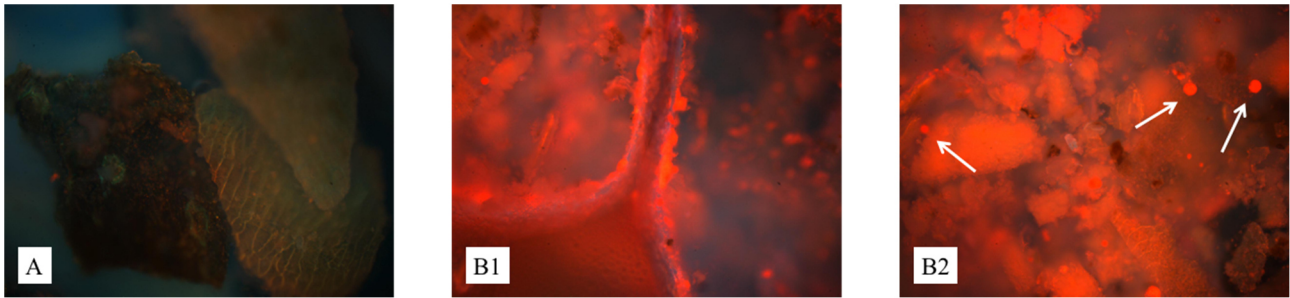


Fig. 2.3. Light-microscope images of coarse (A) and fine (B1; B2) buckwheat brans stained with Sudan Orange.

On the contrary, micronization seemed to destroy the original structure, so that lipids completely covered the bran surface and thus emitted a fluorescence orange-red light when marked with the stain (Fig 2.3B1 and 2.3B2). Single lipid droplets are also recognized (Fig. 2.3B2, indicated by arrows). The high lipid content in buckwheat bran lipids mostly arise from the presence of embryo tissues. Germ is mainly composed of triacylglycerides (Steadman et al., 2001). Severe mechanical treatment of bran can help to further crush embryo, promoting the release of lipid droplets and the bran particles covering.

2.4 Conclusions

A part from the decrease in particle size, buckwheat bran micronization promoted relevant changes as the increase in SDF and different lipid droplets distribution that might be further influence the behavior of bran when supplemented to flour in dough or bread products.

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3. Physical mechanisms describing the influence of buckwheat bran enrichment varying in granulometry on the thermo-mechanical behavior and bread-making performance of wheat dough

3.1 Introduction

In the last decades, the demand for healthy foods has grown due to increased consumers awareness of the role of nutrition in preventing or lowering the risk of developing chronic diseases such as cardiovascular disease, cancer or type 2 diabetes (Who and Consultation, 2003). Cereal and pseudo-cereal products, being a staple food category, may represent a valid resource to provide adequate amount of nutrients such as non-digestible cell wall polymers (dietary fiber, DF) and related compounds with relevant bio-activity (Vitaglione et al., 2008).

Common buckwheat (*Fagopyrum esculentum*) is a nutritional-relevant pseudocereal, being an important source of dietary fiber and antioxidant compounds (Steadman et al., 2001a). In particular, buckwheat is rich in polyphenols, including the flavonoid rutin, which is a strong health protective compound thanks to its anti-inflammatory and anticarcinogenic activity (Zhang et al., 2012). Moreover, buckwheat proteins have high biological value and balanced amino acid composition containing a relatively high amount of lysine – the limiting amino acid in wheat – (Dziadek et al., 2016).

Buckwheat flour is traditionally used in a number of products including pancakes (Mazza and Dave Oomah, 2005), crêpes (Biacs et al., 2002), Italian pasta “Pizzoccheri” and noodles, e.g. Soba in Japan (Bonafaccia et al., 2003; Marti et al., 2011; Pagani et al., 2007). Buckwheat is also milled into groats and used as ingredient for porridge (Mazza and Dave Oomah, 2005). Recently, buckwheat flour has gained popularity as a functional ingredients in gluten-free products, as reviewed by Alvarez-Jubete et al. (2010), or in wheat-based products (such as bread) in order to obtain an economically advantageous enrichment in naturally derived antioxidants (Dziki et al., 2014; Vogrinčič et al., 2010).

The outermost layers of buckwheat groats contain most of the nutritional compounds (Steadman et al., 2001b) but they are usually discarded during the production of refined flour and collected into feeding material. The enrichment of wheat-based products with buckwheat bran provides an opportunity to improve nutritional profile and valorize the side stream material.

Many studies deal with the technological impact of wheat bran on bread quality, indicating detrimental effects, resulting in decreased loaf volume, increased crumb hardness and changes in sensory properties (Ktenioudaki and Gallagher, 2012). The observed negative effects have been largely associated with changes in gluten development and quality resulting from wheat bran incorporation (Heiniö et al., 2016; Schmiele et al., 2012; Sivam et al., 2010). The mechanisms by which bran negatively impacts dough quality have been ascribed by authors to gluten dilution (Gan et al., 1992) and physical hindrance (Lai et al., 1989), reduced gluten development due to bran competition for water (Hemdane et al., 2016), and by chemical interactions between wheat bran components and gluten proteins which affects network formation (Noort et al., 2010).

Changes in the dynamics of gluten hydration is among the proposed mechanisms by which wheat bran interferes with gluten development. The hypothesis derives from the changes in water absorption and dough mixing time observed by using empirical tests such as Farinograph and Mixograph (Dobraszczyk, 2016). The effects on the dynamics of water absorption can be mainly related to the amount of bran added, its particle size and the sorption properties of bran. In fact, under external stresses as in a dough mixing, water sorption from bran has been mainly related to the hydrogen bonding (Jacobs et al., 2015), which are described by the sorption properties of the material and are largely dependent on the molecular composition (van der Sman, 2013).

Reduction in bran particle size, obtained with severe grinding as micronization, has been often associated with more detrimental effects on dough and bread quality compared to coarse bran. The detrimental effects have been related to: i) increased water absorption resulting from higher specific surface; ii) increased physical hindrance due to a larger number of particles at similar addition level; iii) increased chemical interactions with cell components which interfere with gluten network formation (Noort et al., 2010). On the contrary, some researchers have suggested an optimum wheat bran size for bread-making or even no significant effects of variations in particle size based on evaluations of bread volume. Variations in bread-making protocols and definition of optimal water absorption and mixing times may explain the observed differences (Hemdane et al., 2016).

While extensive research has been performed on wheat bran, little information is available with regards to the technological impact of buckwheat bran. For such reason, the present study aimed at evaluating the mechanisms by which addition of buckwheat bran may impart negative effects to wheat dough bread-making quality and how much the dough worsening is related to particle size. For this purpose, the influence of coarse and fine buckwheat bran addition at different levels on small and large deformation rheology and on thermo-mechanical properties of wheat dough were evaluated. Front-face spectroscopy in flour-water mixtures was performed to evaluate the effect of bran addition on gluten structural arrangements. The insights on dough properties were complemented with the evaluation of wheat-buckwheat mixture baking performance and textural quality of bread.

3.2 Materials and methods

3.2.1 Materials

Coarse (CB) and fine (FB) buckwheat bran: refer to Section 2.2.1. A commercial wheat flour (WF) for bread making application (protein: 11.7 g/100 g) was provided by Meneba (Rotterdam, The Netherlands).

3.2.2 Methods

3.2.2.1 Dough preparation

Doughs were prepared with coarse or fine bran by adding 5 g, 10 g, and 20 g of bran to 95, 90 and 80 g of flour, respectively. Coarse bran-enriched doughs were labeled as CB5, CB10, and CB20, whereas micronized

bran-enriched doughs were labeled as FB5, FB10 and FB20 with numbers indicating the level of addition. A reference dough with no addition of bran was prepared as control (WF).

Dough samples were prepared in a Farinograph-E (Brabender, Duisburg, Germany) equipped with a 50 g mixing bowl. The ICC standard method 115/1 (ICC-Standards, 2006) was used with few modifications. Briefly, 1 g of sodium chloride (Merck, The Netherlands) was added to 50 g of wheat flour or buckwheat bran-enriched mixture and pre-mixed for 2 min. Distilled water was added in quantity required for producing a consistency of 420 ± 20 FU (Farinograph Units). Dough was mixed until reaching the maximum consistency range (420 ± 20 FU).

3.2.2.2 Dynamic Mechanical Thermal Analysis (DMTA)

Dough viscoelastic properties were measured by using a DHR2 hybrid rheometer (TA Instruments, New Castle, USA) equipped with 25 mm steel parallel Peltier plate. Approximately 1 g of dough was placed between plates (loading gap: 20 mm) and compressed until 1.025 mm. Dough excess was removed and silicon oil was applied to prevent sample drying and dough was compressed until 1 mm. Before the measurement, the dough was rested for 5 min at 25 °C to allow relaxation. Samples were oscillated at a frequency of 1 Hz and heated from 40 to 120 °C with a ramp of 5 °C/min. Before analysis, oscillation amplitude test was performed from $1.0e-4$ to 10 to select the linear viscoelastic range. Thus the strain amplitude was kept at $0.5e-3$ for all samples. Key parameters related to physical transitions in the dough were derived from the analysis of the G' and $\tan(\delta)$ curves in the DMTA curves by using the analysis functions in TA Trios v3.3 (TA Instruments, New Castle, USA): initial value (at 40 °C) of $\tan(\delta)$; G' onset temperature (calculated as the intersection of the tangents of the peak with the baseline); $\tan(\delta)$ value at onset G' ; peak G' and temperature. The analysis was carried out at least in triplicate on different dough.

3.2.2.3 Modelling the influence of composition on key parameters derived from DMTA curves

According to the Flory-Huggins equation for biopolymer melting, the starch gelatinization temperature in a water solution is function of the volume fraction of water (Φ_{water}) present in the food matrix (Renzetti and Jurgens, 2016), following the equation:

$$\frac{1}{T_m} - \frac{1}{T_m^{\circ}} = \frac{R}{\Delta H_U} \frac{v_U}{v_W} [\Phi_{water} - \chi \Phi_{water}^2] \quad (1)$$

Where T_m is the melting temperature of starch in the system under consideration, T_m° the melting temperature of the dry crystalline starch, ΔH_U is the melting enthalpy per mole of the repeat unit of the biopolymer, i.e. starch, v_U is the molar volume of the starch repeat unit, v_{water} is the molar volume of the diluent, i.e. water, Φ_{water} is the volume fraction of water, χ is the Flory-Huggins solvent-biopolymer interaction parameter and R is the universal gas constant. The theory can also apply to a system composed of water and flour since the ratio between gluten and starch is constant and hence water will partition between the two components in a similar manner, irrespective of its volume fraction. However, the addition of bran changes the partitioning of water in the system as it will compete with starch and gluten to absorb the available water. According to Flory-

Huggins theory, the partitioning of water can be described as the chemical potential of water among the different polymer phases following (Van der Sman and Meinders, 2011):

$$\frac{\mu_w}{RT} = \ln(1 - \Phi) + \left(1 - \frac{1}{N}\right)\Phi + \chi\Phi^2 \quad (2)$$

Where μ_w is the chemical potential of water, Φ the volume fraction of the biopolymer, N is the ratio of the molar volume of biopolymer and water and χ is the interaction parameter water-biopolymer. From the equation, it follows that the partitioning of water will change with increasing volume fraction of bran Φ_{bran} , thus reducing the amount of water available for starch gelatinization. When such approach holds for the wheat dough system and the χ of bran is unaffected by micronization (i.e. the moisture sorption properties are the similar for fine and coarse bran), the onset of starch gelatinization should be mainly a function of both Φ_{water} and Φ_{bran} .

Following on the model proposed by Taylor and Bagley (1979; 1977; 1974), Steeneken (1989) demonstrated that the rheological properties of swollen starch granules in water suspensions are determined by the volume fraction occupied by the particles and by their rigidity. In the dough system under study, the volume fraction of starch particles changes with addition of bran, while the rigidity of the particles can be assumed constant. Therefore, the contribution of starch swelling to the rheology, i.e. peak G' , of the dough should be mainly a function of Φ_{starch} . However, it should be noted that the model of Taylor and Bagley applies to a binary water-starch system, where the variation in Φ_{starch} reflects the variation in water to starch ratio. That is not the case of the complex dough formulation under study due to the addition of bran in replacement of flour and to the adjustments in water levels. Therefore, a scaling factor accounting for the volume fraction of water over Φ_{starch} seems necessary.

In order to validate the proposed interpretation of data, the volume fraction of ingredients in the dough formulations were computed from the mass fraction using the mass density ρ_i of each ingredient. For water we have taken ρ_{water} 1000 kg/m³ while for polysaccharide it holds that $\rho_{polysaccharide}$ 1550 kg/m³, and for proteins $\rho_{proteins}$ 1330 kg/m³ (Van der Sman, 2008). Bran is largely composed of polysaccharide macromolecules being dietary fibers and starch, hence the mass density was assumed that of polysaccharides. In order to compute the volume fraction of wheat starch and gluten, their mass fraction in the flour was obtained from supplier specifications and converted into volume fraction by using the mass densities for polysaccharides and proteins.

As already indicated, in a complex system like the wheat dough under study, the variation in water volume fraction Φ_{water} are not fully representative of the variation in the water to starch ratio as in the case of the water-starch system described by equation (1). For such reason, Φ_{water} was rescaled over the volume fraction of starch Φ_{starch} in dough by using the following equation:

$$\Phi_w = \frac{\Phi_{water}}{1 - \Phi_{starch}} \quad (3)$$

3.2.2.4 Differential Scanning Calorimetry (DSC)

Starch gelatinization in doughs was measured using a DSC Q2000 (TA Instruments, New Castle, USA). Sample (10-15 mg) was placed in sealed aluminum pans, equilibrated at 2 °C for 5 min, and scanned to 160 °C at a rate of 7.5 °C/min. Starch gelatinization temperatures (onset, maximum peak) were determined by using the analysis functions in Universal Analysis software (TA Instruments, New Castle, USA). The analysis was carried out at least in triplicate on different doughs.

3.2.2.5 Kieffer extension test

Dough rheology at large deformations was assessed by Kieffer extension test. A texture analyzer equipped with a 50 N load cell and SMS/Kieffer dough and gluten extensibility rig was used (TA.HDi 500, Stable Micro Systems, Surrey, UK). Dough was placed in a teflon form, compressed into strips of constant dimension, and rested for 40 min at room temperature. Then, stripes were carefully removed using a spatula, placed on the sample plate and pulled until breakage at 3.3 mm/s by the hook probe. Dough was prepared in duplicate, obtaining five stripes respectively from each dough.

3.2.2.6 Protein structural data

Protein surface hydrophobicity was assessed through titration of minimally mixed dough of various composition with increasing amounts of 1,8-aniline-naphthalen sulfonate (ANS), added in the water used for mixing, as reported by Bonomi et al. (2004). Front-face (solid state) spectrofluorimetric measurements were carried out in an LS-50 spectrofluorimeter (Perkin-Elmer Waltham, MS) by recording emission fluorescence spectra (from 400 to 600 nm, with excitation at 390 nm, emission and excitation slits set at 5 nm) on small amounts of individual dough samples containing 0-0.5 mmol L⁻¹ ANS.

Standard binding algorithms were used to calculate F_{\max} (i.e., the fluorescence at saturating probe concentration, related to the number of surface hydrophobic sites available for binding of the probe), and K_d (i.e., the apparent dissociation constant of the assumedly bi-molecular probe/protein complex) from the ANS titration data. These two parameters may be conveniently combined in a protein surface hydrophobicity index (PSH), calculated as the ratio $(F_{\max}/\text{protein content})/K_d$ (Huschka et al., 2012; Iametti et al., 2006).

A similar solid-state spectrofluorimetric approach was used to assess the extent of protein solvation in various minimally mixed dough samples containing 0.3 mM ANS and prepared with a water content ranging from 40 to 55%, as described in Bonomi et al. (2004). All samples for spectrofluorimetric measurements were prepared in duplicate, and multiple emission spectra ($n = 3$) were averaged for each individual sample.

3.2.2.7 Bread making

Small-scale puffy loaves were produced according to Hemery et al. (2010) with slight modifications. Dough was prepared in a 300 g Farinograph mixing bowl at 20 °C and speed of 63 rpm. Instant yeast (1.67%; Fermipan red, AB Mauri), salt (2%; EFP, Akzo Nobel), and calcium propionate (0.1%; Sigma-Aldrich, Germany) were added to either wheat flour or buckwheat bran-enriched mixture and pre-mixed in the mixer bowl for 2 min. Then, distilled water was added according to Farinograph water absorption. After mixing until development

time, dough was divided in pieces containing 47.9 g of flour, to correct the loaf weight for the different amount of water added, manually rounded, and fermented two time at 30 °C for 15 min. Subsequently loaves were molded (Betrand Euro 2000, Nevers, France) and placed in loaf tins (top: 10.5*4.5 cm; bottom: 9.5*3.5 cm; height: 3.5 cm). Final proof was carried out in a fermentation cabinet (custom made by TNO) at 30 °C and 90% RH for 40 min, corresponding to the time needed to produce 200 mL CO₂ as measured in a SJA-fermentograph (Nässjö, Sweden). Finally, loaves were baked in a custom made swing oven (TNO) at 230 °C for 20 min. Loaf volume and weight were determined 2 h after cooling with a rapeseed displacement method and a technical scale, respectively. Specific volume was calculated as loaf volume divided by loaf weight. Moisture content of crumb was measured according to AACC method (44-15.02, 2001). Water activity (a_w) was measured by electronic hygrometer (AquaLab Water Activity Meter 4TE, Decagon Devices, Pullman, WA).

Cylindrical crumb samples were collected from fresh bread and stored for 1, 2 and 4 days at controlled temperature (18 °C) in sealed polyethylene containers until analysis. This operation was made to assess staling as influenced by starch retrogradation and crumb structure while eliminating the contribution of moisture loss and water migration from crumb to crust.

For baking tests, 4 different doughs were made, obtaining in total 24 loaves of which 6 (deriving from 2 different baking) were used for each analysis time. Weight loaf and specific volume were carried out on 4 loaves. From every loaf, 2 central slices were used for Texture Profile Analysis (TPA), 1 for moisture content and 1 for a_w evaluation.

3.2.2.8 Texture Profile Analysis (TPA)

Texture Profile Analysis (TPA) was carried out using a texture analyzer (TA-XT2i Texture Analyser, Stable Micro Systems, Surrey, UK) equipped with 30 kg load cell and a 75 mm compression plate. Crosshead speed and trigger force were set respectively to 3.30 mm/s and 9.81 mN. Three loaves were analyzed for each sample. Two central slices (thickness: 20 mm) were obtained from each loaf, from which, a cylindrical specimen (height: 20 mm; diameter: 25 mm) was cut in the crumb middle part. Before testing, sample weight was measured by a technical scale (Mettler Toledo, Tiel, The Netherlands). Sample underwent two cycles of compression until 40% of deformation. Sample height recorded by the instrument was used to calculate crumb density, considering the specimen as a cylinder with constant diameter (25 mm). The parameters recorded by the software (Exponent, Stable Micro Systems, Surrey, UK) were hardness and cohesiveness.

In cellular solids, the hardness of the material is related to its density, according to the Ashby-Gibson theory (Ashby and Medalist, 1983). In order to correct the instrumental hardness for variations in density, an adapted Ashby-Gibson theory was applied following:

$$E_{\text{crumb}} = CE_{\text{film}}(\rho)^n \quad (4)$$

where E_{crumb} is the elastic moduli of the crumb, E_{film} is the elastic moduli of the solid crumb matrix, ρ is the crumb density, C is a constant and n is the parameter describing the cellular structure, i.e. $n = 3$ for a foam and $n = 2$ for a sponge. For bakery products, the crumb structure can be assumed to be that of a sponge.

3.2.2.9 Statistical Analysis

Analysis of variance (one-way ANOVA) was used for analyze dough rheology and baking test data. Different dough samples were considered as factors for ANOVA. When a factor effect was found significant ($p \leq 0.05$) significant differences among the respective means were determined using Fischer's Least Significant Difference (LSD) test. Both tests were performed by using XLSTAT Version 2016.02 (Addinsoft, Paris, France). Linear regression analysis of dough rheology and textural data as function of composition was also performed using XLSTAT.

3.3 Results and discussion

3.3.1 Dough properties

Addition of buckwheat coarse bran to wheat dough resulted in a progressive increase in water absorption with increasing bran level (Table 3.1). The increase in water absorption was coupled with a progressive decrease in mixing time (Table 3.1). On the contrary, the addition of buckwheat fine bran showed a slight increase in water absorption which was similar for all dough samples, independently from the level of bran inclusion.

Table 3.1. Water absorption and mixing time of dough samples

Sample	Water absorption (%)	Mixing time (min:s)
WF	57.4	11:48
CB5	58.6	11:12
CB10	59.6	9:30
CB20	61.6	8:00
FB5	58.2	9:18
FB10	58.0	9:12
FB20	58.1	10:42

Generally, the incorporation of increasing amount of bran results in higher water absorption values (Sudha et al., 2007) due to the greater number of hydroxyl groups of fiber that allow more water interaction through hydrogen bonding (Rosell et al., 2001). Usually, when particle size is decreased, no changes in water absorption are observed, since when bran is subjected to kneading forces, the water weakly bound is released (Hemdane et al., 2016). Since no differences were observed in the sorption properties of coarse and fine bran (refer to Section 2.3.2), the lower water absorption of dough enriched with micronized bran – in comparison

with coarse bran - may be explained by the fact that the Farinograph method highlights the behavior of the whole dough system, that considers not only the bran properties but also the properties of the gluten network (Noort et al., 2010). No clear trend was instead observed concerning the dough development time.

The effect of enrichment in coarse or fine bran on the thermo-mechanical behavior of wheat dough was investigated by DMTA during a temperature sweep. This technique provides insights on the influence of phase transitions, e.g. starch gelatinization, on the mechanical properties of the dough at small deformations (Erickson et al., 2014). Fig. 3.1A and 3.1B show the evolution of the storage modulus during heating of wheat doughs containing respectively coarse and fine buckwheat bran. In all samples G' initially decreased going from 40°C to 50°C approximately due to the softening of the dough. In the temperature range between 50 and 56 °C all dough samples showed a sharp increase in G' , which can be associated with the onset of starch gelatinization (Dreese et al., 1988; Jekle et al., 2016; Xie et al., 2008).

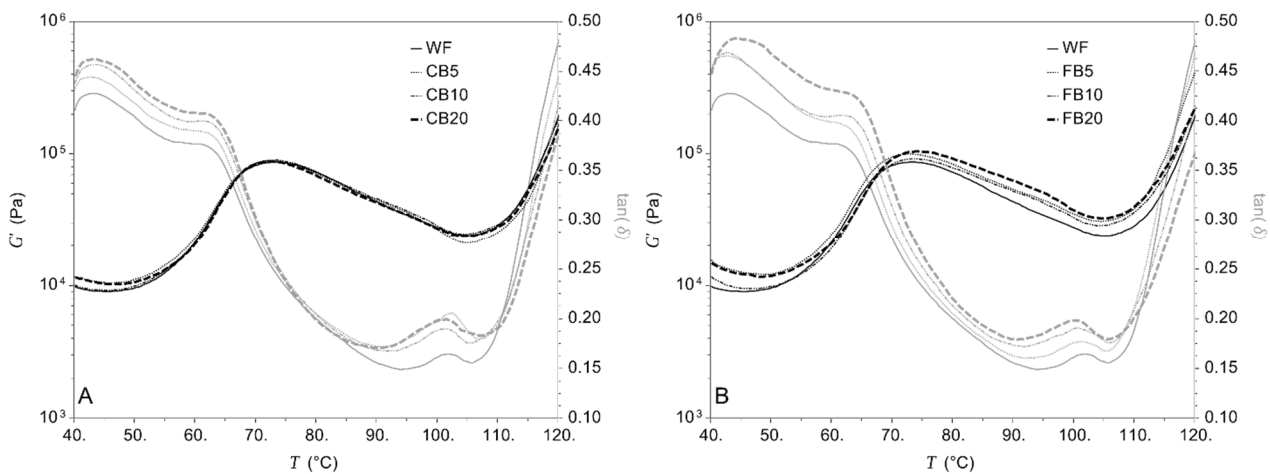


Fig. 3.1. DMTA profiles for wheat dough enriched in different coarse (CB) and fine (FB) buckwheat bran levels. Black lines: G' modulus; grey lines: $\tan(\delta)$.

In fact, the onset temperatures of starch gelatinization as derived from G' (Table 3.2) was found to be highly correlated with those obtained by DSC analysis of the dough ($R^2 = 0.868$; $p < 0.00$). This result confirms that the mechanical transition observed in the 50-56 °C was the result of heat-induced gelatinization as a consequence of water absorption and swelling by starch granules. Consequently, the further increase in G' could be associated with the increased hydration of the starch granules and the gelling of the leached starch, reaching a maximum around 70-75°C, characteristic for the maximum gel strength (Jekle et al., 2016). After the maximum, the typical decrease in the gel strength with increasing temperature was observed.

Table 3.2 DMTA parameters for wheat dough enriched in coarse (CB) and fine (FB) buckwheat bran.

Sample	Onset temperature (°C)	Peak G' (Pa)	Peak temperature (°C)	tan(δ) at onset
WF	54.20 \pm 0.22 ^c	88711 \pm 3548 ^{bc}	73.37 \pm 0.04 ^{bc}	0.388 \pm 0.004 ^d
CB5	54.9 \pm 0.3 ^{bc}	85991 \pm 3004 ^c	73.2 \pm 0.3 ^c	0.41 \pm 0.02 ^{bcd}
CB10	54.8 \pm 0.2 ^{bc}	93383 \pm 7900 ^{abc}	73.0 \pm 0.3 ^c	0.41 \pm 0.01 ^{abc}
CB20	55.3 \pm 0.1 ^{abc}	88517 \pm 1819 ^{bc}	72.38 \pm 0.02 ^d	0.418 \pm 0.003 ^{ab}
FB5	54.9 \pm 0.5 ^{bc}	93751 \pm 6406 ^{abc}	73.4 \pm 0.5 ^{bc}	0.39 \pm 0.02 ^{cd}
FB10	56 \pm 1 ^{ab}	95989 \pm 6962 ^{ab}	73.6 \pm .3 ^{bc}	0.412 \pm 0.007 ^{abc}
FB20	56 \pm 1 ^a	101689 \pm 4387 ^a	74.2 \pm 0.2 ^a	0.43 \pm 0.01 ^a

Different letters in the same column indicate statistically significant differences (LSD; $p \leq 0.05$).

Bran enrichment of wheat dough resulted in a progressive increase in the onset of starch gelatinization, which was significant only for the 10% and 20% level of inclusion of fine bran (Table 3.2). The changes in gelatinization temperature could be better understood considering the main mechanisms that influence the melting process. According to thermodynamic theories describing the state diagram of starch in water mixtures (Van der Sman and Meinders, 2011), the starch gelatinization temperature is function of the volume fraction of water in the system. Consequently, the applied variations in the amount of water added in the dough (Table 3.1) contribute in modulating the starch gelatinization process. However, this alone would not explain the observed increase onset temperature, as extra water was added in the enriched doughs, which would lower the considered parameter. As recently described by Jekle et al. (2016), starch gelatinization in the presence of other biopolymers such as gluten, is modulated by a competitive hydration between the polymers. The addition of bran changes the partitioning of water in the system, as it will compete with starch and gluten to absorb the available water. As described in equation (3), the partitioning of water is function of the volume fraction of bran, Φ_{bran} , and the specific water-bran interaction parameter χ . Sorption properties were similar for coarse and fine bran (Refer to Section 2.3.2). Therefore, the increasing level of bran alone could describe the changes in water partitioning in the dough, thus slowing down the hydration of starch. As shown in Fig. 3.2A, the interplay between the water volume fraction Φ_w and the volume fraction of bran Φ_{bran} , could well explain the observed variation ($p < 0.00$ for both Φ_w and Φ_{bran}).

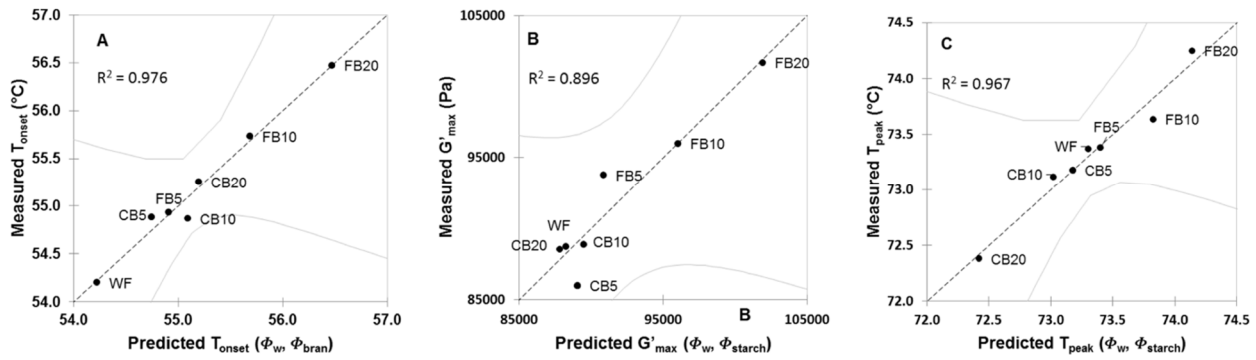


Fig. 3.2. Correlation between the DMTA parameters measured and those predicted from compositional data. **A)** correlation between T_{onset} measured and T_{onset} predicted from Φ_w and Φ_{bran} ; $p < 0.00$ for both Φ_w and Φ_{bran} ; **B)** correlation between G'_{max} measured and G'_{max} predicted from Φ_w and Φ_{starch} ; $p < 0.00$ for both Φ_w and Φ_{starch} ; **C)** correlation between T_{peak} measured and T_{peak} predicted from Φ_w and Φ_{starch} ; $p < 0.00$ for both Φ_w and Φ_{starch} .

Variations in G'_{max} were observed as function of bran addition and type of bran (Table 3.2). In particular, the increasing addition of fine bran resulted in a progressive increase in G'_{max} . On the contrary, no clear trend could be observed with the addition of coarse bran. It should be noted that all fine bran enrichment level had a similar amount of water added during mixing, while the progressive coarse bran enrichment required higher water addition. The interplay of several factors may explain these results. First, the addition of solid bran particles increased the volume fraction of solids, resulting in higher particle interactions (Le Bleis et al., 2015). However, the particle interactions are also the results of the concentration of the suspended particles, which was modulated by the amount of water present. When computing the total volume fraction of solids in the wheat dough, the addition of coarse and fine bran thus not resulted in an increased in solids, due to the adjustments in water level. It should be noted that under the heating conditions applied in DMTA, the wheat dough is a suspension of swelling starch granules, with G'_{max} representing the strength of the starch paste. As already indicated, the rheology of a swollen starch granules in a binary water-starch system is a function of Φ_{starch} . In the enriched dough, the volume fraction of water available for starch swelling should be also taken into account. The contribution of the described mechanism on the observed G'_{max} values was confirmed by the strong correlation with the predicted G'_{max} (Fig. 3.2B). An increase in Φ_{starch} resulted in an increase in G'_{max} while an increase in Φ_w resulted in a decrease in G'_{max} .

As observed in Fig. 3.2C, buckwheat bran inclusion significantly decreased peak temperature with 20% addition of coarse bran ($p \leq 0.05$). On the contrary, a significant increase in peak temperature was observed with 20% addition of fine bran. The variation in peak temperature could be also well described by the interplay of Φ_w and Φ_{starch} (Fig. 3.2C). An increase in Φ_{starch} resulted in an increase in peak temperature while an increase in Φ_w resulted in a decrease in peak temperature.

The $\tan \delta$ plots of wheat dough enriched in coarse (Fig. 3.1A) and fine bran (Fig. 3.1B) provided information on the contribution on of the viscous and elastic modulus to the viscoelastic behavior during heating. The $\tan \delta$ values for all samples were smaller than 1, suggesting that elastic properties predominated. However, a progressive inclusion in buckwheat bran – either coarse or fine bran – promoted an increase in $\tan \delta$ with increasing level of bran enrichment, which was evident at the onset of starch gelatinization (Table 3.2). The

increase in $\tan \delta$ indicated an increase in the viscous behavior of the dough relative to the increase in elastic-like behavior. During mixing, water was absorbed by the gluten and the soluble and insoluble fibers molecules. The water absorption is a dynamic process that occurs during mixing. The addition of bran promoted a competition for water, resulting in a lower gluten hydration and, therefore, a lower gluten network development. Furthermore, addition of bran resulted in a dilution of gluten as well as physical hindrance to gluten networking. Altogether, these mechanisms may well explain the increase in $\tan \delta$ with bran addition. After T_{onset} , the progressive decrease in $\tan \delta$ during heating could be related to the structural change from dough to semi-solid matrix of gelatinized starch and polymerized gluten.

Kieffer extensibility test was used to provide information on dough behavior at large deformations. Figure 3.3A and 3.3B show the effects of coarse and fine bran addition on the viscoelastic behavior of wheat flour dough.

Bran incorporation into dough generally decreased resistance to extension with progressive coarse and fine bran enrichment (Fig. 3.3A), in agreement with previous studies (Hartikainen et al., 2014; Rieder et al., 2012). The effect was significant for both coarse and fine bran enrichment ($p \leq 0.05$). Increasing level of fine bran resulted in higher extensibility (Fig. 3.3A). Since dough extensibility assessed with the Kieffer test is quite sensitive to non-homogeneities present in the dough strips, the large bran particles present in the coarse bran may facilitate an earlier rupture of the dough strips.

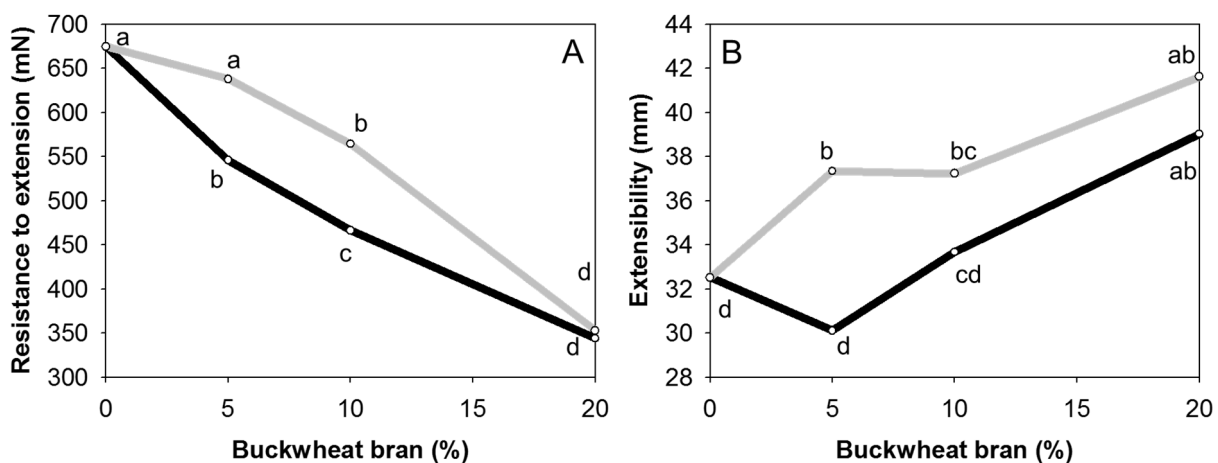


Fig. 3.3. Kieffer parameters: resistance to extension (A) and extensibility (B) in function of enriching levels of bran. Black line: dough enriched in coarse buckwheat bran (CB); grey line: dough enriched in fine buckwheat bran (FB). Interaction between type and % of bran is significant ($p \leq 0.05$). Different letters indicate significant differences for corrected hardness parameter (LSD; $p \leq 0.05$).

Gluten dilution may well explain the lower resistance to extension, as a strong negative correlation could be observed between resistance to extension and gluten volume fraction ($R^2 = 0.927$, $p < 0.00$). However, the effect of particle size is unclear. Wang et al. (2016) incorporated wholegrain flour with different particle size in wheat dough, and found less detrimental effects on maximum resistance using fine particles (median diameter: 90 μm) compared to coarse ones (median diameter: 180 μm). On the other hand, as previously indicated, fine bran particles should result in large detrimental effects on gluten development, due to the

increase in number of particles, the gluten dilution being equal. Similarly, when chemical interactions between bran and gluten would occur, the high particle surface promoted by micronization should impair the gluten development (Noort et al., 2010).

Gluten solvation studies as well as the exposure of hydrophobic sites were studied for further clarifying the influence of coarse and fine bran on gluten development and dough quality. The number of surface-exposed hydrophobic sites, their accessibility to the fluorescent hydrophobic probe ANS, and their affinity towards the probe were assessed by spectrofluorimetric titration. Dough samples of appropriate composition were prepared adding water according to Farinograph water absorption at increasing ANS concentrations. Data processing through standard ligand binding (such as the Scatchard plot proposed in Fig. 3.4) gave the binding parameters presented in Table 3.3.

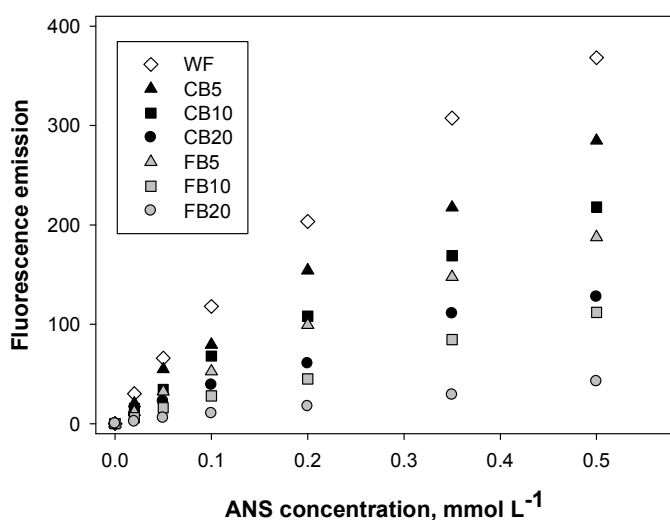


Fig. 3.4. Scatchard plot: intensity of ANS fluorescence at 470 nm as a function of ANS concentration in dough samples enriched in coarse (CB) and fine (FB) buckwheat bran.

As previously reported by Bonomi et al. (2004), proteins in common wheat dough have a high number of surface hydrophobic sites available for ANS probe binding, as indicated by F_{max} (Table 3.3). Enrichment in coarse buckwheat bran resulted in a gradual decrease of the number of protein sites available for the binding of the probe, but had only a modest effect on their average affinity for the ANS (as indicated by the apparent dissociation constant, K_d). Addition of micronized buckwheat bran had far more dramatic effects on both F_{max} and K_d , especially when high bran levels were considered (> 10%). By combining the number of hydrophobic sites and their affinity to the probe, the PSH index dropped from ~ 196 in wheat dough to ~ 44 in the presence of 20% coarsely ground buckwheat bran, falling to ~ 11 in the presence of 20% fine buckwheat bran. As pointed out in recent studies (Jazaeri et al., 2015; Quayson et al., 2016), hydrophobic interactions are among the main forces involved in network formation in weak wheat dough.

Table 3.3. ANS binding parameters for wheat dough enriched in coarse (CB) and fine (FB) buckwheat bran at optimal water content.

Sample	K_d^{app} ($\mu\text{mol ANS/g flour}$)	F_{max}	F_{max} (corrected for the protein content)	Surface hydrophobicity index (PSH)
WF	0.344	716	68	196
CB5	0.399	582	51	128
CB10	0.460	476	39	84
CB20	0.468	286	21	44
FB5	0.525	458	40	77
FB10	0.908	383	32	35
FB20	1.065	159	12	11

Fluorescence intensity at saturating ANS concentration (F_{max}) and the apparent dissociation constant of the ANS/flour complex (K_d^{app}) were calculated from ANS-titration experiments analyzed through the Scatchard plots. The protein surface hydrophobicity index (PSH) is defined as [F_{max} (corrected for the protein content) $\times (K_d^{app})^{-1}$].

Since the addition of either type of buckwheat bran did not alter the protein profile in the systems under investigation (not shown), it appears reasonable to attribute the effects previously discussed to the fact that proteins in the system did not undergo the structural rearrangements required to bring hydrophobic regions from the interior of proteins (or of the protein aggregates) to their surface. These rearrangements largely depend on water availability and gluten hydration, as demonstrated by a number of spectroscopic solvation studies (Bonomi et al., 2013, 2004). As observed in Fig. 3.5, common wheat proteins were almost fully solvated at 45% water in the dough, as assessed by the Farinograph analysis.

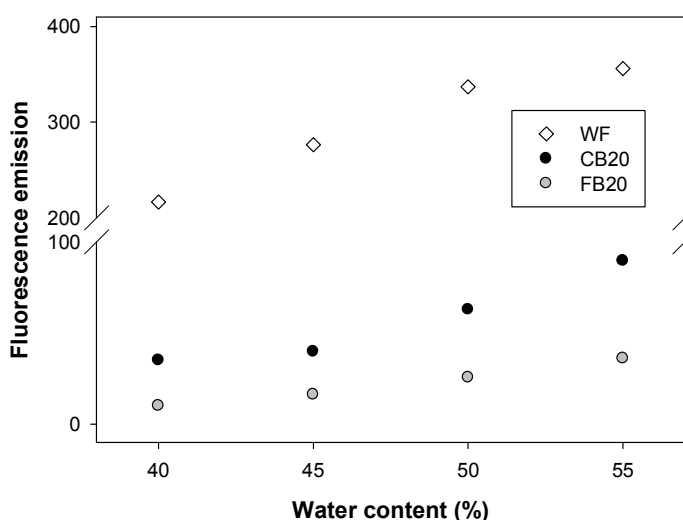


Fig. 3.5. Intensity of ANS fluorescence at 470 nm as a function of the amount of meal enriched in coarse (CB) and fine (FB) 20% buckwheat bran. Excitation was at 390 nm.

In the presence of buckwheat bran, gluten protein solvation was highly delayed. In fact, proteins in bran-enriched dough did not complete their solvation (and therefore, the exposure of ANS-binding hydrophobic

sites) even at water contents as high as 55%, as indicated by the continuous and progressive increase in fluorescence in Fig. 3.5. Nevertheless, these hydration values were incompatible with the formation of a dough but were associated to the production of a batter. Fig. 3.5. made also evident a more pronounced impairment of protein solvation when small-sized bran is used. The differences observed between fine and coarse particle bran could be explained by the higher dispersion of fine particles compared to the coarse one in the mass, accounting for a lower rate of gluten hydration with the fine bran due to the competition for water. The different competition for water could indeed explain differences in water absorption levels as observed in the Farinograph test (Table 3.1).

3.3.2 Baking properties

Either coarse or fine buckwheat bran were added to wheat flour to produce bread in small-scale trials. Already from a visual quality standpoint (Fig. 3.6.), it can be seen that incorporation of micronized bran had higher worsening effect on loaves volume and slices height and conferred darker and redder color to crust and crumb. The high content in phenolic and proteins in buckwheat bran promoted the formation of more Maillard reaction compounds, responsible for change in product color (Heiniö et al., 2016). The reaction seemed to be more pronounced when bran was subject to severe grinding likely due to the increase in bran surface and the exposure of reactive compounds.



Fig. 3.6. Loaves and bread slices of samples enriched in coarse (CB) or fine (FB) buckwheat bran.

The effect of buckwheat bran enrichment on bread quality is summarized in Table 3.4. All bran-enriched bread samples had lower specific volume than the control, except for sample CB5 that showed no statistically differences ($p \leq 0.05$) from the wheat reference. At similar enrichment level, FB5 caused a significant decrease (about 15%) in bread specific volume compared to CB5.

Although the detrimental effect of fiber-material on bread volume is well known (Lai et al., 1989; Pomeranz et al., 1977), the effect of particle size on this parameter is still controversial: some authors demonstrated that enrichment in wheat fine bran had no effect (Curti et al., 2013; Sanz-Penella et al., 2012) on loaf volume. Coda et al. (2014) identified 160 μm as the optimal bran particle size for bread production. Conversely, Noort et al. (2010) found a negative effect of bran particle size reduction on bread volume.

The decrease in specific volume could be explained by the detrimental effect of bran addition on gluten development as indicated by dough rheology results. As suggested by the protein structure traits, bran with

lower particle size enhanced competition for water and could explain the worsening effect of fine bran particles compared to coarse bran ones.

Crumb density significantly decreased with either coarse or fine bran enrichment as a result of the low specific volume of the bread (Table 3.4). As regard moisture content of fresh crumb, the progressive dough enrichment in coarse bran resulted in more wet samples (Table 3.4). On the contrary, no significant differences were observed among samples added with fine bran particles. The moisture content was likely due to the higher water content of dough formulation ($R^2 = 0.983$; $p < 0.00$).

Table 3.4. Properties of wheat bread enriched in coarse (CB) and fine (FB) buckwheat bran.

Sample	Specific volume (mL/g)	Crumb Density (g/mL)	Moisture content (%)	a_w	Hardness (N)	Cohesiveness	Springiness	Resilience
WF	3.5 ± 0.6 ^a	0.23 ± 0.01 ^d	43.7 ± 0.1 ^e	0.966 ± 0.002 ^{bc}	1.2 ± 0.1 ^e	0.88 ± 0.01 ^a	0.988 ± 0.006 ^a	0.50 ± 0.01 ^a
CB5	3.6 ± 0.2 ^a	0.22 ± 0.01 ^d	44.30 ± 0.09 ^c	0.968 ± 0.002 ^{ab}	1.10 ± 0.09 ^e	0.88 ± 0.09 ^a	0.988 ± 0.002 ^a	0.497 ± 0.006 ^a
CB10	3.3 ± 0.2 ^b	0.25 ± 0.01 ^c	44.6 ± 0.2 ^b	0.967 ± 0.002 ^{abc}	1.5 ± 0.1 ^d	0.87 ± 0.02 ^{ab}	0.981 ± 0.008 ^a	0.486 ± 0.005 ^b
CB20	2.7 ± 0.2 ^c	0.31 ± 0.02 ^b	45.5 ± 0.1 ^a	0.969 ± 0.002 ^a	3.0 ± 0.3 ^c	0.83 ± 0.01 ^c	0.96 ± 0.01 ^b	0.454 ± 0.007 ^c
FB5	3.3 ± 0.2 ^b	0.25 ± 0.01 ^c	44.19 ± 0.02 ^{cd}	0.9673 ± 0.0008 ^{abc}	1.7 ± 0.1 ^d	0.86 ± 0.01 ^b	0.98 ± 0.01 ^a	0.494 ± 0.005 ^a
FB10	2.60 ± 0.09 ^c	0.30 ± 0.02 ^b	44.2 ± 0.1 ^{cd}	0.965 ± 0.003 ^c	3.6 ± 0.6 ^b	0.82 ± 0.02 ^c	0.95 ± 0.01 ^b	0.458 ± 0.009 ^c
FB20	2.3 ± 0.1 ^d	0.397 ± 0.008 ^d	44.13 ± 0.06 ^d	0.966 ± 0.001 ^{bc}	7.3 ± 0.4 ^a	0.746 ± 0.007 ^d	0.91 ± 0.01 ^c	0.37 ± 0.01 ^d

All data are expressed as the mean ± standard deviation (Specific volume: n = 8; Hardness; Corrected hardness and Cohesiveness: n = 12; Moisture: n = 4; all analyses were performed on 2 independent baking tests). Different letters in the same column indicate statistically significant differences (LSD; p ≤ 0.05).

The TPA test confirmed the effects on specific volume of either buckwheat bran type or level on bread quality (Table 3.4). Bran addition resulted in detrimental effects on bread crumb texture, as indicated by a general increase in crumb hardness and decrease in its cohesiveness with higher enrichment with either coarse and fine bran (Table 3.4). In cellular solids, the hardness of the material is related to its density, following on the Ashby-Gibson theory (Renzetti and Jurgens, 2016). In fact, a strong correlation between crumb hardness and crumb density was observed at day 0 (Fig. 3.7, $p < 0.00$).

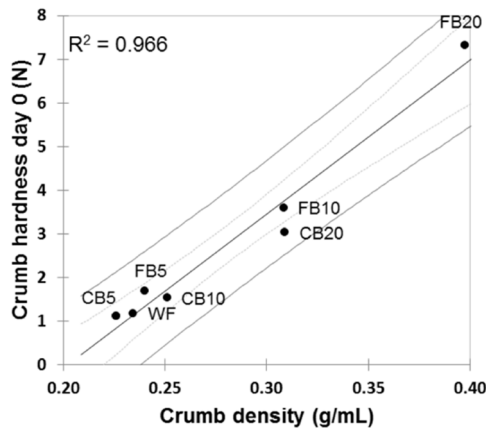


Fig. 3.7. Correlation between crumb hardness and crumb density at day 0.

The textural properties of cellular food are controlled by the volume fraction of the air (Renzetti and Jurgens, 2016), as observed also in this case. Therefore, the adapted Ashby-Gibson model was applied to correct for differences in crumb density (Figure 3.8).

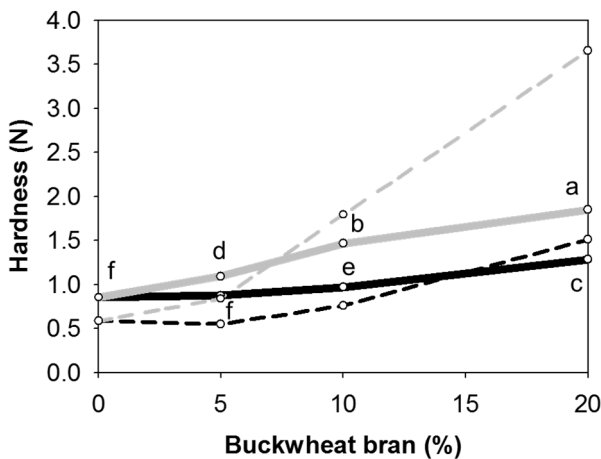


Fig. 3.8. Crumb hardness of bread enriched in buckwheat bran with (solid line) and without applying (dash line) the correction for crumb density. Black line: coarse bran (CB). Grey line: fine bran (FB). Interaction between type and % of bran is significant ($p \leq 0.05$). Different letters indicate significant differences for corrected hardness parameter (LSD; $p \leq 0.05$).

Significant differences among samples enriched in coarse bran (solid black line) and samples enriched in micronized bran (solid grey line) persisted even though the distance between the lines was decreased, confirming that the changes in fresh bread crumb texture could be only partially described by the density differences. Enrichment in fine bran clearly resulted into deeper texture changes. Any deviation from the

model, can be ascribable to modification of the cellular structure by the incorporation of fiber. The increase in the volume fraction of bran Φ_{bran} in the solid crumb matrix may enhance the elastic moduli due to stronger particle interactions. Additionally, the significant variations in crumb moisture content (Table 3.4) can also affect the textural properties of the crumb. The interplay between these two mechanisms seemed to relate with the corrected hardness at day 0, as indicated by the high correlation between measured and predicted hardness after correction (Fig. 3.9A).

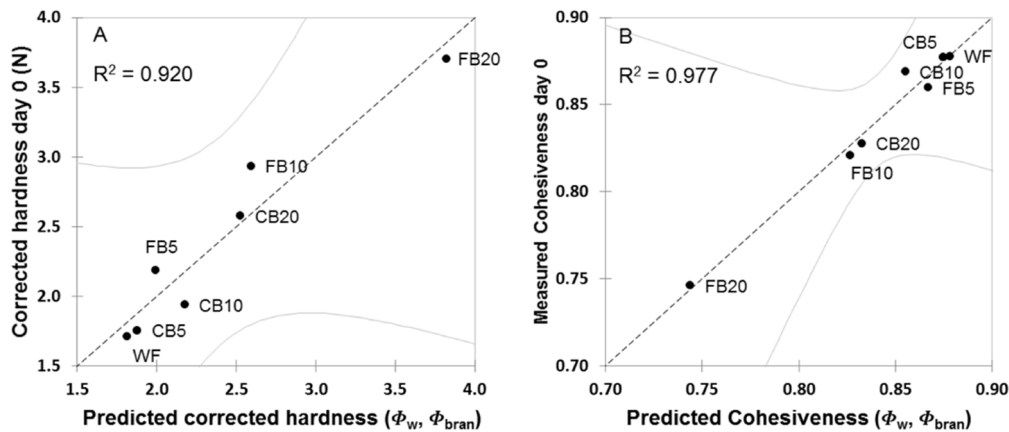


Fig. 3.9. Correlation between the TPA parameters measured and those predicted from compositional data. **A)** correlation between corrected hardness measured and corrected hardness predicted from Φ_w and Φ_{bran} ($p < 0.00$ and $p < 0.02$ for Φ_{bran} and Φ_w , respectively); **B)** correlation between measured cohesiveness and cohesiveness predicted from Φ_w and Φ_{bran} ($p < 0.00$ for both Φ_w and Φ_{bran}).

Fiber reduced the crumb ability of recovery after the first compression (springiness and resilience, Table 3.4), worsening its structural integrity when subjected to compressive forces (cohesiveness). A reduction in cohesiveness indicated an enhanced micro-fracturing of the solid lamellae around the air cells during the compression. Such micro-fractures were enlarged by discontinuity in the polymeric crumb network resulting from the increased volume of solid particles, i.e. Φ_{bran} . Furthermore, crumb moisture also played a role. Consequently, the observed variation in cohesiveness could be well described by the interplay of these two mechanisms (Figure 3.9B). Bread enriched in 5% coarse buckwheat bran (CB5) had overall the best baking performances, always comparable ($p \leq 0.05$) to that of wheat bread, showing the possibility of enriching bread in fiber content, without decreasing product quality.

During storage, a progressive increase in crumb hardness was observed for all breads as a result of staling (Fig. 3.11). The differences in hardness observed at day 0 among wheat bread reference and the bran-enriched bread samples persisted during storage. It should be noted that the crumb samples were stored in sealed containers to distinguish the effects of starch retrogradation from moisture redistribution between crumb and crust. All hardness data were corrected for density by applying the Ashy-Gibson theory. The corrected hardness values were strongly correlated with the melting enthalpy of starch during storage (Fig. 3.10, $p < 0.00$). Hence, the changes in hardness during storage for all bread types could be mainly related to the variations in density and the starch retrogradation.

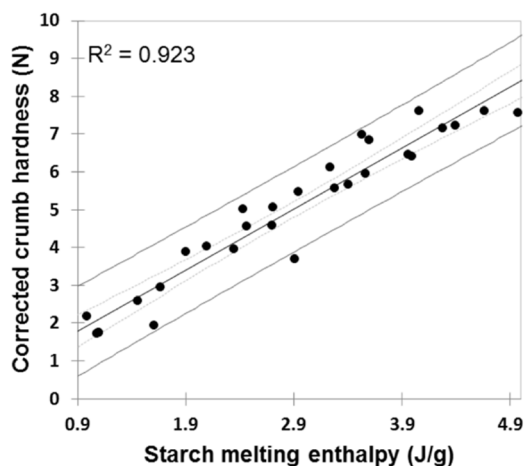


Fig. 3.10. Correlation between corrected crumb hardness and starch melting enthalpy during storage.

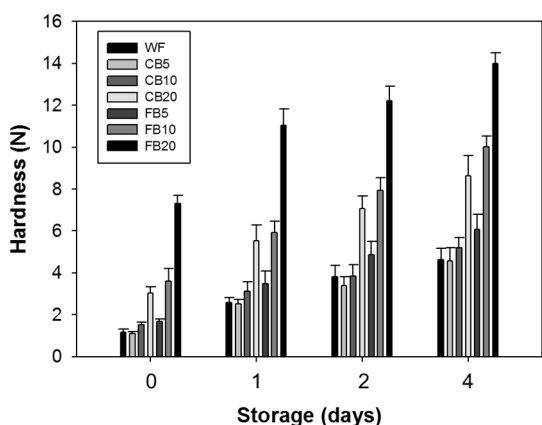


Fig. 3.11. Crumb hardness during storage.

3.4 Conclusions

Wheat bread enrichment with high-fiber material generally results in altered rheological and baking properties in respect to products derived from refined flour. The thermo-mechanical behavior of dough was found to be mainly related to starch phase transitions during heating. The enrichment in coarse or fine bran influenced key transitions such as the onset of starch gelatinization and the peak gel strength of the starch paste. The onset of starch gelatinization was controlled by the interplay of the volume fractions of water and bran, while the peak gel strength (as well as the temperature at peak) was controlled by the interplay of the volume fractions of starch and water. With regards to the extensional properties of the dough, the detrimental effects with fiber enrichment seemed mainly related to the decrease in gluten volume fraction, irrespective of bran particle size. However, gluten surface hydrophobicity properties clearly pointed out at a distinct effect of particle size on gluten structural arrangements for similar level of bran addition. The larger detrimental effects of fine bran compared to coarse bran ones may be related to a more homogeneous dispersion of bran particles thus enhancing the effects on gluten hydration and gluten proteins interactions. The negative effects of fiber-enrichment on dough rheology and gluten development were reflected on the bread baking quality, as indicated

by the specific volume decrease. Fine bran addition resulted in lower volume compared to coarse bran, following on the described mechanisms. With regards to texture, variations in the perceived crumb hardness were mainly related to changes in crumb density. The application of an adapted Ashby-Gibson model to correct hardness values for the variation in density revealed that the mechanical properties of the solid crumb matrix were controlled by the variation in moisture and in bran volume fraction. Similarly, moisture and bran volume fraction in the crumb were the main factors explaining the variations in crumb cohesiveness. Addition of bran enhanced the elastic moduli of the solid crumb matrix, thus increasing its hardness and affecting its structural integrity during compression. Finally, changes in hardness during storage could be mainly related to starch retrogradation.

Overall, this study identified a number of physical mechanisms that can describe the influence of buckwheat bran addition on the rheological and baking performance of wheat dough and the textural properties of the resulting breads.

3.5 References

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4. Superheated Steam (SS) treatment on buckwheat bran: effects on enriched-wheat dough and bread characteristics

4.1 Introduction

Common buckwheat (*Fagopyrum esculentum*) is a pseudocereal that has recently gained attention for the production of enhanced cereal-based products thanks to the high content in fiber, antioxidant compounds (such as rutin) and proteins with well-balanced amino acid compositions (Lin et al., 2009; Zhang et al., 2012).

In buckwheat seeds, as for cereal grains, the nutritional-relevant compounds (e.g. fiber and antioxidants) are mainly located in the outermost layers of grain as in the pericarp (hull) or in the seed coat (bran fractions) (Steadman et al., 2001a; Steadman et al., 2001b). Despite the several nutritional advantages associated with the consumption of bran-rich or whole-grain products, it is well-known that the incorporation of high-fiber fractions results into technological issues and negative effects on the overall acceptability by consumers (Foschia et al., 2013).

Hydro-thermal treatments (e.g. microwave, autoclaving, toasting/roasting and steam-cooking) have been used in few works on wheat bran as suitable approaches to modify bran structure with the aim of counteracting the detrimental effect on bread quality (De Kock et al., 1999; Nelles et al., 1998). Although it is known that these treatments generally lead to inactivation of heat-sensitive components (e.g. enzymes) and may induce change in bran hydration properties (Jacobs et al., 2016), no consistent view can be summarized from the literature on bread making performance of bran treated in a such way (Hemdane et al., 2016).

As regard buckwheat, autoclaving of achene increased the amount of bound phenolics and decreased the rutin conversion into quercetin, while retaining cooking quality of whole wheat pasta enriched in 10-30% buckwheat (Jambrec et al., 2015). Moreover, the comparison of roasting and extrusion of dark buckwheat flour resulted in no changes in total phenolic content or a slight decrease in antioxidant activity (only for extrusion) in respect to untreated sample (Şensoy et al., 2006).

Among heating processes, Superheated Steam (SS) drying is an innovative drying technology used to remove excess water from the material exploiting steam beyond its boiling point. It has been tested on different food matrices with the aim of drying sample limiting collateral effect on product quality together with saving energy (Sehrawat et al., 2016).

Regarding to cereals, SS has been employed in recent studies to improve either the storage stability (Wu et al., 2016b) or the nutritional attributes (Wu et al., 2016a) of milled rice. SS was applied also to oat as an alternative method for groats stabilization in replace of wet steam conditioning and kiln drying, to obtain improved sensory quality (Head et al., 2011). Moreover, SS has been found to be an effective method in reducing contamination by mycotoxin and spores in wheat grains (Cenkowski et al., 2007). Finally, SS treatment was successfully used on β -glucans extracted from oat bran to increase β -glucans water solubility in respect to conventional methods (Izydorczyk et al., 2014). Recently SS has been proposed in a patent as a process to modify wheat or corn bran in order to enhance the nutritional value of enriched food (Diaz et al., 2015).

Apart from these work, the effect of SS on bran techno-functionality for facilitating its addition in baking products has not been tested yet. Therefore, this study aimed at evaluating the effect of SS on buckwheat bran properties and its influence on the rheological properties and baking quality of high-enriched wheat dough/bread.

4.2 Materials and methods

4.2.1 Materials

Both coarse and micronized buckwheat bran (Refer to Section 2.2.1) were treated by Superheated Steam (SS) in a pilot apparatus designed and manufactured by the TNO. Samples were treated at different combination of temperature (120, 140 or 160 °C), water activity (0.4, 0.6 or 0.7) and time (20 or 40 min). The parameters ranges were set based on preliminary trials on different cereals.

SS settings are shown in Table 4.1. Before treatment, samples were conditioned at 50°C for 5 min to minimize condensation of vapor at the initial stage of drying. SS-treatment was carried in single for each condition.

In the second part of the work, the following sample were considered: SS120 (temperature: 120 °C; a_w : 0.7; time: 40 min), SS140 (temperature: 140 °C; a_w : 0.7; time: 40 min) and SS160 (temperature: 160 °C; a_w : 0.7; time: 40 min).

4.2.2 Methods

4.2.2.1 Water Binding Capacity (WBC)

Refer to Section 2.2.2.5.

4.2.2.2 Water solid extraction: charge level and supernatant sugar composition

Refer to Section 2.2.2.6.

4.2.2.3 Compositional traits

Ash, protein, total starch content, SDF, IDF, susceptibility to α -amylase (Damaged starch): refer to Section 2.2.2.4. Moisture content of bran was measured as weight-loss after drying sample at 105 °C for 18 h.

The level of furosine was determined in bran by HPLC according to the conditions described in Standard ISO 18,329:IDF 193 (ISO-IDF, 2004), as detailed in (Stuknyte et al., 2014): 500 mg of sample were hydrolysed at 110 °C for 23 h, purified by solid-phase extraction and then submitted to HPLC analysis. Furosine was quantified using furosine dihydrochloride (NeoMPS, PolyPeptide Laboratories, Strasbourg, France) as external standard. The results are expressed as milligrams of furosine/100 g of protein.

4.2.2.4 Color evaluation

Refer to Section 2.2.2.1.

4.2.2.5 Dynamic water vapor sorption experiments

Refer to Section 2.2.2.3.

Table 4.1. SS treatment conditions on buckwheat bran with different particle size

Code	Buckwheat bran sample	Temperature (°C)	a_w	time (min)	Pressure (bars ABS)
C-Ref	Coarse	-	-	-	-
C12-4-20	Coarse	120	0.4	20	0.79
C12-4-40	Coarse	120	0.4	40	0.79
C12-6-20	Coarse	120	0.6	20	1.19
C12-7-20	Coarse	120	0.7	20	1.39
C12-7-40	Coarse	120	0.7	40	1.39
C14-4-20	Coarse	140	0.4	20	1.45
C14-4-40	Coarse	140	0.4	40	1.45
C14-6-20	Coarse	140	0.6	20	2.17
C14-7-20	Coarse	140	0.7	20	2.53
C14-7-40	Coarse	140	0.7	40	2.53
C16-4-20	Coarse	160	0.4	20	2.47
C16-4-40	Coarse	160	0.4	40	2.47
C16-7-20	Coarse	160	0.7	20	4.33
C16-7-40	Coarse	160	0.7	40	4.33
F-Ref	Fine	-	-	-	-
F12-4-20	Fine	120	0.4	20	0.79
F12-4-40	Fine	120	0.4	40	0.79
F12-6-20	Fine	120	0.6	20	1.19
F12-7-20	Fine	120	0.7	20	1.39
F12-7-40	Fine	120	0.7	40	1.39
F14-4-20	Fine	140	0.4	20	1.45
F14-4-40	Fine	140	0.4	40	1.45
F14-6-20	Fine	140	0.6	20	2.17
F14-7-20	Fine	140	0.7	20	2.53
F14-7-40	Fine	140	0.7	40	2.53
F16-4-20	Fine	160	0.4	20	2.47
F16-4-40	Fine	160	0.4	40	2.47
F16-7-20	Fine	160	0.7	20	4.33
F16-7-40	Fine	160	0.7	40	4.33

4.2.2.6 Protein solubility in buckwheat bran fractions

The solubility of proteins in various buckwheat samples was determined in triplicate by using various buffers as described by Barbiroli et al. (2013). Proteins were extracted by dispersing 0.5 g of finely ground samples in 10 mL of 0.05 M sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl. After stirring at room temperature for 60 min and removal of insoluble materials by centrifugation (10000 x g for 20 min at 20 °C), the protein content in the supernatant was assessed by a dye-binding method (Bradford, 1976). Where indicated, the buffer used for protein extraction also contained 6 M urea or 6 M urea and 10 mM dithiothreitol (DTT). Results are expressed as mg soluble proteins/g sample.⁹

4.2.2.7 SDS PAGE

A known amount of sample (containing 0.015 mg protein in buffer) was mixed with an equal volume of SDS–PAGE denaturing buffer (0.125 M Tris-HCl, pH 6.8, 50% Glycerol, 1.7% SDS; 0.01% Bromophenol Blue) containing 1% 2-mercaptoethanol when indicated, and heated at 100 °C for 10 min. As described in previous studies (Barbiroli et al., 2013) SDS–PAGE was carried out on a fixed porosity gel (12% monomer), by using a MiniProtean apparatus (Bio-Rad, Richmond, VA, USA). Gels were stained with Coomassie Blue.

4.2.2.8 Dough preparation

Either untreated (BW) or SS treated (SS120, SS140 or SS160) buckwheat bran was added to commercial wheat flour at 20% replacement level. Since superheated steam treatment led to a progressive sample drying, the bran quantity for dough preparation was rescaled according to bran moisture content.

Dough preparation: refer to Section 3.2.2.1.

4.2.2.9 Dynamic Mechanical Thermal Analysis (DMTA)

Refer to Section 3.2.2.2.

4.2.2.10 Kieffer extension test

Refer to Section 3.2.2.5.

4.2.2.11 Bread making

Refer to Section 3.2.2.7.

Cylindrical crumb samples were collected from fresh bread and stored at controlled temperature (18 °C) for 1 day in sealed polyethylene containers.

4.2.2.12 Texture Profile Analysis (TPA)

Refer to Section 3.2.2.8.

4.2.2.13 Statistical Analysis

Principal Components Analysis (PCA) was used as an “explorative” method to verify the effect of SS-treatment on the overall bran characteristics. PCA was carried out considering for each sample the average

values from each analysis. Data were normalized for the standard deviation and centered for the average. The first two components were considered in the results discussion.

Analysis of variance (one-way ANOVA). Different bran, dough or bread samples were considered as factors for ANOVA. When a factor effect was found significant ($p \leq 0.05$) significant differences among the respective means were determined using Fischer's Least Significant Difference (LSD) test. Both tests were performed by using XLSTAT Version 2016.02 (Addinsoft, Paris, France).

4.3 Results and discussion

4.3.1 Set-up of Superheated Steam (SS) treatment conditions on buckwheat bran properties

Different physical characteristics of bran were chosen to screen the effect of SS treatment conditions. Color was chosen as an indicator of the appearance of material that can be greatly modified by severe heat treatments. Since the behavior between bran (fiber) and water plays an important role in defining the characteristics of dough and therefore the quality of baked products, Water Binding Capacity (WBC) was used as a rapid method to assess the hydration properties of enriching material. WBC includes only the water strongly absorbed by bran material and it can be linked to the amount of water retained by the fiber (Lebesi and Tzia, 2012). Finally, the sugar composition of supernatant was assessed to verify whether intense treatments might produce any evident changes in the insoluble material (e.g. hydrolysis of non-starch polysaccharides).

The overall effect of superheated steam on physical characteristics of buckwheat bran was evaluated by Principal Component Analysis (Fig. 4.1). The first two components described 61.87% of the overall variability. As regard color, samples were divided in two directions mostly along F1 axes: samples in the left part of the plan tended to have higher L^* values (lightness), while samples in the right part showed higher a^* (redness) values. Both coarse (C-REF) and micronized (F-REF) bran were located at the bottom of the plot, having higher L^* values and lower a^* (redness) and b^* (yellowness) values than SS-treated samples. Color changes were mainly due to non-enzymatic browning as Maillard reaction, occurring at high temperature drying, highly promoted by the richness in lysine (Bonafaccia et al., 2003) and of soluble carbohydrate - that are most that concentrated in bran (Steadman et al., 2001b) - can promote Maillard reaction. Temperature was more effective on color changes than a_w and time and micronized bran samples had in general low L^* and high a^* and b^* values (Table 6.2). To conclude, the higher the temperature, the darker and redder the sample.

In PCA bi-plot, differences in hydration property (WBC) were shown along the F2 axes. In respect to reference samples, the trend in WBC followed a semi-circular distribution (dotted line L1): an initial increase was found in all samples treated at 120 °C and in some of them treated at 140 °C. On the contrary, 160 °C-treated samples were clearly located in the bottom right part of the plot, along axes F1, indicating a decrease in WBC. Jacobs et al. (2016) studied the effect of dry treatment on wheat bran hydration properties and found that both strong and weak binding capacity were not affected by heat treatment. Anyway, any difference observed could be likely due to the different material used or as the result of interfering phenomena, such as the partial starch gelatinization during the initial stages of SS treatment that can modify hydration properties. Fine-bran samples

tended to have slight lower WBC values in respect to coarse bran samples. As observed in wheat samples, larger particles were able to retain more water (Jacobs et al., 2015) when bran was subjected to external force (centrifugation).

Supernatant obtained from WBC analysis was collected to verify any effect caused by SS-treatment on soluble components of bran. No univocal trend occurred in total amount of soluble compounds present in supernatant: in coarse bran sample SS caused a general slight increase when respectively the lowest (120 °C) and highest (160 °C) temperatures were applied, while in fine bran, the increase was observed only in the case of sample treated at 160 °C for 40 min ($a_w = 0.7$). However, micronization of bran, contributed to a general increase in solid in supernatant when compared with coarse bran treated at the same conditions, likely due to a more water extractability caused by highest particle surface. Considering the latter parameter, samples were distributed on PCA plot along the F2, in the opposite part respect to WBC trend.

Again, the electrical conductivity of supernatant (charge level) was influenced by SS treatment. A drastic drop (around -30%) in charge level was observed with mildest conditions (140 °C). The application of more intense conditions – in particular the temperature – increased the electric charge of supernatant over the initial values of untreated bran. Since buckwheat bran is rich in mineral as potassium, magnesium, calcium and sodium (Christa and Soral-Šmietana, 2008), the change in electric behavior of water extracts could be addressed to lower minerals solubilization.

The effect of SS treatment on bran soluble compounds was even more evident when sugar compositions of supernatant was considered (Table 4.4, Table 4.5). A progressively general increase in total sugar amount was observed when more severe conditions were applied (e.g. temperature), reaching a maximum (approximately 2-fold in respect to reference sample) in micronized bran treated at 160 °C for 40 min ($a_w = 0.7$). SS treatment caused a remarkable increase in glucose and in galactose content respectively in almost all samples. Rhamnose content had a general but more limited increase in respect to glucose. Xylose content in supernatant increased starting from sample treated at 140 °C while the amount in arabinose was higher than reference bran only when severe treatment conditions were applied. Mannose and fucose were found only in small amount.

Non-starch polysaccharides of buckwheat contain a high amount of pectic polysaccharides, especially arabinans with high degree of ramification. According to the study of Wefers and Bunzel (2015), the more abundant monosaccharide in buckwheat insoluble fiber are glucose (39.2%) and arabinose (27.7%), whereas in the soluble fraction are mannose (24.5%), galacturonic acid (22.1%) and arabinose (18.4%). The increase in sugar content in supernatant (that should be assumed to contain soluble fiber) may likely due to a partial hydrolysis of the insoluble fiber (very abundant in both coarse and fine buckwheat bran, refer to Section 2.3.3) favored by the hydrothermal treatment. Again, the highest temperature (160 °C) was found to be more effective in the modification of buckwheat bran. Therefore, the application of more drastic conditions (e.g. temperature) produced a continuous increase in sugars in the supernatant. Considering the results related to the total water extractable solids, it can be stated that other relevant components such as soluble proteins (up to 80% in buckwheat flour; Tomotake et al., 2002) were affected in a different way than sugars. The application of higher

temperatures likely promoted proteins polymerization, lowering their solubility and therefore the content in total water extractable decreased reaching a minimum (Table 4.2 and Table 4.3).

PCA analysis of data (Fig. 4.1) put in evidence that all sugars content, except mannose, seemed to have the same load in distributing samples. At the same conditions, fine bran did not appear to cause relevant differences in respect to coarse bran as showed by the proximity of samples of the series C and F in the plot.

For this reason, looking at either WBC and supernatant characteristic and supernatant sugar composition, coarse bran was chosen for the following research activity (Refer to Section 4.3.2; 4.3.3 and 4.3.4), considering that buckwheat coarse bran had less detrimental effect on the quality of wheat dough and bread enriched (Refer to Section 3.3.1 and 3.3.2). Furthermore, the preliminary tests on processing conditions showed that temperature - rather than a_w or time - promoted the most important modifications on bran technological (e.g. WBC) and compositional characteristic (e.g. water extractable sugar composition). For this reason, temperature was varied from 120 to 160°C, while a_w and time were kept constant ($a_w = 0.7$, time = 40 min).

Variables (axes F1 and F2: 61.87 %)

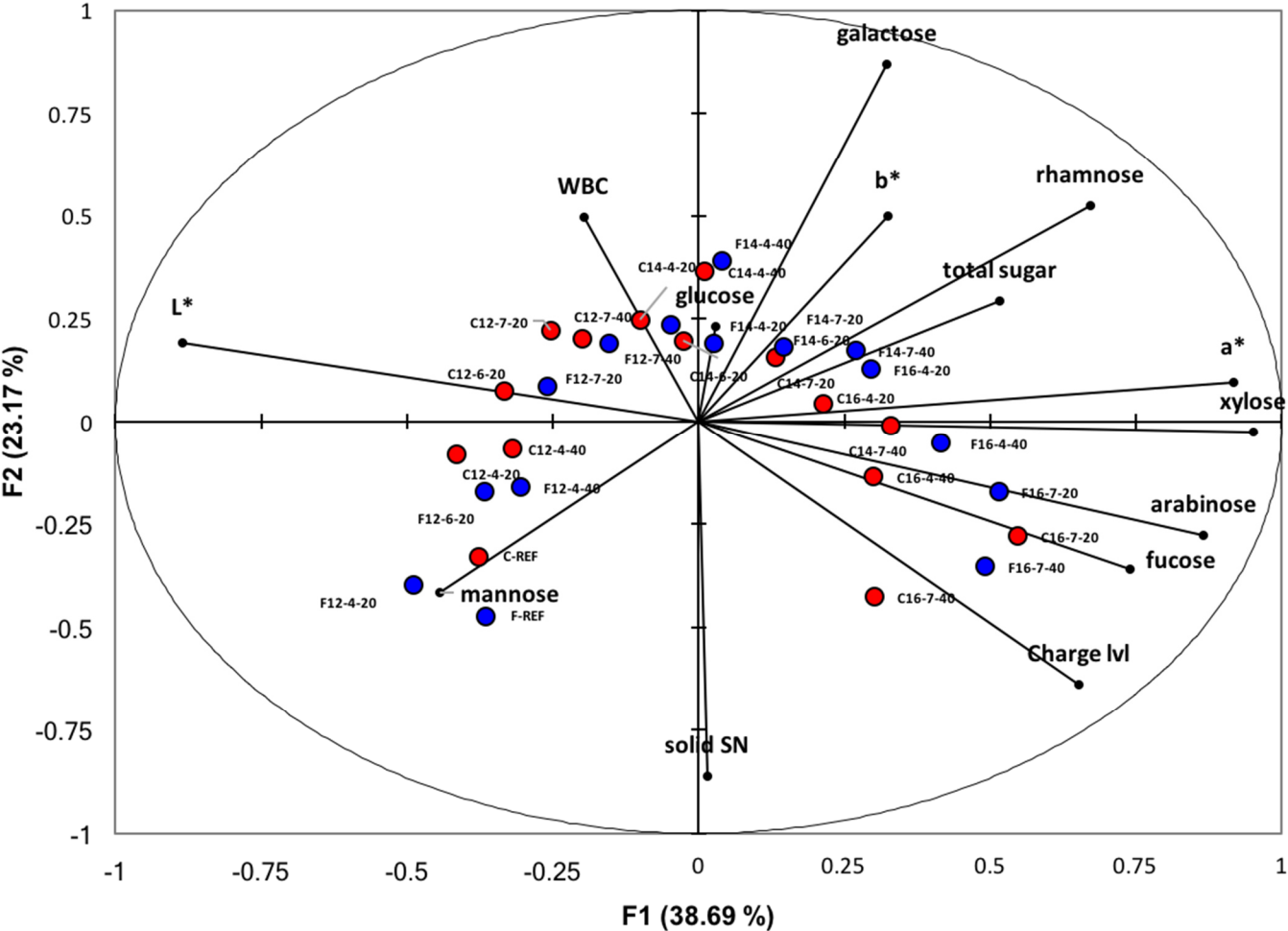


Fig. 4.1. Principal Components Analysis: overall effect of superheated steam on buckwheat bran.

Table 4.2. Color indices (L*, a* and b*), WBC and supernatant characteristics of coarse buckwheat bran treated by SS.

Sample	L*	a*	b*	WBC (g water/g sample)	Solid SN (mg/mL)	Charge level (μ S/cm)
C-Ref	61.9 \pm 0.3	3.33 \pm 0.09	13.0 \pm 0.1	2.8 \pm 0.1	5.3 \pm 0.1	1151 \pm 22
C12-4-20	57.8 \pm 0.7	5.0 \pm 0.1	15.72 \pm 0.08	3.1 \pm 0.2	5.95 \pm 0.05	830 \pm 10
C12-4-40	56.7 \pm 0.9	5.7 \pm 0.1	15.8 \pm 0.2	2.95 \pm 0.03	5.56 \pm 0.02	919 \pm 2
C12-6-20	55.0 \pm 0.4	6.6 \pm 0.1	15.9 \pm 0.1	3.12 \pm 0.06	5.37 \pm 0.03	758 \pm 3
C12-7-20	56.8 \pm 0.9	6.4 \pm 0.1	16.2 \pm 0.2	2.81 \pm 0.07	4.66 \pm 0.03	772 \pm 9
C12-7-40	54 \pm 1	7.4 \pm 0.3	15.87 \pm 0.06	2.9 \pm 0.1	4.6 \pm 0.1	801 \pm 21
C14-4-20	55 \pm 1	7.26 \pm 0.07	16.9 \pm 0.2	2.89 \pm 0.07	4.78 \pm 0.01	855 \pm 9
C14-4-40	52.3 \pm 0.7	8.4 \pm 0.1	16.5 \pm 0.2	4.5 \pm 0.2	4.68 \pm 0.09	913 \pm 15
C14-6-20	52.8 \pm 0.6	8.32 \pm 0.07	16.7 \pm 0.2	2.66 \pm 0.08	4.81 \pm 0.08	862 \pm 17
C14-7-20	49.4 \pm 0.7	9.16 \pm 0.05	16.5 \pm 0.2	2.61 \pm 0.05	4.85 \pm 0.03	917 \pm 3
C14-7-40	46.0 \pm 0.8	9.8 \pm 0.2	15.3 \pm 0.2	3.9 \pm 0.2	5.23 \pm 0.05	1074 \pm 7
C16-4-20	48.6 \pm 0.6	9.3 \pm 0.1	15.31 \pm 0.05	2.29 \pm 0.03	5.22 \pm 0.01	1037 \pm 42
C16-4-40	38.3 \pm 0.7	10.4 \pm 0.1	15.7 \pm 0.3	2.6 \pm 0.1	5.47 \pm 0.01	1194 \pm 13
C16-7-20	41.4 \pm 0.9	9.4 \pm 0.2	13.02 \pm 0.09	2.28 \pm 0.09	5.93 \pm 0.05	1222 \pm 41
C16-7-40	35.1 \pm 0.7	9.08 \pm 0.08	11.9 \pm 0.1	2.4 \pm 0.1	7.09 \pm 0.03	1354 \pm 8

All data are expressed as the mean \pm standard deviation (color indices: n = 5; WBC: n = 3; Solid SN and charge level: n = 3 deriving from 3 different water extractions).

Table 4.3. Color indices (L*, a* and b*), WBC and supernatant characteristics of fine buckwheat bran treated by SS.

Sample	L*	a*	b*	WBC (g water/g sample)	Solid SN (mg/mL)	Charge level (μ S/cm)
F-Ref	66.4 \pm 0.5	3.73 \pm 0.05	12.55 \pm 0.04	2.18 \pm 0.06	6.52 \pm 0.10	1251 \pm 38
F12-4-20	58.89 \pm 0.07	5.94 \pm 0.05	16.55 \pm 0.06	2.52 \pm 0.07	7.41 \pm 0.8	899 \pm 13
F12-4-40	59.3 \pm 0.1	5.91 \pm 0.05	16.87 \pm 0.03	2.53 \pm 0.04	6.279 \pm 0.004	978 \pm 13
F12-6-20	56.3 \pm 0.5	6.61 \pm 0.09	16.23 \pm 0.06	2.49 \pm 0.05	6.48 \pm 0.08	842.7 \pm 0.6
F12-7-20	56.4 \pm 0.3	6.7 \pm 0.1	17.40 \pm 0.07	2.58 \pm 0.03	5.26 \pm 0.04	867 \pm 3
F12-7-40	57.3 \pm 0.1	7.07 \pm 0.05	17.62 \pm 0.09	2.51 \pm 0.08	5.04 \pm 0.04	904 \pm 3
F14-4-20	56.3 \pm 0.2	6.80 \pm 0.09	19.05 \pm 0.05	2.58 \pm 0.08	5.11 \pm 0.05	933 \pm 7
F14-4-40	56.0 \pm 0.4	7.57 \pm 0.09	19.61 \pm 0.03	3.98 \pm 0.08	4.80 \pm 0.04	953 \pm 10
F14-6-20	54.5 \pm 0.4	7.73 \pm 0.07	19.74 \pm 0.09	2.24 \pm 0.05	5.20 \pm 0.05	955 \pm 11
F14-7-20	50.7 \pm 0.2	9.16 \pm 0.05	20.91 \pm 0.07	2.19 \pm 0.03	5.07 \pm 0.03	942 \pm 23
F14-7-40	49.4 \pm 0.5	10.24 \pm 0.05	21.7 \pm 0.1	3.54 \pm 0.06	5.24 \pm 0.06	1086 \pm 8
F16-4-20	49.5 \pm 0.4	10.34 \pm 0.04	22.12 \pm 0.08	2.22 \pm 0.09	5.16 \pm 0.09	1074 \pm 5
F16-4-40	39.6 \pm 0.3	12.37 \pm 0.02	21.56 \pm 0.08	2.01 \pm 0.07	5.41 \pm 0.06	1158 \pm 7
F16-7-20	40.5 \pm 0.5	11.61 \pm 0.04	20.3 \pm 0.1	2.17 \pm 0.08	6.00 \pm 0.06	1218 \pm 38
F16-7-40	34.7 \pm 0.3	12.28 \pm 0.06	18.7 \pm 0.1	1.72 \pm 0.02	7.66 \pm 0.02	1366 \pm 5

All data are expressed as the mean \pm standard deviation (color indices: n = 5; WBC: n = 3; Solid SN and charge level: n = 3 deriving from 3 different water extractions).

Table 4.4. Solid extracted sugar composition of coarse buckwheat bran treated by SS.

Sample	Sugar total	Fucose	Rhamnose	Arabinose	Galactose	Glucose	Xylose	Mannose
C-REF	12.5 ± 0.4	0.04 ± 0.01	0.65 ± 0.06	1.9 ± 0.3	5.1 ± 0.1	4.0 ± 0.2	0.55 ± 0.02	0.26 ± 0.03
C12-4-20	18.1 ± 0.2	0.018 ± 0.007	0.63 ± 0.08	0.37 ± 0.03	5.22 ± 0.07	11.2 ± 0.2	0.43 ± 0.01	0.22 ± 0.04
C12-4-40	19.3 ± 0.3	0.024 ± 0.009	0.67 ± 0.07	0.644 ± 0.005	5.53 ± 0.09	11.7 ± 0.4	0.52 ± 0.05	0.25 ± 0.04
C12-6-20	20 ± 1	0.019 ± 0.004	0.65 ± 0.08	0.303 ± 0.008	5.5 ± 0.3	12.6 ± 0.9	0.346 ± 0.004	0.17 ± 0.01
C12-7-20	22.3 ± 1.0	0.018 ± 0.004	0.74 ± 0.05	0.468 ± 0.007	6.2 ± 0.2	14.2 ± 0.9	0.45 ± 0.03	0.19 ± 0.04
C12-7-40	22.1 ± 0.9	0.020 ± 0.001	0.73 ± 0.08	0.66 ± 0.01	6.2 ± 0.2	13.8 ± 0.9	0.50 ± 0.05	0.19 ± 0.03
C14-4-20	23.7 ± 0.6	0.03 ± 0.02	0.8 ± 0.1	0.90 ± 0.06	6.7 ± 0.1	14.4 ± 0.8	0.61 ± 0.09	0.21 ± 0.04
C14-4-40	23 ± 1	0.02 ± 0.01	0.89 ± 0.06	1.82 ± 0.04	6.6 ± 0.3	12.5 ± 0.9	0.76 ± 0.07	0.17 ± 0.01
C14-6-20	22.7 ± 0.3	0.023 ± 0.007	0.7 ± 0.3	1.3 ± 0.2	6.5 ± 0.2	13.1 ± 0.8	0.90 ± 0.09	0.16 ± 0.02
C14-7-20	22.05 ± 0.03	0.04 ± 0.02	0.8 ± 0.1	2.12 ± 0.05	6.36 ± 0.01	11.5 ± 0.3	1.06 ± 0.07	0.16 ± 0.03
C14-7-40	19.89 ± 0.04	0.096 ± 0.001	0.8 ± 0.1	3.50 ± 0.07	5.93 ± 0.03	8.2 ± 0.2	1.19 ± 0.04	0.16 ± 0.03
C16-4-20	21 ± 1	0.029 ± 0.005	0.87 ± 0.04	3.5 ± 0.1	6.4 ± 0.4	8.7 ± 0.8	1.09 ± 0.01	0.17 ± 0.03
C16-4-40	20 ± 1	0.05 ± 0.04	0.8 ± 0.1	3 ± 1	5.9 ± 0.1	8.19 ± 0.02	1.1 ± 0.1	0.17 ± 0.03
C16-7-20	18.6 ± 0.2	0.11 ± 0.07	0.92 ± 0.07	5.1 ± 0.1	5.9 ± 0.2	4.9 ± 0.1	1.51 ± 0.05	0.16 ± 0.03
C16-7-40	24 ± 3	0.054 ± 0.005	0.76 ± 0.07	2.87 ± 0.08	4.92 ± 0.09	14 ± 3	1.06 ± 0.07	0.178 ± 0.008

All data are expressed as mg/g and represent the mean ± standard deviation (n = 2).

Table 4.5. Solid extracted sugar composition of fine buckwheat bran treated by SS

Sample	Sugar total	Fucose	Rhamnose	Arabinose	Galactose	Glucose	Xylose	Mannose
F-REF	14.0 ± 0.2	0.023 ± 0.03	0.7 ± 0.1	2.41 ± 0.07	4.7 ± 0.1	5.5 ± 0.3	0.52 ± 0.02	0.24 ± 0.04
F12-4-20	13.9 ± 0.2	0.013 ± 0.002	0.5 ± 0.1	0.34 ± 0.06	4.08 ± 0.07	8.4 ± 0.5	0.31 ± 0.08	0.19 ± 0.04
F12-4-40	18.5 ± 0.1	0.020 ± 0.003	0.7 ± 0.1	0.83 ± 0.06	5.33 ± 0.02	10.9 ± 0.4	0.54 ± 0.06	0.24 ± 0.06
F12-6-20	16.0 ± 0.3	0.014 ± 0.005	0.58 ± 0.07	0.339 ± 0.007	4.68 ± 0.03	9.9 ± 0.3	0.35 ± 0.02	0.130 ± 0.001
F12-7-20	18.87 ± 0.08	0.011 ± 0.001	0.7 ± 0.1	0.44 ± 0.03	5.45 ± 0.03	11.8 ± 0.3	0.42 ± 0.05	0.12 ± 0.02
F12-7-40	21.1 ± 0.4	0.017 ± 0.003	0.8 ± 0.1	0.66 ± 0.02	6.18 ± 0.03	12.9 ± 0.5	0.50 ± 0.03	0.14 ± 0.04
F14-4-20	21.7 ± 0.5	0.03 ± 0.03	0.82 ± 0.09	0.86 ± 0.05	6.32 ± 0.09	12.8 ± 0.6	0.67 ± 0.05	0.126 ± 0.007
F14-4-40	21.8 ± 0.4	0.025 ± 0.003	0.9 ± 0.2	1.40 ± 0.02	6.7 ± 0.2	11.7 ± 0.3	0.9 ± 0.3	0.15 ± 0.03
F14-6-20	21.3 ± 0.3	0.021 ± 0.002	0.8 ± 0.1	1.30 ± 0.06	6.21 ± 0.04	12.0 ± 0.5	0.83 ± 0.04	0.122 ± 0.004
F14-7-20	20.9 ± 0.4	0.025 ± 0.001	0.8 ± 0.1	2.02 ± 0.02	6.2 ± 0.1	10.7 ± 0.4	1.03 ± 0.02	0.12 ± 0.02
F14-7-40	20.05 ± 0.06	0.033 ± 0.001	0.8 ± 0.1	3.4 ± 0.2	6.10 ± 0.05	8.4 ± 0.4	1.2 ± 0.1	0.138 ± 0.005
F16-4-20	20.4 ± 0.5	0.032 ± 0.003	0.8 ± 0.1	3.3 ± 0.1	6.3 ± 0.2	8.6 ± 0.3	1.1 ± 0.2	0.15 ± 0.02
F16-4-40	21.11 ± 0.08	0.05 ± 0.03	0.8 ± 0.1	3.6 ± 0.4	5.8 ± 0.1	9.7 ± 0.4	1.0 ± 0.1	0.16 ± 0.01
F16-7-20	19.3 ± 0.4	0.06 ± 0.04	0.8 ± 0.1	5.1 ± 0.3	5.7 ± 0.2	6.0 ± 0.2	1.43 ± 0.09	0.18 ± 0.01
F16-7-40	34.1 ± 0.1	0.06 ± 0.04	0.6 ± 0.01	2.6 ± 0.2	4.6 ± 0.5	25.1 ± 0.5	1.02 ± 0.04	0.14 ± 0.03

All data are expressed as mg/g and represent the mean ± standard deviation (n = 2).

4.3.2 Effect of selected SS conditions on bran chemical-physical traits

Buckwheat bran was composed approximately by one third of fiber (almost insoluble) and one third of proteins, whereas starch accounted for 21.5% (Table 4.6). SS treatment led to a general decrease in starch content between 8 and 11% with no univocal trend in respect to temperature applied (Table 4.6). This change could be due to the partial gelatinization of the starch, to the formation of amylose-lipid complexes or of resistant starch as observed in brewer's and distillers spent grains SS- drying (Pronyk et al., 2004). In fact, greater changes were observed as the temperature increased in the starch fraction susceptible to α -amylase activity: this damaged fraction was about the half of total starch content in sample treated at 160 °C. These traits agreed with the observations of Wu, McClements, Chen, Hu, & Liu (2016a) who found that approximately 48% of starch in partial milled rice (3% bran layer removal) stabilized by superheated steam was rapidly digestible, caused as a consequence of the partial gelatinization of starch due to water vapor condensation during initial stages of processing.

The SS did not lead to any remarkable change in dietary fiber content. On the contrary, SS-treatment led to considerable change in buckwheat bran color (Fig. 4.2.): sample lightness was progressively hardly decreased from 61.9 of untreated bran to 35.1 of buckwheat bran treated at 160 °C, while a slight increase in redness values was observed from 3.33 to a maximum of 9.08. More intense brown and red hues were the results of non-enzymatic browning reaction and Maillard's compounds production accelerated by temperature (Jamradloedluk et al., 2007). In fact, non-treated bran contained only low amount of furosine (32 mg/100 g protein) (Table 4.6), a marker of the early stage Maillard reaction. The value roughly doubled and tripled in sample respectively treated at 120 °C and 140 °C (58 and 88 mg/100 g protein). Furosine content in SS-160 was approximately 6-fold of that of untreated bran, confirming that non-enzymatic browning reactions could explain the color changes in treated bran. However, furosine levels were in line with the values reported for other cereal products toasted at 180 °C for 10 min (Rufián-Henares et al., 2009).

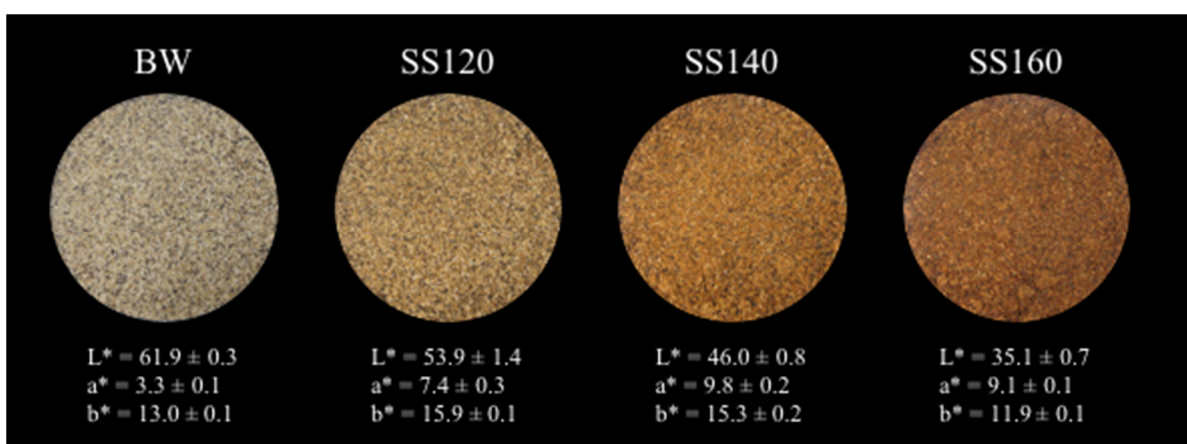


Fig. 4.2. Images and color indices of untreated and SS-treated buckwheat bran. All data are expressed as the mean \pm standard deviation (n = 5)

Table 4.6. Compositional characteristics of untreated and SS-treated buckwheat bran.

	Protein	Total starch	Starch susceptible to α -amylase	SDF	IDF	Moisture	Furosine
BW	31.74 \pm 0.08 ^a	21.5 \pm 0.3 ^a	0.84 \pm 0.02 ^d	3.48 \pm 0.03 ^b	32.5 \pm 1.7 ^a	13.5 \pm 0.2 ^a	32.3 \pm 0.1 ^d
SS120	29.0 \pm 0.2 ^b	19.8 \pm 0.3 ^b	2.05 \pm 0.05 ^c	3.2 \pm 0.2 ^{ab}	33.6 \pm 0.5 ^a	6.69 \pm 0.02 ^b	58.1 \pm 0.3 ^c
SS140	29.50 \pm 0.05 ^b	19.1 \pm 0.4 ^c	5.5 \pm 0.2 ^b	3.4 \pm 0.1 ^a	33.2 \pm 0.3 ^a	5.1 \pm 0.1 ^c	88.7 \pm 0.2 ^b
SS160	28.6 \pm 0.3 ^b	19.6 \pm 0.2 ^b	9.0 \pm 0.2 ^a	3.7 \pm 0.1 ^{ab}	33.8 \pm 0.2 ^a	3.73 \pm 0.06 ^d	196.3 \pm 0.5 ^a

Compositional data are expressed as g/100 g sample (d.b.). Furosine content is expressed as mg/100 g protein. All data are expressed as the mean \pm standard deviation (n = 2).

Even though SS did not promote relevant modification on protein content (Table 4.6), important changes were observed in the protein structural organization. In fact, information on the nature of the bonds that stabilize the protein association in the treated samples could be provided by assessing the amount of protein solubilized in the presence/absence of denaturants and/or disulfide reducing agents (Fig. 4.3). The amount of proteins solubilized in all media decreased at increased intensity of treatment. However, treatment at temperatures above 140°C resulted in the formation of protein aggregates that were almost insensible to the combined solubilizing action of chaotropes and a disulfide-reducing agent. Below the 120°C threshold, the temperature treatment greatly favored the formation of disulfide-linked aggregates. The structural compactness of aggregates formed at temperatures above 140 °C confirmed the results of SS-screening conditions evaluation.

The nature of the proteins involved in the temperature-induced aggregation events was assessed by SDS-PAGE. As shown in the two panels of Fig. 4.4, treatments above the 120°C threshold caused the formation of aggregates insensitive to the treatment with SDS and 2-ME, confirming solubility data. The proteins solubilized from materials treated at 120°C were disulfide-linked aggregates, mainly composed of 25 kDa and 12 kDa proteins, with minor component at higher molecular mass. Thus, the largest proteins in the original material were the most temperature-sensitive. The progressively overall changes promoted by high temperatures and observed in molecular studies, may suggest the formation of a more “inert” structure towards proteins and water.

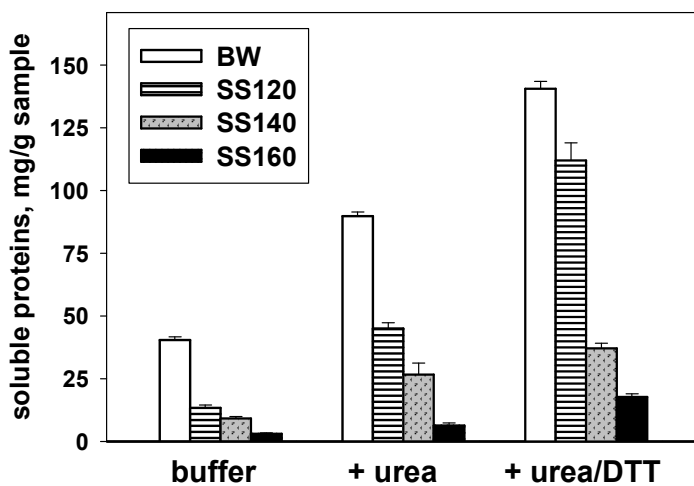
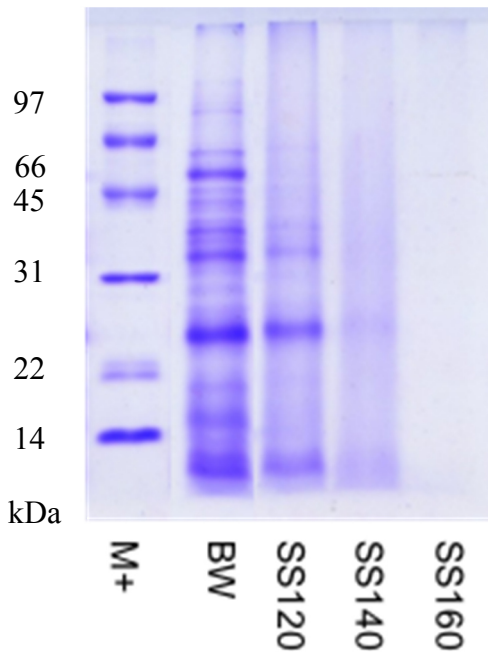


Fig. 4.3. Solubility of proteins from buckwheat bran samples in phosphate/saline buffer in the absence or in the presence of urea and of urea/DTT. Standard deviation is given for each sample.

DEN +



DEN -

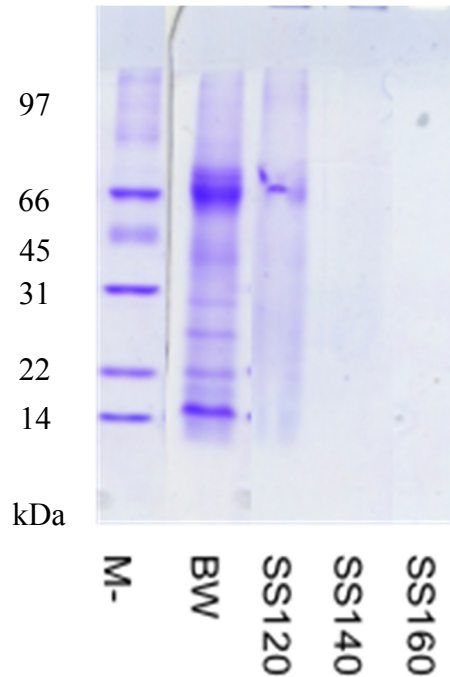


Fig. 4.4. SDS-PAGE patterns of proteins solubilized in different media from the buckwheat bran samples. Samples were denatured in the absence (A) or in the presence (B) of 2-mercaptoethanol. M: molecular mass markers.

Definitely, SS-treatment had a clear influence on the water vapor sorption properties of buckwheat bran (Fig. 5). According to Brunauer et al. (1940) classification, both untreated and treated samples displayed the characteristic sigmoid shape of a type II isotherm as classified by, which is common for dehydrated food. Increasing in temperature process resulted in a decrease of water absorbed, especially in high water activity region ($RH > 50\%$), suggesting a lowering in water affinity of bran components.

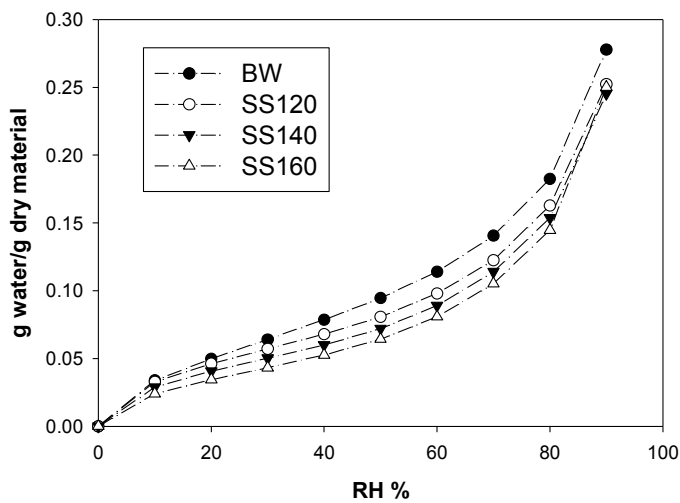


Fig. 4.5. Water vapor sorption of untreated and SS-treated buckwheat bran.

4.3.3 Effect of SS-treated bran on wheat dough properties

SS-treatment of buckwheat bran increased water absorption of enriched doughs up to 64.4% in the case of sample treated at 140 °C (Table 4.7). It has been observed that drying processes as steam-cooking, autoclaving, and roasting increased water absorption and mixing time of dough enriched in 20% of wheat bran (Caprez et al., 1986). Buckwheat bran treatment seemed to slightly improve dough stability to mixing, suggesting a less interaction between fiber and gluten (Zhang and Moore, 1997). SS-treatment slightly prolonged the mixing time of the dough as observed by Caprez et al. (1986).

Table 4.7. Farinograph parameters for doughs enriched in 20% untreated (BW) or SS-treated (SS120; SS140; SS160) buckwheat bran.

	Water absorption (%)	Mixing time (min:s)	Stability (min:s)
BW	61.6	8:00	9:30
SS120	62.8	9:12	10:24
SS140	64.4	9:18	10:06
SS160	61.9	8:48	10:18

Dynamic Mechanical Thermal Analysis (DMTA) profiles for wheat dough enriched in untreated (BW) or buckwheat bran SS-treated (SS120; SS140; SS160) are shown in Fig. 4.6. After initial decrease of G' , due to the softening of the dough, all dough samples showed a sharp increase in the temperature range between 55.5 and 57 °C. This phenomenon can be associated with the onset of starch gelatinization as stated before (Refer to Section 3.3.1). Nevertheless, the absence of correlation and significance between the G' onset temperature obtained by DMTA and those obtained by DSC analysis of the dough ($R^2 = 0.445$; $p = 0.555$) indicated that other phenomena occurred during the temperature increase. In fact, as observed by Rouillé et al. (2005), other phenomena can be responsible for the dough/crumb transition, as gluten aggregation. Any difference observed between results of Chapter 3 and 4 has to be ascribed to the different treatment considered in these two chapters.

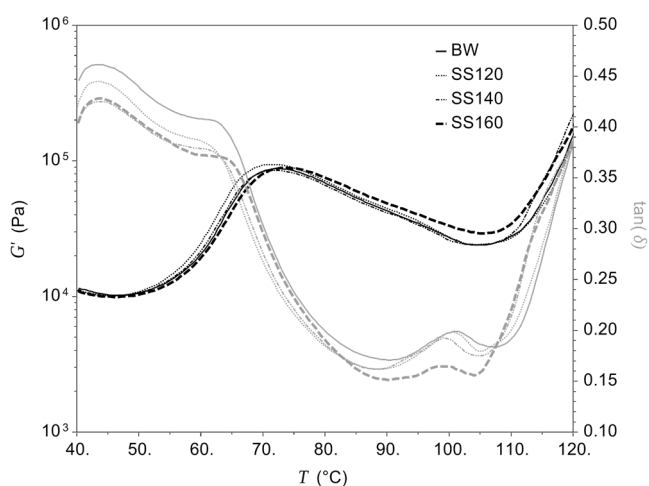


Fig. 4.6. DMTA profiles for wheat dough enriched in 20% untreated (BW) or SS-treated (SS120; SS140; SS160) buckwheat bran. Black lines: G' modulus; grey lines: $\tan(\delta)$.

At temperature lower than starch gelatinization/protein aggregation (e.g 40 °C), no relevant differences were observed between SS-treated samples and the control BW, except for a slight lower $\tan \delta$ values in SS-samples (Table 4.8). The general decrease in $\tan \delta$, was more evident during heating, starting from the onset of mechanical transitions and the effect on samples treated at the highest temperatures (140 and 160 °C) was more marked. Since $\tan \delta$ values were always smaller than 1, elastic properties predominated over viscous ones. In general, the enrichment in SS160 led to lower $\tan \delta$ values, implying in this sample a greater predominance of the elastic component. Since no significant difference was seen in G' values (Table 4.8), the observed changes in viscoelastic properties of dough were due to decrease in viscous modulus (G''). Loss moduli at the beginning of structural changes (onset) and at peak showed only slight differences for samples enriched in SS-treated bran. Addition of insoluble fiber to wheat dough generally increases viscoelastic modulus due either to competition for water absorption and consequent reduction of water lubricant role, or to filler-like effect in the matrix (Bonnand-Ducasse et al., 2010). Therefore, decrease in loss modulus may suggest respectively a less hydrophilic nature of SS-treated buckwheat bran or more inert bran structure, in agreement with the results of water sorption and of proteins macromolecular structure.

The Kieffer dough extensibility test was performed to provide information on the effect of SS-treated bran incorporation on wheat dough behavior at large deformation. Addition of bran treated at progressively high temperatures, increased the resistance to extension in comparison with the dough enriched in untreated bran (Table 4.9), showing a clear positive trend depending on temperature (sample SS160 over 40% increase). Adding treated bran to wheat decreased dough extensibility and the change was less evident as high temperature has been applied. Generally, the resistance and extensibility decrease in samples in which refined wheat flour is replaced by high level of fiber (Boita et al., 2016; Foschia et al., 2013). This behavior has been attributed to gluten dilution or hindrance of bran in the network development that weakens extensible characteristics (Boita et al., 2016). Dough resistance to extension appeared to be highly negatively correlated with the $\tan \delta$ measured at 40 °C DMTA tests ($R^2 = -0.964$; $p < 0.05$). A decrease in $\tan \delta$ resulted in an increase in elastic over viscous behavior which reflected the progressive increase in the resistance to extension. This observation may suggest the less detrimental effect of SS-treated bran on gluten development in comparison with untreated buckwheat bran.

Table 4.9. Kieffer parameters for doughs enriched in 20% untreated (BW) or SS-treated (SS120; SS140; SS160) buckwheat bran.

	Resistance to extension (mN)	Extensibility (mm)
BW	344 ± 69 ^a	39 ± 5 ^c
SS120	431 ± 37 ^b	26.3 ± 0.8 ^a
SS140	446 ± 37 ^b	27.0 ± 0.9 ^a
SS160	488 ± 25 ^c	29 ± 2 ^b

All data are expressed as the mean ± standard deviation (n = 15, performed on 3 different doughs). Different letters in the same column indicate statistically significant differences (LSD; $p \leq 0.05$).

Table 4.8. DMTA parameters for doughs enriched in 20% untreated (BW) or SS-treated (SS120; SS140; SS160) buckwheat bran.

	Elastic modulus (G')		Viscous modulus (G'')			tan(δ)	
	Onset Temperature	Peak Temperature	Onset Temperature	Peak Temperature	Peak G''	at 40 °C	at G' onset
	(°C)	(°C)	(°C)	(°C)	(Pa)		
BW	55.3 ± 0.1 ^b	72.38 ± 0.02 ^b	56.4 ± 0.2 ^b	69.3 ± 0.5 ^b	25931 ± 504 ^a	0.443 ± 0.005 ^a	0.418 ± 0.003 ^a
SS120	54.5 ± 0.1 ^d	71.7 ± 0.03 ^c	55.57 ± 0.08 ^c	68.31 ± 0.01 ^d	25065 ± 1100 ^{ab}	0.418 ± 0.006 ^b	0.397 ± 0.004 ^b
SS140	54.9 ± 0.3 ^c	71.85 ± 0.02 ^c	55.7 ± 0.2 ^c	68.82 ± 0.01 ^c	23834 ± 1016 ^b	0.408 ± 0.005 ^b	0.385 ± 0.004 ^c
SS160	55.7 ± 0.1 ^a	73.34 ± 0.03 ^a	57.0 ± 0.3 ^a	70.33 ± 0.01 ^a	24078 ± 576 ^b	0.408 ± 0.006 ^b	0.380 ± 0.003 ^c

All data are expressed as the mean ± standard deviation (n = 3). Different letters in the same column indicate statistically significant differences (LSD; $p \leq 0.05$). G' onset, G' peak and G'' onset were not statistically significant difference at 95.0% confidence level.

4.3.4 Effect of SS-treated bran on bread quality

Either SS-treated or untreated buckwheat bran was added to wheat flour to produce bread in small-scale trials. Already from a visual quality standpoint (Fig. 4.7), it can be seen that incorporation of different treated bran had dissimilar effect on loaves volume and slices height. Differently from samples treated at 120 °C and at 140 °C, the sample treated at 160 °C showed better performance than the control in terms of volume and slice height. The increase in crust/crumb redness in respect to bread by untreated bran was more evident as much the treatment temperature increased.



Fig. 4.7. Loaves and bread slices of samples enriched with untreated (BW) or SS-treated (SS120; SS140; SS160) buckwheat bran

The effect of the incorporation of SS-treated bran on baking properties is reported in Table 4.9. SS-treatment improved specific volume of loaves only when the highest temperature (160 °C) was applied. Specific volume appeared correlated with G' onset and peak temperature (respectively: $R^2 = 0.989$, $p < 0.05$; $R^2 = 0.962$, $p < 0.05$): the higher the temperature of dough/crumb transition occurs, the higher loaf maximum expansion and then consequently the loaf volume (Rouillé et al., 2010). This result could be influenced by either modification to starch component (as seen in bran compositional analyses) and gluten.

As regard the relationship with Kieffer parameters, in refined wheat dough, bread volume is well estimated by resistance to extension rather than extensibility (Kieffer et al., 1998); when fiber material is incorporated into dough, the weakening in extensible characteristics provides impact as lower retention of gas and lower volume of bread (Boita et al., 2016). The simultaneous higher resistance and extensibility of SS160 dough in respect to the other treated samples may explain its better making behavior, likely due to a less interfering activity, as stated before.

When considering the texture of crumb, the better effect in terms of softness is already evident starting from sample treated at 140 °C. Generally, bran incorporation increases crumb firmness and hardness, not only due to the decrease of loaf volume but also to a number of factors as gluten dilution proteins (assumption not usable

in the case of dough enriched with the same amount of SS-treated bran) and to physical, chemical or biochemical properties of bran (Hemdane et al., 2016).

The positive effect of bran SS treatment over 140 °C on bread texture were also evident when considering the crumb cohesiveness (Table 4.9). The increase of this parameter indicated a less micro-fracturing of the solid lamellae around the air cells during the compression, due to a more continuous crumb network likely resulting from a less interfering role of bran solid particles, as observed before.

Positive effect on texture was mainly due to the decrease in crumb density of samples SS-140 and SS160. Moisture seemed to have not a clear influence in crumb hardness or in the correlation with the amount of water added during mixing.

Table 4.9. Baking properties of bread enriched with SS-treated bran.

	Specific volume (mL/g)	Hardness (N)	Corrected Hardness (N)	Cohesiveness	Crumb moisture (%)
BW	2.7 ± 0.2 ^b	5.6 ± 0.7 ^b	5.5 ± 0.3 ^a	0.73 ± 0.01 ^b	45.36 ± 0.09 ^a
SS120	2.45 ± 0.09 ^a	6.7 ± 0.4 ^c	5.9 ± 0.5 ^c	0.723 ± 0.004 ^c	44.88 ± 0.07 ^b
SS140	2.5 ± 0.2 ^a	5.1 ± 0.7 ^a	5.5 ± 0.2 ^{ab}	0.746 ± 0.008 ^a	45.30 ± 0.09 ^a
SS160	2.83 ± 0.08 ^c	5.3 ± 0.5 ^{ab}	5.8 ± 0.3 ^{bc}	0.749 ± 0.005 ^a	44.16 ± 0.06 ^c

All data are expressed as the mean ± standard deviation (Specific volume: n = 8; Hardness, Corrected hardness and Cohesiveness: n = 12; Moisture: n = 4. All analyses were performed on 2 independent baking tests). Different letters in the same column indicate statistically significant differences (LSD; p ≤ 0.05).

Only few studies were conducted on the effect of bran heat-treated on baking properties. Among these, De Kock et al. (1999) and Nelles et al. (1998) observed an increase in loaf volume when wheat bran was treated by dry autoclave (121 °C for 90 min) or boiled (97.4 °C for 15 min), respectively due to the inactivation of detrimental enzymes or small reactive compounds and to improved hydration meal components and decrease in oxidizable substances, rather due to a wash-out effect.

On the contrary, Jacobs et al. (2016) did not obtain any significant differences when adding bran toasted (170 °C for 30 min) in wheat bread. Differences in results might be attributed to differences in bread and dough preparation/making conditions (Jacobs et al., 2016).

Since in bread, texture is controlled by the elastic properties of the matrix - a discontinues air phase - in a continuous hydrophilic matrix (the crumb cell walls), hardness was corrected for crumb density values. Although the correction produced differences among samples (Table 4.9), the diversity for the hardest sample (SS120) in respect to the other samples was attenuated, suggesting that the response to TPA test could be mainly described by crumb differences.

4.4 Conclusions

SS treatment deeply affected the chemical/physical properties of buckwheat bran. In particular, changes in water affinity were progressively observed with increasing temperature, also due to the formation of protein aggregates. These modifications influenced also the thermo-mechanical properties of high-bran enriched doughs that reflected baking behavior. When appropriate temperature setting was chosen (temperature: 160 °C) SS treatment increased buckwheat bran performances. Loaf specific volume and crumb softness were improved in comparison with untreated bran.

4.5 References

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5. Enzymatic treatments on buckwheat bran: effects on dough rheology and bread-making performance

5.1 Introduction

The importance of wholegrain or of cereal-based product enriched in dietary fiber on human health has been well documented over the years (King, 2005; Lappi et al., 2013; Weickert and Pfeiffer, 2008). Besides the beneficial effects of fiber-rich products on nutrition, the adverse effect on the final product quality it is also well known (Foschia et al., 2013; Heiniö et al., 2016). Among strategies aimed at improving the processing and/or quality of dough and bread, the application of enzymes is well established (Corke et al., 2008). A whole range of enzymes belonging to different classes are nowadays used in bread-making (Table 5.1). Pre-treatment of bran from different cereals with cell-wall degrading enzymes has been observed to lessen problems in bread supplementation with high fiber amount (Autio et al., 1996; Laurikainen et al., 1998; Lindahl and Eliasson, 1992). The use of pretreated ingredients has also permitted to minimize some of the problems associated with the addition of enzymes directly in the bread formulation, thus facilitating the exploitation of enzymatic treatment (Marti et al., 2014).

In wholemeal rye, the use of xylanase for the fragmentation and depolymerization of cell-wall resulted in softer dough and in positive effect on bread volume and texture, likely due to the modification of water distribution between pentosans and starch (Autio et al., 1996). Enzyme preparation containing hemicellulase was used to lower problems caused by the presence of insoluble pentosans thanks to the partial solubilization of non-starch polysaccharides resulting in less interference with the gluten network (Krishnarau and Hosene, 1994). Pentosans play a significant role in dough rheology and bread quality, affecting gluten formation indirectly or directly. The competition for water or the effect of limiting the movement of glutenin proteins towards a larger aggregate are ascribable to the first way, whereas the covalently linkage or association of pentosans to glutenin proteins can be an interpreted as a direct effect (Wang et al., 2003). Buckwheat bran is richer in pentosans than wheat (Drobot et al., 2014 and is mainly composed of highly branched arabinans (Wefers and Bunzel, 2015).

Cell-wall degrading enzymes have been largely used in buckwheat bran processing as pre-treatment to produce proteins isolates (Grossman et al., 1980; Wang et al., 1999); nevertheless, they have been never used as a strategy aimed at improving the technological quality of this by-product as fiber-enriching material for dough and bread supplementation. Therefore, the aim of this study was to determine the effects of polysaccharide hydrolases on the functionality of buckwheat treated bran in bread-making process.

Table 5.1. General effect of enzymes in bread making. Modified from Corke et al. (2008).

Enzyme (<i>Substrate</i>)	Flour standardization	Increased loaf volume	Antistaling	Improved dough handling and machinability	Improved crumb texture	Increased dough strength and/or stability	Crumb bleaching
Amylolytic enzyme (<i>Starch</i>)	X	X	X		X		
Endoprotease (<i>Gluten proteins</i>)				X			
Endoxylanase (<i>Arabinoxylans</i>)		X	X	X	X		
Lipase (<i>Flour lipids and glycolipids</i>)		X			X	X	
Glucose oxidase (<i>Mono- and oligosaccharides</i>)		X			X	X	
Lipoxygenase (<i>Polyunsaturated fatty acids</i>)				X			X
Transglutaminase (<i>Proteins</i>)		X				X	

5.2 Materials and methods

5.2.1 Materials

Common buckwheat (*Fagopyrum esculentum*) bran was provided by Filippini s.p.a. (Teglio, Italy).

Bran was treated with either pectinolytic (named “Pect”; Pectinex Ultra SP-L; Novozymes A/S, Bagsvaerd, Denmark) or cellulolytic (named “Cell”; Celluclast 1.5L; Novozymes A/S (Bagsvaerd, Denmark) commercial preparations. Main and side activities of enzymes are reported in Table 5.2.

Table 5.2. Activities of Pectinex Ultra SP-L and Celluclast 1.5L.

Activity (nkat/mL)	Pectinex Ultra SP-L	Celluclast 1.5L
endo-1,3(4)- β -glucanase	8235	26860
β -glucosidase	4.27	221
cellulase	1342	19040
polygalacturonase	175436	272
endo-1,4- β -xylanase	427	8840
protease	18.3	nd

Modified from Rommi et al. (2014). nd = not detected.

Each enzymatic preparation (1 mL/100 g of bran) was added to bran and mixed with water (2.5 mL/1 g of bran). Then, sample was incubated for 17 h at 18 °C keeping the suspension in the dark without mixing. Either coarse buckwheat bran or bran after superheated steam treatment (SS140) (Refer to Section 4.2.1) were used.

5.2.2 Methods

5.2.2.1 Dough and bread preparation

After enzymatic treatment, bran samples were directly added at 20% level to a commercial wheat flour (protein: 11.7 g/100 g, provided by Meneba; Rotterdam, The Netherlands) with salt (2 g/100 g) and mixed in the Farinograph bowl for 2 min. Then, the rest of the water needed to reach the optimal consistency (420 ± 20 FU) was added. For dough and bread analyses, fiber pre-hydrated without enzyme addition was considered as blank to verify the effect on the product quality of this required step. Bread was prepared as described above (Refer to Section 3.2.2.7).

5.2.2.2 Kieffer extension test

Refer to Section 3.2.2.5.

5.2.2.3 Bread characterization

Refer to Section 3.2.2.8.

5.2.2.4 Statistical Analysis

Analysis of variance (one-way ANOVA) was performed by using XLSTAT Version 2016.02 (Addinsoft, Paris, France). Different enzyme treated sample were considered as factors for ANOVA. When a factor effect was found to be significant ($p \leq 0.05$), significant differences among the respective means were determined using Fischer's Least Significant Difference (LSD) test.

5.3 Results and discussion

5.3.1 Dough properties

The effects of enzymatic treatment of buckwheat bran on the mixing properties of enriched dough are summarized in Table 5.3.

Table 5.3. Water absorption, dough development time and stability of coarse or SS treated buckwheat bran.

Sample	Water absorption (%)	Dough development time (min:s)	Stability (min:s)
BW-Ref	61.6	08:00	9:30
BW-Water	60.6	08:42	15:12
BW-Pect	55.7	07:30	15:06
BW-Cell	56.8	06:00	9:30
SS-Ref	64.4	09:18	10:06
SS-Water	64.1	08:00	12:06
SS-Pect	60.6	08:00	11:54
SS-Cell	60.4	07:00	9:24

Ref: Coarse bran (BW-A) or SS140 as such. Water: pre-hydrated (without enzymes) fiber. Pect: bran treated by Pectinex Ultra SP-L. Cell: bran treated by Celluclast 1.5L.

In native bran, pre-hydration resulted in a slight decrease in water absorption, while the same pre-processing on SS-treated bran seemed not to affect water absorption. Our results agreed with Nelles et al. (1998) who found that prehydration treatments decreased the water absorption during the farinograph test. Generally, bran hydration increases the optimum water absorption of whole wheat flour (Cai et al., 2015); bran fully hydrates during the long enzymatic treatment before its addition to wheat flour and mixing. Consequently, the competition with flour for water during dough development is lowered and the complete gluten hydration assured (Cai et al., 2015). The higher values of dough stability suggested less interference of fiber on gluten development in pre-hydrated samples (BW-Water and SS-Water) and the formation of stronger dough when compared with the corresponding references (Zhang and Moore, 1997).

Enzymatic treatments of bran resulted in a decrease in water absorption for both untreated and SS-treated samples. Similar results have been reported with other cereals bran enzymatically treated (Haros et al., 2002;

Marti et al., 2014). In fact, the hydrolysis of high water-binding polymers (e.g. pentosans) reduced the amount of water necessary to hydrate these macromolecules (McCleary et al., 1986). Enzymatic treatment increased the dough stability only when Pectinex Ultra SP-L was used but no differences were observed in comparison with bran pre-hydrated without adding enzymes. When considering the overall mixing properties summarized in Table 5.3, enzymatic and SS bran treatment alone or in combination, could modulate water absorption and dough development.

The dough extensibility parameters evaluated with the Kieffer test are reported in Fig. 5.1.

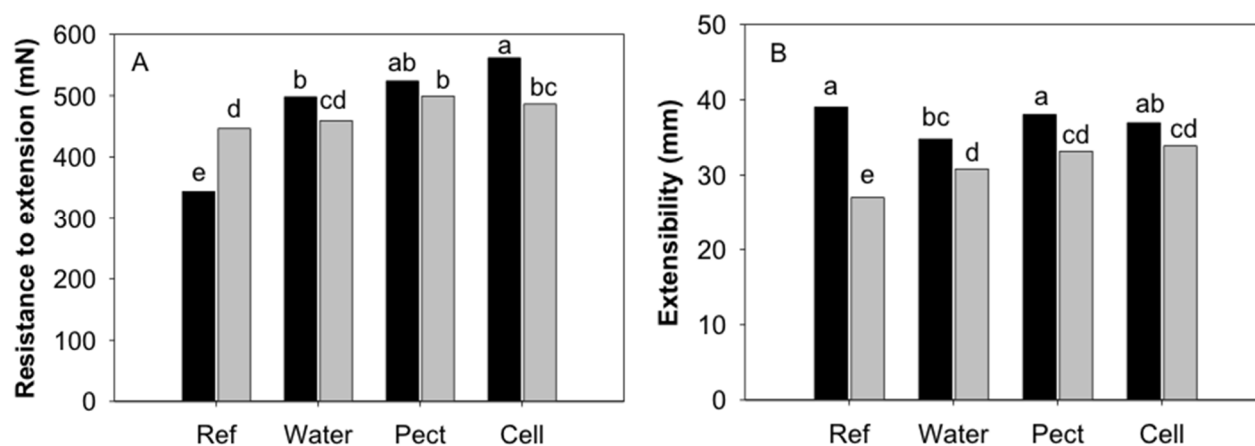


Fig. 5.1. Resistance to extension (A) and Extensibility (B) of coarse or SS treated buckwheat bran. Black bars: coarse bran series; grey bars: SS140 series. Bars represent the average of 15 measurements performed on 3 different doughs. Different letters indicate statistically significant differences (LSD; $p \leq 0.05$).

Pre-hydration of buckwheat bran (BW-Water) increased the resistance to extension (Fig 5.1A) and decreased the extensibility (Fig. 5.1B), whereas both enzymatic formulations had the same positive impact on dough resistance to extension, suggesting the development of a more compact reticular structure that could stand to mechanical stress, in agreement with the higher farinograph stability.

The use of cell-wall degrading enzyme (xylanases) was observed to increase the resistance to extension in wheat doughs (Hartikainen et al., 2014; Selinheimo et al., 2006) due to the solubilization of arabinoxylans. Buckwheat is lacking of cross-linking polysaccharides as arabinoxylans, however it contains highly branched arabinans (Wefers and Bunzel, 2015): thus, improved resistance to extension was likely linked to the development of a more loose fiber network, thanks to enzymes action.

The Pectinex Ultra SP-L action could be mainly ascribable to polygalacturonase and endo-1,3(4)- β -glucanase (Table 5.1), whereas Celluclast 1.5L contains predominantly and endo-1,3(4)- β -glucanase and cellulases and has been found to be effective for recovering pectin from plant cell walls (Yuliarti et al., 2011). Despite the well-known high content of buckwheat in pectin polysaccharides, only Hozová et al. (2007) reported the content of insoluble β -glucans in buckwheat, founding values much lower than that found in common cereal grains.

The enhancement of resistance to extension was more pronounced in BW series than in SS series: the first samples in fact generally showed higher ability to stand to large uniaxial deformation, whereas no synergy was observed between the SS-treatment and the use of enzymes. However, the pre-hydration or the enzymatic treatment increased the resistance to extension to values similar to those of dough enriched with 5% of bran (in the case of BW-series) or with 10% of bran (in the case of SS-series) (Refer to Section 3.3.1).

As regard extensibility, the enzymatic treatment increased this rheological property (in respect to the reference) only when SS bran was used, whereas when considering untreated bran, no significant differences were observed (Fig 5.2B).

As observed in dough enriched in wheat bran, the use of cell-wall degrading enzymes may have caused a redistribution of water from fiber phase to gluten phase, increasing the gluten volume fraction and providing more extensibility (Katina et al., 2006). This observation could be also supported by the decrease in water absorption in dough made by enzymatic-treated bran (Table 5.3). Even for extensibility, inclusion in native bran led to a better rheological behavior. Modified rheology, with overall improvement in viscoelastic properties can suggest a less detrimental effect on gluten properties, resulting in a better baking behavior.

5.3.2 Baking properties

Either native or SS140 buckwheat bran was added to wheat flour after enzymatic treatment and used to produce bread in small-scale trials. Already from a visual quality standpoint (Fig. 5.2), incorporation of different treated bran had a highly different effect on loaves volume and slices height.

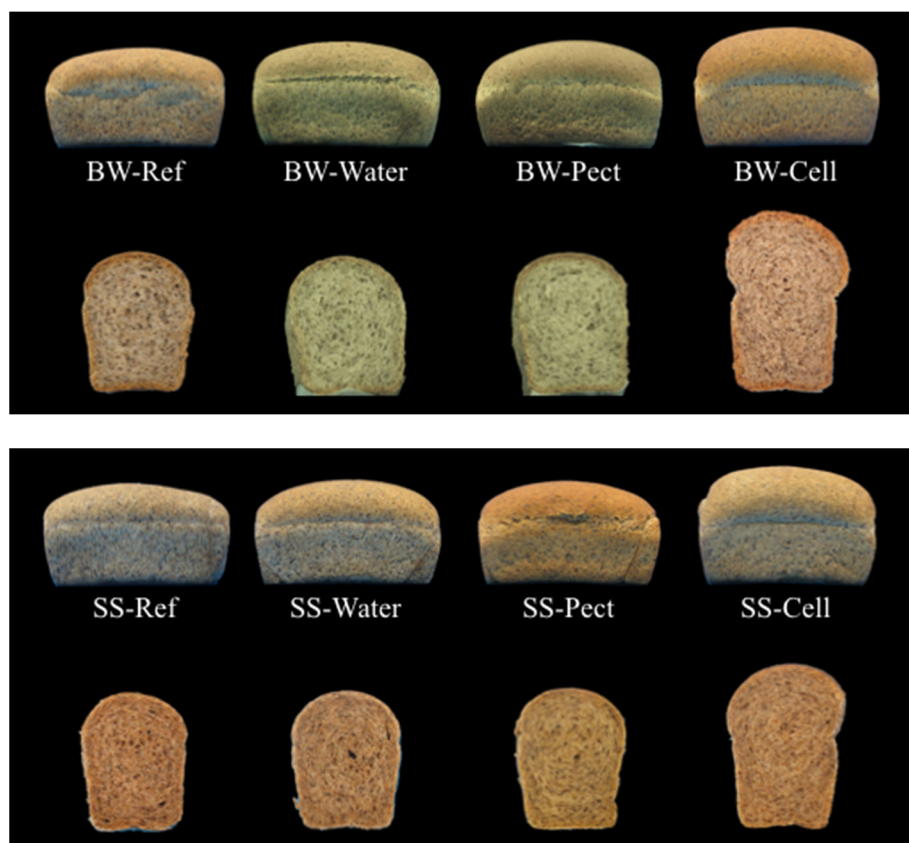


Fig 5.2. Loaves and bread slices of samples enriched with untreated (BW) or SS-treated buckwheat bran

Bran pre-hydrated (BW-Water and SS-Water) or treated by pectinase (BW-Pect and SS-Pect) seemed to have no or slight effect on bread expansion compared with the correspondent reference sample. Conversely, improved bread characteristics were observed when cellulase was used. Noteworthy was the effect of Celluclast that allowed to increase the specific volume by almost 30% of the breads containing BW or SS bran (Fig. 5.3A), reaching values comparable to those of not-enriched wheat bread (Refer to Section 3.3.2). Pectinex assured a slight higher (about 9%) specific volume in comparison with the reference, only when bran as such (BW-Pect) was used. The positive effects of cellulases could not be totally explained by the higher dough resistance to extension or by the dough water content, differently from what observed with untreated or fine bran (Refer to Chapter 3). In fact, Kieffer test did not show any differences between the samples treated by Pectinex and by Celluclast and the water absorption of the dough was approximately the same. Although it is known the correlation between Kieffer parameters and the loaf volume (Dobraszczyk and Morgenstern, 2003), the uniaxial deformation could not completely predict a tridimensional deformation occurring during fermentation. As suggested by Pritchard (1992), when cellulases are present, dough could expand more, due to the higher fluidity that assure a greater expansion.

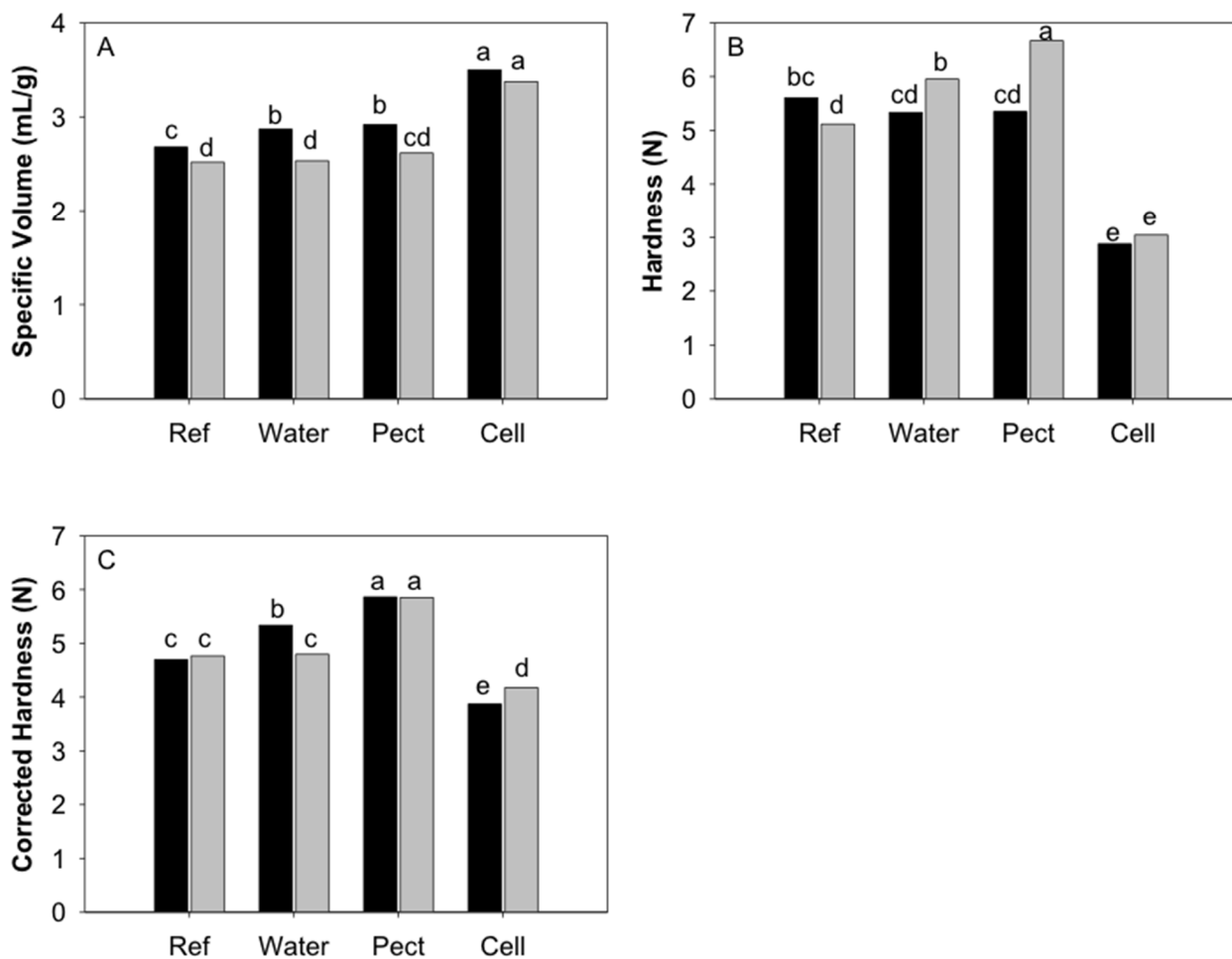


Fig. 5.3. Specific volume (A), Hardness (B) and Corrected hardness (C) of coarse or SS treated buckwheat bran. Black bars: coarse bran series; grey bars: SS140 series. Bars represent the average of 8 (Specific volume), 12 (Hardness and corrected hardness performed on 2 independent baking tests) measurements. Different letters indicate statistically significant differences (LSD; $p \leq 0.05$).

Crumb hardness (Fig. 5.3B) followed the same trend of specific volume only when BW-series was considered. In SS-series, only samples enriched in pre-hydrated bran or pectinase-treated bran showed improved baking quality parameters. The use of cellulase was confirmed to produce the best result in terms of softness, regardless the type of bran. Pre-hydration or enzymatic treatment promoted differences in crumb density that could be only partially explained by a different moisture content (Table 5.4) which was, in turn, related to water utilized in dough production (Table 5.3, water absorption).

Finally, added enzymes were also active during bread making, thus any positive effects or different results from rheological tests, could be also due to the interaction between the enzymes and the endosperm components during the long leavening step (Hemdane et al., 2015).

Table 5.4. Water activity, moisture content and density of crumb.

Sample	a_w	Moisture content (%)	Density (g/mL)
BW-Ref	0.970 ± 0.001^a	45.4 ± 0.1^a	0.31 ± 0.01^{ab}
BW-Water	0.966 ± 0.003^{bc}	44.75 ± 0.05^c	0.29 ± 0.01^d
BW-Pect	0.961 ± 0.004^d	42.9 ± 0.2^s	0.27 ± 0.01^e
BW-Cell	0.9663 ± 0.0007^{bc}	43.3 ± 0.2^f	0.246 ± 0.008^f
SS-Ref	0.9706 ± 0.0007^a	45.30 ± 0.09^a	0.30 ± 0.02^{cd}
SS-Water	0.968 ± 0.001^{ab}	45.00 ± 0.06^b	0.318 ± 0.007^a
SS-Pect	0.964 ± 0.002^{cd}	43.8 ± 0.2^d	0.31 ± 0.02^{bc}
SS-Cell	0.964 ± 0.002^{cd}	43.57 ± 0.09^e	0.24 ± 0.01^f

All data are expressed as the mean \pm standard deviation ($n = 3$). Different letters in the same column indicate statistically significant differences (LSD; $p \leq 0.05$). ns = there is not a statistically significant difference at 95.0% confidence level.

However, differences in density were not exhaustive in describing crumb firmness. In fact, the application of the Ashby-Gibson theory revealed that each treatment (pre-hydration, pectinase or cellulase hydrolysis) had a distinct effect on crumb texture (Fig 5.3C), probably corresponding to a different organization of the cellular structure by the incorporation of fiber. The better behavior of bran treated by Celluclast 1.5L was likely due to the most detrimental effect of cellulose on bread properties, although the content in this non-starch-polysaccharide is quite modest in buckwheat bran. Moreover, the absence of protease side-activity in the commercial preparation could represent issue for gluten development.

5.4 Conclusions

In conclusion, from a technological standpoint, a preliminary bran treatment with cellulases (as Celluclast 1.5L) could be a feasible solution to produce bread enriched in buckwheat bran. The enzymatic pre-treatment of bran set-up in this work could be easily included in every bread-making process (both at industrial and artisan scale) as the partially hydrolyzed-bran requires no further processing as drying and can represents an ingredient of the final dough recipe.

Since no synergistic effect was observed between SS and enzymatic treatment, the use of SS-treated bran is recommended only when superheated steam could be associated with relevant modifications on nutritional or sensorial aspects.

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General conclusions

The production of foods rich in dietary fiber represents one of the most important driving forces in the development of innovative cereal-based products. This PhD thesis showed that the use of ingredients rich in fiber and other bio-active compounds could be a practicable strategy only if combined with adequate technology to modify the negative effect(s) associated with the presence of macromolecules weakening the gluten network.

An overview of the effects of the treatments on nutritional quality as well as rheological properties and final product quality of enriched products is shown in Table 1.

To summarize this PhD thesis findings:

The debranning processing allows to collect biocompound-rich fractions from purple wheat kernels that can be successfully incorporated into pasta. This process seems to be relevant also to other grains (cereals and non-cereals) that contain bio-active molecules in their external layers.

The relevant changes promoted by micronization of buckwheat bran need to be considered when supplementing flour with bran. In fact, the size of bran particle and the changes associated with strong mechanical stresses can be pivotal in influencing dough water absorption.

The identification of physical mechanisms describing the influence of buckwheat bran addition to the rheological and baking performance of wheat dough could help to explain the detrimental effect of bran incorporation into bread products.

Superheated steam could be used to improve bran technological behavior when high temperatures are used (e.g. SS treatment at 160 °C).

Treatment with cellulases (as Celluclast 1.5L) could be a feasible solution to produce bread enriched in buckwheat bran. The positive role of this type of enzymes can be related to the peculiar composition of buckwheat bran.

Future studies will include the assessment of the effects of bran-treatments on the macromolecular structure of fiber, to define not only its rheological performance but also its nutritional functionality.

Table 1. Overview of the effect on bran properties of several treatments applied on cereal and pseudocereal.

Treatment	(Pseudo) Cereal	Nutritional effect	Technological effect
Debranning	Purple wheat	<ul style="list-style-type: none"> - Higher TDF (8.5 g/100 g) in pasta enriched in debranning fractions compared to commercial wholegrain pasta - Higher anthocyanins content in pasta enriched in debranning fractions compared to pasta from conventional bran - Lower enrichment level of F1 led to the same antioxidant capacity of pasta from bran 	<ul style="list-style-type: none"> - Fair cooking quality of pasta enriched in debranning fractions allowed by the mild forming process (i.e. roll-sheeting) - Pasta enriched in debranning fractions had comparable cooking losses to conventional bran pasta
Micronization	Buckwheat	<ul style="list-style-type: none"> - Higher SDF content in fine bran (FB) in respect to coarse bran (CB) 	<ul style="list-style-type: none"> - Better or similar rheological properties of FB-enriched dough (e.g. elasticity, resistance to extension, extensibility) - Better baking quality of CB-enriched bread (e.g. specific volume, crumb softness)
Superheated Steam (SS)	Buckwheat	<ul style="list-style-type: none"> - Higher susceptibility to α-amylase activity (<i>in vitro</i>) - Progressive increase in furosine content with temperature, although values were in line with other cereal products toasted 	<ul style="list-style-type: none"> - Increase in dough resistance to extension and elastic properties in function of treatment temperature - Decrease in dough extensibility, less evident at high temperatures - Increase in loaf specific volume for the most extreme condition - Increase in crumb softness starting from samples treated at 140°C
Enzymes	Buckwheat	<ul style="list-style-type: none"> - To be assessed. Potentially variations in non-starch polysaccharides composition and organization 	<ul style="list-style-type: none"> - Increase in dough resistance to extension. No relevant changes in extensibility - Increase in bread volume. Cellulases improved the crumb softness
SS + Enzymes	Buckwheat	<ul style="list-style-type: none"> - To be assessed. Potentially variations in non-starch polysaccharides composition and organization 	<ul style="list-style-type: none"> - No synergistic effect between SS and enzymatic treatment



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Debranning of purple wheat: recovery of anthocyanin-rich fractions and their use in pasta production



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ABSTRACT

Debranning is a pre-milling treatment that partially removes the external coats and the aleurone layer of the kernel, allowing the selective recovery of bioactive compounds, such as fiber and phenolic compounds. A two-step debranning process was applied to purple wheat, a naturally antioxidant-rich variety, that removed 9.7% of the material. Debranned fractions from the first (F1; 3.7% of the whole grain) and the second (F2; 6.0% of the debranned grain after the first step) step were used separately to produce fiber-enriched pasta. Bran from conventional milling (CB) was also used as a control. F1 and F2 had a higher or comparable content in total and soluble fiber than CB. Moreover, both samples exhibited a higher ferric reducing-antioxidant power (FRAP) than CB, whereas the highest amount of anthocyanins was found in F1 (695 ± 64 µg/g). When compared with CB-enriched pasta, samples enriched with either F1 or F2 had similar FRAP values (2.6 ± 0.1 and 2.3 ± 0.2 µmol Fe(II)/g for pasta with F1 and F2, respectively), and a higher amount of anthocyanins (67.9 ± 0.9 and 60 ± 1 µg/g for pasta with F1 and F2, respectively), while retaining a fair cooking quality.

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1. Introduction

Whole wheat grain is a good source of dietary fiber and antioxidants which can promote health benefits towards several chronic diseases usually associated with oxidative stress (Yu, 2008). Although most of the cultivated cultivars are white- or red-grained, some varieties – such as purple and blue wheat grains – have drawn the attention of researchers and food industry due to their high content in anthocyanin pigments and to their antioxidant properties (Abdel-Aal, Young, & Rabalski, 2006; Escribano-Bailón, Santos-Buelga, & Rivas-Gonzalo, 2004; Zeven, 1991).

Anthocyanins are the largest group of water-soluble natural

pigments that provide many fruits, vegetables, and cereal grains with red, violet, and blue color (Escribano-Bailón et al., 2004; Mazza & Miniati, 1993). These bioactive compounds not only scavenge free radicals, they also have a detoxifying effect towards heavy metals (Jan et al., 2015). Various fruits and vegetables are good sources of anthocyanins (Mazza & Miniati, 1993). However, all of these foods are less frequently consumed in comparison with cereal products. Consequently, blue and purple grains would be potential candidates for the development of bioactive food ingredients. At present, these grains are underutilized, and their contribution to the human diet is very little. For this reason, only limited data are available about their functional characteristics.

Currently, blue and/or purple corns are used for the production of naturally colored blue tortillas. As for wheat-based products, anthocyanin-rich biscuits (using whole purple wheat; Pasqualone et al., 2015), muffins (using bran from purple wheat; Li, Pickard, & Beta, 2007) and pasta (from purple durum wheat; Ficco et al., 2016) have been recently studied, focusing on the effects of processing conditions on the antioxidant properties.

In the case of pasta from durum purple wheat, the technological process led to a dramatic decrease in nutritionally-relevant antioxidant compounds (Ficco et al., 2016), suggesting that greater

Abbreviations: CB, bran from conventional milling; CF, refined flour from conventional milling; F1, fraction removed with the first step of debranning; F2, fraction removed with the second step of debranning; FRAP, Ferric reducing-antioxidant power; IDF, insoluble dietary fiber; M-CB, CB-enriched wheat mixture; M-F1, F1-enriched wheat mixture; M-F2, F2-enriched wheat mixture; P-C, control pasta from refined wheat flour; P-CB, CB-enriched wheat pasta; P-F1, F1-enriched wheat pasta; P-F2, F2-enriched wheat pasta; SDF, soluble dietary fiber; TDF, total dietary fiber.

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Article

Soybean-Enriched Snacks Based on African Rice

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Abstract: Snacks were produced by extruding blends of partially-defatted soybean flour with flours from milled or parboiled African-grown rice. The interplay between composition and processing in producing snacks with a satisfactory sensory profile was addressed by e-sensing, and by molecular and rheological approaches. Soybean proteins play a main role in defining the properties of the protein network in the products. At the same content in soybean flour, use of parboiled rice flour increases the snack's hardness. Electronic nose and electronic tongue discriminated samples containing a higher amount of soybean flour from those with a lower soybean flour content.

Keywords: e-nose; e-tongue; extrudates; rice; soybean enriched snacks

1. Introduction

Changes in eating patterns in West Africa are occurring at an increasing rate due to urbanization, globalization, economic, and demographic trends. This is further fueled by changes in the social structure as a result of the increase in the number of mothers working outside their home and in the increasing demand for convenience foods [1]. These changes in eating patterns include an increasing consumption of snacks (cookies, nuts, extruded snacks) in all age groups. Snacks provide a significant part of the nutrient and calorie intake for many African consumers [2]. Currently, high snack consumption in Ghana is associated to their widespread presence in open markets, supermarkets, petty trading, and restaurants in both urban and rural areas [3]. Extrusion-cooking has found widespread application in the cereal-based snack food industry, because of its ease of operation and of its ability to produce a number of consumer-appealing textures and shapes [2]. Though wheat- and corn-based snacks are the most popular products in Ghana, rice flour has become an attractive ingredient for the production of extruded snacks due to its bland taste, hypoallergenicity, availability, and high digestibility [4].

Rice has become a staple in most West African countries [5], including both rural and urban areas [6,7]. The strong increase in the local rice production is yet not sufficient to meet the increasing demand. In addition, African consumers prefer imported rice to locally-produced rice, due to low grain quality, low head yield, high chalkiness, poor cooking performance, and taste of locally-grown

Conference's abstracts

**19th Workshop on the *Developments in the Italian PhD Research on Food Science Technology and Biotechnology*
Bari, 23-25 September 2014**

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Biotechnological actions to improve the quality of enriched cereal foods

This PhD project is aimed to obtain enriched foods with enhanced overall quality by applying biotechnological approaches that would modify the structural properties of specific macromolecules (such as non-starch-polysaccharides, polyphenols, etc.) present in cereals and pseudocereals.

Interventi biotecnologici per migliorare la qualità di alimenti derivati dai cereali arricchiti in specifici componenti

Questo progetto di dottorato si propone di definire e applicare a cereali e pseudocereali con differenti peculiarità compositive, approcci biotecnologici mirati a modificare le proprietà di alcune macromolecole, quali polisaccaridi non amido e polifenoli, al fine di ottenere prodotti arricchiti con elevate caratteristiche qualitative.

14th European Young Cereal Scientists and Technologists Workshop, Copenhagen, 15-17 April 2015

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Debranning as a tool for developing innovative milling fractions: application to purple common wheat

The production of food enriched in dietary fiber or antioxidant molecules is a current challenge to satisfy the recommended daily intake of these micronutrients. Cereal products, due to their consumption as staple foods, might be effective for achieving this purpose. However, this kind of incorporation may be detrimental to the properties of final products. Also, conventional milling processes does not consider the selective recover of these compounds, that are frequently lost in bran or germ. In this study, we investigated the effects of a debranning pre-treatment on the antioxidant and fiber content of commercial purple common wheat, an antioxidant-rich variety.

Grains were hydrated and underwent two subsequent debranning steps that removed 3.7% and 9.7% of the starting material. Debranned grains were then milled in a lab-scale conventional milling system to obtain three fractions: flour, middlings, and bran. Non-debranned grains were milled as a control.

When compared to conventional milling, milling of debranned grains led to a decreased content of dietary fiber in the bran and flour fractions, whereas an increase in dietary fiber was assessed in the middlings. The pre-treatment increased the ratio between soluble and insoluble fiber in both middlings and bran from the milling step, and both fractions were also rich in total phenolic compounds.

Debranning did not affect the level of starch damage. After debranning and milling, alpha-amylase activity was associated mostly with the bran, and was essentially absent in the removed layers.

Debranning allows removal of the outer-most layers of the grain that are reportedly the most detrimental for the technological performance of grains. Milling of debranned grains provided fractions with a very high concentration of functional compounds. Thus, these fractions may be used in low amounts as ingredients to exploit their positive nutritional properties while minimizing their detrimental technological effects.

6th International Dietary Fibre Conference, Paris, 01-03 June 2015

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Characterization of new milling fractions and use of biocomponents-enriched fractions in pasta production

The conventional milling process usually does not allow for the selective recovery of bioactive compounds, such as fiber or antioxidants, that are collected in bran and germ. Dry fractionation processes may represent a convenient method for achieving this goal. Debranning is a widespread dry-process for covered cereals as rice, consisting in sequential and alternate steps of friction and abrasion. If applied to wheat, it allows the partial removal of the external coats and of the aleurone layer, allowing to recover tissues with different composition and functional activities.

The debranning was applied to purple wheat, a naturally antioxidant-rich variety. Wheat was hydrated and then debranned, removing the 3.7% of the whole grain (DF1). The step was repeated to obtain a final 9.7% of debranned material (DF2).

A mixture of commercial wheat flour and debranned fractions were used to produce enriched rolled sheeted pasta (tagliatelle-shape; width: 11 mm; thickness: 1 mm) dried at 40°C for 10 hours. The addition of the debranned fractions was calculated in order to have the same total dietary fiber (TDF) content in the final pasta. Therefore DF1 (TDF: 63% d.m.; SDF: 4% of TDF) was added at a 16% level, and DF2 (TDF: 44% d.m.; soluble: 11% of TDF) at 20% level. The two formulations were also similar in terms of phenolic content and antioxidant capacity, both highly greater than the control (pasta without addition of debranning fractions). Pasta was characterized before and after cooking with particular attention to textural parameters and functional properties in term of phenolic content and antioxidant activity.

Thanks to the roll sheeting process, the addition of debranned layers was not associated with a worsening of textural properties, but instead contributed to a major pasta elasticity, suggesting the presence of a good structure and an uniform distribution of fiber into the matrix, as showed by the higher values of tensile strength and Young's modulus.

Moreover, the enrichment led to a decrease in antioxidant activity only for DF2 and for control pasta that, respectively, lost about 25% and 5% of the original scavenging capacity, whereas DF1 showed a gain around 20%, probably due to an effect of concentration of insoluble compounds as fiber-bound phenolics.

Our results suggest that the debranning fractions may represent interesting ingredients for the incorporation of fiber and antioxidant compounds into pasta products.

**20th Workshop on the *Developments in the Italian PhD Research on Food
Science Technology and Biotechnology*
Perugia, 24-26 September 2015**

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Biotechnological actions to improve the quality of enriched cereal foods

In the first part of the present PhD thesis project, a dry fractionation process was applied to common wheat kernels. In particular, a pigmented (purple) commercial variety underwent a pre-milling debranning treatment to separate and selectively recover tissues with different composition and functional properties. The removed fractions were then used for the production of enriched pasta by a roll-sheeting process and a drying cycle at LT.

**Interventi biotecnologici per migliorare la qualità di alimenti derivati dai cereali
arricchiti in specifici componenti**

La prima parte di questo progetto di tesi di dottorato riguarda l'applicazione di un processo di frazionamento a secco su grano tenero. In particolare, un processo di decorticazione pre-macinazione è stato condotto su una varietà di grano tenero pigmentato di origine commerciale per separare e recuperare selettivamente tessuti con diversa composizione e proprietà funzionali. Le frazioni di recupero sono state quindi utilizzate per la produzione di pasta arricchita tramite un processo con formatura per laminazione ed essiccazione LT.

AACC (American Association of Cereal Chemists) Annual meeting Minneapolis, 18-21 October 2015

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A multidisciplinary approach to define the molecular requirements for production of enriched pasta from pigmented wheat

Functional foods derived from main crops, such as wheat and corn, are of interest within a balanced and varied diet. Among these, pasta has the advantage to be obtained through a consolidated and relatively simple technological process. However, producing a “functionally enriched” pasta may be tricky, in particular when using species other than wheat. The aim of the present research is to develop new types of pasta, naturally rich in phenolics and in fiber, through the application of appropriate technologies. Attaining this goal calls for an accurate characterization of the raw materials and for defining the physical pre-treatments necessary for selective enrichment in specific bioactive compounds. This study addresses the overall molecular and physical properties of the outermost fractions collected by debranning pigmented wheat grains, as well as those of the bran obtained from milling the same grains. Grains were hydrated and underwent two subsequent debranning steps that removed 3.7% and 9.7% of the starting material. Debranned grains and controls were then milled in a lab-scale conventional milling system. Analytical profiling of the phenolics in bran components/fractions separated by physical techniques was carried out by advanced LC methods, and indicated that the outermost fraction obtained through debranning represents a very rich (and convenient) source of phenolics (and fiber, as assessed by independent measurements). From a practical standpoint, this fraction can be incorporated into pasta with negligible or minimal detrimental effects on dough rheology, on microstructure, on appearance, and on the cooking properties of the final pasta as measured by appropriate physical measurements. In conclusion, this multidisciplinary approach may allow to define proper formulation and processes for the production of naturally enriched pasta.

49th Miami Winter Symposium Miami, 24-26 January 2016

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Phenolics from pigmented grains have remarkable immunomodulating properties

Pigmented grains are of interest in functional foods because of their antioxidant properties, due to their high content in phenolics and anthocyanins. These compounds have been often associated with protection against chronic diseases.

The purpose of this study was to investigate the anti-inflammatory activities of extracts from the outermost parts of pigmented grains using intestinal epithelial Caco-2 cells as models.

When required and as appropriate, pigmented grains were de-branned in a pilot debranning plant, and milled in a lab-scale milling system. Phenolics were extracted with an ethanol/water mixture (65:35 v/v) containing 0.01% HCl. Analytical profiling of the phenolics was carried out by HPLC on the extracts. A DPPH assay was used to investigate the anti-oxidant activity of individual fractions. The anti-inflammatory properties of extracts were assessed by using cytokine-stimulated biosynthesis of a luciferase-labeled reporter of cytokine-related gene activation in transformed Caco-2 cells, using red grape cyanidines as a positive control.

Analytical profiling of the phenolics indicated that the outermost fraction of debranned pigmented grains represents a rich and convenient source of phenolics (and fiber, as assessed by independent measurements). The anthocyanin-rich fraction decreased the immune response of Caco-2 cells in a dose-responsive manner, and grain phenolics were apparently more effective than grape cyanidins, that reportedly possess a strong anti-inflammatory activity.

Our results indicate that the phenolics-rich fraction from pigmented grains appears to be at least twice more effective than phenolics from other sources (such as grape skin) in impairing the expression of NF- κ B in appropriately stimulated transformed cells. From a practical standpoint, it is noteworthy that these bioactive-rich fractions can be incorporated with minimal efforts in ready-to consume staple foods, such as pasta. Further studies will also address the bioavailability of grain-derived anthocyanins and the amount of metabolites formed/released in the gastrointestinal tract, also in consideration of the activity of the local microflora and of its interactions with phenolics-enriched foods.

15th International cereal and bread congress, Istanbul, 18-21 April 2016

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Effect of buckwheat bran enrichment on wheat dough and bread properties

The production of foods rich in dietary fiber represents one of the most important driving forces in the development of innovative cereal-based products. However, inclusion of high levels of fiber is still technologically challenging, as for the texture and the sensory quality.

Buckwheat is a nutritionally-relevant pseudo-cereal and is traditionally used in Europe and Asia for pasta production, but rarely exploited in bakery applications.

In this study, we investigated the effects of enriching wheat flour with increasing levels of buckwheat bran (5, 10, 20%), with different particle size (d_{av} bran as such: 360 μm ; d_{av} micronized bran: 110 μm).

Dynamic Mechanical Thermal Analysis (DMTA) and differential scanning calorimetry (DSC) allowed evaluating the effects of bran on the thermo-mechanical transition during processing. Dough rheology at large deformations was assessed by the Kieffer test. Standard baking tests and texture profile analysis were performed on bread in small-scale trials (60g puffy loafs).

As for the dough properties, both DMTA and DSC approaches indicated that bran enrichment resulted in a progressive increase in the onset temperature of starch gelatinization. DMTA also provided evidence as for changes in the viscoelastic behavior during heating associated with bran enrichment. As for the dough behavior at large deformations, dough resistance to extension decreased with increasing bran inclusion. On the contrary, no univocal behavior was assessed as for the dough extensibility increase.

As for baking quality, the decrease in loaf volume due to buckwheat bran enrichment clearly depended on the level of inclusion and on bran particle size. Crumb moisture content of fresh bread reflected the behavior in dough water absorption for both types of bran: gradual enrichment with buckwheat as such led to a wet product, whereas the substitution level with micronized bran has no significant effect. Crumb firmness increased as the bran content increased and was positively correlated with the increase in crumb density. For this reason, the Ahsby-Gibson theory for cellular solid foods was applied. Bran as such increased the corrected hardness for addition level higher than 10%. On the contrary, the addition of micronized bran deeply altered crumb firmness already at 5%.

The inclusion of buckwheat bran greatly affected dough rheology and thermal transitions during baking, which resulted in considerable changes in bread quality. Such changes could be related not only to the enrichment degree but also to the size of the bran particles.

15th European Young Cereal Scientists and Technologists Workshop, Bergamo, 26-29 April 2016

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Including buckwheat bran in wheat dough and bread: what happens?

- DMTA and DSC allowed evaluating the effects of buckwheat bran addition on the thermo-mechanical transitions during processing
- At similar composition of buckwheat bran, variations in particle size resulted in distinct effects on dough thermo-mechanical properties and bread quality
- The Ashby-Gibson theory for cellular solid foods allowed to reveal the influence of bran on the mechanical properties of the solid crumb matrix

Development of products rich in ingredients with high nutritional value –as dietary fiber– is currently one of the most important goals for food companies. However, inclusion of high levels of fiber in cereal-based products is still technologically challenging, as for the texture and the sensory quality.

Buckwheat is a nutritionally-relevant pseudo-cereal and is traditionally used in Europe and Asia for pasta production, but rarely exploited in bakery applications.

In this study, we investigated the effects of enriching wheat flour with increasing levels of buckwheat bran (5, 10, 20%), before and after micronization.

DMTA and DSC allowed evaluating the effects of bran on the thermo-mechanical transition during processing while dough rheology at large deformations was assessed by the Kieffer test. Standard baking tests and TPA were performed on bread.

Regarding dough properties, both DMTA and DSC showed that bran enrichment resulted in increase in the temperature of starch gelatinization. The size of the effect depended on bran amount and particle size. DMTA also provided evidence as for changes in the viscoelastic behavior during heating. As for Kieffer test results, increasing bran inclusion led to a decrease of resistance to extension.

Concerning the baking quality, the decrease in loaf volume due to buckwheat enrichment clearly depended on the level of inclusion and on bran particle size. Moisture content of fresh bread reflected the behavior in dough hydration for both types of bran: gradual enrichment with buckwheat as such led to a more wet product, whereas the substitution level with micronized bran had no significant effect. Crumb firmness increased as the bran content increased and was positively correlated with crumb density. The Ashby-Gibson theory for cellular solid was applied to correct for the effect of density. Bran as such increased the corrected hardness for addition level higher than 10%. On the contrary, micronized bran deeply altered crumb firmness already at 5% substitution level.

Overall, buckwheat bran greatly affected dough rheology and thermal transitions during baking, which resulted in considerable changes in bread quality. Such changes could be related not only to the enrichment degree but also to the size of the bran particles.

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Effect of (bio-)technological approaches on bran to improve the quality of cereal products

This PhD thesis dealt with the application of different physical or biotechnological approaches to modify the structural properties of specific macromolecules (such as non-starch-polysaccharides) present in cereals and pseudocereals, in order to enhance the quality of fiber-enriched cereal foods.

Effetto di interventi (bio-)tecnologici sulla fibra per migliorare la qualità di alimenti derivati dai cereali

Questa tesi di dottorato ha riguardato l'applicazione di diversi interventi (bio-)tecnologici per la modificazione delle proprietà di alcune macromolecole (quali polisaccaridi non amido) presenti in cereali e pseudocereali, al fine di migliorare la qualità di prodotti arricchiti in fibra.