Bread making aptitude of mixtures of re-milled semolina and selected durum wheat milling by-products

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Running title

Bread from mixtures of re-milled semolina and durum by-products

Abstract

We evaluated the bread-making ability of meals composed of re-milled semolina and either 10% or 20% of i) residuals of the second and third debranning steps of durum wheat (DB), ii) the micronized and air-classified thin fraction obtained from the same residuals (MB), or iii) coarse bran obtained from conventional roller milling of non-debranned durum wheat (B). Dietary fibers, proteins, total soluble phenolic compounds, ferulic acid, and antioxidant activity were significantly higher ($P < 0.05$) in MB and DB than B. The addition of by-products to re-milled semolina decreased the alveograph W and increased the P/L ratio, with stronger effects at higher doses. Particularly negative were the effects of B on P/L and farinograph dough-development time. Bread containing 10% MB did not show significant differences ($P < 0.05$) in specific volume, crumb hardness, resilience, and chewiness with pure re-milled semolina bread but had higher dietary fiber, phenolics and antioxidant activity.

Key words: durum wheat, bread, milling by-products, debranning, bioactive compounds

1. Introduction

Refined wheat flour, derived almost exclusively from endosperm, has very low levels of some valuable healthy compounds, such as dietary fiber, vitamins, mineral salts, and antioxidant phenolic compounds, which are abundant in bran. Bran represents about 15% of the grain and is a composite, multi-layered adhesive tissue composed of the outer and inner pericarps, the testa, hyaline and aleurone layers, plus some starchy endosperm residues (Hemery et al., 2011). Depending on the mill, bran ends up in various bran-rich streams, including coarse bran (regular bran), coarse weatings (fine bran), fine weatings (middlings or shorts), and low-grade flour (red dog), distinguished by particle size and residual endosperm content (Hemdane, Jacobs, Dornez, Verspreet, Delcour, & Courtin, 2015).

Debranning, or pearling, is a dry separation technique consisting of progressive bran removal by consecutive abrasion of cereal kernels. Usually applied to rice and barley (Dexter & Wood, 1996), debranning recently has been extended to wheat prior to roller milling, to improve flour yield (Bottega et al., 2009), increase luminosity (L^*), and decrease redness (a^*) (Singh & Singh, 2010). Reciprocal kernel-to-kernel friction (peeling) and abrasion by rough surfaces (pearling) take place in debranning machines (Hemery et al., 2011). These mechanical treatments allow the consecutive detachment of the outer, intermediate, and inner (the closest to the aleurone) layers of pericarp, leading to different by-product classes, namely the first, second, and third debranning fractions. These fractions are removed separately by pressurized air flowing through the screens and outlets of the debranner with a technology that can be applied to both common (*Triticum aestivum* L.) and durum wheat (*Triticum durum*, Desf.). Several patents cover the process, reviewed by Hemery et al. (2011).

Many studies demonstrated the impact, usually negative, of wheat bran on bread quality (see the recent review by Hemdane et al., 2015). A few studies focusing on common wheat evaluated the potential of wheat pearling. In particular, when meal from flour and milling by-products were composed of the same overall starch level, the specific volume of the bread decreased more markedly with fine

weatings and low-grade flour than with coarse bran and weatings, suggesting that the properties of the former were intrinsically more detrimental to bread-making than those of the latter (Hemdane, Leys, Jacobs, Dornez, Delcour, & Courtin, 2015). Gan, Galliard, Ellis, Angold, & Vaughan (1992) noted that the most marked depression in loaf volume occurred when the outermost bran fraction (about 1% of the grain weight) was incorporated into the bread-making recipe. Blandino et al. (2013) observed that levels of 10% enrichment with the second debranning fraction made antioxidant bioactive compounds and dietary fiber increase, without negatively affecting bread physical properties in a significant way.

The residuals of the second and third debranning steps involve minor risks of mycotoxin contamination compared to the first debranning fraction and contain higher levels of proteins and lysine, due to the presence of some aleurone cells (Brouns, Hemery, Price, & Mateo Anson, 2012; Rizzello, Coda, Mazzacane, Minervini, & Gobbetti, 2012). In addition, these two fractions can be mixed and submitted to dry fractionation by micronization and a subsequent air classification treatment (Hemery et al., 2007, 2011), to give sub-fractions with different particle size and chemical composition, the thinner of which is poorer in dietary fiber than the coarser, but richer than the starting mixture of untreated residuals and retains at least the same protein content (Rizzello et al., 2012).

A previous study evaluated the bread-making impact of adding 1-5% micronized and air-fractioned residuals from the second and third debranning of durum wheat to common wheat flour. Such low levels produced bread with improved health features without compromising quality, whereas higher pearling amounts excessively altered the rheological properties of the dough (Rizzello et al., 2012).

Durum wheat semolina and re-milled semolina are the main refined products of durum wheat milling, both extracted from the endosperm but having different granulometry. Re-milled semolina, in particular, is characterized by smaller particle size (about 70% of particles below 180 μm) and a higher hydration rate than semolina, and is traditionally used in bread-making in the Mediterranean area (Pasqualone, 2012; Pasqualone, Caponio, & Simeone, 2004; Quaglia, 1988). The

end-product, durum wheat bread, is characterized by a prolonged shelf-life (Raffo et al., 2003), appreciated sensory features (Pasqualone, Summo, Bilancia, & Caponio, 2007; Raffo et al., 2003), and interesting nutritional attributes due to the presence of carotenoid pigments with provitamin A activity (Pasqualone et al., 2004). The addition of durum wheat milling by-products, such as bran and debranning fractions, could increase further the nutritional and health value of durum wheat bread while adding value to underutilized by-products that are usually destined to animal feed.

Until now, no study has evaluated the bread-making ability of re-milled semolina mixed with selected durum wheat milling by-products. Moreover, the typically dense structure of durum wheat bread (Raffo et al., 2003) could tolerate the alteration in consistency induced by fiber contained in the bran and debranning fractions better than soft wheat bread.

Hence, we evaluated the bread-making ability of meals composed of re-milled semolina and i) residuals of second and third debranning steps of durum wheat, ii) the micronized and air-classified fine fraction obtained from the same residuals, or iii) coarse bran obtained from conventional roller milling of non-debranned durum wheat. These durum wheat milling by-products were added at the 10% and 20% levels.

2. **Materials and methods**

2.1. Production of selected durum wheat milling by-products, composite meals with re-milled semolina, and corresponding breads

Re-milled semolina and three durum wheat (*T. durum*, Desf.) milling by-products, all derived from the same grain lot, were kindly furnished by a local durum wheat milling industry (Molini Tandoi S.p.A., Corato, Italy). The experimental plan was repeated three times at intervals of approximately 1.5 months. The by-products were i) coarse bran obtained by non-debranned wheat, ii) the second and third debranning fractions mixed together, and iii) the fine sub-fraction obtained by

micronization and air-classification of the second and third debranning fraction mix.

The sampling plan and milling processing steps are schematized in Fig. 1. Each grain lot was subdivided into two halves. The pre-milling Peritec debranning system (Satake Europe Ltd, Bredbury, Stockport, England), described in Hemery et al. (2011), was applied to a half lot of grain to obtain three consequential debranning fractions. A fraction accounting for 6% of the kernel weight was removed and discarded in first debranning step, then a further 3% was removed and collected (second debranning step) and, finally, also a further 3% was detached and collected (third debranning step), up to 12% of the kernel weight in total. The second and third fractions were mixed together (DB) and submitted to micronization and air classification treatments carried out as in Rizzello et al. (2012) to obtain two sub-fractions with different particle size, the thinner of which was collected (MB). In parallel, coarse bran (B) and re-milled semolina were obtained by conventional roller-milling, without debranning pre-treatment, of the second half lot of grain.

The main particle size of B, DB, and MB was respectively coarse (80-90% > 500 µm, 5-10% 500-425 µm, 2-4% 425-300 µm, 0-5% 300-180 µm, and 0-2% < 180 µm), thin (5-25% > 500 µm, 0-20% 500-425 µm, 20-40% 425-300 µm, 20-40% 300-180 µm, and 0-20% < 180 µm), and very thin (0-2% > 500 µm, 0-1% 500-425 µm, 5-25% 425-300 µm, 25-50% 300-180 µm, and 75-95% < 180 µm). The particle size distribution of re-milled semolina was 2% > 300 µm, 27% 300-180 µm, 41% 180-126 µm, and 30% < 126 µm.

Subsequently, 10% and 20% (w/w) of B, DB, and MB were added to re-milled semolina to obtain a series of composed meals that were coded 10B, 20B, 10DB, 20DB, 10MB, and 20MB. Both re-milled semolina and composed meals were then used to prepare bread, coded 10B-Br, 20B-Br, 10DB-Br, 20DB-Br, 10MB-Br, and 20MB-Br, where ‘Br’ indicates bread. The breads were prepared without using shortenings, malt, ascorbic acid, and potassium bromate to follow the procedure usually adopted in Italy for durum wheat bread making (Pasqualone, 2012). The formula contained 1 kg of composed meals or re-milled semolina, 20 g

fresh baker's yeast, 20 g NaCl, and the optimal water amount (reported in Table 3), previously determined by farinograph (Brabender, Duisburg, Germany). According to straight-dough method, the ingredients were mixed in the farinograph chamber for 13 min, then dough was put in baking pans (9 cm × 4.5 cm and 6 cm deep), leavened at 30 °C and a relative humidity of 85% for 90 min, and baked (Bon Cuisine 520 oven, Ariete De Longhi, Campi Bisenzio, Italy) at 220 °C for 30 min.

2.2. Basic analyses of re-milled semolina and milling by-products

Protein content (total nitrogen × 5.7), ash, and moisture content were determined according to the AACC methods 46-11.02, 08-01.01, and 44-15A, respectively (AACC, 2000).

2.3. Determination of total dietary fiber and β-glucans

The determination of total dietary fiber of re-milled semolina, milling by-products, and bread was carried out by means of the enzymatic-gravimetric procedure according to the AOAC method 991.43 (AOAC, 1995). The total β-glucan concentration of re-milled semolina, milling by-products, and breads was determined according to the AOAC method 995.16 (McCleary & Mugford, 1997), using the Megazyme mixed-linkage β-glucan assay kit (Megazyme International Ltd., Bray, Ireland).

2.4. Colorimetric evaluations

Colorimetric evaluations of the red (*a**), yellow (*b**), and brown (BI, defined as 100-*L**) indices of bread crumb, re-milled semolina, and milling by-products were carried out under D65 illuminant by using a spectro-colorimeter CM-700d (Konica Minolta Sensing, Osaka, Japan) equipped with a pulsed xenon lamp. Re-milled semolina and milling by-products were placed in the granular materials

attachment (Konica Minolta Sensing, Osaka, Japan) to obtain a smooth surface suitable for color readings.

2.5. Farinograph and alevograph analyses of re-milled semolina and composite meals

The mixing properties of doughs were determined by farinograph (Brabender, Duisburg, Germany) according to the AACC method AACC 54-21 (AACC 2000). Water absorption capacity (i.e., the percentage of water required to yield a dough consistency of 500 Brabender Units), dough development time (i.e., the time needed from the first addition of water to reach the maximum consistency, corresponding to the greatest torque), and dough stability (i.e., the elapsed time at which dough consistency is kept at 500 Brabender Units) were measured.

Alveograph analysis was performed according to the UNI 10453 method (UNI, 1995) using an alveograph (Tripette et Reanaud, Chopin Technologies, Villeneuve-la-Garenne Cedex, France). Dough strength, the resistance to deformation (W), and tenacity/extensibility ratio (P/L) were determined.

2.6. Texture Profile Analysis (TPA) of breads

The Texture Profile Analysis (TPA) of the bread samples was determined according to Giannone et al. (2016) by means of a TVT-300XP Texture Analyzer (TexVol Instruments, Viken, Sweden) equipped with a P-Cy25S cylindrical probe and Texture Analyzer TVT-XP 3.8.0.5 software (TexVol Instruments, Viken, Sweden). Hardness, cohesiveness, springiness, resilience, and chewiness were determined.

2.7. Specific volume of the bread

The specific volume of the bread was determined using a laser-based volume measuring instrument BVM TxTVol (TexVol Instruments, Viken, Sweden) according to manufacturer's instructions.

2.8. Quantitative analysis of carotenoid pigments

Total carotenoid pigments were determined according to the AACC approved method 14–50.01 (AACC, 2000) with slight modifications: 1 g of bread (lyophilized and ground in a mortar), re-milled semolina, and milling by-products was extracted with 5 mL of water-saturated *n*-butyl alcohol on an orbital shaker for 3 h at 260 rpm. Samples were centrifuged for 7 min at 2400 × *g*, and the absorbance of water-saturated *n*-butyl alcohol extracts was measured at 435.8 nm by a Cary 60 UV-Vis spectrophotometer (Agilent Technologies Inc., Santa Clara, CA, USA). Total carotenoid content was expressed as mg kg^{-1} β-carotene, and calculations were made based on the extinction coefficient of 1.6632 for a solution of 1 mg β-carotene in 100 mL water-saturated *n*-butyl alcohol.

2.9. Quantitative analysis of soluble phenolic compounds

The soluble phenolic compounds (composed of free phenolic acids and phenolics bound to low molecular mass components) were extracted from breads, re-milled semolina, and milling by-products by methanol and spectrophotometrically determined (Cary 60 UV-Vis, Agilent Technologies Inc., Santa Clara, CA, USA) at 765 nm after Folin-Ciocalteu reaction, as in Pasqualone et al. (2014). A calibration curve was built by methanol solutions of ferulic acid (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) at concentrations between 0.1 and 2 g L^{-1} ($y = 0.0007x + 0.0089$; $r^2 = 0.9985$). The results were expressed as mg g^{-1} ferulic acid.

2.10. HPLC quali-quantitative analysis of total phenolic acids (sum of soluble and insoluble fractions)

The total phenolic acids (sum of soluble and insoluble fractions) were extracted from breads, re-milled semolina, and milling by-products as described in Laddomada et al. (2016) and quali-quantitatively analyzed using an Agilent 1100 Series HPLC-DAD system (Agilent Technologies, Santa Clara, CA, USA). equipped with a reversed phase C18(2) Luna column (Phenomenex, Torrance, CA, USA) (5 μm, 250 × 4.6 mm) at a column temperature of 30 °C. A mobile phase consisting of acetonitrile (A) and 1% (v/v) water solution of H_3PO_4 (B) was utilized for the following elution program: isocratic elution, 100% B, 0-30 min; linear gradient from 100% B to 85% B, 30-55 min; linear gradient from 85% B to 50% B, 55-80 min; linear gradient from 50% B to 30% B, 80-82 min; and post time, 10 min before the next injection. The flow rate of the mobile phase was 1 mL min^{-1}, and the injection volume was 20 μL. The column temperature was maintained at 30 °C. Peaks were identified by comparing their retention times and UV-Vis spectra to those of authentic phenolic standards. All phenolic acids were quantified via a ratio to the internal standard (3,5-dichloro-4-hydroxybenzoic acid) added to every sample and using calibration curves of phenolic acid standards.

2.11. Determination of in vitro antioxidant activity

The *in vitro* antioxidant activity was assessed in whole-meal flour by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay in the conditions reported in Pasqualone et al. (2014). The antioxidant activity of the samples was expressed as percent capacity of scavenging the DPPH radical (SC%) according to the equation SC% = (1 – Abs of $sample_{t=30'}$/Abs of $control_{t=0}$) × 100, where Abs of $sample_{t=30'}$ was the absorbance of DPPH radical solution + sample at t = 30 min; Abs of $control_{t=0}$ was the absorbance of the DPPH radical solution at t = 0 min.

2.12. Statistical analyses

Each analysis was performed in triplicate. One-way analysis of variance (ANOVA), followed by Tukey HSD test for *post hoc* comparison of means, was performed by using XLStat software (Addinsoft SARL, New York, NY, USA) for Windows.

3. Results and discussion

3.1. Characteristics of re-milled semolina and milling by-products

Ash, moisture, and protein content of re-milled semolina fulfilled the legal requirements (Italian Presidential Decree no. 187/2001) (Table 1). Protein content was markedly lower in re-milled semolina than in DB and MB, but was higher than in coarse bran, whereas ashes, dietary fiber and β-glucan content were noticeably lower in re-milled semolina than in all the by-products examined. Slightly higher β-glucan values were observed in MB and DB than in B, but without significant difference. Dietary fiber and proteins of the different milling by-products increased significantly ($P < 0.05$) in the order B < DB < MB, reflecting both the composition of the kernel portions and the processing conditions from which they were derived. In fact, the micronization and air-classification processes are reported to allow the selection of fractions having increased protein and fiber content (Rizzello et al., 2012). Moreover, the outer and inner pericarp are rich in branched heteroxylans and cellulose (insoluble dietary fiber), the testa is a hydrophobic layer rich in lipidic compounds, and the aleurone layer contains bioactive compounds (tocols, phenolic acids, and B vitamins), proteins and dietary fibers such as linear arabinoxylans and β-glucans (Hemery et al., 2011). Proteins were positively correlated with ash ($r = 0.9039$; $P < 0.001$), which were significantly higher in MB and DB than in B, and with fiber content ($r = 0.8295$; $P < 0.01$).

The results of colorimetric analysis evidenced that re-milled semolina had markedly lower brown (100-L^*) and red (a^*) indices than milling by-products,

whereas the yellow index (b^*) was similar. Among the by-products, DB was significantly ($P < 0.05$) more red and brown than B, whereas the MB color indices were intermediate, without significant differences with DB and B. The brown and red indices were correlated with dietary fiber ($r = 0.8383$; $P < 0.01$ and $r = 0.7077$; $P < 0.05$, respectively). The brown index was correlated also with ash ($r = 0.7195$; $P < 0.05$). No significant differences in the yellow index were observed among the by-products.

Both phenolic compounds and the antioxidant activity of the re-milled semolina (Table 2) were within the range observed in previous studies (Pasqualone et al. 2014; 2015) and were lower than in the by-products considered. Among the single phenolic acids assessed by HPLC, the most abundant was ferulic acid, followed by sinapic, *p*-coumaric, and vanillic acid, which agrees with previous work (Laddomada et al., 2016). The content of ferulic acid was significantly higher in MB, followed by DB and B, implying that the by-products richer in dietary fiber and ash also were richer in phenolic compounds. Different trends were observed for the other, less abundant, phenolic acids, and for the total soluble phenolic compounds determined by the Folin Ciocalteu reaction. The latter did not show significant difference between MB and DB, but both these by-products showed higher values of total soluble phenolic compounds than B. Phenolics, indeed, are known to be more concentrated in the outer layers of the kernel (Lempereur et al., 1997; Acquistucci et al., 2013; Pasqualone et al., 2013), especially in the aleurone (Brouns et al., 2012). The contents of total soluble phenolic compounds and the antioxidant activity agreed with Beta, Nam, Dexter, & Sapirstein, (2005). The antioxidant activity of the by-products paralleled the trend of ferulic acid, with values significantly higher ($P < 0.05$) in DB and MB than in B. The total soluble phenolic compounds were positively correlated with antioxidant activity ($r = 0.7060$; $P < 0.05$), as reported by Yu (2008) and Soobrattee et al. (2005), as well as with ash ($r = 0.6794$; $P < 0.05$). Phenolics also were correlated with the brown index ($r = 0.7115$; $P < 0.05$), confirming their known tendency to form brown quinones by oxidation (Taranto, Delvecchio, Mangini, Del Faro, Blanco, & Pasqualone, 2012).

The carotenoid pigments did not show any significant differences among the by-products and were notably higher than in semolina. Despite their correlation with yellowness (*b**), reported in case of semolina (Pasqualone et al., 2004), carotenoids were not correlated with this colorimetric index, probably because the prevailing brown tone masked the yellow hue in the by-products.

3.2. Rheologic characteristics of meals composed of re-milled semolina and milling by-products

The bread-making quality of re-milled semolina (Table 3) fulfilled the requirements of the "ordinary" quality category based on the Italian voluntary ranking of flours (Pagani et al., 2006), as modified for re-milled semolina, i.e. by tolerating P/L ratio values up to 2.00 (Pasqualone et al., 2004; 2011).

All by-products caused important alterations in the rheological properties of dough when added to re-milled semolina. A significant ($P < 0.05$) decrease of alveograph W (resistance to deformation) was observed, due to the fiber interference with the gluten network (Tudorica et al., 2002; Aravind et al., 2012), as well as to the diluting of gluten-forming proteins causing dough weakening. W decreased proportionally and significantly ($P < 0.05$) as the level of by-product increased, but at the same level of enrichment, there were no significant differences in W among the three composed meals. Additionally, the P/L ratio significantly increased ($P < 0.05$) by adding 10% of the three by-products to re-milled semolina, and further significant increases were observed at higher percentages. At the same enrichment level, B showed significantly higher P/L values than MB and DB, the latter without significant differences between them. The increase in P/L was due to the relevant hydrophilicity of fiber (Rosell, Santos, & Collar, 2010), that rendered more compact and less extensible the dough, as well as to the already mentioned interference of fiber with gluten formation (Tudorica et al., 2002; Aravind et al., 2012). Hence, all the by-products worsened the alveograph characteristics, with stronger effects at higher doses, with particularly negative effects of B on P/L.

Overall, farinograph characteristics of the composite meals showed that more water was absorbed than in the re-milled semolina, and increasing the enrichment level made water absorption rise significantly ($P < 0.05$), due to the contribution of dietary fibers, which are very hygroscopic. At the same addition level, the composite meals containing DB showed significantly higher water absorption capacity than those added of B (at 10%) and MB (at 20%).

No significant differences in dough-development time were observed between the composite meals containing micronized by-product and re-milled semolina. However, compared to pure re-milled semolina, the dough-development time significantly increased after adding coarse bran at both addition levels (10B and 20B) or DB at 20%. Again, these findings were due to the added fiber, which competed for water with the flour proteins and starch (Rosell, Santos, & Collar, 2010), and had negative physical and mechanical effects on the formation of the gluten network (Noort, Van Haaster, Hemery, Schols, & Hamer, 2010), especially in case of coarser particle sizes. These results were confirmed by a significant correlation observed between dough development time and water absorption capacity ($r = 0.6005$; $P < 0.001$), as well as between water absorption capacity and alveographic P/L ($r = 0.6005$; $P < 0.001$). In common wheat, an increase of dough-development time was observed when increasing amounts of pearled fractions were added to refined flours (Blandino et al., 2013).

Dough stability significantly increased when the by-products were added to re-milled semolina, except for 10MB, 20MB, and 10DB. By comparing the composite meals at the same addition level, dough stability was significantly different ($P < 0.05$) in the order B > DB > MB. For the same by-product type, significant increases always were observed by raising the added percentage. These increases, apparently positive, were actually attributed to the stiffening effect of dietary fiber, which allowed dough to maintain the consistency of 500 B.U. for longer time, more than to a real strengthening effect on gluten, as evidenced by the decrease of alveograph W. In fiber-enriched wheat dough, the replacement of flour generally implicates a change in dough stability, although the reported effects often are disputed (Noort et al., 2010; Rosell et al., 2010). Blandino et al.

(2013) did not observe significant variations in dough stability when pearled fractions were added to refined flour.

3.3. Characteristics of breads containing milling by-products

As predicted by the rheological characteristics of the dough, the specific volume of bread added of milling by-products was lower, or at most equal, compared to that of bread from re-milled semolina alone (Table 4), with the only exception of 10B-Br. The specific volume of 10B-Br, however, together with 20B-Br, was overestimated due to the presence of defects such as internal fractures and holes. In fact, the same B-Br samples showed high hardness values when bread slice portions devoid of defects were submitted to a texture analysis. Interesting results were obtained by employing MB in bread making, particularly at 10%, a level that did not cause significant differences ($P<0.05$) with re-milled semolina bread. DB-Br samples were the least voluminous breads, without significant differences when the addition level increased. A significant, depressing effect of intermediate pearled fractions on common wheat bread volume is reported (Blandino et al., 2013; Gan et al., 1992; Hemdane et al., 2015). In general, the reduction in bread volume was related to the high amount of dietary fiber present in the bran, which diluted the gluten protein and interfered with the formation of an optimal gluten matrix during fermentation and baking. The addition of fiber made dough stiff and barely extensive, reducing the ability to retain gas (Wang, van Vliet, & Hamer, 2004).

The results of texture analysis revealed significant differences among the bread samples (Table 4). In agreement with the specific volume data (and excluding volume-overestimated B-Br samples), 10MB-Br was the softest bread, not significantly different ($P < 0.05$) than bread from re-milled semolina alone, followed by the 20MB-Br and 10DB-Br samples. Crumb hardness, i.e. resistance to compression, increased significantly by raising the amount of each by-product added, with the hardest value in 20DB-Br. Hardness values were in the range observed in a previous study on durum wheat bread leavened by compress yeast

(Giannone et al., 2016), but were higher than in bread obtained by using sourdough (Raffo et al., 2003). Blandino et al. (2013) observed increases in bread hardness when the refined flour of intermediate pearled fractions was added.

Springiness, i.e. the elastic recovery, of bread of re-milled semolina did not show significant differences with 10MB-Br bread. All other bread samples were less elastic. Significant decreases in springiness were observed when the addition levels increased, with the exception of breads containing coarse bran.

The highest cohesiveness values were observed in bread of pure re-milled semolina and, interestingly, in 20B-Br bread. Cohesiveness of breads containing milling by-products increased at higher addition levels. However, considering the lower dough alveograph and farinograph properties, these findings were probably more attributal to an increase in crumb moisture than to an improvement of bread crumb structure.

Resilience was the only parameter that did not show significant differences between bread of pure re-milled semolina and those containing milling by-products when the level of addition was 10%. However, resilience, which indicates how well a product fights to regain its original position after a stress (Abdelghafor, Mustafa, Ibrahim, & Krishnan, 2011), decreased when the enrichment level increased to 20%, in agreement with Blandino et al. (2013). Overall, the resilience of the examined samples was high, considering that values as low as 0.35 indicate hardened, stale, durum wheat bread, with an inelastic and fragile crumb (Giannone et al., 2016).

Chewiness, is the product of hardness, resilience and springiness and expresses the intensity of chewing needed before swallowing, was lower in bread of re-milled semolina, with no significant differences between the 10MB-Br, 20MB-Br, and 20DB-Br breads.

Color is another fundamental characteristic, strongly influencing consumer choice. Bread crumb became browner as the amount of milling by-product increased, irrespective of the type used (Table 4). The highest brown index values were observed in DB-Br and MB-Br. Red and yellow indices followed the same trend. Blandino et al. (2013) observed that increased substitution of pearled

fractions resulted in a reduction in L^* and an increase in a^* (redness). Previous studies ascertained that the high amount of phenolic compounds in whole meals negatively affects the color of whole meal and dough (Taranto et al., 2012; Pasqualone et al., 2014). Thus, the observed color alterations were contributed by the phenolics in the added milling by-products (Table 2).

Several bioactive compounds also were analyzed and were expected to vary with the addition of milling by-products. The β-glucans (Table 4) significantly increased when raising the enrichment level. By adding 20% of any milling by-product, the β-glucan content doubled in comparison with bread of re-milled semolina alone, but the levels observed could barely allow to reach the suggested daily intake of 3 g needed to maintain normal blood cholesterol levels (European Commission, 2012). No reduction in β-glucans during the bread-making process was observed, which is in agreement with Blandino et al. (2013). Dietary fiber content also significantly increased after raising the enrichment level, but it was more sensitive than β-glucans to the type of by-product added. The highest value was observed in 20MB-Br bread, where the amount of fiber fulfilled the requirements for a "source of fiber" nutrition claim (fiber > 3% of fresh weight), and was close to the value required for a "high fiber" claim (fiber > 6% of fresh weight), according to Annex to Regulation (EC) No 1924/2006 (European Parliament and Council, 2006).

The addition of milling by-products caused an increase in carotenoid pigments compared to that of pure re-milled semolina bread (Table 5). These results were observed previously at the 10% level, irrespective of the milling by-product considered. Further increases made raised the carotenoid level significantly. The carotenoid pigment content in the breads roughly agreed with the expected values, which were calculated by considering the contribution of the raw materials, with modest decreases during bread making. However, other studies conducted on pasta and bread, reported higher carotenoid losses due to the lipoxygenase-mediated oxidation of the polyunsaturated fatty acids during kneading, which in turn starts the oxidation of carotenoids (Borrelli, Troccoli, Di Fonzo, & Fares, 1999; Hidalgo, Brandolini, & Pompei, 2010). This loss is also attributed to non-

enzymatic oxidations during baking, although some single compounds could increase due to isomerization and hydroxylation of carotenes at high temperatures (Hidalgo et al., 2010).

The phenolic compounds (both the soluble fraction, determined by Folin Ciocalteu reaction, and the phenolic acids of the soluble and insoluble fractions together, determined by HPLC) were significantly lower in bread of pure re-milled semolina bread than in all the other bread samples (Table 5). Among the single phenolic acids, ferulic acid was the most abundant in all bread samples, followed by sinapic, *p*-coumaric, and vanillic acid, reflecting the same composition observed in the raw materials. The addition of 10% milling by-products caused an increase of total soluble phenolics and ferulic acid, with further significant increases at 20%. The highest levels of soluble phenolics and ferulic acid were observed in 20DB-Br and 20B-Br, which were not significantly different.

Phenolics markedly decreased during bread making, compared with the values calculated from starting raw materials, probably due to oxidative phenomena during the kneading and leavening steps. The variation resembled that observed during bread making by Menga, Fares, Troccoli, Cattivelli, & Baiano (2010). Interestingly, among the raw materials, the MB milling by-product showed the highest content of ferulic acid but, after baking, MB-Br breads (particularly at 10% addition level) showed the lowest amount among all breads made from composite meals. These findings were probably due to the increased surface area exposed to oxygen in such a small particle-sized by-product. A smaller decrease in total soluble phenolics, and no variation in single phenolic acids, was observed when pure re-milled semolina was processed into bread, probably due to the low polyphenoloxidase activity of re-milled semolina. This enzyme, responsible for the oxidation of phenolics, is localized in the pericarp , particularly in the aleurone, where phenolic compounds also are found (Okot-Kotber, Liavoga, Yong, & Bagorogoza, 2001; Rani, Rao, Leelavathi, & Rao, 2001). Hence, the milling by-products contributed phenolic compounds, but probably also contained enzymes that oxidized a large part of them, especially when the particle size was

small. However, despite the decrease during bread-making, phenolics remained higher in breads containing milling by-products than in re-milled semolina bread.

The addition of milling by-products caused an increase of *in vitro* antioxidant activity compared to that of bread of pure re-milled semolina. By raising the enrichment level from 10% to 20%, the antioxidant activity increased significantly. This trend roughly resembled the variations of total soluble compounds, but better paralleled those of ferulic acid, with the highest values in 20B-Br and 20DB-Br breads. In addition, antioxidant activity increased during bread making, even with the above reported decrease in bioactive compounds, probably due to contribution of Maillard reaction products, which arise during baking and are characterized by antioxidant properties (Osada & Shibamoto, 2006).

4. **Conclusions**

The use of milling by-products in bread making contributed an end-product with an array of functional compounds, such as dietary fiber, β-glucans, phenolics, and carotenoids. Debranning fractions, both micronized and air-classified (MB) or not (DB), did not cause excessive alterations in the textural properties compared with those of bread from re-milled semolina alone. In particular, 10MB-Br did not show significant differences ($P < 0.05$) in specific volume, crumb hardness, resilience, and chewiness with pure re-milled semolina bread, but had higher dietary fiber, phenolics, and antioxidant activity. Modern consumers are aware of the importance of increasing fiber consumption, thus, even bread with 20% added bran could be well accepted, in spite of some structural defects such as crumb fractures.

These results could help with establishing new bakery products enriched with those durum milling by-products that are usually destined to animal feed, while helping to increase the daily intake of fiber and antioxidants.

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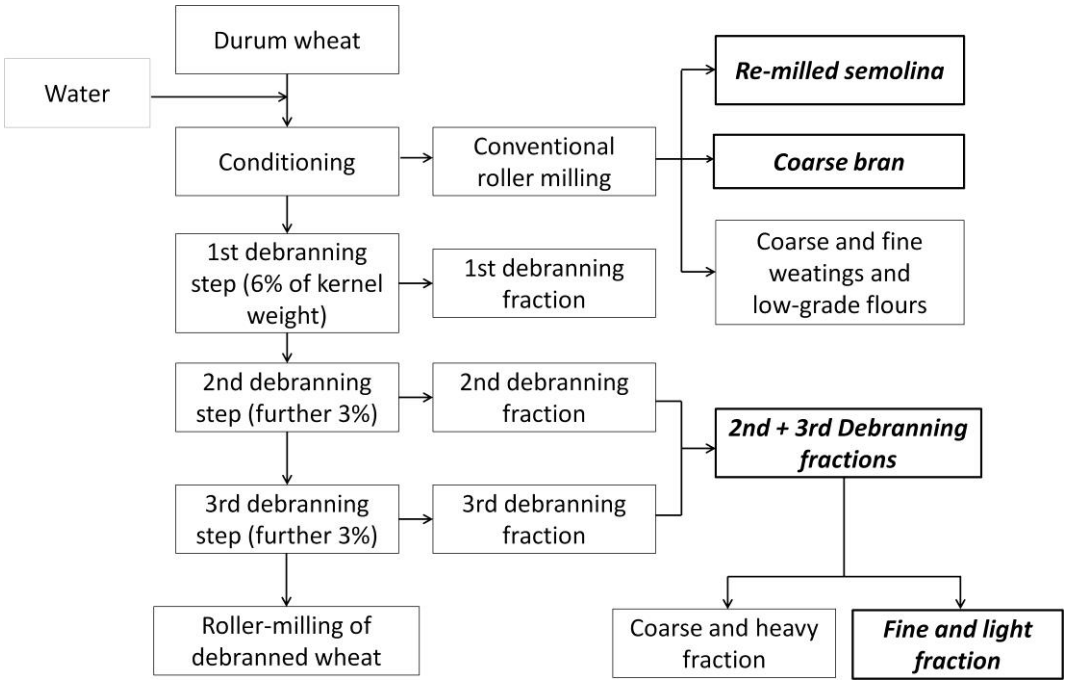
565
566 **Figure captions**
567
568 **Fig. 1.** Flow-chart of productive process and sampling plan. The collected
569 samples are indicated in bold, italics.
570



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** indicates key references, as required by the authors' Guidelines, for the reasons below.*

1) *Hemery et al. (2011) is a key reference because it extensively reviews the debranning of cereals and its several patented processes.*
2) *Hemdane et al. (2015) is a key reference because it extensively reviews the effect of wheat bran on bread quality, although focusing only on Triticum aestivum L.*

3) *Brouns et al. (2012) is a key reference because it extensively reviews the composition, separation, health aspects, and potential food use of wheat aleurone and milling fractions containing it.*
4) *Rizzello et al. (2012) is a key reference because it describes the use of micronized by-products from debranned durum wheat in bread-making, although focusing only on bread obtained from Triticum aestivum L.*
5) *Pasqualone et al. (2004) is a key reference because it describes the quality characteristics of durum wheat re-milled semolina typically used for bread-making in the Mediterranean area.*

Table 1

Content of moisture, protein, ash, dietary fiber, β-glucans, and color characteristics of re-milled semolina and durum wheat milling by-products. B = coarse bran; DB = second and third debranning fractions mixed together; MB = thin sub-fraction from micronized and air-classified DB mix. Different letters in column indicate significant differences ($P < 0.05$).

Type of milling product	Moisture (g 100 g^{-1})	Protein (g 100 g^{-1})	Ash (g 100 g^{-1})	Dietary fiber (g 100 g^{-1})	β-glucans (g 100 g^{-1})	Brown index (100 – *L**)	Red index (*a**)	Yellow index (*b**)
Re-milled semolina	12.7±0.1	12.8±0.1	0.82±0.02	3.0±0.1	0.23±0.01	12.90±0.20	-0.17±0.01	21.65±0.11
B	15.6^{a}±0.1	11.5^{c}±0.3	5.39^{b}±0.14	17.1^{c}±0.6	1.24^{NS}±0.20	27.58^{b}±2.56	3.26^{b}±0.62	19.81^{a}±0.74
DB	13.9^{b}±0.3	17.2^{b}±0.4	6.80^{a}±0.19	24.2^{b}±0.3	1.39^{NS}±0.12	33.46^{a}±1.67	4.53^{a}±0.47	20.42^{a}±0.22
MB	10.5^{c}±0.3	18.7^{a}±0.5	6.59^{a}±0.21	43.5^{a}±2.0	1.36^{NS}±0.20	30.64^{ab}±0.90	3.96^{ab}±0.40	21.23^{a}±0.61

Table 2

Carotenoid pigments, phenolic compounds, and antioxidant activity of re-milled semolina and durum wheat milling by-products. B = coarse bran; DB = second and third debranning fractions mixed together; MB = thin sub-fraction from micronized and air-classified DB mix. Different letters in column indicate significant differences ($P < 0.05$).

Milling product	Carotenoid pigments (mg kg^{-1} β-carotene d.m.)	Total soluble phenolic compounds* (mg g^{-1} ferulic acid d.m.)	Phenolic acids** (μg g^{-1} d.m.)						*In vitro* antioxidant activity (DPPH SC%)
			p-Hydroxy benzoic acid	Vanillic acid	Siringic acid	*p*-Coumaric acid	Sinapic acid	Ferulic acid	
Re-milled semolina	5.46±0.04	1.92±0.01	n.d.	1.90±0.09	0.75±0.19	3.00±0.13	16.62±1.98	107.00±4.58	12.7±0.4
B	9.64^{a}±1.04	4.08^{b}±0.14	12.16^{b}±0.05	29.03^{a}±2.69	11.09^{a}±0.54	39.19^{a}±3.43	192.93^{a}±10.91	1281.31^{c}±14.87	20.7^{b}±1.3
DB	9.69^{a}±0.24	4.56^{a}±0.16	11.52^{b}±0.95	22.75^{b}±1.33	5.34^{b}±0.78	27.16^{b}±1.52	171.80^{b}±10.81	1713.88^{b}±35.66	26.0^{a}±0.5
MB	8.98^{a}±0.85	4.51^{a}±0.37	21.69^{a}±2.12	33.48^{a}±4.69	11.10^{a}±0.68	36.82^{a}±6.89	199.14^{a}±33.30	1894.59^{a}±35.35	26.3^{a}±0.6

*Total phenolics of the soluble fraction determined by Folin Ciocalteu reaction; **Single phenolics of the sum of soluble and unsoluble fractions determined by HPLC.
DPPH = 2,2-diphenyl-1-picrylhydrazyl radical; SC% = percent capacity of scavenging the DPPH radical; n.d. = not detected.

Table 3

Alveograph and farinograph characteristics of dough obtained from meals composed of re-milled semolina and durum wheat milling by-products. 10B and 20B = re-milled semolina added of coarse bran at 10% and 20% (w/w), respectively; 10DB and 20DB = re-milled semolina added of a mix of second and third debranning fractions at 10% and 20% (w/w), respectively; 10MB and 20MB = re-milled semolina added of the thin sub-fraction from micronized and air-classified mix of second and third debranning fractions, at 10% and 20% (w/w), respectively. Different letters in column indicate significant differences ($P < 0.05$).

	Alveograph indices			Farinograph indices	
Type of meal	**W (10^{-4} J)**	**P/L**	**Water absorption capacity (%)**	**Dough-development time (min)**	**Dough stability (min)**
Re-milled semolina	242^{a}±3	1.56^{e}±0.14	63.2^{e}±0.3	2.3^{cd}±0.3	5.2^{d}±0.4
10B	164^{b}±7	4.21^{c}±0.41	65.0^{d}±0.9	3.6^{b}±0.2	11.0^{a}±0.3
20B	118^{c}±38	8.64^{a}±2.73	67.1^{ab}±1.0	4.9^{a}±0.8	8.9^{b}±0.4
10DB	182^{b}±3	2.63^{d}±0.35	66.2^{bc}±0.7	2.7^{cd}±1.0	6.0^{d}±0.3
20DB	134^{c}±7	6.55^{b}±1.11	67.8^{a}±0.4	4.6^{a}±0.7	7.2^{c}±1.4
10MB	170^{b}±3	2.58^{d}±0.34	65.3^{cd}±0.3	1.9^{d}±0.2	3.8^{e}±0.5
20MB	132^{c}±3	6.08^{b}±0.16	66.6^{b}±0.6	3.4^{bc}±0.2	5.5^{d}±0.4

B.U. = Brabender Units.

Table 4

Specific volume, texture and color characteristics, dietary fiber and β-glucan of bread obtained from meals composed of re-milled semolina and durum wheat milling by-products. 10B-Br and 20B-Br = breads obtained from re-milled semolina added of coarse bran at 10% and 20% (w/w), respectively; 10DB-Br and 20DB-Br = breads obtained from re-milled semolina added of a mix of second and third debranning fractions at 10% and 20% (w/w), respectively; 10MB-Br and 20MB-Br = breads obtained from re-milled semolina added of the thin sub-fraction from micronized and air-classified mix of second and third debranning fractions, at 10% and 20% (w/w), respectively. Different letters in column indicate significant differences ($P < 0.05$).

Type of bread	Specific volume (mL g^{-1})	Hardness (N)	Springiness (mm)	Cohesiveness	Resilience	Chewiness (N*mm)	Brown index ($100 - L^*$)	Red index (a^*)	Yellow index (b^*)	Dietary fiber (g 100 g^{-1})	β-glucans (g 100 g^{-1})
Re-milled semolina bread	2.18^{b}±0.02	25.7^{e}±0.3	8.80^{a}±0.10	0.60^{a}±0.01	0.71^{a}±0.01	160.1^{b}±11.1	21.89^{d}±0.46	0.50^{d}±0.09	21.25^{d}±0.16	2.0^{f}±0.1	0.22^{c}±0.01
10B-Br	2.32^{a}±0.06*	36.8^{c}±0.2	7.54^{d}±0.11	0.51^{c}±0.01	0.70^{ab}±0.03	194.2^{a}±9.0	26.89^{c}±0.66	2.39^{c}±0.26	21.92^{c}±0.44	3.7^{e}±0.1	0.35^{b}±0.01
20B-Br	2.19^{ab}±0.09*	43.9^{b}±0.1	7.37^{d}±0.13	0.60^{a}±0.01	0.66^{b}±0.02	213.5^{a}±15.2	30.88^{b}±0.94	3.86^{b}±0.33	23.22^{b}±0.44	5.4^{c}±0.2	0.46^{a}±0.02
10DB-Br	1.82^{d}±0.02	31.5^{d}±0.2	8.08^{c}±0.11	0.37^{d}±0.01	0.69^{ab}±0.02	175.6^{b}±10.3	26.02^{c}±0.23	2.71^{c}±0.15	21.86^{c}±0.38	4.4^{d}±0.2	0.34^{b}±0.02
20DB-Br	1.86^{d}±0.02	47.1^{a}±0.1	7.28^{d}±0.10	0.53^{bc}±0.02	0.64^{b}±0.01	217.7^{a}±13.1	34.60^{a}±0.66	4.43^{a}±0.25	25.95^{a}±0.69	6.8^{b}±0.3	0.42^{a}±0.03
10MB-Br	2.16^{b}±0.01	25.4^{e}±0.2	8.93^{a}±0.16	0.49^{c}±0.02	0.73^{a}±0.02	165.6^{b}±9.3	29.76^{b}±0.92	2.74^{c}±0.16	24.10^{b}±0.58	6.3^{b}±0.3	0.32^{b}±0.01
20MB-Br	1.96^{c}±0.03	31.6^{d}±0.2	8.44^{b}±0.14	0.55^{b}±0.02	0.66^{b}±0.03	176.0^{b}±14.6	34.49^{a}±0.50	4.22^{a}±0.06	25.92^{a}±0.49	10.7^{a}±0.6	0.47^{a}±0.01

*Overestimated data due to the presence of crumb defects, such as internal fractures and holes.

Table 5

Carotenoid pigments, phenolic compounds, and antioxidant activity of bread obtained from meals composed of re-milled semolina and durum wheat milling by-products. 10B-Br and 20B-Br = breads obtained from re-milled semolina added of coarse bran at 10% and 20% (w/w), respectively; 10DB-Br and 20DB-Br = breads obtained from re-milled semolina added of a mix of second and third debranning fractions at 10% and 20% (w/w), respectively; 10MB-Br and 20MB-Br = breads obtained from re-milled semolina added of the thin sub-fraction from micronized and air-classified mix of second and third debranning fractions, at 10% and 20% (w/w), respectively. Different letters in column indicate significant differences ($P < 0.05$).

Type of bread	Carotenoid pigments (mg kg^{-1} β-carotene d.m.)	Total soluble phenolic compounds* (mg g^{-1} ferulic acid d.m.)	Phenolic acids** (μg g^{-1} d.m.)						*In vitro* antioxidant activity (DPPH SC%)
			p-Hydroxy benzoic acid	Vanillic acid	Siringic acid	*p*-Coumaric acid	Sinapic acid	Ferulic acid	
Re-milled semolina bread	5.02^{c}±0.30	0.10^{d}±0.01	0.74^{c}±0.12	2.03^{c}±0.21	1.04^{c}±0.23	4.32^{d}±0.23	11.80^{e}±0.83	122.51^{e}±10.33	15.56^{f}±1.03
10B-Br	6.12^{b}±0.21	0.14^{c}±0.01	0.83^{c}±0.10	4.36^{b}±0.35	1.80^{b}±0.04	9.20^{cd}±0.11	43.11^{c}±1.49	329.78^{c}±3.51	27.87^{d}±0.65
20B-Br	7.11^{a}±0.26	0.19^{b}±0.01	1.37^{bc}±0.53	8.67^{a}±0.89	2.85^{ab}±0.66	12.65^{bc}±0.61	61.87^{b}±2.01	485.36^{a}±30.19	35.74^{ab}±0.94
10DB-Br	6.13^{b}±0.24	0.20^{b}±0.02	3.03^{ab}±0.77	9.54^{a}±0.22	2.26^{b}±0.65	15.58^{ab}±0.71	67.67^{ab}±0.91	408.11^{b}±7.94	30.41^{c}±1.30
20DB-Br	7.08^{a}±0.29	0.22^{b}±0.02	3.66^{a}±0.06	11.71^{a}±2.44	3.53^{a}±0.08	15.98^{ab}±2.45	70.38^{a}±6.60	538.68^{a}±6.60	39.30^{a}±3.20
10MB-Br	6.03^{b}±0.14	0.13^{c}±0.01	1.15^{c}±0.50	5.21^{b}±0.95	1.65^{b}±0.17	10.12^{c}±0.92	27.52^{d}±4.09	152.42^{d}±4.26	23.43^{e}±1.29
20MB-Br	6.85^{a}±0.05	0.29^{a}±0.02	3.45^{a}±0.68	9.33^{a}±0.15	3.47^{ab}±0.55	17.7^{a}±0.52	33.92^{cd}±2.44	325.14^{c}±16.79	33.66^{b}±1.31

*Total phenolics of the soluble fraction determined by Folin Ciocalteu reaction. **Single phenolics of the sum of soluble and unsoluble fractions determined by HPLC.
DPPH = 2,2-diphenyl-1-picrylhydrazyl radical; SC% = percent capacity of scavenging the DPPH radical.