Nutritional and technological quality of bread enriched with an intermediated pearled wheat fraction

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Title: Nutritional and technological quality of bread enriched with an intermediated pearled wheat fraction

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Authors: Massimo Blandino[a*], Valentina Sovrani[a], Federico Marinaccio[a], Amedeo Reyneri[a], Luca Rolle[a], Simone Giacosa[a], Monica Locatelli[b], Matteo Bordiga[b], Fabiano Travaglia[b], Jean Daniel Coïsson[b], Marco Arlorio[b]

Affiliation:

[a] Università di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari, via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy.

[b] Università del Piemonte Orientale "A. Avogadro", Dipartimento di Scienze del Farmaco, Largo Donegani 2, 28100, Novara (NO), Italy.

* Corresponding author: Tel: +39-011-6708895; fax +39-011-6708798.

E-mail address: massimo.blandino@unito.it

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Abbreviations: ARs, alkylresorcinols, DF, dietary fibre; DDT, dough development time; DON, deoxynivalenol; dw, dry weight; TPC, total phenolic content; TAA, total antioxidant activity; TE, trolox equivalents; TPA, Texture Profile Analysis.

Abstract

A strategy to maximize the health benefits of wheat-based products enriched with refined flour and selected fractions of kernel, obtained by sequential pearling, has been tested. Five mixtures of refined commercial flour with an increasing replacement of a pearled wheat fraction were used to prepare bread and were compared with a control for the dough rheological properties (Mixolab® parameters), the bioactive compound content, deoxynivalenol (DON) contamination and the physical properties (volume, crust color, instrumental crunchiness and crumb texture profile analysis parameters). The antioxidant and dietary fibre contents increased linearly as the flour was enriched with the pearled fraction. The dietary fibre, β-glucan, total phenolic, alkylresorcinol content and the antioxidant activity increased significantly at a replacement level of 10%, while the technological properties were not significantly different from those of the control. It has been shown that refined flour could be enriched through the addition of a selected wheat pearled fraction and the bioactive compound content of composite bread could be improved, while few rheological and technological differences could be obtained and the risk for DON contamination could be limited.

1. Introduction

The consumption of whole grains, which are rich in dietary fibre (DF), vitamins, minerals and bioactive compounds, has been reported to offer many health benefits and help to reduce the incidence of several diseases. The regular consumption of whole-grain foods reduces coronary heart disease rates and several forms of cancer and helps to regulate the blood glucose level (Slavin, Jacobs, Marquart & Wiemer, 2001). Moreover, several phytochemicals of wheat grain, such as phenolic compounds and alkylresorcinols (AR), have been reported to exert antioxidant and anti-inflammatory effects (Anson et al., 2011). However, consumers' acceptance of whole grain foods is limited, due to their lower volume and coarser texture than refined ones (Zhang & Moore, 1999); thus, refined white bread remains the most commonly consumed type of bread.

The replacement of refined flour with a percentage of bran fractions, obtained during the roller milling process, is actually the main way of increasing the bioactive compound concentration in wheat flour and the derived products (Noort, van Haaster, Hemery, Schols & Hamer, 2010). Bran is a kernel fraction that contains the external kernel layers, in addition to hyaline and aleuronic layers rich in DF, phenolic compounds and ARs and shows high antioxidant properties (Beta, Nam, Dexter & Sapirstein, 2005). Nevertheless, the use of wheat bran in wheat flour for bread-making also results in changes in the dough properties, processing techniques and bread quality characteristics (lower volume, darker crust and denser crumb texture) (De Kock, Taylor & Taylor, 1999; Noort et al., 2010). Pre-treatments of bran, such as pre-hydration, fermentation or heat treatments, can be employed to modify its structure and composition, in order to enhance the bio-accessibility of nutritional compounds and minimize the negative effects related to dough rheology and bread volume (Nelles, Randall, & Taylor, 1998; de Kock et al., 1999). The addition of gluten or the use of surfactants and enzyme in the bread formula could partially

compensate the negative effects of bran replacement on the bread-making performance and bread quality (Sanz Penella, Collar & Haros, 2008).

Another concern regarding the use of bran to partially replace refined flour is that this by-product is the wheat fraction, that is most contaminated by mycotoxins, or heavy metals and pesticides (Cheli et al., 2010). Among mycotoxins, deoxynivalenol (DON), that belongs to type-B trichothecenes and that could contaminated wheat kernel and cereal products, is associated with serious mycotoxicosis in humans and animals since it inhibits protein biosynthesis (Bottalico & Perrone, 2002). The present EU admissible maximum levels for DON are 1250 $\mu g\ kg^{-1}$, 750 $\mu g\ kg^{-1}$ and 500 $\mu g\ kg^{-1}$ for unprocessed wheat kernel, wheat flour or bran and bread, respectively (EC 2006),

An alternative strategy to maximize the health benefits of wheat-based products, and avoid the defects derived from the use of whole grain or bran, could be to enrich refined flour with fractions of external kernel layers, obtained using grain fractionation technologies in order to separate the negative and positive elements (Delcour, Rouau, Courtin, Poutanen & Ranieri, 2012). Among the fractionation processes, sequential pearling has provided interesting results: the degree of pearling could efficiently be modulated to separate the external bran fractions, which are characterized by a safety risk and coarse fibre, from the fractions with potential high health benefits (Sovrani et al., 2012). Thus, an intermediate wheat kernel pearled fraction could be used as a functional ingredient in order to enrich wheat-based products in bioactive compounds but also to the risks associated with the use of bran layers. In this work, a pearled fraction was chosen in order to offer the best compromise between high nutritional value and low contamination risk. The first external fraction (8% of kernel weight) was separated and the fraction resulting from a second pearling (another 8% of kernel weight) was used for bread-making.

The aim of the study was to determine the effect of replacing refined white wheat flour with

increasing percentages of the selected pearled wheat fraction on the content of the bioactive compounds and the antioxidant activity of composite bread. Moreover, in order to analyze the impact of the wheat fractions derived from sequential pearling in the bread-making process, the effect of their refined flour replacement on the dough rheological parameters, DON contamination and the technological properties of composite bread has been investigated.

2. Materials and methods

2.1 Wheat grain pearling

The Taylor winter wheat variety, classified as an improver on the basis of the Synthetic Index of Quality method for the quality classification of common wheat (Foca et al., 2007), was used. The pearling process consisted of an abrasive action on the wheat and pearled wheat in an abrasive-type grain testing mill (TM-05C model, Satake, Tokyo, Japan) at a constant speed of 55 Hz. The pearling process was monitored by means of time control. After each pearling passage, the laboratory pearler was thoroughly cleaned with dust aspiration and compressed air, to minimize equipment contamination.

The most appropriate pearled fraction was selected on the basis of results obtained in a previous work (Sovrani et al., 2012). Starting from unprocessed grain, kernels were initially pearled to remove 8% of the original grain weight and this most external fraction was discarded. The remaining kernels were then pearled to remove a second 8% fraction of the original grain weight (8-16%) and this fraction, presumably containing the aleurone layer (Sovrani et al., 2012; Laca Mousia, Diaz, Webb & Pandiella, 2006), was used to replace refined commercial flour for bread-making at different percentages.

The particle size of the selected pearling fraction resulted similar to that of refined commercial flour: in both cases more than 80% of the particles were distributed within the size range < 200 μm.

2.2 Bread making procedure

Five mixtures of refined commercial flour for bread-making with an increasing replacement rate (5%, 10%, 15%, 20%, 25%) of the selected pearled wheat fraction were obtained and employed for bread preparation. Refined flour and the selected pearled wheat fractions were accurately mixed using a rotary laboratory blender (Beccaria S.r.l., Cuneo, Italy). The Chopin® alveograph parameters of the used commercial refined flour were: deformation energy (W) 325 J 10^{-4} and curve configuration ratio (P/L) 0.52.

The breads were prepared according to the AACC Method 10-10.03 for 3 kg of flour (AACC, 2008). The formula contained salt (2% of flour weight), brewer yeast (3% of flour weight) and the optimal water absorption. The dough was then mixed in a spiral mixer (Esmach®, Bonpard Group, Vicenza, Italy) for 15 minutes and divided into three pieces of approximately 400 g/piece, to obtain three loaves, which were placed in baking pans (10.5 x 6 cm^2 and 6.5 cm deep). After fermentation (at 30°C and a relative humidity of 85% for 120 min), the loaves were baked at 215°C for 45 min.

The three composite loaves for each replacement level were used as replicates for chemical and technological analyses.

2.3. Chemicals

The total Dietary Fibre (DF) and Mixed-Linkage β-Glucan kits for enzymatic determinations were supplied by Megazyme (Megazyme International Ireland Ltd, Wicklow, Ireland). The solvents (HPLC or GC grade) and formic acid (50%, LC–MS grade) were purchased from Sigma–Aldrich (Milan, Italy). The water was obtained from Milli-Q instrument (Millipore Corp., Bedford, MA, USA). The antibody-based immunoaffinity columns were supplied by VICAM (Waters Corporation, Watertown, MA, USA). The analytical standards (purity ≥ 95%) and all the other chemicals (reagent-grade level) were purchased from Sigma–Aldrich (Milan, Italy).

2.4 Chemical analyses

2.4.1. Sample preparation

The refined commercial flour and the selected wheat pearled fraction were analysed without any pre-treatment. The whole Taylor cv kernels were ground using a laboratory centrifugal mill (ZM-100; Retsch GmbH, Haan, Germany) with a 1 mm opening. Bread samples were ground in the previous laboratory mill, and in the case of DF, the total phenolic content (TPC) and the total antioxidant activity (TAA) determinations, they were also freeze-dried (Heto Drywinner 8, Copenhagen, Denmark) according to the following procedure: pre-freezing – 25 °C for 1 hour; primary drying -10 °C for 16 hours and 0 °C for 16 hours; secondary drying 10 °C for 30 hours and 20 °C for 10 hours. Finally, the lyophilized samples were ground in an oscillatory mill (Mixer Mill MM440, Retsch GmbH, Hann, Germany).

2.4.2. Proximate composition

All the methods had previously been applied to characterize the wheat grain pearling fractions (Sovrani et al. 2012). Briefly, the moisture content, which was determined in order to express the results on a dry weight (dw) basis, was obtained using a Sartorius MA30 thermo-balance (Sartorius AG, Goettingen, Germany). The total nitrogen content and total protein content (conversion factor: 5.70) were obtained according to the Kjeldahl method, using Kjeltec system I (Tecator, Sweden). The ash content was determined in a muffle furnace. The total DF was measured using the Megazyme total dietary fibre analysis kit, according to the enzymatic-gravimetric method; the determination was performed employing the Fibertec 1023 system (FOSS Italia S.p.A., Padova, Italy). β-glucan

determination was performed using the Megazyme mixed-linkage β -glucan assay kit, according to the instructions provided by the manufactures.

2.4.3. Total phenolic content (TPC)

Finely ground samples (0.5 g) were treated with 10 mL of an NaOH 4N solution, under stirring and nitrogen flushing, at room temperature, for 4 hours. The solutions were adjusted to pH 1.35 with HCl 6N and then extracted with ethyl acetate. The organic layers were evaporated to dryness and the residue dissolved in 1 mL methanol. The extracts (30-100 µL according to the expected concentration) were added to 100 µL of Folin-Ciocalteu reagent and neutralised with 350 µL of sodium carbonate (5%), and distilled water was then added to a total volume of 2900 µL. After incubation at room temperature for 1 h, the absorbance was measured at 760 nm using a Kontron UVIKON 930 Spectrophotometer (Kontron Instruments, Milan, Italy). A solution containing all the reagents, but without the samples, was used as a blank. The results were expressed as ferulic acid equivalents (calibration curve linearity range: 10–90 µg; $r = 0.9935$).

2.4.4 Total antioxidant activity (TAA)

The TAA was determined adapting the classical DPPH radical scavenging method to the QUENCHER approach (direct measurement of antioxidant activity on solid samples), as previously described in Sovrani et al., 2012. Briefly, 5 milligrams of flour samples (white flour, pearled fraction and whole Taylor wheat) or 20 milligrams of freeze-dried bread samples were weighted, then 700 µL of methanol and 700 µL of a 100 µM $DPPH^{\bullet}$ methanolic solution were added. The samples were vortex-mixed and the reaction was then carried out in the dark under stirring for 25 min. The samples were promptly centrifuged for 1 min at 14000 rpm (Microcentrifuge 5417 R, Eppendorf Italia, Milan, Italy) and the absorbance was then measured at 515 nm after exactly 30 min of reaction

(Kontron UVIKON 930 spectrophotometer, Kontron Instruments, Milan, Italy). A control solution (without sample) was tested under the same conditions, in order to calculate the $DPPH^{\bullet}$ inhibition percentage. The final results were expressed as mmol of trolox equivalents (TE) per kg of sample (dw) through a calibration curve.

2.4.5. Total alkylresorcinol (AR) content

Alkylresorcinols (AR) were extracted with ethyl acetate from flour and ground bread samples and analyzed by means of gas chromatography (GC), according to Ross et al. (2003). Briefly, 200 µL of 0.5 mg/mL methyl behenate solution was added as an internal standard to the samples (0.5 g of flour or 2.5 g of bread), which were extracted with 40 mL of ethyl acetate for 24 h through continuous shaking at 20 °C. The samples were then centrifuged at 20,800 g for 20 min at 4 °C and subsequently filtered through 0.45 mm filters before the derivation step.

Aliquots (100 µL) of the AR extract were placed in a glass-stoppered test tube and trimethylsilyl ether derivates of the AR were prepared by adding 100 µL of BSA+TMCS+TMSI silylating reagent. The tubes were shaken to properly mix the sample in the reagent and then heated at 65 °C for 30 min. Any excess reagent was then removed under nitrogen, and the residue was re-dissolved in hexane (1 mL) and stored at -20 °C for no more than one week. All the working standard solutions were freshly prepared daily prior to use.

The qualitative/quantitative characterization of the AR extracts was obtained on a GC-17A Shimadzu gas chromatograph, coupled to a flame ionization detector (FID). The separation was performed on a TR-5MS capillary column (5% Phenyl Polysilphenylene-siloxane; length 15 m, inner diameter 0.25 mm, film thickness 0.25 µm; Thermo Fisher Scientific) with the following temperature program: 50 °C (0 min), raised by 10 °C min^{-1} to

300 °C and held for 20 min at 300 °C. H_2 was used as the carrier gas at an inlet pressure of 0.7 bar and with a constant column flow rate of 1.0 mL min^{-1}. The injector and detector temperatures were 250 and 350 °C, respectively. The used apparatus was equipped with a split/splitless injector. Individual compounds (5-*n*-heptadecylresorcinol, 5-*n*-nonadecylresorcinol, 5-*n*-heneicosylresorcinol, 5-*n*-tricosylresorcinol and 5-*n*-pentacosylresorcinol) were quantified against the internal standard by automatically integrating the peak areas. Finally, the results were expressed as the sum of the identified ARs.

2.4.6. DON contamination

The DON content was analysed using a high performance liquid chromatography (HPLC-MS-MS) method (range 80-4000 μg kg^{-1}). The bread was milled using a laboratory centrifugal mill (ZM-100; Retsch, Haan, Germany) with a 1 mm opening. 25 g of milled sample was extracted with 100 mL of water in a blender at a high speed for 30 minutes; the extract was then filtered and collected. Antibody-based immunoaffinity columns (DON test™ WB Columns VICAM) were used for the clean-up of the sample extracts. Before sample loading, the column was conditioned with 1 mL of deionized water. 1 mL of the sample was loaded into the column at a rate of approximately 1-2 drops s^{-1}. The column was washed with 5 mL of distilled water. DON was eluted from the column using 2 mL of methanol. DON was quantified by the injection of 10 μL of diluted eluate into the HPLC-MS-MS system, which consisted of a Varian 212-LC Chromatography Pump and a 310-MS TQ Mass Spectrometer. The analytical column was a reverse Varian Polaris C18-A (100 x 2.00 mm, 3 μm) while the mobile phase was a gradient mixture of methanol and water fed at a flow rate of 0.2 mL/min. The DON retention time was 4.1 min with a runtime of 13 min. Mass spectrometric analyses were performed in the negative ion mode. The nebulising gas was N_2 (20 psi), the drying gas was air (300 °C, 25 psi), the needle voltage

was -4500 V, the shield voltage was -600 V, the detector voltage was 1650 V, the capillary voltage was -60 V and the collision energy voltage was 16 V. The deprotonated molecule (m/z = 295) was fragmented to its product ion (m/z = 265) and used for quantification purposes. The deprotonated molecule (m/z = 295) was fragmented to its product ion (m/z = 138) and used for identification purposes.

The percentage of recovery, obtained using a Certified Reference Materials (CRM Trilogy® Analytical Laboratory, 2.1 mg kg^{-1} ± 0.2 mg kg^{-1}) was 79% (Relative Standard Deviation = 6%). The limit of detection (LOD) and the limit of quantification (LOQ) were 5 µg kg^{-1} and 16 µg kg^{-1}, respectively.

2.5 Technological quality analyses

2.5.1. Rheological properties of the flour

The mixing and pasting behaviours of the control and different replaced flours was studied using a Mixolab® analyser (Chopin Technologies, Paris, France), according to the ICC Standard Method 173 (ICC, 2010). The instrument allows specific information to be obtained about the behaviour of flour constituents (starch, protein, water) by continuously measuring the torque (Nm) produced by the passage of the dough between two kneading arms submitted to both shear stress and a temperature constraint. The resulting Mixolab® curve is separated into five different stages. A detailed description of the physical changes that occur during a Mixolab measurement has been reported in Rosell, Collar & Haros (2007). The key parameters derived from the Mixolab curve are water absorption (g kg^{-1}), or percentage of water required for the dough to produce a torque (peak C1) of 1.1 Nm, Dough Development Time (DDT, min), or the time necessary to reach the maximum torque (C1) at 30°C, dough stability, protein weakening i.e. decrease in dough consistency due to shear and temperature stress (C2, Nm), starch gelatinization i.e. the starch

granules absorb water and this results in an increase in viscosity (C3, Nm), amylase activity (C4, Nm) and starch gelling (C5, Nm).

2.5.2. Crust color

The chromatic characteristics of the bread crust were determined using a Minolta Chroma Meter (Model CR-400, Minolta Co., Osaka, Japan) reflectance spectrophotometer. Standard illuminant C was used as the reference. The analysis was performed in triplicate at 3 different point for each loaf. The color values of L*, a*, b*, C and h* were determined directly by the instrument in accordance with Commission Internationale de L'Eclairage (1986).

2.5.3. Combined acoustic-mechanical analysis of the bread crust

A penetration test was carried out to assess the mechanical and acoustic properties of the bread crust, using a TA-XT Plus Texture Analyzer (SMS-Stable Micro Systems, Surrey, UK), combined with an AED Acoustic Envelope Detector supplied by from the same manufacturer. Force and acoustic emission acquisitions of were made simultaneously using the Texture Exponent software (Stable Micro Systems), with a data rate of 500 points per second during a compression/penetration test (Piazza, Gigli & Ballabio, 2007). Each loaf was penetrated by a P/6 6-mm steel cylindrical probe, a deformation of 20 mm was applied with a test speed of 1 mm s^{-1} and an instrumental trigger of 0.05 N was used. The microphone was placed at a fixed distance of 10 mm from the sample for the acoustic measurements. In order to minimize the noise, the acoustic measurements were filtered through an integrated 1-kHz high pass filter, and a 24 dB instrumental gain was applied.

The analysis was performed in triplicate at 3 different points for each loaf. The following mechanical and acoustic parameters were determined from the force-distance and acoustic spectra according to Piazza et al. (2007) and Chen, Karlsson & Povey (2005).

Total energy (mJ), the maximum acoustic emission, (dB (SPL)), the acoustic emission peak average (dB (SPL)) using two different threshold values of 5 and 15 dB (SPL), respectively, during data integration, in order to discriminate between the total and "high" acoustic emission peaks (Rolle, Giacosa, Torchio & Río Segade, 2012).

2.5.4. Bread volume

Loaf volume was determined 1 h after baking, by means of a rapeseed displacement method, AACC Standard 10-05 (AACC, 2008).

2.5.5. Breadcrumb texture profile analysis

Texture measurements were performed on two slices (20 mm thick), cut out from the central part of the three replicated loaves for each mixture of refined flour and pearled fraction, 4 h after baking. On average, six measurements per slice were made. The bread slices were compressed in the central area using a SMS P/35 flat probe (Stable Micro Systems) for a 50% deformation of the slice (Wang, Rosell & Benedito de Barber, 2002) with a waiting time between the two bites of 30 seconds, using 1 mm s^{-1} as the speed test (Caballero, Gómez & Rosell, 2007). An instrumental trigger of 0.05 N was applied. The typical texture profile analysis parameters were determined from the Force-Distances curves and calculated by the software: hardness (N), cohesiveness (adimensional), gumminess (N), springiness (mm), chewiness (mJ), and resilience (adimensional) (Fik & Surówka, 2002).

2.6. Statistical analysis

The results of the chemical and technological analyses are reported as the means of the three loaf replicates, with the exception of the Mixolab® analyses, which was only performed on one replicate for each dough replaced mixture, according with the quantity of sample available and required by the analyses.

The analysis of variance (ANOVA) was used to compare the chemical content and the technological properties of the composite breads at different replacement levels with refined commercial flour and the pearled wheat fraction. The residual normal distribution was verified using the Kolmogorov-Smirnov test, while variance homogeneity was verified using the Levene test. Multiple comparison tests were performed according to the Student-Newman-Keuls test on treatment means. The SPSS for Windows statistical package, Version 17.0 (SPSS Inc., Chicago) was used for the statistical analysis.

3. Results and Discussion

3.1 Chemical characterization of the flour and pearled fraction

First, the refined commercial flour, the selected pearled fraction and the whole wheat kernel were characterized before the pearling process for their chemical composition (protein, DF, β-glucan, total phenolic, AR and ash contents), TAA and DON contamination (Table 1). The selected fraction, rich in outer kernel layers, but which was obtained through a sequential pearling to discard the most external fraction, resulted in a higher concentration of bioactive compounds than both the refined flour and the original whole kernel, confirming previously reported data (Beta et al., 2005; Sovrani et al., 2012). The proteins, DF, β-glucans, total phenols, ARs, ash and TAA were 1.2, 2.9, 1.4, 2.3, 2.5, 2.9 and 3.4 times higher in the pearled fraction than in the corresponding whole kernel, respectively. The pearled fraction had a 15, 7, 10 and 27 higher content of DF, β-glucans, TPC and ARs, respectively, than the refined commercial flour. The TAA of the pearled fraction was 11.4 times higher than in the refined flour.

As far as the AR composition is concerned, the main identified compound was 5-*n*-heneicosylresorcinol, which accounted for about 52% of the white flour and about 49% of both the whole grain and pearled fraction of the Taylor cv. However, the internal percentages of the individual compounds of the refined flour were slightly different from those obtained for the Taylor cv samples (data not shown). In particular, 5-*n*-nonadecylresorcinol was higher in the Taylor wheat samples (about 32%) than in the commercial flour (about 18%), although the latter was richer in 5-*n*-pentacosylresorcinol (about 9% and 3% in the commercial flour and Taylor cv samples, respectively). These differences can be related to the specific characteristics of the considered cultivar.

As far as DON contamination is concerned, several studies have reported that the outermost kernel layers had the highest DON content, which decreased from the external to the internal layers showing biphasic behavior (Sovrani et al., 2012). The first external fraction (8% of kernel weight) was therefore separated and only the fraction resulting from the second pearling level was used for bread-making. Nevertheless, compared to the whole kernel, the selected pearled fraction concentrated DON by 2.5 times and the pearled fraction content of this mycotoxin was 68% higher than the refined flour. A careful control of raw material contamination, especially of the pearled kernel fraction, is required to minimize the DON level in derived products and to respect the maximum permitted DON levels (European Commission, 2006).

3.2 Rheological parameters of the replaced flours

Five mixtures of refined flour for bread-making with an increasing replacement of the selected pearled wheat fraction (5%, 10%, 15%, 20%, and 25%) were obtained and characterized for their rheological properties; the refined flour (no replacement) was analyzed as the control.

The refined flour control in the Mixolab test required a water supply of 59.8% (14% basis) to reach the adequate dough consistency requested by the instrument. The progressive replacement of flour with the pearled fraction increased the amount of water required for the hydration process of the flour, 60.2%, 61.4%, 62.8%, 64.4%, 66.2% for flour substitutions of 5%, 10%, 15%, 20% and 25%, respectively.

The dough development time (DDT) was similar for the control and the 5% replacement level, while it increase clearly at 10% replacement level (Fig. 1); after the increasing replacement of refined flour with the pearled fraction proportionally delayed the DDT. For example, the DDT increased by 2 min compared to the control at 10% replacement level.

On the other hand, dough stability did not vary with the increase in pearled fraction (data not shown).

Increasing the replacement level of refined flour with the pearled fraction did not affect the C2 point (protein weakness), but it did decrease the dough consistency at the C3 (starch gelatinization) and C5 (starch gelling) points. Compared to the control without pearled fraction addition, the differences for these parameters are more pronounced for higher replacement levels than 15%. The C4 point (amylase activity) was unaffected by the flour substitution with the pearled kernel fraction percentage (data not shown).

The collected data confirm the effects on the rheological properties of dough when refined flour is replaced by wheat bran (Sanz Penella et al., 2008; Gómez, Jiménez, Ruiz & Oliete, 2011). This different rheological dough behaviour is mainly related to the competition for water of the added wheat fibres with the flour proteins and starch (Rosell, Santos & Collar, 2010) and to the physical and mechanical negative effect of fibre on the formation of the gluten network (Noort et al., 2010). However, the addition of the pearled fraction seems to lead to a higher DDT than that obtained with bran addition (Gómez et al., 2011). On the other hand, Schmiele, Jaekel, Patricio, Stelle, & Chang (2012) reported that a substitution of refined flour with wheat bran up to 20% decreased DDT, while DDT only increased at 40%-replacement level. Bonnand-Ducasse, Della Valle, Lefebvre & Saulnier (2010) have reported that the water absorption and water-holding capacity of aleurone are higher than those of the outer bran fractions and this effect could be related to a lower arabinose/xylose ratio, These data have been also confirmed by Noort et al. (2010).

Moreover, in fibre enriched wheat dough, the fibre replacement of flour generally implicates a change in dough stability, although the reported effects are often discordant, and a higher dough weakening as a consequence of a gluten-diluting effect (Rosell et al., 2010; Noort et al., 2010; Gómez et al., 2011). However, in the present work, dough

stability and protein weakness were not affected by the replacement of refined flour, probably because the added pearled fraction provided a non-negligible protein content that could have prevented an excessive gluten dilution.

3.3 Bread technology properties

The control (no replacement) and the 5%-, 10%-, 15%-, 20%-, 25%-substituted breads were produced using the previously described flours and were first analyzed for their technology properties: crust color, bread crunchiness and volume, TPA test.

3.3.1 Crust color

ANOVA showed significantly differences in the L*, a*, b*, C and h values for the crusts of bread made with different replacement levels of the refined flour with the pearled wheat fraction (Table 2). An increase in substitution with the pearled fraction resulted in a reduction in the L* (lightness) and h values and an increase in the a* (redness) and Chroma values. Less significant changes were found in the blue-yellow component (b*). In the bread made at replacement levels of 5% and 10%, the lightness (L*), redness (a*) and h values of the crust were significantly different from those of the bread made only with the refined flour, and no differences were observed for Chroma. Modifications in the crust color increased drastically in the enriched bread for higher replacement levels of the refined flour than 10%.

Jiang, Martin, Okot-Kotber, & Seib (2011) suggested that the high amount of TPC in whole wheat flour could negatively affect the color of whole grain foods. In order to obtain the pearled fraction, white wheat cultivars with a brighter appearance could be used to reduce the impact of TPC on bread color. These varieties, mostly soft, although recently hard white wheat cultivars presented a growing interest, especially in Australia and North

America, have agronomical characteristics and bread-making quality similar to that observed for red wheat lines (Ransom, Berzonsky & Sorenson, 2006).

3.5.2 Bread crust crunchiness

The results of the mechanical and acoustical properties of the composite bread crust are reported in Table 2. The total break energy, that is the energy necessary to break the crust and to continue the compression until 20 mm of penetration, was found to be higher in the 0% and 5% flour substituted samples, with a descending trend when the flour replacement level was increased (from 149 mJ to 99 mJ, -35.6%).

The acoustic emission released from crushed food is one of the most important parameters for the evaluation of crispness (Saeleaw & Schleining, 2011) and is related to the consumers' acceptance of the product. The acoustic emission measurements showed that the addition of the pearled fraction influenced the crunchiness "sound" of the product. The maximum acoustic peak only showed significant differences for the high substituted formulations (20 and 25 %). Different behaviour was detected for both the 5 and 15 dB (SPL) thresholds between 0-10% and 15-25% formulations. All the acoustic parameters measured showed a high dispersion of the values, in accordance with the data reported in scientific literature (van Nieuwenhuijzen, Primo-Martín, Meinders, Tromp, Hamer & van Vliet, 2008). The instrumental crust crunchiness parameters are related with the structure of the product, with the chemical composition and with the water activity/water content (Duizer, 2001; van Nieuwenhuijzen, Primo-Martín, Meinders, Tromp, Hamer & van Vliet, 2008).

There were no significant differences in the total break energy or acoustic emissions during compression from the control made with only refined flour until the 10%-

replacement level of refined flour with the pearled fraction. Thus the tactile and acoustic sensation of crispness can be considered the same in these samples.

3.5.3 Bread volume

ANOVA showed a significant effect of the decrease in bread volume that corresponded to the increasing percentage of refined flour replacement (Table 3). This effect began at 10% of replacement of refined flour with the pearled fraction.

Gómez et al. (2011), replacing the refined flour with bran, observed a greater loaf volume reduction: bran reduced the loaf volume by 22% and 31% for a replacement level of 10% and 20%, respectively.

The reduction in bread volume seems to be related to the high amount of DF present in the bran, which diluted the gluten protein and interfered with the optimal gluten matrix formation during fermentation and baking. Moreover, the resulting gluten with fibre addition became stiffer and less extensible, and this led to a lower ability of the dough to retain gas (Wang, van Vliet & Hamer, 2004).

Gan, Gallieard, Ellis, Angold, & Vaughan (1992) showed that the outer layers of bran, especially the epicarp hairs, are responsible for loaf volume depression authors demonstrated that removing the outer kernel layers by pearling significantly improves the baking performance of the resulting flour.

In our experiment, the outer pearled fraction was discarded and this could explain the moderate impact of the intermediate pearled layer of the wheat kernel added at 10%-level on the loaf volume.

Noort et al. (2010) reported that the addition of an aleurone kernel fraction to flour causes a more adverse effect on bread volume than wheat bran. The authors suggested that the higher ferulic acid content of the aleurone fraction, which results in interactions between

the arabinoxylan chain and gluten proteins, is reflected in ability of the glutens to agglomerate. Han and Koh (2011) reported that the addition of purified phenolic acids, mainly ferulic and caffeic, decreased the extension of dough and the bread volume.

3.3.4 Breadcrumb texture profile analysis

The TPA instrumental test reflects the sensory analysis of bread (Gåmbaro, Varela, Giménez, Aldrovandi, Fizman, & Hough, 2002), and it can therefore be used for an objective assessment of the impact of the flour replacement on the perceived quality and attributes of bread. In the TPA variable data (Table 3), small and insignificant differences were found for the three less flour-substituted samples (0%, 5% and 10%), but when the substitution was increased the bread crumb texture characteristics showed very important changes and a great modification of all the mechanical parameters. The greatest differences were found for the bread hardness parameter, with a very high increment (from 1,82 N to 7,00 N) between the 0% and 25% flour-substituted bread. Similar trends and values of crumb hardness were reported by Schmiele et al. (2012) for an increasing replacement of refined flour with wheat bran.

The importance of this parameter is related to the hardness of the crumb, especially in relation to the gumminess and the chewiness parameters (Gómez et al., 2011), which are even higher in more flour-substituted bread crumbs. The 25%-substituted bread crumbs has showed therefore a greater hardness and also less ability to regain their original height after the first compression (resilience).

3.4 Chemical characterization of bread

The protein, DF, β-glucan, TPC, AR, ash content and TAA of bread increased linearly and significantly as the refined flour was replaced with the selected pearled wheat fraction (Table 4).

The maximum increase in the bioactive compounds was recorded at 25% of flour replacement with the pearled fraction: 25%-substituted bread resulted in the highest content of DF (+ 4.4 times higher than refined bread), β-glucans (+3.6 times), TPC (+ 3.6 times), ARs (+35 times), ash (+14%) and TAA (+2.2 times).

Considering the technological information, the 10%-substituted bread, compared to that made with only refined flour, showed significantly higher contents of DF, β-glucans, TPC, ARs and ash (2.7,1.9, 2.0, 13 and 1.1 times higher, respectively). Moreover, the bread made with 10% of pearled fraction could have been classified as a “Source of Fiber” (DF > 3%), and was close to being a “Good Source of Fiber” (DF > 6%), according to the guidelines for the use of nutrition and health claims (Codex Alimentarius, 1997). At this level of replacement, the TAA of bread was 42% higher than the control.

Ragaee, Guzar, Dhull & Seetharaman (2011) used wholegrain wheat flour to replace a portion of flour in the bread-making process. They reported that, at a 30% replacement level, DF, TPC and TAA (measured as $DPPH^{\bullet}$ scavenging capacity) increased 1.7, 2.0 and 1.6 times, respectively, compared to control recipe without enrichment. In our study, a greater increase in DF (+2.7 times) and similar increase in TPC and TAA were obtained at a 10% replacement level, using the pearled fraction. The same increase in protein and ash contents in the bread as that of Ragaee et al. (2011) at 30% of substitution, was recorded for the inclusion of the pearled fraction at a 20% level. Both DF (+ 1.6 times) and β-glucans (+2.8 times) content of the breads was significantly increased with the inclusion at 15% level of pearled barley middlings (Sullivan, O’Flaherty, Brunton, Arendt & Gallagher, 2011).

These results confirm the potential of using the intermediate fraction obtained from sequential pearling in bread-making as a functional ingredient, as it is an important concentrated dietary source of natural wheat antioxidants and DF. The addition of this

functional ingredient, rich in the aleurone layer, seems to lead to a greater nutritional improvement of composite bread than wheat bran or other milling fractions. Zhou, Laux & Yu (2004) reported that aleurone extracts from intermediate kernel wheat layers had 19% and 48% higher TAA and TPC contents than total bran fractions; this information could explain the high nutritional improvement observed in the present study.

The DF, β-glucans and TPC were not reduced during the baking process, thus confirming previously reported data (Ragaee et al., 2011). The TPC resulted in an increase in bread compared to the raw materials, which was probably related to the newly generated intermediate phenolic products, through Maillard reactions (Michalska, Amigo-Benavent, Zielinski & del Castillo, 2008). Han & Koh (2011) reported that, at the end of bread-making process, the TAA and residual free phenolic acid contents of bread are similar to those of flour, although their values are reduced during mixing phases, but are increased by fermentation and baking.

The AR content in breads was lower than that expected from the values obtained for both the refined commercial flour and the pearled wheat fraction. In fact, in processed cereal products, ARs could be trapped in starch-lipid complexes and not completely extracted from the matrix (Ross et al., 2003), thus leading to an underestimation of their content in bread samples.

3.5 DON contamination of bread

The DON contamination of bread made with the refined flour or with the five mixtures is reported in Table 4.

The DON content was not reduced through the bread-making process, when the flour and bread data were corrected on dry weight basis, thus confirming previous reports (Scudamore, Hazel, Patel & Scriven, 2009), although other research works reported

opposite findings related to real industrial production processes (Bergamini et al., 2010). The contamination of this mycotoxin in bread increased linearly as wheat flour was replaced with the selected pearled fraction. The increase in DON content after bread-making was 21% and 67%, respectively, compared to the control, for 10% and 25% of substituted refined flour. Moreover, bread samples with higher levels of replacement returned higher final DON contaminations than expected: Bergamini et al. (2010) suggested that this increment could be due to the release of DON from some bound forms that occur in the flour and which could be cleaved or solubilized during fermentation. In our experiment, this effect might have been more effective on the DON content in the pearled fraction. More research is required to investigate the effect of the bread-making process on DON when bran or intermediate kernel layers are added to the refined flour.

4. Conclusion

The study has demonstrated the importance of enriching wheat bread with pearled fractions from intermediate layers of wheat kernel in order to improve natural antioxidant compound contents, e.g. phenolics, ARs, DF and β-glucans. As expected, the DON content increased linearly in the composite bread as the refined flour was replaced with the pearled fraction, while the dough rheology and the physical and technological properties of the bread changed compared to the control.

Overall, the addition of an intermediate fraction obtained from pearling could lead to a greater nutritional improvement but, at the same time, a generally lower impact on technological quality of composite bread than wheat bran or other milling fractions.

The nutritional value of composite bread improved significantly at a 10%-substitution level, while the increase in DON contamination was moderate and the rheological and technological properties were acceptably similar to the control. Thus, refined flour could be replaced at this level and this would lead to few differences in the quality properties generally required on the market.

Since the food industry is presently focused on producing functional foods that incorporated various cereals, according to a multigrain approach, the fractionation technology proposed for wheat in the present work, with the inclusion of a functional ingredient obtained by sequential kernel pearling, could also be applied to other cereals.

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5. References

AACC International (2008). Approved methods of the American Association of Cereal Chemistry (11th ed., St. Paul, MN).

Anson, N.M., Aura A., Selinheimo, E., Mattila I., Poutanen, K., Van den Berg, R., Havenaar, R., Bast, A., & Haenen, G.R.M.M. (2011). Bioprocessing of wheat bran in whole wheat bread increases the bioavailability of phenolic acids in men and exerts anti-inflammatory effects in vivo. *The Journal of Nutrition*, *141* (1), 137-143.

Bergamini, E., Catellani, D., Dall'Asta, C., Galaverna, G., Dossena, A., Marchelli, R., & Suman, M. (2010). Fate of *Fusarium* mycotoxin in the cereal product supply chain: the deoxynivalenol (DON) case within industrial bread-making technology. *Food Additives and Contaminants*, *27*, 677-687.

Beta, T., Nam, S., Dexter, J. E., & Sapirstein, H. D. (2005). Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. *Cereal Chemistry, 82*, 390-393.

Bonnand-Ducasse, M., Della Valle, G., Lefebvre, J., & Saulnier, L., (2010). Effect of wheat fibres on dread dough development and rheological properties. *Journal of Cereal Science*, *52*, 200-206.

Bottalico, A., Perrone, G., (2002). Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. *European Journal of Plant Pathology*, *108*, 611-624.

Caballero, P.A., Gómez, M., & Rosell, C.M. (2007). Improvement of dough rheology, bread quality and bread shelf-life by enzymes combination. *Journal of Food Engineering*, *81*, 42-53.

Cheli, F., Campagnoli, A., Ventura, A., Brera, C., Berdini, C., Palmaccio, E., & Dell'Orto, V. (2010). Effects of industrial processing on the distribution of deoxynivalenol, cadmium

and lead in durum wheat milling fractions. *LWT-Food Science and Technology, 43*, 1050-1057.

Chen, J., Karlsson, C., & Povey, M. (2005). Acoustic envelope detector for crispness assessment of biscuits. *Journal of Texture Studies*, *36*, 139-156.

Codex Alimentarius (1997). Codex guidelines for use of nutrition and health claims (CAC/GL 23-1997).

Commission Internationale de L'Eclairage (1986). Colorimetry. Publication CIE n° 15.2, Vien.

Delcour, J.A., Rouau, X., Courtin, C.M., Poutanen, K., &Ranieri, R. (2012). Technologies for enhanced exploitation of the health-promoting potential of cereals. *Trends in Food Science and Technology*, 25, 78-86.

de Kock, S., Taylor J., & Taylor J.R.N. (1999). Effect of heat treatment and particle size of differet brans on loaf volume of brown bread. *Lebensmittel-Wissenschaft und-Technologie*, *32*, 349-356.

Duizer, L. (2001). A review of acoustic research for studying the sensory perception of crisp, crunchy and crackly textures. *Trends in Food Science and Technology*, 12, 17-24.

European Commission (2006). Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. Official Journal of the European Union, L255, p. 14-17.

Fik, M., & Surówka, K. (2002). Effect of prebaking and frozen storage on the sensory quality and instrumental texture of bread. *Journal of the Science of Food and Agriculture, 82*, 1268-1275.

Foca, G., Ulrici, A., Corbellini, M., Pagani, M. A., Lucisano, M., Franchini G. C., & Tassi, L. (2007). Reproducibility of the Italian ISQ method for quality classification of bread

wheats: an evaluation by expert assessors. *Journal of the Science of Food and Agriculture, 87*, 839-846.

Gåmbaro, A., Varela, P., Giménez, A., Aldrovandi, A., Fizman, S.M., & Hough, G. (2002). Textural quality of white pan bread by sensory and instrumental measurements. *Journal of Texture Studies*, 33, 401-413.

Gan, Z., Gallieard, T., Ellis, P.R., Angold, R.E., & Vaughan, J.G. (1992). Effect of the outer bran layers on the loaf volume of wheat bread. *Journal of Cereal Science*, *15*, 151–163.

Gómez, M., Jiménez, S., Ruiz, E., & Oliete, B. (2011). Effect of extrude wheat bran on dough rheology and bread quality. *LWT-Food Science and Technology*, *44,* 2231-2237.

Han H.-M., & Koh, B.-K. (2010). Antioxidant activity of hard wheat flour, dough and bread prepared using various processes with the addition of different phenolic acids. *Journal of Science Food Agriculture*, *91*, 604-608.

ICC (2010). Standard Methods of the International Association for Cereal Chemistry 110/1, 105/2, 106/2, 104/1, 173. International Association for Cereal Science and Technology, Vienna.

Jiang, H., Martin, J., Okot-Kotber, M., & Seib, P.A. (2011). Color of whole-wheat foods prepared from a bright-white hard winter wheat and the phenolic acids in it coarse bran. *Journal of Food Science*, *75*, 846-852.

Laca, A., Mousia, Z., Diaz, M., Webb, C., & Pandiella, S. S. (2006). Distribution of microbial contamination within cereal grains. *Journal of Food Engineering, 72*, 332–338.

Michalska, A., Amigo-Benavent, M., Zielinski, H., & del Castillo, M.D. (2008). Effect of bread making on formulation of Maillard reaction products contributing to the overall antioxidant activity of rye bread. *Journal of Cereal Science*, *48*, 123-132.

Nelles, E.M., Randall, P.G., & Taylor, J.R.N. (1998). Improvement of brown bread quality by prehydration treatment and cultivar selection of bran. *Cereal Chemistry*, *75*, 536-540.

Noort, M.M.J, Van Haaster, D., Hemery, Y., Schols, H.A., & Hamer, R.J. (2010). The effect of particle size of wheat bran fraction on bread quality - evidence for fibre-protein interactions. *Journal of Cereal Science, 52*, 59-64.

Piazza, L., Gigli, J., & Ballabio, D. (2007). On the application of chemometrics for the study of acoustic-mechanical properties of crispy bakery products. *Chemometrics and Intelligent Laboratory Systems*, *86,* 52–59.

Ragaee, S., Guzar, I., Dhull, N., & Seetharaman, K. (2011). Effects of fiber addition on antioxidant capacity and nutritional quality of wheat bread. *LWT-Food Science and Technology*, *44*, 2147-2153.

Ransom, J.K., Berzonsky, W.A. & Sorenson, B.K. (2006). Hard white wheat: Producing North Dakota's next market opportunity*. NDSU Ext. Serv. Publ. A-1310, North Dakota State University*.

Rolle, L., Giacosa, S., Torchio, F., & Río Segade, S. (2012). Changes in acoustic and mechanical properties of Cabernet Sauvignon seeds during ripening. *American Journal of Enology and Viticulture,* 63 (3), 413-418.

Rosell. C.M., Collar, C., & Haros, M. (2007). Assessment of hydrocolloid effects on the thermo-mechanical properties of wheat using the Mixolab. *Food Hydrocolloids, 21*, 452-462.

Rosell, C.M., Santos, E., & Collar, C. (2010). Physical characterization of fiber-enriched bread doughs by dual mixing and temperature constraint using the Mixolab®. *European Food Research and Technoogy,231*, 535-544.

Ross, A.B., Shepherd, M.J., Schüpphaus, M., Sinclair, V., Alfaro, B., Kamal-Eldin, A., & Åman P. (2003). Alkylresorcinols in cereals and cereal products. *Journal of Agricultural and Food Chemistry*, *51*, 4111-4118.

Saeleaw M., & Schleining G. (2011). A review: Crispness in dry foods and quality measurements based on acoustic–mechanical destructive techniques. *Journal of Food Engineering*, 105, 387-399.

Sanz Penella,J, J.M., Collar, C., & Haros, M. (2008). Effect of wheat bran and enzyme addition on dough functional performance and phytic acid levels in bread. *Journal of Cereal Science, 48*, 715-721.

Schmiele, M., Jaekel, L.Z., Patricio, S.M.C., Stelle, C.J., & Chang, Y.K. (2012). Rheological properties of wheat flour and quality characteristics of pan bread as modified by partial addition of wheat bran or whole grain wheat flour. *International Journal of Food Science and Technology*, *47*, 2141-2150.

Scudamore, K.A., Hazel, C.M., Patel, S., & Scriven, F. (2009). Deoxynivalenol and other *Fusarium* mycotoxins in bread, cake and biscuits produced from UK-grown wheat under commercial and pilot scale conditions. *Food Additives and Contaminants*, *26*, 1191-1198.

Slavin, J.L., Jacobs, D., Marquart L., & Wiemer, K. (2001). The role of whole grains in disease prevention. *Journal of the American Dietetic Association*, *101* (7), 780-785.

Sovrani V., Blandino M., Scarpino V., Reyneri A., Coïsson J.D., Travaglia F., Locatelli M., Bordiga M., Montella R., & Arlorio M. (2012). Bioactive compound content, antioxidant activity, deoxynivalenol and heavy metal contamination of pearled wheat fractions. *Food Chemistry*, *135*, 39-46.

Sullivan, P., O'Flaherty, J., Brunton, N., Arendt, E., Gallagher, E. (2011). The utilization of barley middlings to add value and health benefits to white breads. *Journal of Food Engineering*, 105, 493-502.

van Nieuwenhuijzen, N.H., Primo-Martín,C., Meinders, M.B.J., Tromp, R.H., Hamer, R.J., & van Vliet T. (2008). Water Content or Water Activity: what rules crispy behavior in bread crust? *Journal of Agricultural and Food Chemistry*, *56*, 6432-6438.

Wang, M.-W., van Vliet, T., & Hamer, R.J. (2004). How gluten properties are affected by pentosan. *Journal of Cereal Science*, *39*, 395-402.

Wang, J., Rosell, C.M., & Benedito de Barber, C. (2002). Effect of the addition of different fibres on wheat dough performance and bread quality. *Food Chemistry*, *79*, 221-226.

Zhang, D., & Moore, W. R. (1999). Wheat bran particle size effects on bread baking performance and quality. *Journal of the Science of Food and Agriculture, 79*, 805-809.

Zhou, K., Laux, J.J., & Yu, L. (2004). Comparison of Swiss red wheat grain and fractions for their antioxidant properties. *Journal of Agricultural and Food Chemistry*, 52, 1118-1123.

Tables

Table. 1.

Protein, DF, β-glucan, TPC, AR and ash content, DON contamination and TAA in refined commercial flour, enriched fraction obtained through pearling and whole wheat kernel before pearling.

Product	Proteins (%)	DF (%)	β-glucans (%)	TPC (mg kg^{-1})	ARs (g kg^{-1})	TAA (mmol TE kg^{-1})	Ash (μg kg^{-1})	DON (μg kg^{-1})
white flour	15.1	2.3	0.1	614	0.06	0.4	0.5	186
pearled fraction	18.3	34.9	0.9	6361	1.68	5.0	5.5	580
whole kernel for pearling	14.7	12.0	0.6	2812	0.68	1.5	1.9	363

Results are expressed on a dw basis.

Table. 2.

Protein, DF, β-glucan, TPC, AR content and TAA in bread derived from different percentages of substitution of refined flour with an enriched fraction obtained through wheat.

Percentage of substitution	**Proteins** (%)	**DF** (%)	**β-glucans** (%)	**TPC** (mg kg^{-1})	**ARs** (mg kg^{-1})	**TAA** (mmol TE kg^{-1})
0	14.9 d	2.0 f	0.13 e	836 f	4 f	0.33 d
5	15.5 c	2.6 e	0.22 d	1411 e	25 e	0.47 c
10	15.7 bc	5.5 d	0.25 d	1688 d	53 d	0.47 c
15	15.3 abc	6.8 c	0.35 c	1847 c	85 c	0.58 b
20	15.9 ab	8.0 b	0.40 b	2559 b	105 b	0.60 b
25	16.0 a	8.9 a	0.48 a	3066 a	149 a	0.73 a
P (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
sem[a]	0.34	0.19	0.05	123	4.37	0.06

Results are expressed on a dw basis. Means followed by different letters are significantly different (the level of significance is shown in table).

[a] sem: standard error of mean.

Table. 3.

Ash content and DON contamination in bread derived from different percentages of substitution of refined flour with an enriched fraction obtained through wheat kernel pearling.

Percentage of substitution	**Ash** (%)	**DON** ($\mu g\ kg^{-1}$)
0	2.20 d	185 f
5	2.31 c	209 e
10	2.44 b	225 d
15	2.54 a	254 c
20	2.57 a	279 b
25	2.54 a	310 a
P (F)	< 0.001	< 0.001
sem[a]	0.05	14

Results are expressed on a dw basis. Means followed by different letters are significantly different (the level of significance is shown in table).

[a] sem: standard error of mean.

Table 4.

Color, texture and acoustic emission tests in bread crust derived from different percentage of substitution of refined flour with an enriched fraction obtained through wheat kernel pearling.

Percentage of substitution	Crust color					Crust crunchiness			
	L* (C)	a* (C)	b* (C)	C (C)	h* (C)	Total break energy mJ	Maximum acoustic emission dB (SPL)	Acoustic emission peak average threshold 5 dB (SPL)	Acoustic emission peak average threshold 15 dB (SPL)
0	70.1 a	3.2 a	33.8 ab	34.0 a	84.6 a	147 a	77 a	41 a	43 a
5	65.7 b	4.6 b	33.7 a	34.0 a	82.2 b	149 a	76 a	41 a	43 a
10	61.6 c	6.5 c	34.8 ab	35.4 ab	79.4 c	137 a	73 a	40 a	42 a
15	57.9 d	8.9 d	35.3 b	36.5 b	75.8 d	118 b	71 a	29 b	32 b
20	55.8 d	9.4 d	35.2 ab	36.4 b	75.1 d	117 b	66 ab	21 b	22 b
25	52.9 e	9.9 d	35.0 ab	36.4 b	74.3 d	99 c	58 b	24 b	27 b
P (F)	< 0.001	< 0.001	0.01	< 0.001	< 0.001	< 0.001	0.004	< 0.001	< 0.001
sem[a]	3.1	1.2	1.6	1.7	1.7	11	9.4	9.0	9.5

Results are expressed on a dw basis. Means followed by different letters are significantly different (the level of significance is shown in table).

[a] sem: standard error of mean.

Table 5.

Volume and Texture Profile Analysis in bread crumb derived from different percentage of substitution of refined flour with an enriched fraction obtained through wheat kernel pearling.

Percentage of substitution	Bread Volume (ml)	Bread crumb					
		Hardness N	Cohesiveness (-)	Springiness mm	Gumminess N	Chewiness mJ	Resilience (-)
0	2146.7 a	1.82 a	0.88 a	9.99 a	1.60 a	16.00 a	0.52 a
5	2135 a	1.74 a	0.89 a	9.97 a	1.55 a	14.00 a	0.53 a
10	1978.3 b	1.99 a	0.88 a	9.99 a	1.76 a	17.59 a	0.54 a
15	1893.3 c	2.89 b	0.88 a	9.98 a	2.61 b	25.28 b	0.54 a
20	1720 d	4.39 c	0.86 b	10.00 a	3.77 c	37.71 c	0.50 b
25	1506.7 e	7.00 d	0.84 c	10.00 a	5.91 d	57.84 d	0.49 b
P (F)	< 0.001	< 0.001	< 0.001	0.06	< 0.001	< 0.001	< 0.001
sem[a]	24.94	0.62	0.01	0.02	0.44	4.76	0.02

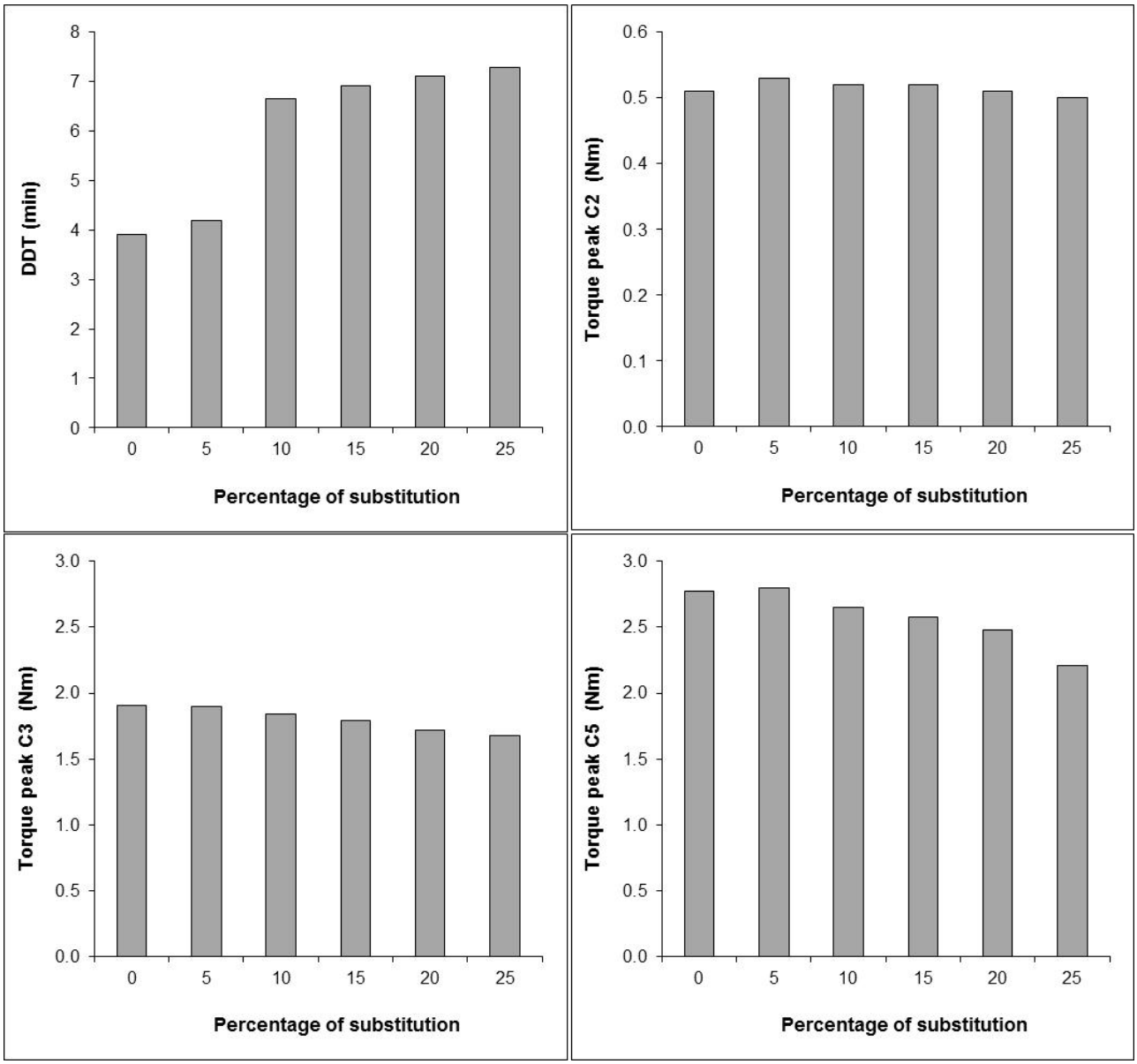
Results are expressed on a dw basis. Means followed by different letters are significantly different (the level of significance is shown in table).

[a] sem: standard error of mean.

Figure legend

Figure 1.

Mixolab parameters[a] for dough with different replacement percentages of refined flour with an enriched fraction obtained through wheat kernel pearling.



[a] Mixolab parameters: DDT = Dough Development Time; C2 = protein weakness; C3 = starch gelatinization; C5 = starch gelling; C2, C3 and C5: end points of the corresponding mixing stages.