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# Properties of whole grain wheat flour and performance in bakery products as a function of particle size

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ABSTRACT

The aim of this study was to evaluate the effect of particle size distribution on composition, properties rheological, pasting, microstructural and baking properties of whole grain wheat flour (WGWF) of three different particles sizes (194.9 μm, 609.4 μm and 830.0 μm). The quantification of free sulfhydryl groups (-SH) of WGWF samples, together with the effects observed in the behavior of the dough and bread showed that particle size influences the functionality of the gluten network in a differentiated way. Firmer and lower breads volume compared to refined wheat flour (RF) were correlated with the quality of the gluten network. In the sample of finer particles, more pronounced adverse effects in quality (dough rheology, bread volume and texture) compared to the medium and coarse particle size sample suggests that the larger contact surface and the increased release of reactive compounds due to cell rupture interact with the gluten-forming proteins changing their functionality.



## 1. Introduction

In whole grain wheat flour (WGWF) all the anatomical components of the grain, such as endosperm, bran and germ are present in the same proportions that exist in the intact form. Thus, WGWF contains substantially more fibers, vitamins, minerals and phytochemicals than refined wheat flour (RF). Accordingly, it is considered as an excellent source of nutritional and functional ingredients for human health with many associated benefits, including the reduction of diseases risk such as diabetes, cardiovascular diseases, obesity, and cancer (Liu, 2007). However, regardless of the health benefits, WGWF can cause structural and sensory changes in food, leading to lower consumer acceptance. As a result, there are difficulties in producing WGWF food that maintains the desired functionality and quality equivalent to refined grain products.

In addition to the qualitative characteristics of the final product, the use of WGWF also provides many changes in the dough properties and processing parameters. The particle size of WGWF is an important factor affecting product quality and functionality of the flour (Kihlberg et al., 2004). Although many studies have already investigated the effect of bran particle size on the technological properties of the flour and baking, there are no studies that have examined the importance of the whole particle size obtained directly (without bran recombination). Most studies have been devoted to the particle size of the bran used at different levels of reincorporation. Moder et al. (1984) and Lai et al. (1989) reported that the reduction of coarse bran particle size slightly improved bread volume. Noort et al. (2010) reported greater negative influence on the quality of baking when the size of the bran particles was reduced. Cai et al. (2014) also found a reduction in bread volume and a higher degree of starch retrogradation during storage of bread made from wheat flour with smaller particle size bran. Hemdane et al. (2015) investigating the deleterious effects of wheat bran in baking across different mills and byproducts, demonstrated that the specific volume of the bread was significantly reduced when fine bran was added.

Heretofore the application of the flour produced by grinding whole wheat grain of different particle sizes on the dough properties and bread making has not been thoroughly examined. In this

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study, the aim was to determine the effects of different sizes of WGWF particles, obtained by grinding, an impact mill, on the technological quality of the flour and the baking performance. For this purpose the diference WGWF were evaluated and compared to RF as regards chemical composition, rheological aspects focusing on mixture and pasta properties, dough microstructure, concentration of free sulfhydryl groups (-SH) and baking performance.

## 2. Materials and methods

### 2.1. Material

Wheat grains (*Triticum aestivum* L.) used in this study was BRS Guabiju, from 2014 harvest in Paraná State, Brazil. The chemicals used were all of analytical grade. The ingredients used for the preparation of the breads were purchased in local market.

### 2.2. Preparation of refined flour (RF) and whole grain wheat flour (WGWF) of different particle sizes

The refined flour was obtained by grinding the wheat grains in the experimental mill (CD1, Chopin, France) according to method 26–10.02 of the American Association of Cereal Chem. (AACCI, 2010), with an extraction rate of 70 g 100 $g^{-1}$.

The whole grain wheat flour was obtained by milling wheat using a laboratory impact mill with a fixed speed of 20.000 rpm, cooled grinding chamber of 250 mL (M20, IKA, Staufen, Germany). The milling time ranged from 5 s, 15 s and 180 s to obtain flour with three different particle sizes.

The distribution of the average particle size of the samples was performed by laser diffraction method on particle size determiner (LV-950, Horiba, Kyoto, Japan) using dry dispersion module. The whole flour with average particle sizes of 194.9 μm, 609.4 μm and 830.0 μm were called fine whole grain wheat flour (FWGWF), medium whole grain wheat flour (MWGWF) and coarse whole grain wheat flour (CWGWF), respectively. The distribution of particle sizes can be seen in Fig. 1.

### 2.3. Physicochemical characterization

The proximate chemical composition of the RF and WGWF (moisture, protein, ash, lipids, dietary fiber), and damaged starch were determined according to the official methods of AACCI (2010), 44–15.02, 46–10.01, 08–12.01, 30-25, 32–07.01 and 76–33.01 respectively.

The gluten content analysis was performed by method nº 38–12.02 (AACCI, 2010), with modifications, determined on Glutomatic equipment (2100, Perten Instruments, USA). For the WGWF, the polyester sieve (88 μm) was initially employed for 120 s until the mass was formed. After this period, was used sieve (840 μm) to elimination of the fibrous layers of the meal during the washing period.

### 2.4. Determination of phenolic compounds

The extracts were prepared according to the procedure of Moore et al. (2006) by stirring 2.5 g sample with 20 mL of 50% acetone (v/v) for 10 min, in vortex equipment. The samples were centrifuged for 10 min at 4500 rpm and the supernatant collected. The extraction procedure was repeated on the precipitate twice more and the supernatants combined. The phenolic compounds content was determined using the Folin-Ciocalteu colorimetric method with modifications (Singleton et al., 1999). Aliquots of 0.2 mL of extracts were diluted in 5.0 mL of deionized water and oxidized with 0.25 mL of Folin-Ciocalteu reagent, 1 M. After 5 min of reaction, the mixture was neutralized with 1.5 mL of sodium carbonate 20% (w/v) and vortexed for 1.0 min. After 30 min incubation in the dark at room temperature, the absorbance was measured by UV-VIS spectrophotometer (Biospectrometer Kinetic, Eppendorf, Germany) at 765 nm. Quantification was done based on a gallic acid standard curve and results expressed in mg of gallic acid equivalents (GAE) per 100 g of sample.

### 2.5. Determination of free sulfhydryl (SH) groups in gluten

The determination of free sulfhydryl groups (-SH) was performed using wet gluten according to the method by Pérez et al. (2005), adapted by Rakita et al. (2014), that included incubation of the sample at two different temperatures, 30 °C and 37 °C for three different time intervals of 45, 90 and 135 min. For the assay, Tris-Glycine buffer (10.4 g Tris, 6.9 g of glycine and 12 g of EDTA in 1.0 L of deionized water) was prepared, with the pH adjusted to 8.0 with NaOH solution 1 M. A guanidine hydrochloride solution (5.0 M) was prepared using Tris-Glycine buffer as solvent (GuHCl/Tris-Gly). Ellman's reagent was prepared by using 40.0 mg of DTNB (5,5'-dithiobis-2-nitrobenzoic acid) in 10 mL Tris-Glycine buffer at the time of analysis.

For the analysis procedure, each wet gluten sample (100 mg) was suspended in 1.0 mL solution of GuHCl/Tris-Gly, vortexed for 5.0 min and then centrifuged at 14,500 rpm for 6.0 min. The volume of 400 μL of supernatant was added to 600 μL of GuHCl/Gly-Tris solution, and 250 μL of Ellman's reagent and stirred for 1.0 min in vortex. The absorbance was read at 412 nm and the results calculated from the standard cysteine curve constructed by successive dilution of a stock solution of 0.83 μmol/mL.

### 2.6. Mixing properties

The mixing properties of RF and WGWF were determined according to method AACC 54–21.02 (2010) in a Promylograph equipment (T6-E, Koloman Egger, Austria).

### 2.7. Extensional properties

Sample preparation was performed in Promylograph equipment using water absorption of the flour by substituting this value of 2% sodium chloride. The dough development time used was the necessary to achieve a consistency of 500 units. Subsequently, the dough produced was left to stand for 45 min, which corresponds to the intended operating conditions to measure the extensional properties of bread dough samples.

The extension resistance and extensibility test was conducted using Texture analyzer (TA.XT.plus, Stable Micro Systems, England), equipped with Exponent 32 software and Kieffer Dough and Gluten Extensibility Rig probe (A/KIE). The speeds applied were: pre-test: 2.0 mm/s; test: 3.3 mm/s; Post-test: 10.0 mm/s; distance of 75.0 mm. For this analysis, 50 g of dough sample was shaped into strips of 7.0 mm in diameter and 60 mm long in a teflon mold that comes with the equipment.

### 2.8. Pasting properties

Pasting properties were analyzed according to method AACC 76–21.01 (2010) using a rapid viscosity analyzer (RVA-3D, Newport Scientific, Australia) equipped with *Thermocline software for Windows*, version 3.1. Samples of 3.5 g of flour (moisture corrected to 14 g for 100 $g^{-1}$), 25 ± 0.1 mL of distilled water and the temperature profile *Standard 1* were used in the assay.

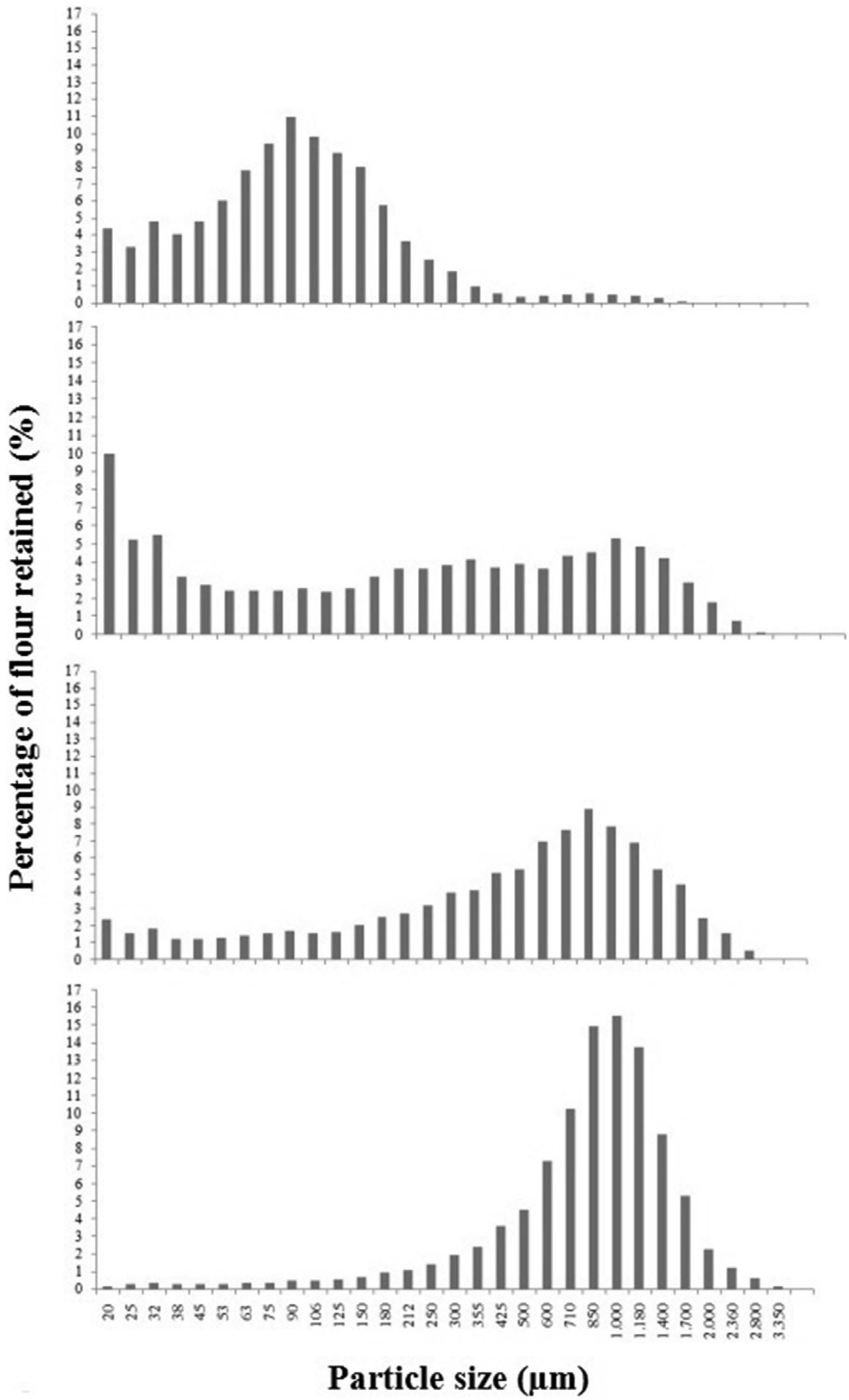


**Fig. 1.** Particle size distribution profile of refined flour and whole grain wheat flour with different particle sizes. Results expressed as mean of three determinations ± standard deviation. RF: refined flour (82.67 μm). FWGWF: fine whole grain wheat flour (194.98 μm). MWGWF: medium whole grain wheat flour (608.44 μm). CWGWF: coarse whole grain wheat flour (830.00 μm).

### 2.9. Scanning electron microscopy (SEM)

The doughs were prepared by the method of Kim et al. (2003), with adaptations, with use of Promylograph equipment (see point 2.7). Samples were dried in freeze drying equipment (LS X.000, Terroni, São Carlos, Brazil). To obtain the micrographs, a scanning electron microscope (LM VEGA3, Shimadzu, Japan) was used at 20 kV and magnification of 2000×.

### 2.10. Small-scale baking instrumental test

The small scale baking instrumental test was performed according to the method developed by Oro (2013) with modifications. The formulation was composed of wheat flour (100 g), hydrogenated vegetable oil (3 g/100 g flour), sodium chloride (12 g/100 g flour), ascorbic acid (0.01 g/100 g flour), sugar (5 g/100 g flour), yeast (3 g/100 g flour) and water at 4 °C, based on the water absorption of flour to obtain 650UF (±20) in a Promylograph equipment. The ingredients were mixtured for 1.0 min. Distilled water was added and the kneading continued during dough development time found in the mixing properties test for each sample.

The dough was divided manually in portions of 35,0 g and fermentation was carried out in a chamber (MP20, Multipão, Brazil), at of 30 ± 1 °C and relative humidity of 80% for 60 min. The baking was performed in a laboratory oven (QA 226, Labor Instruments, Austria) at 150 °C for 12 min. The breads cooled at room temperature and after 1 h the analyses were performed.

### 2.11. Evaluation of quality characteristics of the breads

The volume of the breads was determined by the seed displacement method, according to AACC method 10–05.01 (2010). The breads were weighed and their specific volume calculated. Bread firmness was determined in accordance with AACC method 74–09.01 (2010), using a texturometer (TA.XT.plus, Stable Micro Systems, England). The loaves were sliced in to 25 mm thickness, reducing the size of the slices to 25 mm wide x 25 mm long and the crust removed. An aluminum cylindrical probe P/20 (radius 20 mm) was used and the speeds were: pretest 1.0 mm $s^{-1}$; test 1.7 mm $s^{-1}$; post-test 10.0 mm $s^{-1}$; compressive force of 40%.

### 2.12. Statistical analysis

All analyses were performed in triplicate. Data analysis was performed by analysis of variance (ANOVA). The comparison of means was performed by Tukey test at 5% significance level. For baking quality characteristics (volume and firmness) the Pearson correlation coefficient was calculated to assess the degree of relationship between these variables and dough properties.

## 3. Results and discussion

### 3.1. Physicochemical characterization

The samples of whole grain milling flour presented moisture content significantly lower than in the RF (Table 1). This can be attributed to the difference form of samples preparation, since in RF grinding carried out the previous conditioning of the wheat grain to improve the separation of endosperm of the bran. The WGWF samples were obtained by impact milling, without prior conditioning, which resulted in greater shear and heat. The content of protein, lipid, ash and fiber was significantly higher in WGWF samples in relation to RF ($p < 0.05$). This was due to the presence of the germ rich in lipids and the outer layers (bran and aleurone), rich in proteins, mineral matter and fibers. Small differences in the results of fibers analysis may be attributed to small sample size used in the analysis technique. The wet gluten represents the gluten yield, a measurement indicative of the ability of proteins to aggregate. In baking, highest quantity of wet gluten is desirable. Samples of WGWF with larger particle size (MWGWF and CWGWF) presented significantly lower gluten contents than RF. This is due to the fact that how larger particle size is, greater is the difficulty of the gluten proteins aggregation. These results are related to those described by Liu et al. (2015), on the study of the effect of different milling processes on the quality of wheat flour. The damaged starch content can affect the properties of the dough and final use characteristics. Moderate amounts of damaged starch are good for baking because help to promote more fermentation activity. However, high levels may also permit high water absorption and fast conduction of enzymatic processes, resulting in sticky dough, smaller bread volume, and changes in the crust color (Barak et al., 2014). As shown in Table 1, after passing through the grinding process, all the WGWF samples showed lower damaged starch content compared to the RF. In RF, to separate the bran from the endosperm, the wheat grains are submitted to different passages between pairs of cylindrical rollers which contribute to damage the starch. Milling in the laboratory mill for WGWF production appears to be less aggressive, however, the longer the milling time and the smaller the particle size, the greater the friction increase and the larger the damaged starch content.

Therefore, possible changes in the technological characteristics of the flour and the quality of bakery products found in this study should not be attributed to the damaged starch of the samples used, in which the highest content was 5.97% for RF.

### 3.2. Particle size effect on the content of phenolic compounds

In addition to their antioxidant power, phenolic compounds are considered as reducing agents and as reported in the literature adversely affect the functional properties of the dough, resulting in development time and mixing tolerance reduced (Han and Koh, 2011; Jackson and Hoseney, 1986).

The results obtained for total phenolic compounds content are presented in Table 1 and showed highest concentration of these compounds in the outer layers of the grain and germ, which are removed during milling to obtain the RF. The decrease in the particle size of WGWF showed a positive effect on the availability of

**Table 1**
Effect of particle size on the damaged starch content, proximate chemical composition, gluten content, phenolic content and free sulfhydryl groups of refined flour and whole grain wheat flour.

| Parameters | Samples | | | |
|---|---|---|---|---|
| | RF | FWGWF | MWGWF | CWGWF |
| Moisture (g.100 $g^{-1}$) | $14.08^a \pm 0.12$ | $11.68^c \pm 0.13$ | $12.62^b \pm 0.14$ | $12.44^b \pm 0.10$ |
| Protein (g.100 $g^{-1}$) | $13.85^b \pm 0.06$ | $14.98^a \pm 0.08$ | $14.81^a \pm 0.13$ | $14.72^a \pm 0.16$ |
| Fat (g.100 $g^{-1}$) | $1.59^b \pm 0.08$ | $1.97^a \pm 0.35$ | $1.94^a \pm 0.16$ | $1.93^a \pm 0.12$ |
| Ash (g.100 $g^{-1}$) | $0.56^b \pm 0.02$ | $1.70^a \pm 0.00$ | $1.70^a \pm 0.02$ | $1.67^a \pm 0.01$ |
| Dietary fiber (g.100 $g^{-1}$) | $3.61^c \pm 0.02$ | $12.45^{\ b} \pm 0.71$ | $14.95^a \pm 0.04$ | $15.95^a \pm 0.15$ |
| Wet gluten (%) | $40.64^a \pm 0.22$ | $39.29^a \pm 0.74$ | $34.71^b \pm 0.70$ | $25.03^c \pm 1.38$ |
| Damaged starch (%) | 5.97+ ± 0.11 | $4.09^b \pm 0.14$ | $3.39^c \pm 0.06$ | $2.60^d \pm 0.14$ |
| Total phenolic content (mg GAE.100 $g^{-1}$) | $1.69^d \pm 0.04$ | $3.06^a \pm 0.04$ | $2.23^b \pm 0.01$ | $2.11^c \pm 0.01$ |

Mean values in the same row followed by different letters are significantly different ($p < 0.05$). Results expressed as mean of three determinations ± standard deviation. RF: refined flour (82.67 μm). FWGWF: fine whole grain wheat flour (194.98 μm). MWGWF: medium whole grain wheat flour (608.44 μm). CWGWF: coarse whole grain wheat flour (830.00 μm).

phenolic compounds. This fact can be explained by the greater rupture of the structural components of the grain during the milling process to prepare the flour with smaller particle size. The higher structural breakdown facilitates the release of the phenolic compounds that are present in the form attached to the cell walls of the grain.

### 3.3. Content of free sulfhydryl groups (SH) in the gluten

It is generally considered that -SH groups and SS bonds have significant influence on dough structure formation and dough stability. During dough mixing, the oxidation of sulphydryl groups of cysteine residues within protein (intrachain) and/or between proteins (interchain) occurs. The established S–S bonds are responsible for gluten network formation and, therefore, they are determinants of rheological and baking properties of flour (Delcour et al., 2012). The determination of free -SH groups, analyzed in gluten in this study was performed after incubation periods of the dough in two temperatures (30 and 37 °C). These temperatures were selected based on standard conditions prescribed for most rheological measurements (30 °C), as well as based on favorable conditions for the activity of proteolytic enzymes potentially present (37 °C) (Pérez et al., 2005). Incubation times of 45, 90 and 135 min are the same commonly used for extensography analysis, and could be related to resting and proofing times during processing. The variation in the concentration of sulfhydryl groups (-SH) in the gluten with respect to particle size, temperature and incubation time is shown in Fig. 2. The extension of incubation time and temperature elevation provided an increase in the concentration of free -SH, and are consistent with the results of Pérez et al. (2005). These results indicate that higher incubation temperature and time may promote the process of enzymatic hydrolysis and division of the gluten structure.

Among the WGWF studied, the CWGWF showed higher content of free -SH at the different temperatures and incubation times analyzed, followed by FWGWF and MWGWF. These results indicate that greater depolymerization of the gluten occurred, a process that results in the weakening of the dough. This is in agreement with the results of the rheological and baking properties observed in this study, in which the CWGWF with the highest free -SH content was also the one of lower stability, less resistance of the extension and smaller volume of baking.

The release of free -SH groups in the particle sizes studied demonstrates two mechanisms of action acting on the functionality of the gluten network. In the CWGWF, the physical effects of the external layers of the grain that interfere in the aggregation of the protein network predominate. In FWGWF, the chemical effects, caused by the increase of the contact surface of the particles are predominant, allowing greater interactions between the reactive compounds present in the outer layers of the grain with the gluten proteins. The lower content of free -SH groups, as well as the better baking properties observed, demonstrate the equilibrium between the two mentioned mechanisms acting on the sample of medium particle size of this study.

### 3.4. Effect of particle size on mixing and extensional properties

The study of the effect of different particle sizes in mixing and extensional properties of dough are shown in Table 2. Compared with RF water absorption, WGWF samples showed significantly higher values ($p < 0.05$), probably due to the fiber content (Hemery et al., 2011; Boita et al., 2016). The large number of hydroxyl groups in the fiber structure permits greater interaction with water through hydrogen bonds (Penella et al., 2008). FWGWF sample showed higher water absorption, which may be related to the larger contact surface of the particles, providing greater exposure of hydroxyl groups. The higher dough development time (DDT) ($p < 0.05$) found in WGWF samples classified as MWGWF and CWGWF is due to the larger particles absorbing water more slowly, hindering the development of the gluten network and thereby increasing the time required for the optimal dough development (Rosell et al., 2006). The dough stability was significantly lower ($p < 0.05$) in WGWF samples, indicating that the presence of the outer parts of the grain present in the flour composition led to

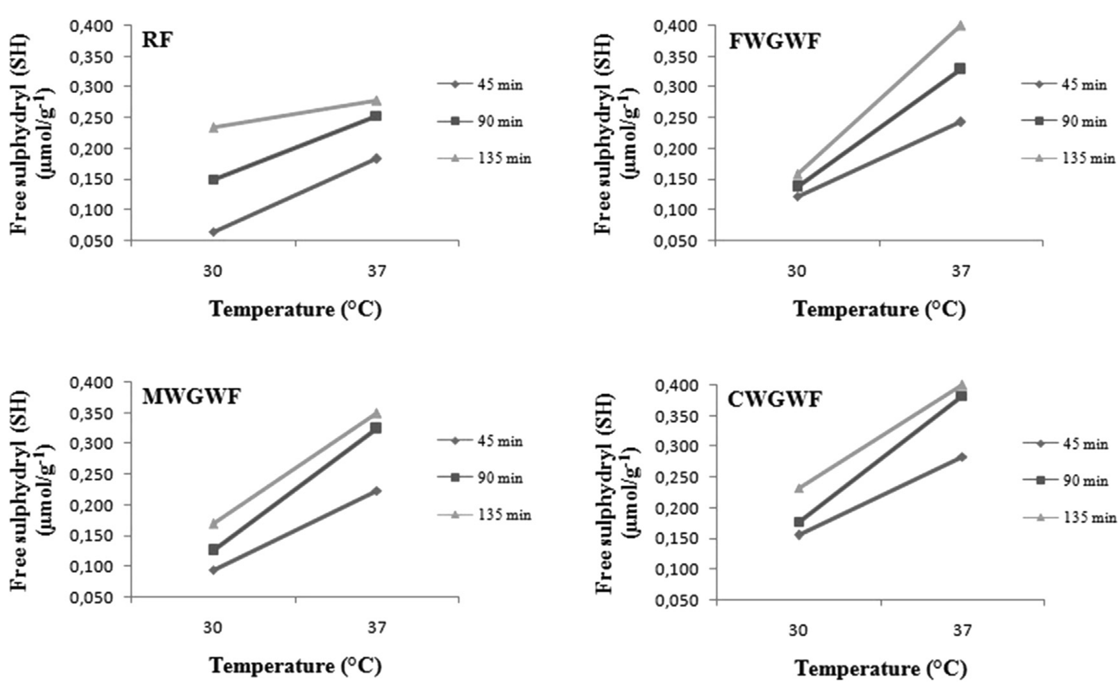


**Fig. 2.** Effect of incubation temperature and time on the free sulphydryl content of wet gluten of refined flour and whole grain wheat flour with different particle sizes. Results expressed as mean of three determinations ± standard deviation. RF: refined flour (82.67 µm). FWGWF: fine whole grain wheat flour (194.98 µm). MWGWF: medium whole grain wheat flour (608.44 µm). CWGWF: coarse whole grain wheat flour (830.00 µm).

**Table 2**
Effect of particle size on mixture properties. extensional properties and paste properties of refined flour and whole grain wheat flour.

| Parameters | Samples | | | |
|---|---|---|---|---|
| | RF | FWGWF | MWGWF | CWGWF |
| Absorption (%) | $65.83^{d} \pm 0.29$ | $76.06^{a} \pm 0.51$ | $72.66^{b} \pm 0.29$ | $70.50^{c} \pm 0.44$ |
| DDT (min) | $9.26^{b} \pm 1.12$ | $7.60^{b} \pm 1.04$ | $11.80^{a} \pm 0.51$ | $12.93^{a} \pm 0.71$ |
| Stability (min) | $17.5^{a} \pm 0.26$ | $12.8^{b} \pm 1.14$ | $12.6^{b} \pm 0.21$ | $11.6^{b} \pm 0.35$ |
| MTI (FU) | $23.0^{c} \pm 2.65$ | $50.0^{a} \pm 6.98$ | $40.0^{ab} \pm 3.54$ | $29.0^{bc} \pm 0.71$ |
| Resistance (g) | $47.86^{a} \pm 0.72$ | $41.12^{b} \pm 0.80$ | $37.44^{c} \pm 0.57$ | $35.46^{d} \pm 0.22$ |
| Extensibility (mm) | $38.63^{a} \pm 0.44$ | $30.22^{b} \pm 1.56$ | $27.17^{b} \pm 2.59$ | $25.13^{b} \pm 3.27$ |
| Max Visc (RVU) | $209.64^{a} \pm 1.87$ | $128.97^{b} \pm 7.53$ | $92.66^{c} \pm 6.58$ | $47.19^{d} \pm 1.04$ |
| Breakdown (RVU) | $73.94^{a} \pm 0.59$ | $48.28^{b} \pm 2.35$ | $24.44^{c} \pm 1.95$ | $3.89^{d} \pm 0.54$ |
| Final Visc (RVU) | $221.44^{a} \pm 3.10$ | $167.25^{b} \pm 6.80$ | $145.77^{b} \pm 7.29$ | $107.73^{c} \pm 14.13$ |
| Retrograd (RVU) | $85.75^{a} \pm 1.32$ | $86.55^{a} \pm 1.56$ | $77.55^{b} \pm 2.68$ | $73.75^{b} \pm 0.80$ |

Mean values in the same row followed by different letters are significantly different ($p < 0.05$). Results expressed as mean of three determinations ± standard deviation. RF: refined flour (82.67 μm). FWGWF: fine whole grain wheat flour (194.98 μm). MWGWF: medium whole grain wheat flour (608.44 μm). CWGWF: coarse whole grain wheat flour (830.00 μm). DDT: dough development time. MTI: misture tolerance index. Max visc: maximum viscosity. Final Visc: final viscosity. Retrograd: retrogradation. RVU: Rapid visco unit.

formation of a weaker gluten network and less stable during extended mixing. For values of mixing tolerance index (MTI), WGWF samples showed higher values than RF and particle size proved to be an interferer because a marked increase in MTI was observed with the reduction of particle size, as noted by Zhang and Moore (1999) and Penella et al. (2008).

As seen in Section 3.3, the results of this study demonstrated that between WGWF the flour with higher content of phenolic compounds (FWGWF) also had the lowest dough development time, without differing from RF and lowest tolerance to mixing, shown by the highest MTI. Table 2 shows the extensional properties of the sample of WGWF and RF doughs. The resistance to extension was significantly ($p < 0.05$) reduced in the WGWF samples in the order CWGWF < MWGWF < FWGWF compared the RF values. This weakening of the dough can be explained by the physical interference mechanism that the presence of the outer layers of the wheat grain provides to the gluten. The larger particles hinder the formation of a more cohesive gluten network able to offer greater resistance to the dough (Schmiele et al., 2012). Moreover, the extensibility of the dough was also significantly lower ($p < 0.05$) in WGWF samples in relation to RF. However, the particle size did not result in significant differences between the values of extensibility.

### 3.5. Effect of particle size on the paste properties

Observing the paste properties, WGWF samples showed lower values of maximum viscosity, breaking and final viscosity (Table 2). The decrease of the maximum viscosity of the WGWF samples with respect to RF ($p < 0.05$) may be due to the higher RF starch content compared with WGWF (Ragaee and Abdel-Aal, 2006), as well as variations in other chemical constituents, by the elimination of bran in RF. In WGWF, interactions with protein, lipids and fibers, may interfere with the water absorption of the starch, leading to different viscosities. This effect is mainly observed in the flours with smaller particle size, in which the constituents are more exposed and the speed of the reactions is increased. Especially in relation to fibers, OH-grouping exhibits higher capacity to absorb water, generating competition for water between fibers and starch, as evidenced in the results of this study, which showed that flours with finer particles showed higher viscosity peak, as well as greater water absorption in the promilography.

The breakdown values are associated with the decrease in viscosity caused by the disruption of swollen starch granules with continuous heating and agitation (Ragaee and Abdel-Aal, 2006). This explains why higher breakdown values were obtained for the samples that presented higher maximum viscosity.

The reassociation between starch molecules, results in the paste structure formation and viscosity increases reaching a final viscosity which is associated with the starch tendency to retrograde (Ragaee and Abdel-Aal, 2006), which explains why the RF sample has the highest value for this parameter. The fibers have the ability to absorb high water content, which makes it less available for retrogradation, as can be observed in this study in the samples WGWF. For the WGWF, important features such as breakdown and retrogradation have are more pronounced, which revealed greater difficulty to obtain important characteristics for the quality of products, such as volume and maintenance of softness.

However, it was observed that when the size of the particles were smaller, these effects were more pronounced, considering the larger contact surface of the fibers and interactions with water by hydrogen bonds. This would produce higher viscosity and softness of the products, and in turn, lower shelf-life due to the availability of water for chemical reactions.

### 3.6. Scanning electron microscopy (SEM)

The microstructures of RF and WGWF are shown in Fig. 3(a–d). In the RF mass (Fig. 3a), a more compact gluten matrix can be observed in relation to the mass structures prepared with WGWF (Fig. 3b, c, d), which was due to the absence of the outer layers in RF, and thus smaller probability of disturbance in protein network formation in the mass (Penella et al., 2008). In the WGWF samples the bran fragments and starch granules can be easily identified (Fig. 3b, c, d), as well as the less cohesive and more porous structure, which may be attributed to the presence of the outer layers, which interferes in theiInteraction between the starch granules and proteins (Pomeranz et al., 1977).

The results indicated that the differences observed in the WGWF gluten matrix were influenced by particle size. The coverage of the starch granules and the thickness of the protein network were reduced when the particle size increased from 608.44 μm to 830.00 μm. However the FWGWF (Fig. 3b) showed a structure with smaller openings relative to samples with higher particle size, but with the same lack of connectivity between starch and gluten matrix.

### 3.7. Effect of particle size on the quality characteristics of breads

The breads prepared with the RF and WGWF samples are shown in Fig. 4 (a). RF bread showed an average specific volume of 3.45 mL (Fig. 4b) and the largest effect on specific volume reduction was observed in the use of CWGWF, presenting 1.25 mL/g. This effect

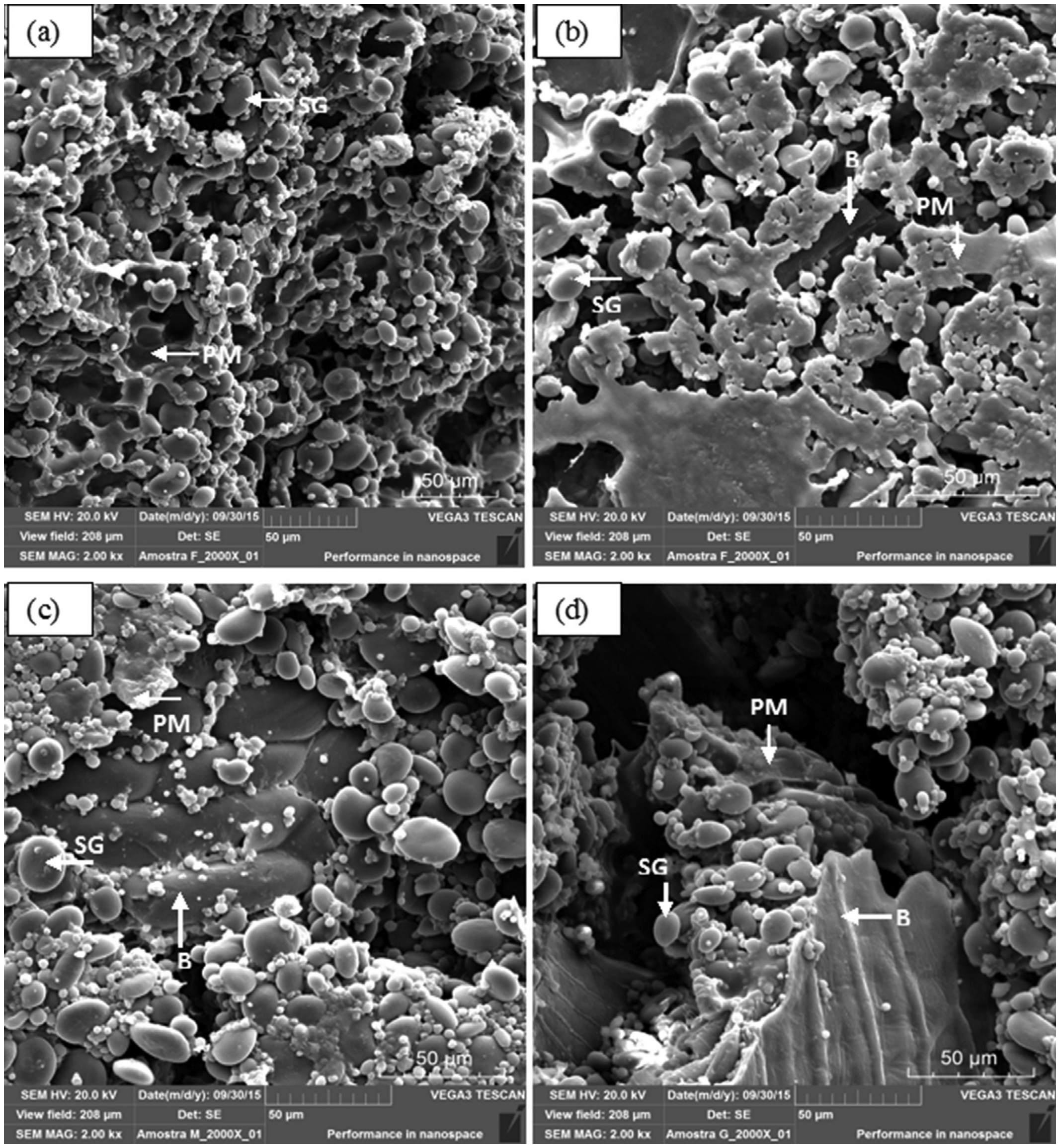


**Fig. 3.** Scanning Electron Microscopy (SEM) images of dough made from refined flour and whole grain wheat flour with different particle sizes. (A): micrograph of refined flour (82.67 μm.) (B): micrograph of fine whole grain wheat flour (194.98 μm.) (C): micrograph of medium whole grain wheat flour (608.44 μm.) (D): micrograph of coarse whole grain wheat flour (830.00 μm.) SG: starch granules. PM: protein matrix. B: Bran. Increased 2000×.

reflects the greater impact on the gluten network due to the action of the fibers, which in turn can provide both steric effects during the development of the dough, as well as destabilize gas cells, resulting in reduction in the specific volume of the breads (Pomeranz et al., 1977). Additionally, bread made with MWGWF (608.44 μm) had a higher specific volume than bread made with coarse (830.0 μm) or fine (194.9 μm) flour. The specific volume reduction (1.62 mL/g) observed in FWGWF in relation to the MWGWF (1.94 mL/g) suggests that the effects of a given particle size may be related to the increased contact surface through physical and chemical interactions, as proposed by Noort et al. (2010).

A comparison of these results based on the literature is difficult because majority of studies are performed with bran reincorporation and given the large conflict that exists with regard to effect of particle size reduction in the specific volume of the loaves. As in the current study, Noort et al. (2010) and Cai et al. (2014) showed that fine bran can exert detrimental effect on the bread specific volume. Pomeranz et al. (1977), Moder et al. (1984) noted otherwise. Moreover, Zhang and Moore (1999) and Coda et al. (2014) found that the use medium particle size resulted in bread of greater specific volume.

Recently, Jacobs et al. (2016) studying the impact of bran hydration capacity on the optimal development of the dough and loaf specific volume demonstrated that the inadequate estimate of mixing time can lead to wrong assessment of the real impact on production of WGWF breads and help explain the contradictions found in the literature. Working on constant mixing time can provide additional benefits in terms of bread volume for samples with smaller particles, assuming larger particles require longer time to develop the dough. In this study, the baking test condition of each sample was used considering the development time and optimum water uptake for the formation of dough. Overall, the results of WGWF specific volume herein can be related to the quality of the gluten network, and there was evidence found by the content of free -SH groups, which proved to be in agreement with

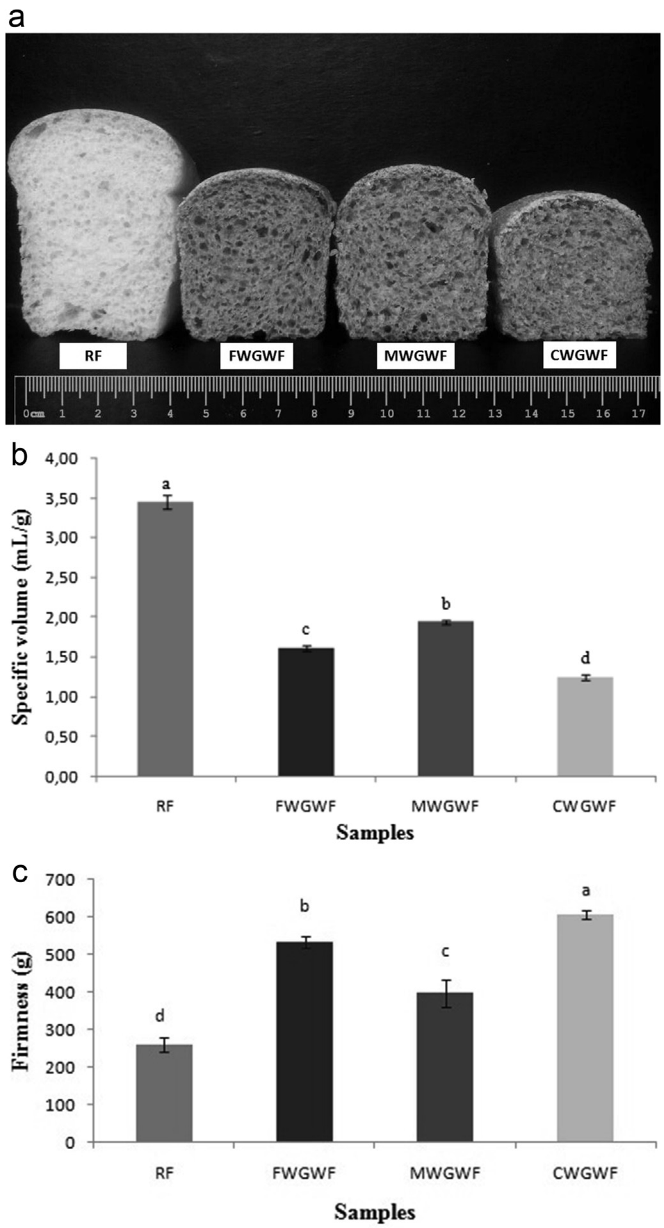


**Fig. 4.** Mean values in the same row followed by different letters are significantly different ($p < 0.05$). Crumb cross-section (a). loaf volume (b). and firmness (c) of white and whole-grain wheat bread with different particle sizes. Results expressed as mean of three determinations ± standard deviation. RF: refined flour (82.67 μm). FWGWF: fine whole grain wheat flour (194.98 μm). MWGWF: medium whole grain wheat flour (608.44 μm). CWGWF: coarse whole grain wheat flour (830.00 μm).

the results of rheological and baking properties observed.

The texture of bread is another important parameter of quality and acceptance by consumers. The deformation force reflects the hardness of the breads, a higher deformation value indicates a harder texture. The WGWF samples produced breads with significantly greater firmness ($p < 0.05$) (Fig. 4c). Among the WGWF samples, breads made from MWGWF had the best specific volume as well as the best firmness compared to the other two WGWFs with larger or smaller particle sizes. In the same way as the WGWF with larger particles is affected by the difficulty of forming the gluten network, resulting in lower volumes, bread texture is also affected by the water absorption capacity of the starch and may be associated with paste properties, where the reduction of maximum viscosity, parameter that provides an indication of the susceptible bulk viscosity, may result in harder textures, as observed with the CWGWF sample breads of this study (Table 2). Flours with smaller particle size showed smaller effect on hardness. However, Fig. 4C also shows that the WGWF with the smallest particle size showed smaller firmness compared to the medium size sample. To explain this phenomenon, we proposed, as well as Noort et al. (2010) and Li et al. (2012), that the release of certain active compounds increased with the WGWF particle size refinement, especially reducing compounds such as phenolic compounds. The particle size refinement also enhances the competition of the fibers by water with the gluten proteins, inhibiting their formation, since sufficient water availability is required for the development of the gluten network. The increase in bread hardness was inversely correlated with the specific volume of bread ($r = -0.950$), indicating that breads with higher hardness were also smaller in specific volume.

## 4. Conclusions

The particle size distribution of flour has an important role on its properties and the quality of end products. The results demonstrate that particle size may have significant differences in mixing, rheological and baking properties largely related to the quality of the gluten network. Coarse particles, such as those represented by WGWF demonstrate greater impact on the gluten network, with less stability, less resistance to extension and lower specific volume of baking. On the other hand, the increase of the effects of FWGWF on baking quality in relation to MWGWF suggests that greater contact surface provides a combination of physical and chemical mechanisms that act on the formation and function of the gluten network in the presence of all grain constituents.

Based on the present results, the authors can report that some traditional parameters of evaluation techniques of dough properties such as mixing properties, extensional properties do not represent the effects observed during baking for FWGWF. While techniques such as determining the concentration of free sulfhydryl groups and of dough microstructure allow better understanding of the gluten network functionality of the flour produced by grinding whole wheat grain. This study provides fundamental and additional information on the technological quality and performance in baking of WGWF with different particle sizes.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jcs.2017.05.001.

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