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Influence of milling whole wheat grains and particle size on thermo-mechanical properties of flour using Mixolab

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Abstract: Whole grain wheat flour (WG) of three different particles sizes (194.9, 609.4, and 830.0 μm) was prepared by milling whole grain. The effect of particle size on the thermo-mechanical properties of flour was investigated using Mixolab equipment and solvent retention capacity (SRC). The results showed that particle size influences the functionality of the gluten network. The SRC test revealed that the water absorption increased from 77.43% to 85.76%, with decrease in particle size. The C2 (protein weakening) values were correlated with the values for water absorption in the SRC and wet gluten test, respectively. The degree of gelatinization of starch (C3) showed that the presence of the fibers in the WG samples limited the availability of water to the starch, and this effect was especially true for flour with smaller particle size. In summary, the Mixolab equipment allowed a better understanding of the functionality of WG with regard to the behavior of protein properties. WG with coarse particles demonstrated a greater impact on the gluten network, indicating a negative effect on the baking quality.

Keywords: flour properties; granulometry; rheology; *Triticum aestivum*; whole grain; whole wheat flour

In recent years there has been an increasing interest in whole-grain products among consumers, especially those most concerned with health, thus directing the development of cereal-based products. In this context, whole grain wheat flour (WG) has become the subject of research around the world. In WG, the main anatomical components of grain, such as the endosperm, bran, and germ are present in the same proportions as that in the grain in its intact form, which characterizes it as an excellent source of nutritional and functional ingredients for human health (Liu 2007). Although WG can provide more health benefits than refined flour (RF), it also presents many baking related quality challenges, making it difficult to produce WG foods that maintain the functionality and quality desired by the consumers. For example, WG is known to reduce bread quality as it produces bread with a lower specific volume and a denser crumb texture (Pomeranz *et al*. 1977).

The particle size of WG is an important factor affecting product quality and functionality of the flour (Kihlberg *et al*. 2004). It is largely unknown how the particle size of the flour made from the milling of whole wheat grain influences the technical properties of the flour and baking characteristics. Especially as most studies refer to particle size of the

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bran used in different levels of reincorporation on the properties of the dough and quality of the finished products, such as breads, pasta, and biscuits (Noort *et al.* 2010; Li *et al.* 2012; Cai *et al.* 2014; Niu *et al.* 2014; Hemdane *et al.* 2015; Wang *et al.* 2016).

The rheological properties of wheat flour are normally used as an indicator of gluten structure and as a means of predicting gluten quality and functional behavior during baking (Jirsa *et al.* 2007). Thus, traditionally, changes in rheological properties during the baking process are studied with equipment that separately controls each step of the process, such as mixing behavior, fermentation stage, rest and temperature variation experienced by the dough throughout the baking process. Devices for assessing the consistency of the dough during mixing, such as the Farinograph, Mixograph, and Consistograph, have been widely used to study the behavior of dough. Likewise, changes in starch behavior associated with thermal processes are evaluated on the basis of paste viscosity during heating and cooling cycles using the Viscomilograph or the Newport Rapid Visco Analyser. However, the temperature range, at which initial protein unfolding as well as water uptake by the starch granules occurs, can not be measured with such equipment (Rosell *et al.* 2010).

The Mixolab technique can be considered an evaluation that, in only one test, predicts in varying degrees the quality of the final product, thereby simulating the steps of the baking process (mixing, cooking and cooling). The Mixolab technique measures the torque associated with the dough during the process with temperature variations, making it possible to measuare information on the amount of water required for dough development, the time of dough development, gluten strength and weakening, starch gelatinization, and the retrogradation, enzymatic activity, and gel resistance of the dough (Dubat 2010; Švec & Hruskova 2015). In addition, the Mixolab technique has been successfully applied to evaluate different aspects of wheat flour quality (Codina *et al.* 2010; Banu *et al.* 2011; Papoušková *et al.* 2011) as well as the impact of particle size on quality attributes of reconstitute WG (Liu *et al.* 2016; Wang *et al.* 2017). Nevertheless, no study has reported the evaluation of wheat flour produced by the milling of the whole grain (without bran recombination) of different particle sizes using the Mixolab technique.

The aim of this study was to evaluate the influence of milling whole wheat grains of different particle size ranges on the thermo-mechanical properties using the Mixolab technique. The influence of particle size on damaged starch and solvent retention capacity (SRC) profiles were also examined and evaluated.

MATERIAL AND METHODS

Material. The wheat (*Triticum aestivum* L.) used in this study was from the Brazilian cultivar BRS Guabiju from the 2014 harvest. All reagents used in the study were of analytical grade.

Milling process for obtaining flours. To obtain refined flour (*RF*), the wheat grains were conditioned to 15% (wet basis) moisture with distilled water and after 24 h were milled at experimental roll mill CD1 (Chopin Technologies, France) according to the method No. 26-10.02 of the American Association of Cereal Chemistry (AACCI, 2010).

Figure 1 illustrates the detailed process for the production of whole grain wheat flour (WG). In order to produce flours with three different average particle sizes, an impact laboratory mill with a speed of 20 000 rpm and a refrigerated grinding chamber (M20;

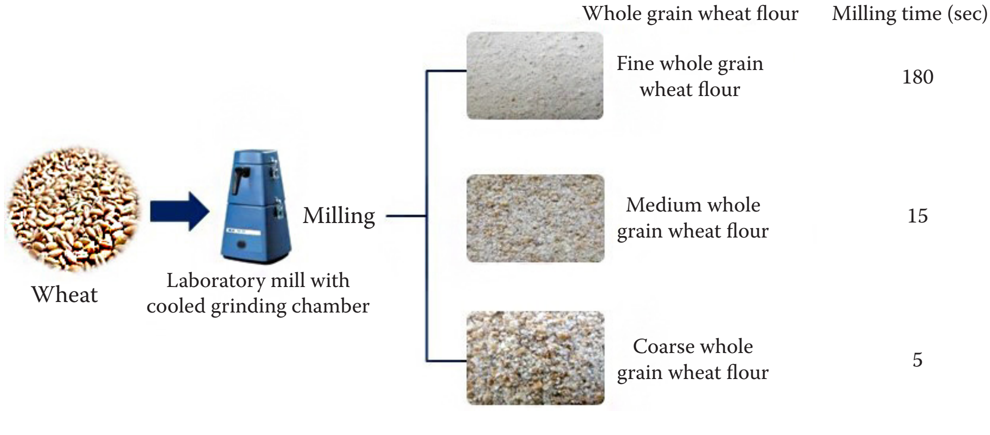

Figure 1. Representation of the milling process and production of whole grain wheat flours with different particle sizes

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IKA, Germany) was used. To obtain flour with fine particle size, the fixed milling time of 180 s was used. The flour with a middle particle size was obtained by milling for 15 s, while the flour of the coarser particle size was obtained by milling for 5 seconds. The distribution of the particle size of the samples was determined using the laser diffraction method on a particle size determinator (LV-950; Horiba, Japan) with the dry dispersion modulus. The average particle size of the flours produced by the milling of the whole grains were the following: 194.9 μm, fine whole grain wheat flour (FG); 609.4 μm, medium whole grain wheat flour (MG); and 830.0 μm, coarse whole grain wheat flour (CG) (Figure 2). The particle sizes used in this study were designed to encompass three particle sizes, considering that in practice WG can be produced by different milling procedures, resulting in various particle sizes and functionalities.

Flour physicochemical analyses. The levels of moisture, protein, ash, lipids and dietary fiber were determined according to the official methods of AACCI (2010), 44-15.02, 46-10.01, 08-12.01, 30-25, and 32-07.01, respectively. The gluten content analysis was performed by the method, No. 38-12.02 (AACCI, 2010), with modifications on a Glutomatic equipment (2100; Perten Instruments, USA). For the WG, a polyester sieve (88 μm) was initially used for 120 s, until a mass was formed. After this period, a larger sieve (840 μm) was used to eliminate the fibrous layers of the meal during the washing period.

Damaged starch was determined using the Chopin SDmatic according to method, 76-33.01 AACCI (2010).

SRC tests that included determining the water SRC (W-SRC), lactic acid SRC (LA-SRC), sodium carbonate SRC (SC-SRC), and sucrose SRC (Su-SRC) values of WGWF of different particle sizes, were performed according to method, 56-11.02 AACCI (2010).

Mixolab analysis. The thermomechanical characteristics of *RF* and WGWF with different particle sizes were evaluated using the Mixolab analyser (Chopin Technologies Inc. Paris, France) according to method 54-60.01 AACCI (2010) and application of the Chopin and Mixolab protocol. The flour mass used was 75 g, and the target consistency (C1) was 1.1 Nm (± 0.05). After an initial mixing for 8 min at 30°C, the dough was heated for 15 min at the rate of 4°C/min until the temperature of the dough reached 90°C. The dough was held at 90°C for 7 min and then cooled to 50°C, at the rate of 4°C/min, and then mixed at 50°C for 5 minutes.

Statistical analysis. All analyses were performed in triplicate. Data analysis was performed using analysis of variance (ANOVA). The comparison of means was performed using Tukey's test at 5% significance level. The Pearson correlation coefficient was used to evaluate the correlation between variables.

RESULTS AND DISCUSSION

Effect of particle size on the physicochemical properties of flour. The whole grain milling significantly reduced ($P < 0.05$) the moisture content of the flours relative to *RF* (Table 1); the reduction was most likely due to not pre-moistening the wheat grains. The small differences in moisture content observed between the WG samples can be attributed to the increase in milling time and thereby greater surface area of the flour. The content of proteins, lipids, ashes and fibers was higher in relation to RF ($P < 0.05$) (Table 1), which was expected because, through the complete milling of the grains, the germ, rich in lipids and the outer layers (bran and aleurone), rich in proteins, mineral matter and fibers, were maintained in the flour composition.

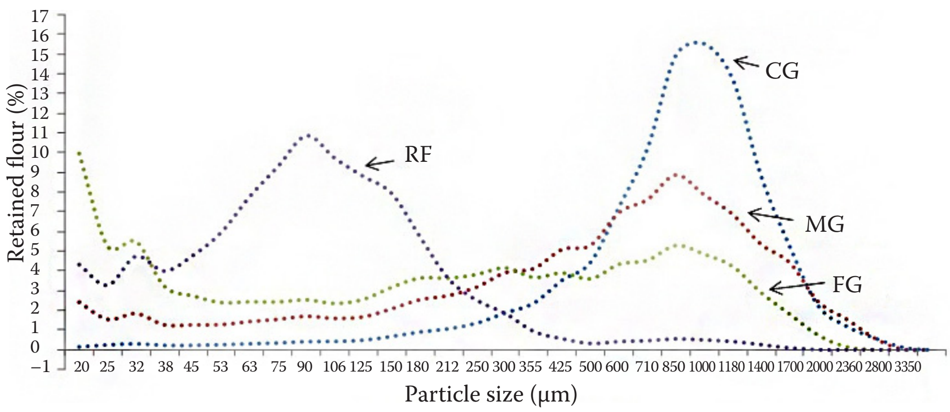

Figure 2. Particle size distribution profile of flour with different particle sizes
Results are a mean ± s.d. ($n = 3$); RF – refined flour (82.67 μm), FG – fine whole grain wheat flour (194.98 μm), MG – medium whole grain wheat flour (608.44 μm), CG – coarse whole grain wheat flour (830.00 μm)

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The wet gluten represents a measure of the ability of proteins to aggregate. The wet gluten values of the flours of medium and coarse particle size were significantly lower in relation to RF (Table 1). This was because larger the particle size, greater the difficulty in aggregation of gluten proteins, a behavior that can be attributed to the larger surface area of the bran in the larger particle size samples. Steglich *et al.* (2015) studied the impact of bran particle size on the mass microstructure and observed that smaller bran particles may be more flexible, and therefore easier to incorporate into the gluten matrix. According to these authors, bran with a larger particle size allows attachment to other bran particles. Therefore, aggregated bran particles that are more rigid, may damage the aggregation of gluten. This change with variation in particle size is consistent with the studies of Liu *et al.* (2015), who observed differences in gluten aggregation, studying different grinding processes in the quality of wheat flour.

For flour intended for bread making, certain levels of damaged starch are essential to provide fermentable sugars, and thus promote fermentation activity. Studies have shown that flour has the best baking performance when the starch damage is between 4.5 and 8.0%. (Gaines 1985; Dexter *et al.* 1994). However, very high amounts accelerate the enzymatic processes, providing changes such as sticky mass, reduced bread volume and changes in the colour of the crust (Barak *et al.* 2014). The damaged starch content of RF (5.97%) was significantly higher ($P < 0.05$) compared to flours obtained by whole grain milling (Table 1). Among the WG samples, the level of damaged starch increased with the degree of particle size reduction (Table 1) from 2.60% in the CG sample to 4.09% in the FG. These results demonstrate that longer the milling time of WG, greater the shear provided, thus causing greater damage to the flour starch.

The SRC test addresses the water absorption contributions of each component of the flour using different solvents. Thus, it relates the quality of flour to specific constituents (Kweon *et al.* 2009). As shown in Table 1, which compares the SRC of the four solvents of the WG samples with different particle sizes with that of the *RF* sample, the milling of the whole wheat grains resulted in a significant increase ($P < 0.05$) in the values of W-SRC and SUC-SRC. W-SRC is related to the overall water retention capacity of the functional components of flour, including gluten proteins, damaged starch, and arabinoxylans (Kweon *et al.* 2011). Table 1 shows the significant impact that the particle size has on the water absorption capacity of the WG samples. With a higher damaged starch content, which absorbs more water, the FG sample presented a higher W-SRC value (85.76%), while the MG and CG samples were not significantly different.

The higher value of W-SRC found for FG is in agreement with the greater water absorption shown by the Mixolab between the samples of whole flour (Table 2). SC-SRC is related to the amount of damaged starch in the flour. The lower values found in the

Table 1. Effect of whole milling of wheat grains and particle size on the physicochemical properties of flour

Parameters		Samples (n = 3)			
		RF	FG	MG	CG
Moisture	(g/100 g)	$14.08^a \pm 0.12$	$11.68^c \pm 0.13$	$12.62^b \pm 0.14$	$12.44^b \pm 0.10$
Protein	(g/100 g)	$13.85^b \pm 0.06$	$14.98^a \pm 0.08$	$14.81^a \pm 0.13$	$14.72^a \pm 0.16$
Fat	(g/100 g)	$1.59^b \pm 0.08$	$1.97^a \pm 0.35$	$1.94^a \pm 0.16$	$1.93^a \pm 0.12$
Ash	(g/100 g)	$0.56^b \pm 0.02$	$1.70^a \pm 0.00$	$1.70^a \pm 0.02$	$1.67^a \pm 0.01$
Dietary fiber	(g/100 g)	$3.61^c \pm 0.02$	$12.45^b \pm 0.71$	$14.95^a \pm 0.04$	$15.95^a \pm 0.15$
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	(%)	5.97a ± 0.11	$4.09^b \pm 0.14$	$3.39^c \pm 0.06$	$2.60^d \pm 0.14$
W-SRC	(%)	$70.02^c \pm 0.50$	$85.76^a \pm 0.20$	$77.61^b \pm 0.64$	$77.43^b \pm 0.41$
SC-SRC	(%)	$100.68^a \pm 0.74$	$88.02^b \pm 0.60$	$78.82^c \pm 0.45$	$77.30^d \pm 0.34$
LA-SRC	(%)	$96.25^a \pm 0.46$	$90.39^b \pm 0.50$	$88.04^c \pm 0.34$	$78.23^d \pm 0.32$
SUC-SRC	(%)	$98.54^c \pm 0.38$	$112.72^b \pm 0.36$	$113.60^b \pm 0.41$	$129.41^a \pm 0.36$

Mean values in the same row followed by different letters are significantly different ($P < 0.05$); results are a mean ± s.d.; RF – refined flour; FG – fine whole grain wheat flour; MG – medium whole grain wheat flour; CG – coarse whole grain wheat flour

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WG samples, compared to *RF*, are attributed to the lower content of damaged starch caused by the milling process (Table 1). Among the WG, the SC-SRC was significantly ($P < 0.05$) affected by the variation in particle size, increasing from 77.30% to 88.02%, as the particle size was reduced. The increase in SC-SRC was positively correlated with the damaged starch content ($r = 0.98$), indicating a higher interaction of sodium carbonate with damaged starch components. LA-SRC refers to the resistance of gluten. The lowest values found for the WG samples, relative to RF, were confirmed by a trend of reduction in the gluten aggregation values (Table 1) and stability found in the Mixolab (Table 2). SUC-SRC provides an indication of the content of arabinoxylans in the flour (Gaines 2000). Arabinoxylans are highly hydrophilic structural carbohydrates that absorb 10–18 times the weight of water (Sanz Penella 2008). With the presence of the outer layers in the composition of WG samples, flours with a higher concentration of arabinoxylans were produced, resulting in higher SUC-SRC indexes, as compared to RF.

Effect of particle size on the WG Mixolab profile. In the Mixolab analysis, two phases were observed: the first phase determined the protein properties during mixing of the dough at 30°C, and the second phase, the properties of starch paste. Different Mixolab curves were obtained for the RF and WG samples (Figure 3). The similarity of the curves observed in the initial part of the process occurred due to the adjustment made by the addition of water to obtain the same dough consistency (1.1 Nm). However, during the heating and cooling phases of the analysis process, the curves showed significant differences in consistency (Table 2).

Under the condition C1 = 1.1 ± 0.05 Nm, the water uptake of the WG samples presented significantly higher values ($P < 0.05$) than *RF*, except for the CG sample. Among the WG, the particle size considerably affected the water absorption, with the FG sample having the highest water absorption, followed by MG and CG. This is due to the higher contact surface of the particles, which provides greater exposure for the hydroxyl groups present in the fiber structure, and thus greater interactions with water through the hydrogen bonds in smaller particle sizes of bran (Sanz Penella 2008). A similar trend was observed by Xiong *et al.* (2017), who evaluated the influence of bran particle size distribution on protein polymerization and water mobility of whole wheat flour. Compared to WG with finer particle sizes, a significant increase was observed in the dough development time parameter which measures the time between the addition of water and the time when the dough attains the optimal elastic and viscous properties for flour with a coarser particle size with respect to the other two WG particle size (Vizitiu & Danciu 2011). This phenomenon was probably due to the interference caused by the presence of bran during gluten development; this interference can give rise to conditions such as water absorption velocity that is slower in larger particle sizes (Sanz Penella 2008), and formation of physical obstacles that lead to a reduction in the connectivity of the gluten network. Similar results were reported by Liu *et al.* (2016) and Wang *et al.* (2017), when they evaluated reconstituted wheat flour of different particle sizes using the Mixolab technique.

The stability of the dough, a parameter related to the flour strength, significantly decreased ($P < 0.05$)

Table 2. Effect of particle size on the Mixolab parameters of refined flour and whole grain wheat flour

Parameters Mixolab		Samples			
		RF	FG	MG	CG
Absorption (%)		$65.03^{c} \pm 0.05$	$70.06^{a} \pm 0.05$	$67.46^{b} \pm 0.05$	$65.03^{c} \pm 0.05$
Development time (min)		$8.84^{b} \pm 0.18$	$7.70^{b} \pm 1.08$	$7.87^{b} \pm 0.25$	$11.65^{a} \pm 0.39$
Stability (min)		$11.33^{a} \pm 0.11$	$10.52^{b} \pm 0.45$	$9.24^{c} \pm 0.12$	$7.83^{d} \pm 0.17$
Torque (Nm)	C2	$0.54^{b} \pm 0.01$	$0.44^{d} \pm 0.01$	$0.46^{c} \pm 0.01$	$0.61^{a} \pm 0.01$
	C3	$1.74^{d} \pm 0.01$	$1.86^{c} \pm 0.01$	$2.07^{b} \pm 0.01$	$2.31^{a} \pm 0.01$
	C4	$1.49^{a} \pm 0.03$	$1.40^{ab} \pm 0.03$	$1.41^{ab} \pm 0.02$	$1.03^{b} \pm 0.29$
	C5	$2.09^{a} \pm 0.03$	$1.90^{b} \pm 0.09$	$1.92^{b} \pm 0.05$	$0.00^{c} \pm 0.00$

Mean values in the same row followed by different letters are significantly different ($P < 0.05$); values are a mean ± s.d.; RF – refined flour; FG – fine whole grain wheat flour; MG – medium whole grain wheat flour, CG – coarse whole grain wheat flour

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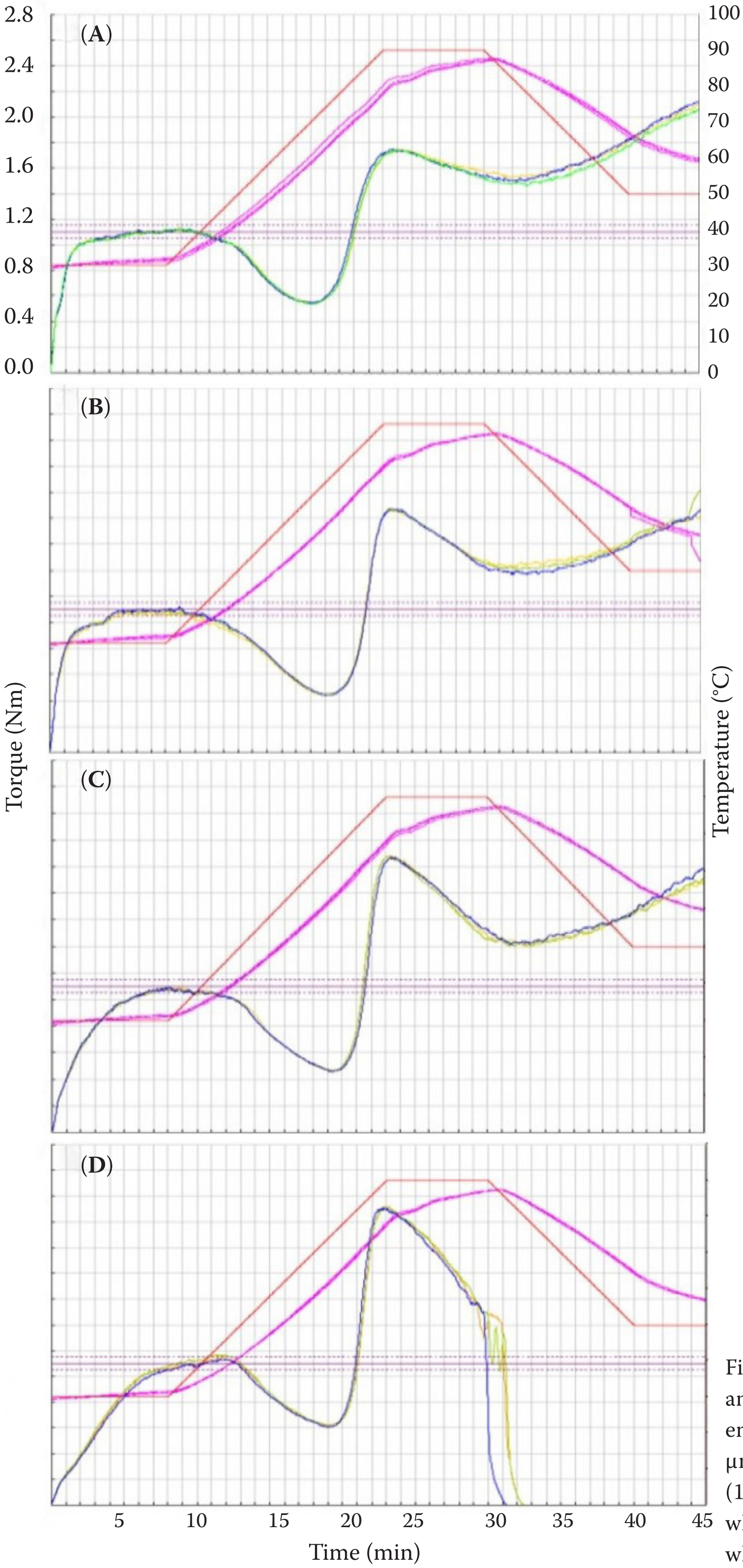

Figure 3. Mixolab curves of refined flour and whole grain wheat flour with different particle sizes: (**A**) refined flour (82.67 μm), (**B**) fine whole grain wheat flour (194.98 μm.), (**C**) medium whole grain wheat flour (608.44 μm), and (**D**) coarse whole grain wheat flour (830.00 μm)

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in the WG samples when compared to the *RF* value. In the WG samples, the competition for water by bran components, such as arabinoxylans, is the main interference in the proper development of gluten, reducing gluten strength (Li *et al.* 2012; Niu *et al.* 2014). As major non-starch polysaccharides in WG samples, arabinoxylans can bind strongly to the water in the dough and reduce its availability, which is ideal for optimal gluten formation. However, the dough stability of WG increased with the reduction in particle size, indicating that WGs of finer particle size are more tolerant to the mixing process and provide stronger dough. Niu *et al.* (2014) studied the effects of fine grinding on the quality attributes of reconstituted whole grain flour, and also observed a significant increase ($P < 0.05$) in the stability time when reducing the average particle size from about 206 to 140 µm.

When the temperature of the dough is raised from 30°C at a rate of 4.0°C/min, the gluten proteins weaken and the consistency of the dough decreases till it reaches the parameter C2. The fine and medium whole grain flours had lower C2 values, with a decrease of 18.51% (FG) and 14.81% (MG), respectively with respect to RF (0.54 Nm) (Table 2). This shows that with increasing temperature and exposure to physical-mechanical stress, the dough strength of whole grain flours decreases due to weakening of the proteins. The highest value of C2 obtained for CG in this study is related to the aggregation difficulty of gluten proteins, a result that is in agreement with the results obtained for the wet gluten parameter (Table 1). In WG with larger particle size, the lack of aggregation provides lower overall gluten yield, resulting in lower protein weakening, and higher torque (C2). Therefore, as observed with CG, where C2 values increased, the particle size effect of WG on C2 may be the result of gluten dilution and fiber interference in protein unfolding.

The last stage of the Mixolab test shows the properties of the starch paste in the dough system. Torque C3 (Nm) represents the degree of gelatinization of the starch and the maximum viscosity, indicating towards the susceptible viscous load produced during the mixing (Huang *et al.* 2010). The value of the peak torque (C3) was significantly affected by particle size of WG and resulted in a linear increase of this parameter with the increase in particle size (Table 2). For CG, a significant increase was observed, 24.19 and 11.59% of peak torque when compared to FG and MG, respectively. These changes were caused by the fact that fine particle size flours retain more water than coarse particle size flours; hence, there is less water available, which would hinder swelling of starch granules. Similar behaviour was reported by Lauková *et al.* (2017) when studying the effects of cellulose fiber length on the rheological properties of wheat dough, and by Blanco Canalis *et al.* (2019) when analyzing the incorporation of dietary fiber in cookie dough. The swelling of starch is greatly dependent on the water available in the medium, which controls the gelatinization behavior (León *et al.* 1997). In the system used in this study, a limited amount of water was available for the starch gelatinization, thus different effects were exerted by the particle size. This modification is the one that can occur in the wheat dough during bread making. In contrast to our results, Wang *et al.* (2017) studied the effects of particle size on the quality attributes of reconstituted whole grain wheat flour, and showed lower C3 values. Liu *et al.* (2016) studied reconstituted whole grain wheat flour, and reported smaller values of C3 with respect to RF, but without a significant difference in the variation in particle size. The results herein showed that the WG can exhibit adverse behavior related to particle size, reaffirming the hypothesis by Drakos *et al.* (2017), that stated that the complexity of the flour matrix and the milling parameters, are important for gel formation as they define the interactions between the various constituents and water.

C4 values indicate the stability of the hot-formed gel. The WG with fine and medium particle sizes showed no significant difference in gel stability with respect to RF. However, the CG presented a marked reduction (69%) when compared to RF. The observed decrease in gel stability for CG suggests that the gel formed is weakened by the presence of fibers in the matrix, in particular by fibers having greater particle size and insolubility. Torque C5 measures the final viscosity, which is reached during the sample cooling process and is generally related to the starch retrogradation (Teng *et al.* 2015). Table 2 shows that the value of C5 significantly decreased for WG of medium and fine particle size, relative to RF, but did not show a significant difference between these two WG particle sizes. The capacity of high fiber to absorb water makes it less available for retrogradation, as can be observed in this study. This reduced rearrangement of the starch may be beneficial for delaying the hardening of bakery products during storage (Cai *et al.* 2014). Despite presenting susceptible viscous

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loading results and gel stability, CG did not show any re-association between the molecules, mainly amylose, to provide a viscosity increase (Figure 3D). Probably, this phenomenon occurred due to the low availability of water in the medium due to the presence of the fibers. The pronounced drop in viscosity and impossibility of torque quantification in the CG reflects the complexity of the WG matrix, as well the interactions between the constituents of the flour and the water, which are strongly influenced by the particle size.

CONCLUSIONS

The integral grinding of wheat grains with different particle size ranges significantly altered the thermo-mechanical properties evaluated by the Mixolab technique. In general, a deleterious effect on time of dough development, gluten strength, starch gelatinization and the retrogradation was intensified by the presence of all constituents of the grain in the wheat mass formulation when compared to RF. Based on the Mixolab curves, the particle size distribution played an important role in the WG thermomechanical properties, presenting different technological qualities. The stages of the Mixolab curves showed that the quality of the protein (C2) and the differences between the WG particle sizes with respect to the stability and development time, are broadly correlated with the quality of the gluten network. Coarse particles, such as those represented by CG, have a greater impact on the gluten network, and present lower stability and longer development time. It also demonstrates that the Mixolab has the ability to generate correlated data for characteristics such as wet gluten and W-SRC. However, the Mixolab curves stage that described the characteristics of starch (C3, C4, and C5) demonstrated that in the WG samples, the presence of fibers limited the availability of water to the starch, and that this effect was especially strong for flour with finer particle size, which also had the highest rate of absorption. Based on the present results, we report that that the Mixolab equipment allows a better understanding of the WG functionality with regard to the behavior of the protein properties. However, properties of starch, such as degree of gelatinization, gel stability, and retrogradation are influenced by the availability of water in the formed mass system. These data are relevant because differences in particle size distribution affect functionality, sensory acceptability, nutritional properties, and shelf life of whole wheat flour.

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