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Effect of Wholewheat Flour Particle Shape Obtained by Different Milling Processes on Physicochemical Characteristics and Quality of Bread

Running head: **Milling Effect on Wholewheat Flour and Bread**

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SUMMARY

Research background. Wholewheat flour is a very good source of nutritional compounds and functional ingredients for human diet. Yet, its use causes negative effect on bread quality. Different milling techniques could be used to obtain wholewheat flour, minimizing the negative effect of both bran and germ on bread quality. The aim of this work was to study the effect of particle size and shape of wholegrain flour on the interaction between the different components, the water distribution, dough rheology and bread volume.

Experimental approach. Wholewheat flour of three varieties (Klein Rayo, Fuste, INTA815) were obtained by cyclonic, hammer and roller mills. The characteristics of wholewheat flour were explored, and the water distribution and rheological properties of dough were determined by thermogravimetric analysis and Mixolab test, respectively. Finally, microscale bread was prepared.

Results and conclusions. The amount of water-soluble pentosans, damaged starch and wet gluten was affected by the milling procedure. Regarding dough rheological properties, wholewheat flour by hammer mill had the lowest water absorption and the highest developing

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time. This result could be mainly attributed to particle shape in these samples with large amount of endosperm attached to the bran, hindering protein unfolding. Thermogravimetric analysis exhibit that both fine and large bran particle size seem to have the same effect on water properties in wholewheat dough during heating. Bread made with Klein Rayo had the highest specific volume, indicating that wheat with high protein content and breadmaking quality is needed to make wholewheat bread. The results of this work showed that particle shape, rather than particle size, affected the quality of wholewheat flour for breadmaking. Thus, the wholegrain milling process should be carefully selected taking into account the shape of particle produced.

Key words: wholewheat flour, milling, particle size, particle shape, thermogravimetric analysis, bread volume

INTRODUCTION

At present, consumers are trying to change their dietary habits in search of gaining health benefits and preventing future diseases. In this sense, consumers are open to explore healthy alternatives that were mostly rejected in the past. Wholegrains are a nutritional option with increasing acceptance, and this is recognised by the food industry. Moreover, whole grain consumption is encouraged by the World Health Organization for a healthy diet with the aim of preventing a range of noncommunicable diseases (*1*).

Wholewheat flour (WWF) is a very good source of nutritional compounds and functional ingredients for human diet. Wholewheat flour, as opposed to refined flour, is rich in fibres, antioxidants, vitamins, minerals and other phytochemicals such as carotenoids, flavonoids and phenolic acids (*2*). In addition, the intake of wholegrains is associated with a decreased risk of cardiovascular diseases, diabetes, obesity and colon cancer (*3,4*).

Many research works have proved that bread elaborated with wholewheat flour shows reduced technological quality as compared to that of refined flour (*5,6*). The main reasons causing this detrimental effect on bread quality have been attributed to: 1- water-holding capacity of bran limiting gluten network development; 2- gluten dilution effect; and 3- disruption of gas cells (*7*). Moreover, Every *et al.* (*8*) corroborated that the germ contains reducing compounds such as glutathione, which depolymerizes gluten network. Pareyt *et al.* (*9*) also found high levels of non-polar lipids, which tend to destabilize gas cells and thus decrease loaf volume.

One strategy that has been explored, is the reduction in bran particle size, thus decreasing its steric hindrance during gluten development. However, the results obtained are controversial and inconclusive. While some studies have found that the reduction of bran particle size improved bread volume (*10*), Noort *et al*. (*5*) reported a negative effect when bran particle size was smaller than or equal to that of starch granules, arguing that fibres negatively affect the formation of gluten network by a combination of both physical and chemical mechanisms. On the other hand, Coda *et al*. (*11*) and Bressiani *et al.* (*4*) reported that there is an optimal particle size for whole flour, and that this allowed producing bread of acceptable quality.

The characteristics of wholewheat flour can be largely influenced by the milling process. Different milling techniques could be used to produce wholewheat flour, minimizing the negative effect of both bran and germ on bread quality. One type of milling is hammer milling, where wheat grains are impacted between wall and hammer to reduce its particle size according to the sieve selected by users (*12*). A further milling procedure is the cyclonic mill. The wheat sample is ground at high speed by impacting the kernels against an abrasive surface. The cyclone cools the wholewheat flour so that its properties are not modified. A further option is the roller mill, which can be used to produce flour while separating bran and germ, and then recombined to obtain wholewheat flour with the same relative proportion as in intact grains.

Liu *et al.* (*13*) studied the effect of different milling processes of wholewheat flour on the quality of steamed bread; however, they obtained the initial flour by using a roller mill and then subjecting germ and brain to different milling types (hammer, stone, ultrafine and recombining processes). Although many studies have already analysed the effect of flour particle size on bread technological properties, few studies examined the effect of the particle shape of wholewheat flour.

Due to this lack of agreement, the objective of this work was to study the effect of particle size and shape of wholegrain flour on the interaction between the different components, dough rheology and bread volume. In this sense, wholewheat flour (WWF) was obtained by different milling processes, and its shape and size were analysed. In addition, the characteristics of WWF were explored and, the water distribution and rheological properties of dough were determined by thermogravimetric analysis (TGA) and Mixolab test, respectively.

MATERIALS AND METHODS

Wholewheat flour

Three varieties of wheat samples, Klein Rayo (KR), Fuste (Fu) and INTA 815 (IN), were provided by Instituto Nacional de Tecnología Agropecuaria (INTA, Marcos Juárez, Argentina), harvested in 2016. The varieties used in this work are classified according to the genetic quality established by the Winter Cereal Committee of the National Seed Commission (Argentina) (*14*) in three quality groups with annual update. The Klein Rayo variety (composition (in % on dry mass basis): proteins 12.65, ash 2.05, lipids 3.41) is a corrector wheat with very strong gluten, while the Fuste variety (composition (in % on dry mass basis):

proteins 10.81, ash 1.85, lipids 3.74) is a good-quality wheat used for long fermentation breadmaking. INTA815 (composition (in % on dry mass basis): proteins 11.42, ash 1.90, lipids 3.03) has high flour yield but low breadmaking quality. Grains were ground to obtain wholewheat flour (WWF) with three different mills, namely cyclonic mill (Cyclotec™ a 1093, FOSS, Hillerød, Denmark) using a 1 mm mesh sieve, hammer mill (Pulverisette 16, Fritsch, Idar-Oberstein, Germany) with a 1 mm mesh sieve, and roller laboratory mill (Mill CD1, Chopin Technologie, Villeneuve-la-Garenne, France). With this last type of milling, all the millstreams (bran, germ, and endosperm) were recovered and recombining them together to obtain wholewheat flour. Thus, all flour samples flour had the same proportion of bran, germ, and endosperm as the original wheat grain. The chemicals used were of analytical grade. The ingredients employed in the preparation of the microscale breads were purchased in the local market.

Particle size determination

Particle size distribution of WWF was determined by laser light diffraction (Horiba LA 960, Kyoto, Japan). The distributions were performed in triplicate from 0.2 g of sample in aqueous suspension. The d_{10}, d_{50}, d_{90} corresponding to the maximum diameter of 10, 50 and 90 % of the particles, respectively (% of total volume) were calculated. In addition, average particle size and span (d_{90}-d_{10}/d_{50}) were calculated, providing information on the amplitude and heterogeneity of the distribution.

Scanning electron microscopy

The microstructure of wheat flour particles was studied using scanning electron microscopy (SEM). Samples were dehydrated with phosphate buffer (0.1 mol/L, pH=6.8), ethanol (30, 50, 70, 80 and 90 % *V*/*V*) and subjected to vacuum. The samples were sprinkled onto double-sided tape attached to the specimen stubs and coated with a thin layer of gold (30 nm thickness) through a cathodic spray coating system. For the observations, an electronic scanning microscope (FE-SEM Σigma LaMARX, FAMAF-UNC) was used under high vacuum conditions (10-4 Pa) at an acceleration voltage of 3.00 kV. Images were obtained with magnifications between 100× and 1000×.

Bran images by stereomicroscopy

Bran particles were resuspended and washed with distilled water. They were then dried in stove for 4 h at 40 °C and observed with a stereo microscope S8AP0 (Leica Microsystems Inc., Bannockburn, USA). The resulting images were analysed using Image J v1.51j8 Software (National Health Institute, USA) (*15*) to calculate and display shape descriptors, such as area, perimeter and circularity.

Characteristics of wholewheat flour

Moisture, ash, and lipid content of the samples were measured according to approved methods 44-15.02 (*16*), 08-12.01 (*17*) and 30-25.01 (*18*), respectively. Briefly, moisture content was determined by weighing the sample prior to and after drying for 2 h at 130 °C (Dry oven model 600 D060602, Memmert, Germany). Ash content was determined by weighing the sample prior to and after igniting for 2 h at 600 °C (Indef model 332, Córdoba, Argentina). Determination of total lipid was done by Soxhlet extraction with petroleum ether (Sintorgan, Buenos Aires, Argentina). After the extraction, lipid content was determined by weighing. Protein content (Kjeldahl method 46-12.01 (N × 5.7) (*19*)) was determined after their digestion with concentrated H_2SO_4 (Sintorgan, Buenos Aires, Argentina). Digest (Raypa digestor, Barcelona, Spain) was used for sample digestion and Distillation (VELP UDK126A) for the distillation (Scientifica, Milan, Italy). The damaged starch (DS) content was evaluated according to AACC 76-31.01 method (*20*). Wet gluten (WG) content was obtained according to the hand washing method 38-10.01 (*21*). The content of total (TP) and water-soluble pentosans (WSP) of flour were quantified following the orcinol-HCl method described in Steffolani *et al.* (*22*) at 670 nm with UV-Vis Spectrometer (JASCO model V-730, Mary's Court Easton, USA). Wholewheat flour was analysed using a prediction test developed for refined flour. The hydration capacity of the proteins in an acidic environment was determined by means of the sodium dodecyl sulphate sedimentation index (SDS-SI) according to Moiraghi *et al*. (*23*).

Evaluation of mixing and pasting properties by Mixolab

The mixing and pasting behaviour tests of WWF were carried out under controlled heating conditions in a Mixolab analyser (Chopin, Tripette et Renaud, Paris, France) according to the method 54-60.01 (*24*). A certain amount of water (water absorption) was added to each sample to reach the maximal 1.1 Nm, representing 500 Brabender units (BU) of consistency for the dough. The parameters obtained from the Mixolab included water absorption capacity and dough properties, such as dough development time (C1), protein weakening (C2), starch gelatinization (C3), stability of hot starch paste (C4) and starch gelling (C5).

Breadmaking procedure

According to Moiraghi *et al.* (*23*), microscale bread tests were carried out with 20 g of flour with minor modifications for wholewheat flour. The ingredients used were (on a flour basis): NaCl, 2 %; sucrose, 1 %; dry baker's yeast, 1 %; and optimum water level (water absorption). Ingredients were mixed for 2 min in a manual mixer (Moulinex Supermix 130, Buenos Aires, Argentina). The resulting dough was taken to a first proof for 20 min at 30 °C in a water-saturated atmosphere. The dough was then manually degassed and sheeted with a Pastalinda® machine (Buenos Aires, Argentina) to form an oval dough piece. This was folded twice into halves. The dough was then divided into 10 g pieces, rolled up and placed in a baking pan (40×25×20 mm). After fermentation of 35 min at 30 °C in a water-saturated atmosphere, dough was baked for 12 min at 200 °C. The volume of each bread loaf was determined by the rapeseed displacement method (method 10-05.01) 2 h after baking (*25*). Specific bread volume (SBV) was obtained as bread volume/bread mass.

Thermogravimetric analysis

The thermal properties of wholewheat dough of KR flour obtained by different milling methods were analysed by thermogravimetric analysis (TGA) in a Discovery TGA (TA Instruments, New Castle, DE, USA). Dough samples were prepared according to bread formulation (without sugar and yeast) and breadmaking procedure. Dough samples (~35 mg) were heated in aluminium pans under a nitrogen atmosphere (nitrogen flow rate 50 mL/min) at a heating rate of 5 °C/min from 25 to 150 °C. Each run was repeated at least twice. All the TG traces, namely mass loss *vs* temperature, were calculated based on the initial water content of each dough sample. From these TG traces, we determined temperature at which samples lost 75, 80 and 90 % of water and percentage of total water loss at several temperatures. TG traces were then analysed for their first derivative, representing the rate of water loss (Derivative thermogravimetry (DTG) (%/°C)) using TRIOS v4.3.1 (TA Instruments–Waters LLC, New Castle, DE, USA) (*26*) to identify specific water loss events. In addition, DTG traces were fitted to a sum of Gaussian functions using PeakFit v4.12 (Systat Software, San José, CA, USA) (*27*) with the aim of determining different water types (*i.e.* free and bound to each major component). The Gaussian peaks were initially added around peak centres and the final location and area of the Gaussians were determined by automatic fitting to get the best fit to the data. Peak area was expressed as a percentage of the total area under the curve. Adjustments with regression coefficient (r) greater than 0.99 were considered.

Statistical analysis

Results were obtained at least in duplicate and expressed as the mean ± standard deviation. The data obtained for the same wheat variety were evaluated by analysis of variance (ANOVA) and results were compared by DGC means-comparison test at a significance level of 0.05. In addition, a variance analysis was performed considering the mean of each treatment (milling process). All analyses were performed using the INFOSTAT statistical software (Faculty of Agricultural Sciences, UNC, Argentina) (*28*).

RESULTS AND DISCUSSION

WWF particle shape and size

The particle size distribution of wholewheat flour was different according to the milling procedure (Table 1). Particle size data could be slightly overestimated due to the hydration of particles. However, all the samples were subjected to the same conditions for measurement. The WWF obtained with the cyclonic mill (CM) was characterized by a relatively small particle size distribution (d_{90} between 519-648 µm) with a span of 3.77-4.44. The d_{90} of the WWF obtained by hammer mill (HM) showed values between 1079-2344 µm with a span of 1.84-2.36. The roller mill (RM) allowed obtaining WWF with large particle size (1534-4167 µm) however, span was grain-variety dependent, where KR presented the highest span and IN, the lowest. In the first step of roller milling process, wheat grains were crushed through serrating rollers that tore and triturated the grain. In the second step, endosperm particles were reduced in size. The particles generated by this milling procedure had a large size since it serrated and inclined rollers to produce histological layers of bran, but with large surface area. The resulting flour had an endosperm reduced in size, but with greater germ and bran particles.

By contrast, the cyclonic mill caused a homogenous reduction of WWF particle size in a single step. The principle of this milling process is a turbine wheel that spins at a very high speed, breaking the sample into pieces and hurling them out to the rim where they are abraded to a fine dust.

The mechanism of hammer mill is intermediate between roller and cyclonic mills; the speed is lower than that of cyclonic mill, with no abrading rim. As a consequence, the WWF particles obtained by hammer mill had intermediate size and a large amount of endosperm attached to the bran (Fig. 1).

In general, the shape of bran particles can be scored as a combination of magnitudes such as area and perimeter or by a single magnitude that indicates the percentage of similarity to a given geometric object such as circularity. This shape descriptor is a measure to a circle and ranges from 0.0 to 1.0, where 1.0 is a perfect circle (*29*).

The analysis of particle shape allowed determining the homogeneity between the WWF obtained with the cyclonic mill and the heterogeneity of the particles obtained by roller mill. In this sense, the WWF obtained by roller mill showed particles with highest perimeter and area surface, whereas circularity was the lowest. In addition, the bran particles obtained by roller mill presented irregular shapes and the typical structure of histological tissues, while the bran particle generated from hammer and cyclonic mill lost part of their original structure (*30*). As Saad *et al*. (*31*) described along the milling process, an erosion phenomenon occurs, the outer surface of the irregular bran particles may undergo a friction causing the small irregularities on the surface to disappear generating particles with more regular shapes.

Wholewheat flour characterization

The content of protein, lipids and ash of the WWF was not affected by the milling type since it was obtained from the same varieties. However, the form in which some specific components appear did depend on the type of milling (Table 2). Damaged starch is caused by mechanical action during wheat milling on starch granules. The damaged granules negatively affect dough behaviour and quality of breadmaking flour (*32*). In this work, the wholewheat flour obtained by hammer mill had the lowest damaged starch content in the three varieties, while WWF obtained by cyclonic and roller mill had similar percentage of damaged starch.

These results confirmed that the hammer mill breaks wheat grain, without tearing it; as a consequence, the endosperm adhered to bran suffers less damage.

The effect of milling on the total pentosan content of WWF was not significantly influenced by the type of milling, and this result was expected since milling was integral. However, the total pentosan content showed significant differences between varieties: INTA815 had the highest value and Fuste, the lowest. On the other hand, the soluble pentosan content depended on the milling type; the WWF from hammer mill showed the lowest soluble pentosan content as compared to other milling procedures. The high water extractable pentosan in WWF obtained by cyclonic and roller mill could be attributed to the rupture of the cell wall, resulting in a release of pentosan polymers entangled in the cell wall matrix. In addition, the friction on the grinding ring of cyclonic mill might result in the cleavage of covalent bonds, turning water unextractable pentosan into water extractable pentosan (*33*).

Wet gluten content was determined by the hand washing method since the glutomatic method did not allow developing good network and full washing. The WWF obtained by cyclonic and roller mill had significantly higher wet gluten content as compared to the WWF produced by hammer mill. This result indicated a significant effect of milling type on the quality of breadmaking flour, whereas the particle size of WWF and wheat variety would have a minor effect. The gluten network is formed and stabilized by covalent disulphide bonds and non-covalent interactions such as hydrogen bonds, ionic bonds and hydrophobic bonds between gliadin and glutenin (*34*), and bran and germ particles interfere during the development of this structure. The SDS-SI is a predictive test of the quality of breadmaking flour. Wholewheat flour had lower sedimentation index as compared to that of white flour, and this result is mainly attributed to the lower gluten content in WWF. In this work, different varieties showed no effect on SDS sedimentation test; yet, the number of samples analysed was very low. The milling type was probably the biggest influence in this test. Morris *et al.* (*35*) examined the SDS sedimentation test on wholewheat flour and observed that this assay was highly sensitive to differences among hexaploid ‘bread’ wheat. In the same work, the authors found no effect between grinding type and particle size. In our work, the WWF obtained by roller mill showed the highest sedimentation index, followed by cyclonic mill, whereas the lowest sedimentation volume was in WWF obtained with the hammer mill. Therefore, the particle shape of hammer-mill flour, with large amount of endosperm attached to the bran, could hinder protein unfolding by SDS and the floccules formed were thus small, unstable and heavier and their volume sedimentation was low.

Rheological properties of dough samples

The properties of dough were analysed with a Mixolab (Table 3). This equipment allows simulating the behaviour of proteins and starch during kneading and cooking, being subjected to mechanical stress and temperature changes (*36*). In a typical curve, the initial steps show the characteristics of gluten, and the last steps show starch properties (*37*). WWF showed higher water absorption compared to white flour as found by Barros *et al*. (*38*). The arabinoxylans present in wheat bran have great capacity to bind water due to the presence of hydrophilic groups, responsible for the increased absorption of water in wholewheat flour (*36*). Water absorption was greater in the samples obtained with roller and cyclonic mills; yet, they showed lower developing time.

On the other hand, the samples obtained by hammer mill had the lowest water absorption and the highest developing time. This result could be mainly due to the particle shape in these samples, which had intermediate surface and were polygonal and coarse with particles of endosperm adhered. As a consequence, the surface was non-porous and with few internal surfaces (*30*). In addition, these samples showed high C3, C4 and C5 values (parameters related to starch pasting properties). Similarly, the characteristics of the particles generated during hammer mill hindered the hydration and gluten developed in dough, thus water was available for starch gelatinization and the consequent retrogradation. According to Mixolab results, the particle size of WWF had no significant effect on water absorption, since roller and cyclonic samples showed similar water absorption while particle size was in opposing extremes. Similar results were reported by Zhang and Moore (*39*) who described that coarse wheat bran (609 mm) had higher water-holding capacity than fine bran (278 mm), but as wheat bran of different particle sizes were mixed into flour, the bran particle size showed no effect on water absorption. Take into account that WWF obtained by cyclonic and roller mill had greater percentage of damaged starch than WWF-HM, it could also play a more important/significant role in determining water-holding properties than that played by bran particle size as Niu *et al.* (*40*) observed when wheat was subjected to superfine grinding.

However, small particle size decreased developing time and increased protein weakening (low C2) due to less interference of bran in the development of gluten network and a larger contact surface between dough components; hence hydration rate of gluten protein was greater and consequently gluten developed quicker (*41*). However, the interaction between polypeptide chains was weaker in WWF. Wang *et al*. (*42*) reported an increase stability time with reduction of flour particle size. In this work, although stability showed no clear trend, WWF-CM had greater stability in relation to other types of flour, but only in Klein Rayo and Fuste varieties, since INTA 815 showed no significant differences between milling types. As opposed to white flour, where high C1, C2 and stability indicate strong gluten and good breadmaking quality, in wholewheat flour these parameters were affected by other factors such as particle size and shape and presence of fibre. The variety effect was negligible. Klein Rayo presented the highest water absorption and lowest protein weakening (stability). The developing time showed no clear effect since the milling process was probably more significant.

Specific bread volume

Fig. 2 shows microscale bread slices made with different WWF. The effect of milling type was significant; the WWF obtained by cyclonic and roller mills had higher specific volume compared to those made with hammer mill WWF. This bread showed a compact crumb insufficiently aerated with small cells. Conversely, RM and CM bread presented larger air cells and crumb was similar to that of white bread. The bread made with Klein Rayo flour had the highest specific volume regardless of milling type, indicating that wheat with high protein content and breadmaking quality is needed to make wholemeal bread. In general, a comparison of these results based on the literature is challenging since most of the studies were carried out with bran reincorporation, modified in particle size. The results reported in this work are opposite to Bressiani *et al.* (*4*), where they informed that WWF with intermediate particle size allowed higher specific bread volume as compared to small and large particle size. However, these authors used an impact mill and different times of milling to obtain WWF of different particle size. The results of this work also differ from those of Noort *et al.* (*5*) since an increase in area surface by grinding did not lead to a decrease in specific bread volume.

Wang *et al.* (*42*) suggested that reducing particle size of WWF from ~160 µm to ~100 µm could be an effective way to improve the quality of whole wheat. In that work, the bran and short obtained with roller mill were further ground 1 to 4 times using a Perten laboratory mill. Thus, the milling process used was different from cyclonic milling.

The better performance noted in bread made with WWF-CM and WWF-RM could be attributed to 30 % higher water-soluble pentosan content on WWF samples compared to WWF-HM. WSP released during the breakdown of the kernel cell-matrix probably played a key role improving bread quality by binding significant amounts of water. Thus, it resulted in less available water for starch gelatinization, allowing the loaves of bread to achieve higher volume before the breadcrumb structure was set.

Dough thermogravimetric analysis

Fig. 3a shows the thermograms of wholewheat dough (WWD) water loss from Klein Rayo wholewheat flour obtained from different milling processes. As the samples had different optimal water absorption, all the TG traces were normalized to the initial water content. When weight loss results from a single process, like dehydration, TG traces show a sigmoid ascending trend with a flexus at some intermediate temperature where water loss rate is maximum (*43*). All samples exhibited a similar pattern. The flexus points were located around 92-95 °C where water loss of 72-74 % took place.

WWD-HM released a total of 75, 80 and 90 % of water at lower heating temperatures. The polygonal and coarse particles of wholewheat flour generated by this type of milling affected water distribution between components in dough samples and the amount of bound water decreased. This effect was also reflected at 90 °C, reaching a maximum temperature bread crumb (*44*), from which samples by HM showed higher percentages of water loss (65 %) compared to that of cyclonic (58 %) and roller mill samples (60 %). An early decrease in water content could lead to premature settling of the crumb structure; therefore, it could limit the development of loaf volume.

Fig. 3b-3d shows the first derivative DTG plot obtained from the TGA data of each sample. The well-defined peaks observed correspond to the flexus points in TG traces and suggest an increase of water evaporation rate was produced (*45*). Maximum water loss rates ranged between 1.48 and 1.61 % / °C. DTG profiles were influenced by the milling process. The WWD-CM and WWD-RM exhibited similar profiles. A mean peak around 95 °C and a secondary peak around 110 °C were observed. On the other hand, the secondary peak was absent in WWD-HM. Therefore, water loss rate at 110 °C was significantly lower in WWD-HM samples (Table 4). It may indirectly indicate how strong water is retained by dough components. Fessas *et al.* (*43*) studied the TGA profile of wheat dough and suggested that the presence of two peaks in the DTG profiles is attributed to water state into the matrix. Free water was absorbed by gelatinization starch while temperature increased, and water strongly bonded to the gluten network could only be evaporated at a higher temperature (> 100 °C). Wholewheat flour is a heterogeneous system comprising polymers with different hydrophilic capacity (starch, non-starchy polysaccharides, fibre, gluten proteins, *etc*.), which therefore form separate aqueous phases, each with a particular composition (*46*). In our work, the deconvolution of each DTG profile of the dough samples allowed distinguishing an overview of water compartmentalization among matrix components (Fig. 3b-3d). The DTG profile of each sample was analysed with a 4-peak model. Adjusted model curves showed r^2 values greater than 0.99. The first peak, whose maximum was around 42 °C, was attributed to adsorbed water or weakly bound water to the bran particle surface, as suggested by Roozendaal *et al*. (*47*). The evaporation ease of this phase is linked to the low affinity of bran with water, which is released when placed under stress (*44*).The second peak, whose maximum was around 65 °C, was related to water associated with starch, in agreement with both Fessas *et al*. (*43*) and Roozendaal *et al*. (*47*). Water is stored into the micro-capillaries of starch granules and junction zones or held by hydrogen bonds between the amylose and amylopectin chains (*48*). Moreover, this water phase can be easily released when placed under mechanical stress or heating (*49*). A third peak, whose maximum was around 92 °C, was associated with water less bound to proteins, free to diffuse from the inside to the surface of the sample. Finally, a fourth peak above 110 °C was observed. According to Lapčíková e*t al.* (*50*), this fourth peak corresponds to water strongly linked to gluten network. The magnitude of this stronger bond results from resistance to the removal of this water from glutamine residues (*51*).

TGA test showed that size and shape of flour particles obtained from different milling processes influenced water redistribution during baking. Table 4 shows average peak temperatures and area percentages. No significant differences in the maximum temperatures of each peak were observed. However, the milling type affected the area (%) of peaks, associated with water content bounded to each component. The dough from hammer mill had a second peak with higher area percentage. This milling type caused less particle damage and lower content of soluble pentosans; therefore, as there was more water available in the system, it increased the hydration of the starch granules during heating and resulting gelatinization. These results are consistent with the C3 values obtained during Mixolab testing. On the other hand, the flour from roller mill showed a third peak with a greater relative area. The particle morphology obtained by this mill type led to the formation of large insoluble protein aggregates and to an increase in the amount of water retained by this phase. In addition, cyclonic and roller mill dough had high area percentage of fourth peak; this result could indicate greater water bound to gluten and a well-developed network.

These findings suggest that particle size and shape of wholegrain flour obtained by different milling processes play a significant role in the water compartmentalization of the dough system. In addition, the magnitude of the events involved in the baking process, which are all governed mostly by water availability, may influence the final quality of baked product. Nevertheless, both fine and large bran particle size seem to have the same effect on water properties in wholewheat flour dough during heating.

CONCLUSIONS

In this work, the study of WWF obtained through different milling types has allowed determining that particle shape in wholegrain milling has a main effect on product quality. In this sense, hammer mill in wheat grains generates intermediate-size particles, but a portion of endosperm is adhered to the bran layers. As a consequence, WWF-HM had lower content of damaged starch, wet gluten and soluble pentosans. In addition, these particle types increased hydration time, modified water distribution between flour components and hindered the accurate development of dough; therefore, specific bread volume was low. On the other hand, in this work we demonstrated that particle size does not significantly influence WWF quality. Both, the small particle of WWF obtained by cyclonic mill and the large particle of WWF obtained by roller mill showed similar properties. These two milling types generate particles with thin layers of bran, completely separated from the endosperm, allowing a better water distribution between dough components and improving gluten development, leading to higher specific bread volume. The effect of milling type and particle shape in WWF was more influential than that in the wheat variety. Thus, the wholegrain milling process should be carefully selected taking to account the shape of particle produced. Nevertheless, further research is needed to identify the main factors and particular components responsible for the detriment effects found on breadmaking quality. This may open new opportunities for developing wholewheat bread with better acceptance by consumers.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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Table 1. Particle size distribution of wholewheat flour (WWF) and bran shape descriptors

Variety	Mill type	WWF particle size distribution		Bran particle shape		
		d_{90}/µm	Span	A/mm^2	P/mm	Circularity
KR	CM	(626±1)^a	(3.77±0.15)^b	(0.38±0.03)^a	(3.48±0.05)^a	(0.39±0.01)^b
	HM	(1079±44)^b	(1.87±0.06)^a	(0.37±0.01)^a	(3.74±0.01)^a	(0.36±0.00)^b
	RM	(1534±10)^c	(11.59±0.33)^c	(2.38±0.26)^b	(10.76±0.30)^b	(0.24±0.03)^a
FU	CM	(648±68)^a	(4.28±0.47)^b	(0.33±0.17)^a	(3.06±0.73)^a	(0.41±0.00)^b
	HM	(2080±58)^b	(1.84±0.07)^a	(0.56±0.19)^a	(4.14±0.32)^b	(0.37±0.04)^a
	RM	(1944±33)^c	(5.33±0.13)^b	(1.73±0.27)^b	(8.72±0.70)^b	(0.29±0.02)^a
IN	CM	(519±13)^a	(4.44±0.05)^c	(0.52±0.03)^a	(5.09±0.03)^a	(0.27±0.02)^a
	HM	(2344±48)^b	(2.36±0.01)^b	(0.46±0.07)^a	(3.69±0.32)^a	(0.42±0.03)^b
	RM	(4167±5)^c	(3.09±0.14)^a	(1.93±0.37)^b	(8.50±0.67)^b	(0.35±0.01)^b
Mean*	CM	(582±71)^a	(4.16±0.38)^b	(0.41±0.10)^a	(3.88±1.07)^a	(0.36±0.08)^b
	HM	(1117±51)^a	(2.02±0.26)^a	(0.46±0.09)^a	(3.86±0.25)^a	(0.38±0.03)^b
	RM	(2699±1130)^b	(6.67±3.94)^b	(2.01±0.33)^b	(9.33±1.24)^b	(0.29±0.05)^a

KR=Klein Rayo, FU=Fuste, IN=INTA815, CM=cyclonic mill, HM=hammer mill, RM=roller mill. *A*=area, *P*=perimeter.

Different letters within a column for the same wheat variety indicate that values are significantly different at the level of $p<0.05$.

*Values of each milling treatment represent the average of the three varieties.

Table 2. Characteristics of wholewheat flour

Variety	Mill type	w(DS)/%	TP/%	WSP/%	WG/%	V(SDS-SI)/mL
	CM	$(7.87\pm0.00)^b$	$(11.4\pm0.1)^a$	$(0.70\pm0.07)^b$	$(26.2\pm0.0)^b$	$(10.55\pm0.35)^b$
KR	HM	$(3.36\pm0.29)^a$	$(11.5\pm0.4)^a$	$(0.53\pm0.01)^a$	$(20.9\pm0.8)^a$	$(7.00\pm0.00)^a$
	RM	$(8.28\pm0.00)^b$	$(11.3\pm0.5)^a$	$(0.77\pm 0.0)^b$	$(24.8\pm0.0)^b$	$(11.75\pm0.35)^c$
	CM	$(8.49\pm0.29)^b$	$(9.4\pm0.9)^a$	$(0.61\pm0.02)^b$	$(24.6\pm0.3)^c$	$(9.25\pm0.35)^b$
FU	HM	$(3.36\pm0.58)^a$	$(10.0\pm1.5)^a$	$(0.43\pm0.01)^a$	$(16.1\pm0.3)^a$	$(5.75\pm0.35)^a$
	RM	$(8.49\pm0.29)^b$	$(10.4\pm0.7)^a$	$(0.57\pm0.01)^b$	$(22.0\pm0.0)^b$	$(11.00\pm0.00)^c$
	CM	$(7.38\pm0.00)^b$	$(13.0\pm2.1)^a$	$(0.85\pm0.04)^a$	$(27.4\pm0.1)^b$	$(10.13\pm0.21)^b$
IN	HM	$(3.57\pm0.29)^a$	$(12.7\pm0.6)^a$	$(0.37\pm0.52)^a$	$(20.0\pm0.8)^a$	$(6.25\pm0.00)^a$
	RM	$(8.18\pm0.14)^b$	$(12.7\pm1.9)^a$	$(0.72\pm0.00)^a$	$(25.2\pm0.2)^b$	$(12.00\pm0.00)^c$
	CM	$(7.93\pm0.51)^b$	$(11.3\pm1.9)^a$	$(0.72\pm0.12)^b$	$(26.1\pm1.2)^b$	$(9.96\pm0.64)^b$
Mean*	HM	$(3.53\pm0.33)^a$	$(11.4\pm1.4)^a$	$(0.56\pm0.23)^a$	$(19.0\pm2.3)^a$	$(6.33\pm0.58)^a$
	RM	$(8.33\pm0.20)^b$	$(11.5\pm1.4)^a$	$(0.73\pm0.09)^b$	$(24.0\pm1.6)^b$	$(11.58\pm0.49)^c$

KR=Klein Rayo, FU=Fuste, IN=INTA815, CM=cyclonic mill, HM=hammer mill, RM=roller mill. DS=damaged starch, TP=total pentosans, WSP=water-soluble pentosans, WG=wet gluten, SDS-SI= sodium dodecyl sulphate sedimentation index. Different letters within a column for the same wheat variety indicate values are significantly different at the level of $p<0.05$.

Results are expressed in dry basis.

*Values of each milling treatment represent the average of the three varieties.

Table 3. Mixolab parameters of wholewheat flour

Variety	Mill type	WA/%	C1/min	S/min	C2/Nm	C3/Nm	C4/Nm	C5/Nm
KR	CM	68.80	$(5.25±0.31)^a$	$(3.54±0.03)^b$	$(0.35±0.01)^a$	$(1.40±0.01)^a$	$(1.07±0.01)^a$	$(1.95±0.20)^a$
	HM	64.70	$(9.39±0.02)^c$	$(1.25±0.68)^a$	$(0.48±0.01)^b$	$(1.92±0.01)^c$	$(1.48±0.01)^c$	$(2.59±0.04)^b$
	RM	71.20	$(7.67±0.38)^b$	$(1.77±0.47)^a$	$(0.46±0.03)^b$	$(1.60±0.01)^b$	$(1.22±0.04)^b$	$(2.05±0.17)^a$
FU	CM	61.30	$(4.32±0.05)^a$	$(5.05±0.31)^b$	$(0.44±0.01)^a$	$(1.75±0.01)^a$	$(1.41±0.00)^a$	$(2.35±0.02)^b$
	HM	56.50	$(10.43±0.13)^c$	$(1.07±0.08)^a$	$(0.54±0.01)^b$	$(2.15±0.04)^b$	$(1.80±0.01)^b$	$(3.03±0.01)^b$
	RM	64.85	$(7.99±0.33)^b$	$(1.62±0.24)^a$	$(0.48±0.01)^a$	$(1.73±0.01)^a$	$(1.34±0.03)^a$	$(2.30±0.05)^a$
IN	CM	62.50	$(3.00±0.30)^a$	$(1.77±0.11)^a$	$(0.37±0.00)^a$	$(1.54±0.00)^a$	$(1.05±0.04)^a$	$(1.94±0.03)^a$
	HM	56.30	$(6.67±0.94)^c$	$(2.60±0.09)^a$	$(0.51±0.00)^b$	$(2.02±0.00)^b$	$(1.66±0.74)^b$	$(2.74±0.00)^b$
	RM	65.80	$(5.59±0.06)^b$	$(1.70±0.35)^a$	$(0.41±0.00)^a$	$(1.57±0.02)^a$	$(1.05±0.06)^a$	$(1.81±0.17)^a$
Mean*	CM	$(64.20±3.6)^b$	$(4.19±1.03)^a$	$(3.45±1.52)^b$	$(0.39±0.10)^a$	$(1.56±0.16)^a$	$(1.17±0.18)^a$	$(2.08±0.24)^b$
	HM	$(59.17±4.3)^a$	$(8.83±1.74)^b$	$(1.64±0.77)^a$	$(0.51±0.03)^c$	$(2.03±0.10)^b$	$(1.65±0.14)^b$	$(2.78±0.20)^b$
	RM	$(67.28±3.1)^b$	$(7.08±1.24)^b$	$(1.70±0.18)^a$	$(0.45±0.03)^b$	$(1.63±0.08)^a$	$(1.20±0.36)^a$	$(2.05±0.25)^a$

KR=Klein Rayo, FU=Fuste, IN=INTA815, CM=cyclonic mill, HM=hammer mill, RM=roller mill. WA=water absorption, C1=dough developing time, S=stability of dough, C2=protein weakening, C3=starch gelatinization, C4=stability of hot starch paste, C5=starch gelling. Different letters within a column for the same wheat variety indicate that values are significantly different at $p<0.05$.

*Values of each milling treatment represent the average of the three varieties.

Table 4. Water loss content (%) during wholewheat dough heating in different types of mill, water loss rate (%/°C) and maximum peak height temperature (°C) and associated area (%) of each peak obtained by 4-peak deconvolution model of the DTG profile

Parameter		CM	HM	RM
75 % WL temperature/°C		$(102.8\pm5.1)^{b}$	$(96.0\pm0.3)^{a}$	$(106.6\pm2.8)^{b}$
80 % WL temperature/°C		$(106.6\pm5.6)^{b}$	$(99.4\pm0.4)^{a}$	$(111.2\pm2.6)^{b}$
90 % WL temperature/°C		$(114.2\pm6.0)^{b}$	$(108.0\pm0.5)^{a}$	$(120.1\pm2.4)^{b}$
WL rate at 110 °C /%/°C		$(1.37\pm0.02)^{c}$	$(095\pm0.00)^{a}$	$(1.04\pm0.03)^{b}$
1st peak-	max peak/°C	$(41.9\pm1.1)^{a}$	$(43.1\pm0.8)^{a}$	$(41.3\pm3.1)^{a}$
	area/%	$(10.3\pm2.8)^{a}$	$(12.3\pm2.0)^{a}$	$(7.8\pm2.0)^{a}$
2nd peak	max peak/°C	$(64.4\pm1.3)^{a}$	$(68.9\pm1.2)^{a}$	$(62.1\pm3.7)^{a}$
	area/%	$(22.1\pm0.8)^{a}$	$(36.7\pm1.1)^{b}$	$(22.8\pm3.0)^{a}$
3rd peak	max peak/°C	$(92.3\pm0.2)^{b}$	$(94.3\pm0.0)^{c}$	$(90.9\pm1.1)^{a}$
	area/%	$(43.7\pm0.4)^{a}$	$(38.3\pm0.2)^{a}$	$(50.4\pm5.4)^{b}$
4th peak	max peak/°C	$(113.7\pm3.4)^{a}$	$(116.4\pm0.4)^{a}$	$(116.2\pm3.0)^{a}$
	area/%	$(23.9\pm3.2)^{b}$	$(12.7\pm0.7)^{a}$	$(19.1\pm0.5)^{b}$

CM=cyclonic mill, HM=hammer mill, RM=roller mill. 75, 80 and 90 % water loss (WL) temperature=temperature at which dough samples lose 75, 80 and 90 % of water content.

WL rate at 110 °C=water loss rate at 110 °C.

Values in the same file with common letter are not significantly different ($p>0.05$)

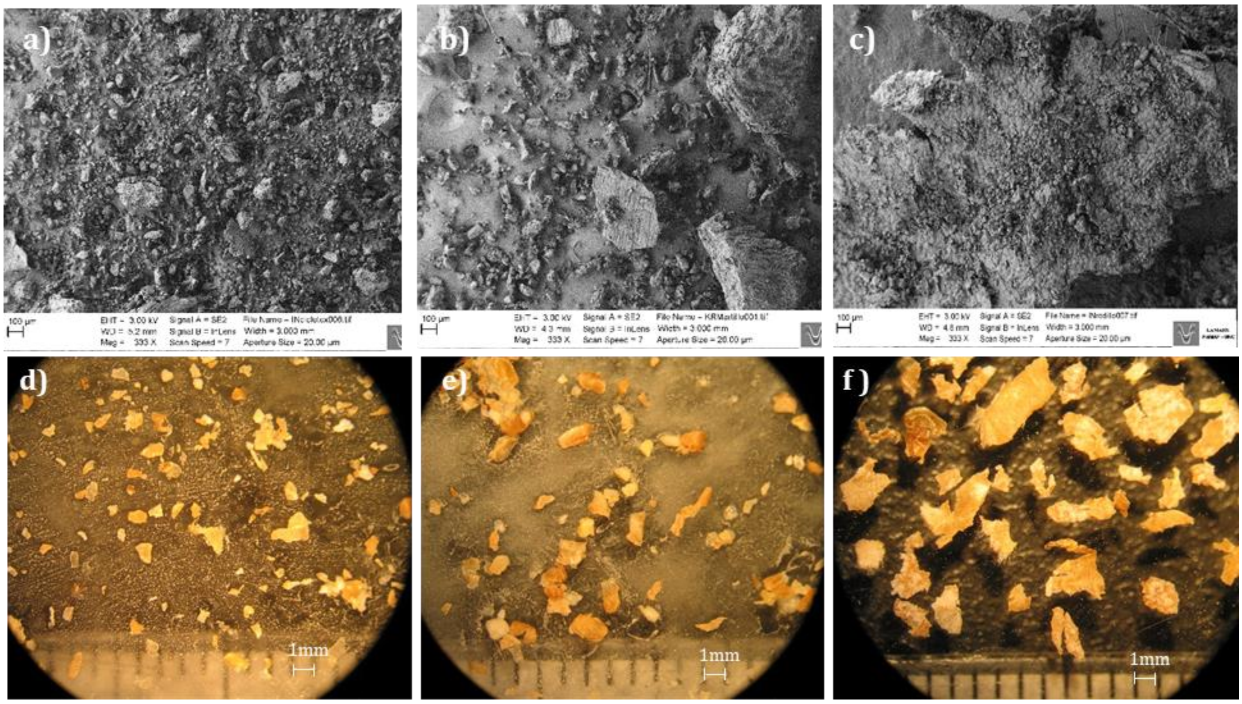

Fig. 1. Scanning electron micrographs of: a-c) wholewheat flour and d-f) stereo microscopy images of bran particles of Klein Rayo variety obtained by cyclonic (a, d), hammer (b, e) and roller (c, f) mill

Sample	Mill type		
	CM	HM	RM
WWF-KR			
SBV (cm^3/g):	(2.43±0.04)^{b}	(2.20±0.01)^{a}	(2.52±0.05)^{b}
WWF-FU			
SBV (cm^3/g):	(2.21±0.20)^{b}	(1.89±0.02)^{a}	(2.32±0.01)^{c}
WWF-IN			
SBV (cm^3/g):	(2.17±0.11)^{b}	(1.74±0.11)^{a}	(2.36±0.07)^{b}

Fig. 2. Representative images of microbread made with WWF obtained by different mills. KR=Klein Rayo, FU=Fuste, IN=INTA815, CM=cyclonic mill, HM=hammer mill, RM=roller mill. SBV=specific bread volume (cm^3/g). Specific bread volumes of the same variety followed by the same letter are not significantly different ($p<0.05$)

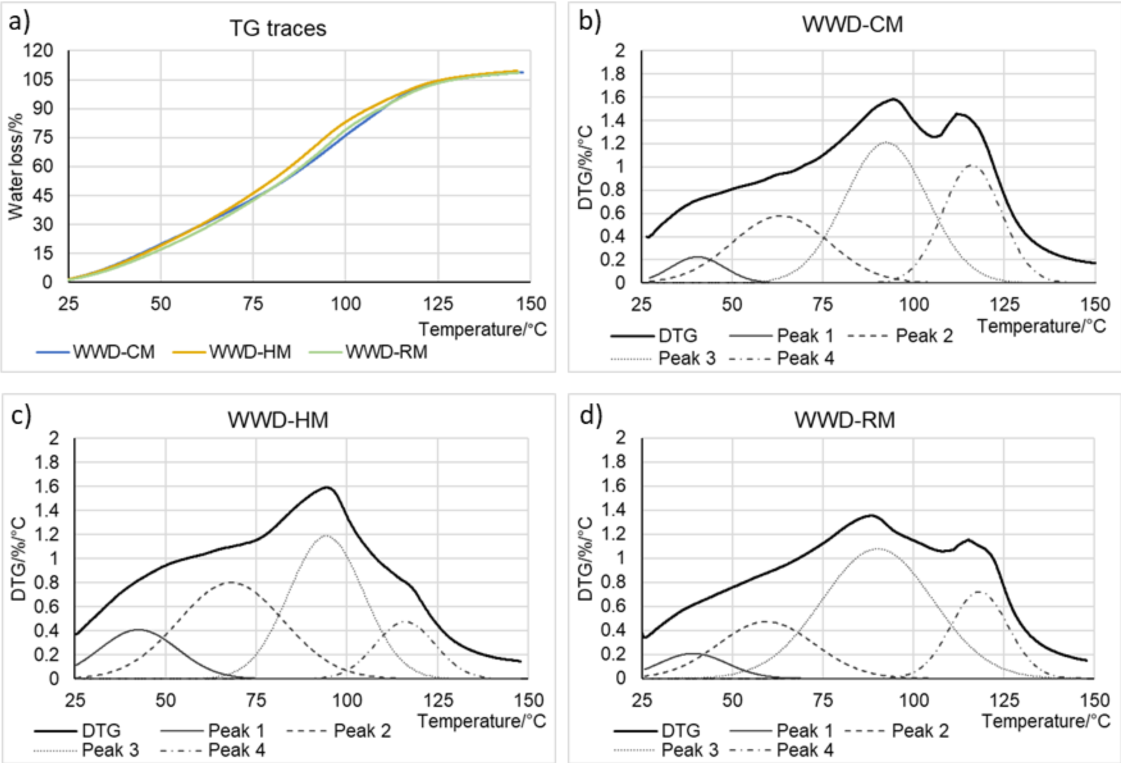

Fig. 3. TG traces show the effect of milling type on water loss (%) in wholewheat dough (WWD) of Klein Rayo variety from 25°C - 150°C (a) and their first derivative (DTG), representing the water loss rate (%/°C) of the WWD obtained by cyclonic (b), hammer (c) and roller (d) mills