Jet milling effect on functionality, quality and *in vitro* digestibility of whole wheat flour and bread

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Abstract

Jet milling is an ultragrinding process in order to produce superfine powders with increased functionalities. The effect of milling pressure, feed rate, vibration rate of feeder and feedback of jet milling on whole wheat flour functionality and the potential of those flours for breadmaking with the goal of improving bread quality and digestibility was investigated. Increasing milling pressure (from 4 to 8 bar), decreasing feed rate (from 0.67 to 5.18 kg/h) and/or using recirculation augmented the severity of the process and reduced flour particle size from 84.15 to 17.02 µm. Breakage of aleurone particles layer and the reduction of particle size in jet milled flours were detected using scanning electron microscopy (SEM). Ash and protein content did not change after jet milling. However, total fiber content and digestible starch increased from 13.01 to 14.72 % and from 33.80 to 43.23 mg/100 mg, respectively, when subjected to jet milling at 8 bar air pressure. Mixolab® data indicated that water absorption increased from 64.1 to 68.0%, while pasting temperature decreased from 63.4 to 66.1 ^{o}C owing to the milling intensity. Referring to bread, jet milled flour addition reduced the specific volume from 2.50 to 1.90 cm^3/g, luminosity, from 60.48 to 55.87 and moisture content from 35.78 to 33.49%, an increased crumb hardness from 707 to 1808 g. Jet milled breads presented a slight decrease in estimated glycaemic index (*eGI*) (from 86 to 81), suggesting that jet milling treatment could also have nutritional benefits.

Keywords: jet milling; whole wheat flour; dough; bread quality; digestibility

Introduction

Wheat is one of the most used cereals for breadmaking. Whole wheat flour (WWF) contains substantially more vitamins, minerals, antioxidants and other nutrients than refined wheat flour, since these compounds are concentrated in the outer portions of the grain (Hemery et al., 2007).

The milling process is considered pivotal in the production of WWFs and it is one of the effective ways to reduce the negative impact of bran and germ on end-use products (Wang et al., 2002). Wheat bran particle size is an important factor influencing gluten network formation and bread quality (Noort et al. 2010). The most traditional milling techniques for reducing the particle size of WWF include burn mill, pin mill and Wiley mill, which allowed to produce fine wheat bran (278 μm) that required shorter dough mixing compared to coarse bran (609 μm) (Zhang and Moore, 1997). Li et al. (2012) reported that the whole-wheat bread made from WWF of average particle size of 96.99 μm, obtained with Waring blender and ultramicro-pulverizer, and had better baking quality with larger volume and specific volume than those made from WWF of two other particle sizes, 50.21 and 235.40 μm.

Jet milling is an alternative process to reduce WWF particle size. It is a fluid energy impact-milling technique commonly used to produce particle sizes less than 40 μm by using high air pressure (Chamayou and Dodds, 2007), and also feeding rate, vibration rate of feeder and feedback can be manipulated to control flour particle size (Protonotariou et al., 2014). Superfine powders are produced by accelerating the particles in a high-velocity air stream, the size reduction being the result of inter-particle collisions or impacts against solid surface (Létang et al., 2002).

Although recently many researches have been conducted for improving WWF breads (Rosell et al., 2009), there is limited information about how jet milling modulates flour properties and starch behavior, as well as bread quality and *in vitro* digestibility of bread.

The aim of the present work was to study the effect of jet milling settings on the characteristics of whole wheat flour and on the physical quality and starch enzymatic digestion of whole wheat breads. In that purpose SEM micrographs, chemical composition, Mixolab® analysis and enzymatic hydrolysis curves of flours were tested. In reference to bread, quality assessment and *in vitro* digestibility were investigated.

Materials and Methods

Whole wheat flour (type T90, with 90% extraction rate), donated by the Company Loulis Mills S.A., was pulverized in a jet mill (Model 0101S Jet-O-Mizer Milling, Fluid Energy Processing and Equipment Company, Telford, Pennsylvania, USA) using four different conditions (Table 1). Two samples were processed for each combination of milling conditions.

Flour analysis

Scanning electron microscopy (SEM)

Wheat flours were stuck on metal stubs with double-sided stick tape and sputter-coated with a 100–200 Å thick layer of gold and palladium by ion sputter (JEE 400, JEOL, Tokyo, Japan). Analysis of the specimens was performed at 10 kV accelerating voltage with a SEM (S-4800, Hitachi, Ibaraki, Japan) equipped with a field emission gun, a backscattered detector of RX Bruker, transmission detector, the QUANTAX 400 programmed for microanalysis and the five motorized axes. This SEM has a spotlight of field emission (FEG) with a resolution of 1.4nm at 1KV. The microstructure analysis was carried out using image analysis software (Image-Pro

Plus 7.0, Media Cybernetics, USA) in the Central Service for Experimental Research of the Universidad de Valencia.

Particle size distribution

Particle size distributions was determined by laser granulometry with a Malvern Mastersizer 2000 diffraction laser particle sizer (Malvern Instruments, Worcestershire, UK), equipped with a Scirocco dry powder unit (Malvern Instruments, Worcestershire, UK). The instrument provides volume weighted size distributions. Particle size parameters, such as volume median diameter (d_{50}), De Brouckere mean diameter ($d_{4.3} = \Sigma n_i\ d_{i4}/\Sigma n_i\ d_{i3}$) and Sauter mean diameter ($d_{3.2} = \Sigma n_i\ d_{i3}/\Sigma n_i\ d_{i2}$) were used to characterize the flour samples, where n_i is the number of droplets and d_i their diameter. Median diameter is the value of the particle size which divides the population exactly into two equal halves i.e. there is 50% of the distribution above this value and 50% below. De Brouckere mean diameter is the volume or mass mean diameter of the particles, and Sauter mean diameter is the surface area weighted mean diameter of the particles. Median diameter is the value of the particle size which divides the population exactly into two equal halves i.e. there is 50% of the distribution above this value and 50% below. The particles were assumed to have a refractive index of 1.53.

Chemical composition

Moisture content was determined by ICC Standard Methods (ICC, 2011). Ash, protein, total fiber and insoluble fiber contents were determined by AACC method (AACCI, 2012). Determinations were carried out in duplicate.

Starch hydrolysis kinetics

Starch hydrolysis was measured following the method described by Gularte and Rosell (2011) with minor modifications. Flour sample (0.1 g) was added to 10 mL of 0.1 M sodium maleate buffer (pH 6.9) containing porcine pancreatic α-amylase (6 U/mL; Type VI-B, ≥10 units/mg solid; Sigma Chemical, St. Louis, USA) and incubated in a shaking water bath at 37 ºC. Aliquots of 200 μL were withdrawn during the incubation period (0.25–16 h) and mixed with 200 μL of ethanol (96%, w/w) to stop the enzymatic reaction and the sample was centrifuged at 10,000 ×*g* for 5 min at 4 ºC. The precipitate was washed with 50% ethanol (200 μL) and the supernatants were pooled together and kept at 4 ºC for further glucose enzymatic release. Supernatant (100 μL) was diluted with 850 μL of 0.1 M sodium acetate buffer (pH 4.5) and incubated with 50 μL amyloglucosidase (33 U/mL) at 50 ºC for 30 min in a shaking water bath.

For resistant starch determination after 16h of hydrolysis the sediment was solubilized with 2 mL of 2 M KOH using a Polytron ultraturrax homogenizer IKA-T18 (IKA works, Wilmington, NC, USA) during 1 min at speed 3. The homogenate was diluted with 8 mL 1.2 M sodium acetate (pH 3.8) and incubated with 100 μL amyloglucosidase (33 U/mL) at 50 ºC for 30 min in a shaking water bath. After centrifuging at 2,000×*g* for 10 min, supernatant was kept for glucose determination.

Digestible starch (DS) was determined in the supernatant after 16 h of incubation.

In order to determine free sugars (FS), flour sample (0.1 g) was suspended in 2 mL of 80% ethanol and was kept in a shaking water bath at 85 ºC for 5 min. Then, centrifuged for 10 min at 2,000×*g*. Supernatant was separated to measure FS released. This was performed twice.

The glucose content was measured using a glucose oxidase–peroxidase (GOPOD) kit (Megazyme, Dublin, Ireland). The absorbance was measured using an Epoch microplate reader (Biotek Instruments, Winooski, USA) at 510 nm. Starch was calculated as glucose (mg) ×0.9. Replicates (n= 4) were carried out for each determination.

Experimental data were fitted to a first-order equation (Eq.1) (Goni, et al., 1997):

$$C_t = C_\infty (1 - e^{-kt}) \qquad (1)$$

Where C_t is the concentration of product at time t, C_∞ is the concentration at the end point, and k is the pseudo-first order rate constant. The plot of $ln\ [(C_\infty - C_t)/ C_\infty] = -kt$ against t was used to estimate the slope that corresponded to $-k$.

Dough rheological characterization by Mixolab®

Wheat flour was poured into the Mixolab® bowl and mixed with the necessary amount of water for reaching optimum dough development (ICC, 2011). Constant consistency was used to compare the rheological behavior of all WWF samples obtained by jet milling. Wheat dough weight was fixed to 75 grams. Water absorption was referred to wheat flour at 14% (d.b.) moisture content. More information about Mixolab® parameters was reported by Rosell et al. (2007).

Breadmaking procedure

The bread dough formula consisted of 300 g flour, 4.5 g salt, 2.1 g dry yeast (Saf-instant, Lesaffre Group, France) and water. Water content was based on flour absorption obtained from Mixolab® results. Dough was mixed for 8 min and divided into 9 hand-rounded pieces (50 g) that were mechanically moulded. One of these was used for calculating dough volume (gassing power) as described by the AACC standard method (AACCI, 2012). The other pieces were proofed for 70 min at 30 °C in a fermentation cabinet (Salva Industrial S.A., Lezo, Guipuzcoa, Spain) and baked into an electric oven (Salva Industrial S.A., Lezo, Guipuzcoa, Spain) for 15 min at 180 °C. Loaves were cooled for 1 h at room temperature and were packaged into polyethylene pouches till further analysis. Two sets of breads were made for each jet-milled flour.

Bread quality parameters

Technological parameters of bread quality included: volume, specific volume (rapeseed displacement, AACCI, 2012), moisture content (AACCI, 2012), crumb color and crumb texture profile analysis (TPA). TPA was measured in a Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, UK) using bread slices of 1-cm thickness, which underwent two double compression tests up to 50% penetration of its original height at a crosshead speed of 1 mm/s and a 30 s gap between compressions, with a cylindrical stainless steel probe (diameter 25 mm). Color of bread crumb coloration was measured in four different slices by using a Minolta colorimeter (Chroma Meter CR-400/410, Konica Minolta, Japan) after standardization with a white calibration plate ($L^*= 96.9$, $a^*= 0.04$, $b^* = 1.84$). The color was recorded using CIE-$L^*a^*b^*$ uniform color space (CIE-Lab), where L^* indicates lightness, a^* indicates hue on a green (-) to red (+) axis, and b^* indicates hue on a blue (-) to yellow (+) axis. Total color difference (ΔE*) was calculated using the equation known as CIE76 formula (Eq. 2).

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \qquad (2)$$

Where L^*, a^*, b^* and L_0^*, a_0^*, b_0^* are the CIE-$L^*a^*b^*$ coordinates of jet milled breads (WF1, WF2, WF3 and WF4) and of control bread (WWF) respectively.

In vitro starch digestibility and estimated glycaemic index of bread

Two slices were dried for determining the *in vitro* digestibility. Enzymatic hydrolysis of bread was determined following the method reported by Gularte and Rosell (2011) using 100 mg of powdered freeze dried breads. The *in vitro* digestion kinetics was calculated in accordance with the procedure established by Goni et al. (1997) as has been described previously for flour. In addition, RS, DS and FS for breads were also determined as previously described for flour. Results were expressed as percentage as is basis.

A non-linear model following the equation Eq.1 was applied to describe the kinetics of starch hydrolysis. The hydrolysis index (*HI*) was obtained by dividing the area under the hydrolysis curve (0–180 min) of the sample by the area of a standard material (white bread) over the same period of time. The estimated glycaemic index (*eGI*) was calculated using the equation described by Granfeldt et al. (1992): $eGI = 8.198 + 0.862HI$, as previously reported Chung et al. (2008). Values are the average of 4 replicates.

Statistical analysis

Experimental data were statistically analyzed using Statgraphics V.7.1 program (Bitstream, Cambridge, MN) to determine significant differences among them. ANOVA test was applied in order to compare the mean values of studied properties at 95% level of confidence. A correlation analysis was also carried out to determine possible relationships among parameters.

Results and discussion

Particle size distribution and Microstructure of flour

Jet milling promoted a decrease in the flour particle size. Particle size distributions of control (WWF) and jet milled flours (WF1, WF2, WF3, WF4) are presented in Fig. 1. In opposition to jet milled samples, WWF's particle distribution presented one great peak at higher particle size value (d50 84.15 µm). When flours were subjected to jet milling, the particle size distribution changed. WF1 displayed a peak at similar particle than control, with a shoulder shifted at lower particle size, suggesting two different particles' populations coexisting. In WF3 samples the higher volume of particle size overlapped with the shoulder of WF1. Conversely, WF2 and WF4 exhibited lower particle size (Table 1) remaining only a small shoulder at higher particle size (Figure 1). The particle size distribution observed in the samples tested suggested that according to the intensity or severity of the milling treatment samples could be listed as WWF>WF1>WF3>WF2≥WF4. WWF was characterized by large heterogeneity in term of size and shape of the particles (Fig. 2A), as also was suggested by the large area of particle size distribution of the samples in Fig. 1. Particles of the aleurone layer and large aggregates of protein matrix embedding groups of cellular components, mainly starch granules, appeared (about 20-180μm). Some A-type starch granules (lenticular shaped) and smaller or B-type granules (spherical shaped) on the surface of the A-type granules can be seen as well. In jet milled flours (Fig. 2B, 2C and 2D) many starch granules were separated from the protein matrix. Similar results have been reported by Létang et al. (2002). As the intensity of milling conditions increased, the size of particles decreased gradually and more separated starch granules were observed. The scheme of particle also changed. The smallest particles seemed more spherical, whereas the largest presented a more polygonal scheme, as shown in wheat flour fractions after intense milling (Protonotariou et al. 2014). WF1 presented both large (about 80 μm) and small (about 20 μm) particles differing significantly from the other jet milled samples because of low air pressure (4 bar) during treatment. Particles of aleurone layer were also detected but were much smaller than those in WWF. WF2 micrographs resembled to WF4 and displayed a more even distribution, also depicted on Fig.1, indicating that although diverse treatment conditions were used they yielded comparable results. Those samples contained small particles (about 15-30 μm) with smooth faces and regular shapes. Landillon et al. (2008) also found that these particle sizes are associated to the presence of isolated starch granules. Data from particle size distribution are in accordance with microscopic observations.

Chemical composition of the flour

Jet milling affected the physicochemical properties of whole wheat flour (Table 2). Moisture content was significantly reduced as the intensity of process increased. Increased milling time, decreased feed rate and/or use of recirculation reduced the moisture content of the samples that were longer exposed to dried air at high flow. Moreover, as the particle size decreased, a higher surface area was available to interact. Again WF2 and WF4 presented similarities in the moisture content. Protein and ash content did not present any trend due to the intensity of treatment. With the gravimetric method used for fiber quantification, total fiber content increased significantly after jet milling, although intensity of the treatment did not show any effect. A hypothesis to explain that fiber increase could include possible interactions between protein and hemicellulose or crosslinking/oxidation among compounds during jet milling that increased the gravimetric determination of fibers.

In general, insoluble fibers content was reduced but the effect was not statistically significant, with the exception of WF4. Chau et al. (2007) have observed that micronization causes a redistribution of fiber components from insoluble to soluble fractions. Similar results had Zhu et al. (2010), who found that ultrafine grinding could effectively pulverize the wheat bran fiber particles to submicron scale; a redistribution of fiber components from insoluble to soluble fractions was observed as particle size decreased.

Enzymatic starch hydrolysis of jet milled whole wheat flour

Even though flours are not consumed directly but as ingredient in food matrices the enzymatic *in vitro* hydrolysis was carried out for WWF and jet milled WF, in order to determine differences in starch susceptibility to enzymatic hydrolysis due to milling. The hydrolysis curves are displayed in Fig. 3. It is evident that jet milled samples showed augmented rate of hydrolysis with differences on the hydrolysis constant (*k*) (Table 3). Low amount of free sugars with no significant difference among samples was obtained. A trend of increasing DS in contrast to RS decrease was noted. As the intensity of milling process increased the particle size of the granules decreased, as displayed in SEM micrographs, leading to higher surface area exposed to enzymatic hydrolysis. Moreover in milled fractions, starch is detached from protein and can be more easily hydrolysed. In fact, the highest amount of hydrolyzed starch at faster hydrolysis rate was presented in sample WF4. These findings agree with de la Hera et al. (2013a) who observed lower hydrolysis rate in the coarse rice flours.

Mixolab® analysis

Different Mixolab® curves were obtained for WWF, WF1, WF2, WF3 and WF4 (Fig 4). The curves at the initial mixing part of the process were rather similar, because the water addition was adjusted for obtaining the same dough consistency (1.1 Nm). However, during the stages of heating-cooling the curves presented significant differences. WF4 curve had significantly lower consistency values, whereas WWF presented the highest ones. The main parameters obtained from Mixolab® curves are collected in Table 4.

Micronization increased the hydration capacity of the flours due to their high specific surface area per unit weight. Thus, in order to reach all doughs the same consistency (1.1Nm), water absorption varied from 64.1 to 68% (WWF- WF4). Gil-Humanes et al. (2012) reported that it was required water adsorption of around 70% to obtain whole wheat doughs of 1.1 Nm consistencies.

The mean value of stability for all jet milled samples was lower when compared with WWF. Moreira et al. (2010) observed the lowest values of stability with the smallest particle size flours when studied the influence of the particle size on the rheological behavior of chestnut flour doughs at the same consistency. Amplitude related to dough elasticity did not show significant differences after treatment. Although protein content for all samples was similar (Table 2), C2, related to protein weakening, differed significantly among samples owing to protein dilution or the implication of other factors in the protein denaturation. Alpha implies protein weakening speed under the effect of heat and was significantly higher at WWF.

Jet milling process resulted in a significantly decrease of C3, related to starch gelatinization Nevertheless, it should be pointed out that as a consequence of the increased water absorption, a dilution effect was induced in the jet milled samples, leading to lower consistency after heating. Further reduction in viscosity (C4) is the result of the physical breakdown of the granules due to the mechanical shear stress and the temperature decrease. C4 differed significantly among all samples and decreased as the intensity of milling augmented, being the greatest effect observed in WF4. Cooking stability range, calculated as the difference between C3 and C4, remained unchanged. Cooling resulted in an increase of the torque, which is referred to setback and corresponds to the gelation process. This last stage is related to the retrogradation (Rosell et al., 2007). The final consistency was higher for WWF and decreased progressively with the severity of the jet milling treatment (WF1>WF3>WF2>WF4). Setback value was almost unaffected from milling process and varied from 0.33 to 0.35 Nm.

Aprodu et al. (2010) suggested that dough consistency was affected by ash content of the flour increasing C3, C4 and C5 torques. However, when jet milling was applied no relationship was found between Mixolab® parameters and the ash content of the flours, which was similar for all samples. Overall, different rheological dough behavior was mainly related to the competition for water of the fibers, proteins and starch, having as significant effect the increased surface area. Water absorption significantly increased with the jet milling and in consequence a dilution of the starch was induced in all the samples owing to the constant dough consistency.

Characteristics of produced bread

Gassing power decreased significantly in jet milled samples (Table 5). Dough with WWF presented the highest gassing power (138.3%) while WF4 and WF2 presented the lowest (94.9% and 93%, respectively). Highly hydrated doughs showed poor dough development characteristics and low gassing power during fermentation (Sanz Penella et al., 2008). The bran-particle size has also great impact, with fine particles having a greater adverse effect on gas retention than coarse ones (Stanley and Young, 2006). Small particles form a weak dough structure, which is probably unable to retain the gas released during fermentation, yielding lower volumes (de la Hera et al., 2013b). Thus, jet milling had as a result a decreased trend in specific volume values.

Despite high amount of water was added for breadmaking when using jet milled flours, the moisture content of the bread did not show significant differences with WWF, with the exception of WF2 and WF4. Likely, the lower particle size favors water released during baking.

Crumb hardness increased in breads obtained from jet milled flours and a steady increase was observed with the intensity of process. Bread hardness correlates with bread volume (Gómez et al. 2011), and thus the explanation of the differences in hardness might be related to differences in the specific volume ($r=-0.9698$, $P<0.05$).

Particle size of bran also affects crumb hardness. Higher hardness values were found in breads made with fine flours compared with coarse ones (Martinez et al. 2014). Bread slices presented close crumb structure characterized by small gas cells, which affected crumb color. Luminosity tended to decrease with a simultaneous increase in redness and yellowness. Influence of jet milled flour on bread color indicated by color difference (ΔE^*), which augmented as the intensity of milling increased. However, only WF1 bread, that had the mildest jet milling treatment, differed significantly from the other fine flours presenting the lowest difference from the control sample (3.34).

Starch digestibility in whole wheat breads

The parameters derived from the *in vitro* digestion of the whole wheat breads are presented in Table 6. In the present study, there was a small amount of RS (1.62- 1.96 mg/100 mg). Mean values augmented because of milling, but no statistical difference was detected among the samples. Generally, starchy foods, like bread, result in rapid degradation in the small intestine as almost all the starch is gelatinized (Parada and Aguilera, 2011).Thus the amount of RS is low. Mechanical and thermal treatments change the structure and digestibility of starch. Thermal treatments, such as the cooking process, completely destroy the semi crystalline structure of native starch granules and cause the loss of RS (Zhang et al., 2006). In agreement with that study, RS content for all samples was reduced after the breadmaking. DS (starch which is absorbed in the human small intestine) values ranged from 42.06 to 50.64 mg/100 mg, as is basis, but no significant difference between samples was detected. Low amount of free sugars were observed with significant differences only between WF2 and WWF bread.

The digestibility curves of the enzymatically treated bread are displayed in Fig. 5. Breads from jet milled flours displayed slower rate of hydrolysis than that from WWF but no trend was observed on the digestible constant (*k*) with the severity of the treatment (Table 6). The maximum hydrolysis (C_∞) was minimum for WF2 (49.4). Most of the wheat products are known to have high *eGI*. WWF breads showed lower *eGI* compared to white bread. Jet milling slightly tended to reduce *eGI* but not in a significant level. Fardet et al. (2006) proposed that it should be produced bread with a more compact food structure or higher density, which is the case in leavened whole wheat bread or bread with intact cereal grain in order to reduce *eGI.* Therefore, the structure of WF2 and WF4 breads, which presented the lowest specific volume and high hardness, could be the reason of the lower hydrolysis, in fact a significant correlation was found between *eGI* and specific volume ($r=0.9711$, $P<0.05$) and crumb hardness ($r=-0.9537$, $P<0.05$). Concerning flour properties, positive significant correlations were found between *eGI* and resistant starch content ($r=0.9784$, $P<0.05$) and negative with protein content ($r=-0.9713$, $P<0.05$) and digestible starch ($r=-0.8830$, $P<0.05$). Yamada et al. (2005) reported that RS affects *eGI*, and proposed the use of RS for lowering the *eGI* value of food products.

Differences observed on the starch enzymatic hydrolysis between flours and baked products suggested that protein-carbohydrate interactions during baking can influence quite differently the hydrolysis of starch.

Conclusions

Whole meal wheat flours with reduced particle size distribution were obtained modifying the severity of the jet milling treatment. The intensity of the process affected the properties of flour and bread. In some cases there was not a clear trend among intensity of process and properties. However, it was evident that WF1 (4 bar, 4.51 kg/h) was rather similar to WWF while samples WF2 (8 bar, 0.67 kg/h) and WF4 (8 bar, 2.54 kg/h), with the higher process intensity, showed significantly different features. The treatment mainly affected moisture content of the flours, which got drier due to both friction and pressure during milling, and there was a shift from insoluble to soluble fibers. The increase in the surface area resulting from particle size reduction increased the susceptibility of the starch granules to be enzymatically hydrolyzed. Regarding whole meal doughs, water absorption significantly increased and in parallel they lost mechanical stability. The resulting whole meal breads obtained from jet milled flours showed a compact structure, which seems to be responsible of the lower *eGI*. Therefore, jet milling is envisaged as a treatment for modifying flour functionality and for obtaining bread with reduced *eGI*. However, much research is necessary in order to optimize the physical properties of produced breads, as jet milled bread were harder with reduced specific volume.

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Figure Captions

Fig.1 Particle size distribution by volume of whole wheat flour of whole wheat flour WWF and jet milled whole wheat flours at different milling conditions WWF (▬), WF1 (▬), WF2 (▬), WF3 (▬) and WF4 (▬).

Fig.2 Scanning electron micrographs of whole wheat flour WWF (A), and jet milled whole wheat flours at different conditions WF1 (B), WF2 (C), WF3 (D) and WF4 (E). Magnification 200x. Particle size order WWF>WF1>WF3>WF2≥WF4.

Fig.3 Effect of different jet milling conditions in the enzymatic starch hydrolysis kinetics of whole wheat flour WWF (▲), WF1 (●), WF2 (×), WF3 (+) and WF4 (■). Particle size order WWF>WF1>WF3>WF2≥WF4.

Fig.4 Mixolab® curves of whole wheat flour WWF and jet milled whole wheat flours at different milling conditions WWF (▬), WF1 (▬), WF2 (▬), WF3 (▬) and WF4 (▬) with temperature (▬). Particle size order WWF>WF1>WF3>WF2≥WF4. Phase (1) - dough development; Phase (2) – weakening of the proteins; Phase (3) - starch gelatinization; Phase (4) – enzymatic activity, constant heating rate; Phase (5) - starch retrogradation. C1 (Nm) - maximum torque during mixing; C2 (Nm) - measures the protein weakening based on the mechanical work and temperature; C3 (Nm) ‑ expresses the starch gelatinization; C4 (Nm) ‑ indicates the stability of the starch gel formed; C5 (Nm) - measures the starch retrogradation during the cooling stage.

Fig.5 Effect of different jet milling conditions in the *in vitro* starch digestibility of whole wheat breads WWF (▲), WF1 (●), WF2 (×), WF3 (♦), WF4 (■).and white bread (+).Particle size order WWF>WF1>WF3>WF2≥WF4.

Tables

Table 1. Settings used for jet milling of whole wheat flour. Particle size (µm) of control (WWF) and jet milled flours (WF1, WF2, WF3, WF4).

Flour Abbreviation	**Air pressure (bar)**	**Feed Rate (kg/h)**	**Vibration Rate of Feeder (%)**	**Feed-back**	**d_{50} (µm)**	**d_{32} (µm)**	**d_{43} (µm)**
WWF	-	-	-	-	84.15 ± 2.45 a	49.23 ± 6.43 a	120.25 ± 2.52 a
WF1	4	4.51	100	No	53.49 ± 3.38 b	18.37 ± 0.17 b	90.62 ± 2.38 b
WF2	8	0.67	70	No	18.11 ± 1.73 c	7.23 ± 2.72 c	57.18 ± 1.11 c
WF3	8	5.18	100	No	29.10 ± 3.09 d	10.57 ± 0.77 d	70.04 ± 1.47 d
WF4	8	2.54	100	Yes	17.02 ± 1.38 c	6.94 ± 1.27 c	57.79 ± 0.53 c

Table 2. Chemical composition of whole wheat flour (WWF) and jet milled whole wheat flours at different milling conditions. Particle size order WWF>WF1>WF3>WF2≥WF4.

Flour Sample	**Moisture (%)**	**Protein (%, db)**	**Ash (%,db)**	**Insoluble Fiber (%, db)**	**Total Fiber (%, db)**
WWF	11.95 ± 0.00 d	15.00 ± 0.18 a	1.31 ± 0.00 a	9.23 ± 0.11 b	13.01 ± 0.53 a
WF1	8.57 ± 0.01 c	15.08 ± 0.32 ab	1.31 ± 0.01 a	8.39 ± 0.34 ab	14.25 ± 0.66 b
WF2	6.64 ± 0.08 a	15.51 ± 0.01 b	1.42 ± 0.00 b	8.89 ± 0.72 ab	14.72 ± 0.16 b
WF3	7.84 ± 0.05 b	15.22 ± 0.09 ab	1.33 ± 0.02 a	8.39 ± 0.06 ab	14.24 ± 0.04 b
WF4	6.61 ± 0.01 a	15.30 ± 0.02 ab	1.33 ± 0.00 a	7.82 ± 0.79 a	14.30 ± 0.10 b

Values followed by different letters in each column indicate significant differences ($P \leq 0.05$).

Table 3. Kinetic parameters of the starch hydrolysis of whole wheat flour (WWF) and jet milled whole wheat flours at different milling conditions. Particle size order WWF>WF1>WF3>WF2≥WF4.

Flour Sample	**Free sugars (mg/100mg, d.b.)**	**RS Starch hydrolysed (mg/100 mg, d.b.)**	**Digestible starch (mg/100 mg, d.b.)**	C_∞	k
WWF	0.24 ± 0.00 a	18.78 ± 0.16 d	33.80 ± 0.82 a	26.9 ± 3.5 a	0.011 ± 0.003 b
WF1	0.22 ± 0.02 a	18.91 ± 1.91 a	36.59 ± 1.43 ab	28.4 ± 4.3 a	0.009 ± 0.001 a
WF2	0.23 ± 0.03 a	13.00 ± 0.86 d	40.63 ± 1.08 cd	24.1 ± 2.2 a	0.021 ± 0.002 d
WF3	0.24 ± 0.02 a	16.53 ± 1.42 bc	39.39 ± 0.74 bc	27.5 ± 0.6 a	0.013 ± 0.000 bc
WF4	0.20 ± 0.04 a	14.61 ± 1.41 ab	43.23 ± 1.28 d	31.3 ± 3.0 a	0.018 ± 0.002 cd

Values followed by different letters in each column and each parameter indicate significant differences ($P \leq 0.05$).

C_∞, equilibrium concentration; k, kinetic constant.

Table 4. Mixolab® parameters for whole wheat flour WWF and jet milled whole wheat flours at different milling conditions WF1, WF2, WF3 and WF4. Particle size order WWF>WF1>WF3>WF2≥WF4.

	Description	WWF		WF1		WF2		WF3		WF4	
Absorption (%)	Amount of water required to obtain 1.10 Nm (C1)	64.1	a	65.1	b	66.6	c	66.9	d	68.0	e
Stability, min	Time during which the upper frame is > C1 – 11%	8.0	b	6.7	ab	4.9	ab	4.5	a	5.3	ab
Amplitude, Nm	Width of curve to C1, Dough elasticity	0.39	a	0.59	b	0.56	ab	0.90	b	0.51	ab
C2, Nm	Dough weakening minimum	0.42	d	0.39	c	0.37	b	0.37	b	0.35	a
alpha, (Nm/min)	Slope of the curve between the end of the period of 30 °C and C2; gives indications about the rate of the proteins' thermal weakening	-0.08	b	-1.03	a	-1.01	a	-1.01	a	-1.01	a
Initial pasting temp, ºC		63.4	a	64.6	ab	64.0	ab	64.7	b	66.1	c
C3, Nm	Dough at the peak of thermal pasting	1.87	d	1.80	c	1.74	b	1.74	b	1.68	a
C4, Nm	Dough viscosity at peak dough Temperature	1.36	e	1.33	d	1.28	c	1.26	b	1.17	a
C5, Nm	Dough viscosity increase at cooling	1.69	d	1.66	cd	1.63	bc	1.58	b	1.50	a
C3-C2, Nm	Starch gelatinization range,	1.47	d	1.42	c	1.37	b	1.38	b	1.33	a
C4-C3, Nm	Cooking stability range,	-0.51	a	-0.48	b	-0.46	b	-0.49	ab	-0.51	a
C5-C4, Nm	Gelling, Setback	0.34	a	0.34	a	0.35	a	0.33	a	0.34	a

Values followed by different letters in each raw indicate significant differences ($P \leq 0.05$).

Table 5. Physical properties of breads made from whole wheat flour (WWF) and jet milled whole wheat flours at different milling conditions. Particle size order WWF>WF1>WF3>WF2≥WF4.

Bread Sample	Gassing power (%)	Specific volume (cm^3/g)	Moisture (%)	Hardness (g)	*L**	*a**	*b**	*ΔE**
WWF	138.3 ± 5.2 c	2.50 ± 0.09 d	35.78 ± 0.49 b	707 ± 98 a	60.48 ± 0.43 b	4.86 ± 0.38 a	19.23 ± 0.12 a	0
WF1	113.0 ± 1.0 b	2.25 ± 0.04 c	34.48 ± 0.09 ab	1066 ± 0 ab	55.87 ± 3.15 a	5.25 ± 0.02 ab	20.45 ± 0.70 b	3.34 ± 0.25 a
WF2	94.9 ± 1.5 a	1.90 ± 0.15 a	33.80 ± 0.51 a	1678 ± 44 c	56.94 ± 0.00 ab	5.96 ± 0.13 c	22.26 ± 0.17 cd	5.11 ± 0.71 b
WF3	106.0 ± 2.8 b	2.15 ± 0.07 bc	34.65 ± 0.21 ab	1281 ±132 b	56.56 ± 0.06 ab	5.77 ± 0.08 bc	21.91 ± 0.44 c	5.07 ± 0.42 b
WF4	93.0 ± 7.0 a	1.98 ± 0.04 ab	33.49 ± 1.16 a	1808 ±296 c	56.06 ± 1.87 a	6.35 ± 0.36 c	22.91 ± 0.02 d	5.39 ± 0.58 b

Values followed by different letters in each column indicate significant differences ($P \leq 0.05$).

Table 6. Kinetic parameters of the *in vitro* starch hydrolysis, estimated glycaemic index and *in vitro* starch digestibility of bread made from whole wheat flour (WWF) and jet milled whole wheat flours at different milling conditions. Particle size order WWF>WF1>WF3>WF2≥WF4

Bread Sample	**WWF**	**WF1**	**WF2**	**WF3**	**WF4**
C_∞	52.2 ± 1.9 ab	56.2 ± 0.9 b	49.4 ± 0.5 a	52.0 ± 1.5 ab	51.9 ± 3.4 ab
k	0.027 ± 0.003 a	0.020 ± 0.004 a	0.025 ± 0.000 a	0.024 ± 0.000 a	0.023 ± 0.002 a
AUC	7411 ± 44 a	7326 ± 399 a	6930 ± 105 a	7213 ± 146 a	7067 ± 310 a
HI	90 ± 1 a	89 ± 5 a	84 ± 1 a	87 ± 2 a	86 ± 4 a
eGI	86 ± 0 a	85 ± 4 a	81 ± 1 a	84 ± 2 a	82 ± 3 a
Free sugars (mg/100mg, as is)	0.12 ± 0.00 a	0.15 ± 0.00 ab	0.16 ± 0.01 b	0.13 ± 0.01 ab	0.13 ± 0.02 ab
Resistant Starch (mg/100 mg, as is)	1.62 ± 0.53 a	1.61 ± 0.28 a	1.83 ± 0.12 a	1.96 ± 0.00 a	1.89 ± 0.05 a
Digestible starch (mg/100 mg, as is)	43.36 ± 1.23 a	42.06 ± 5.62 a	42.20 ± 0.10 a	48.44 ± 3.73 a	50.64 ± 3.20 a

Values followed by different letters in each row and each parameter indicate significant differences ($P \leq 0.05$).

C_∞, equilibrium concentration; *k,* kinetic constant; *HI*, hydrolysis index; AUC 180, area under curve; *eGI*, estimated glycaemic index.

Figure 1

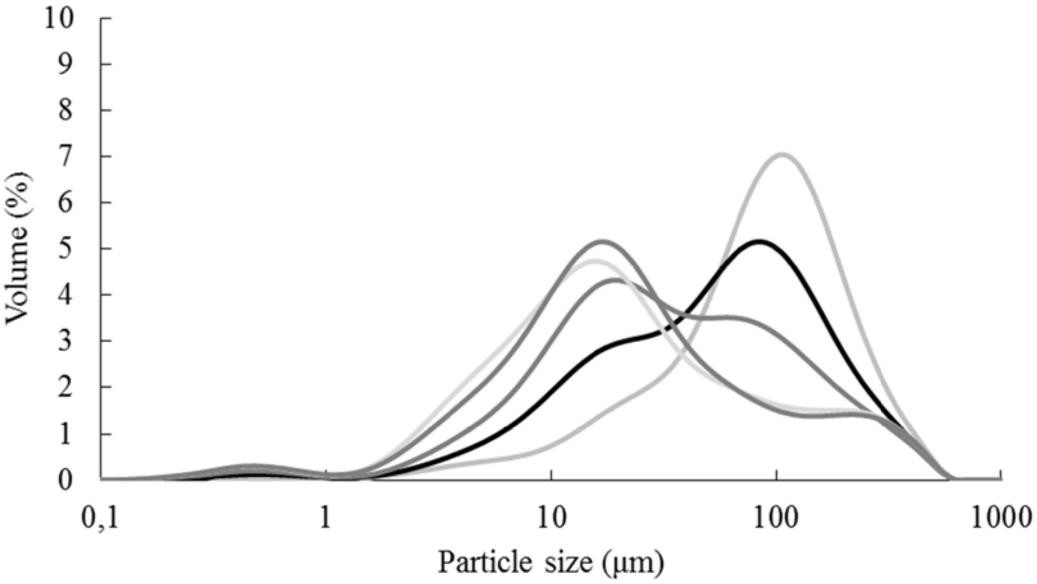
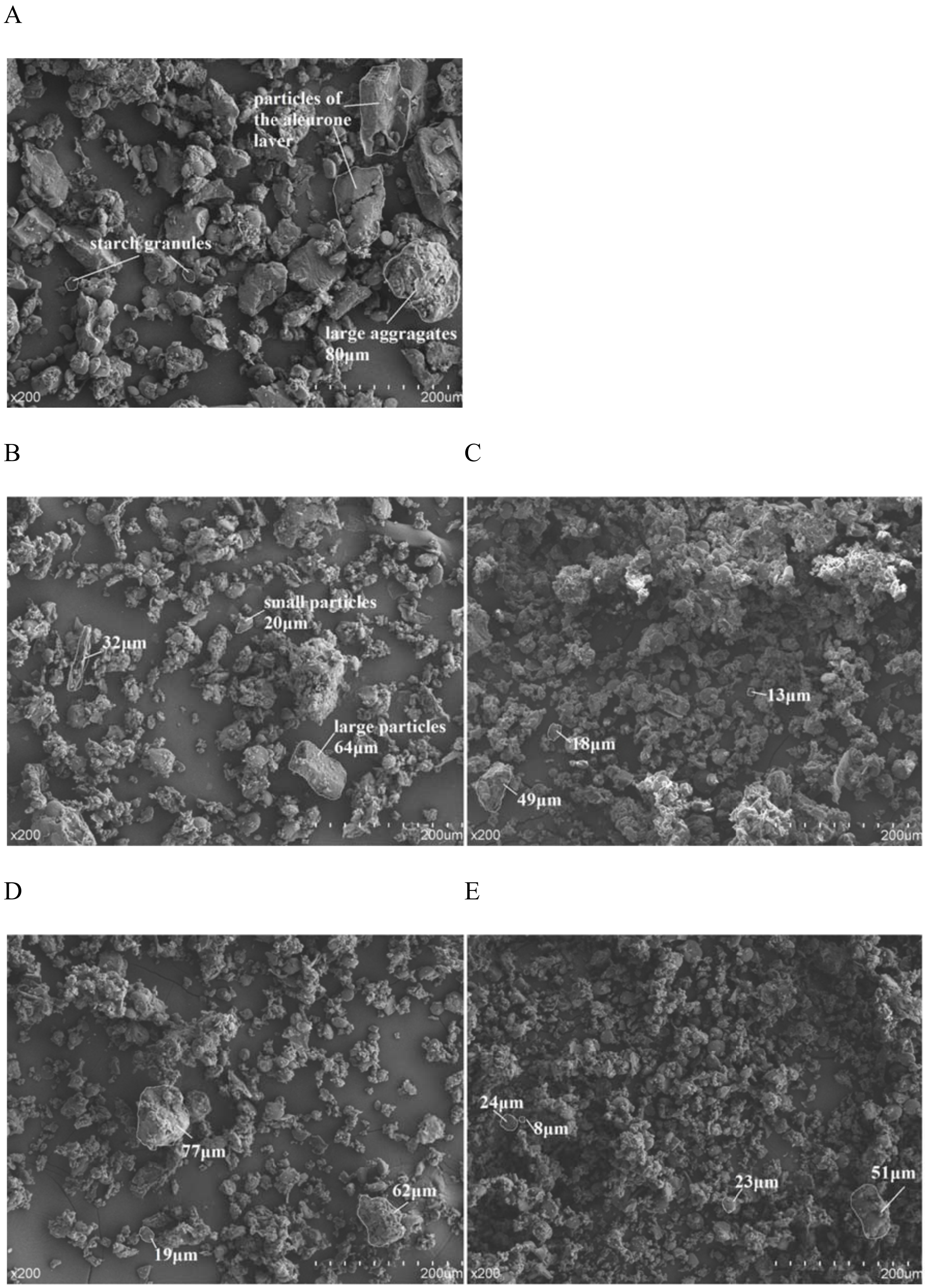


Figure 2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x200
200um
B
32μm
small particles 20μm
large particles 64μm
x200
200um
C
13μm
18μm
49μm
x200
200um
D
77μm
62μm
19μm
x200
200um
E
24μm
8μm
23μm
51μm
x200
200um

544 **Fig. 3**

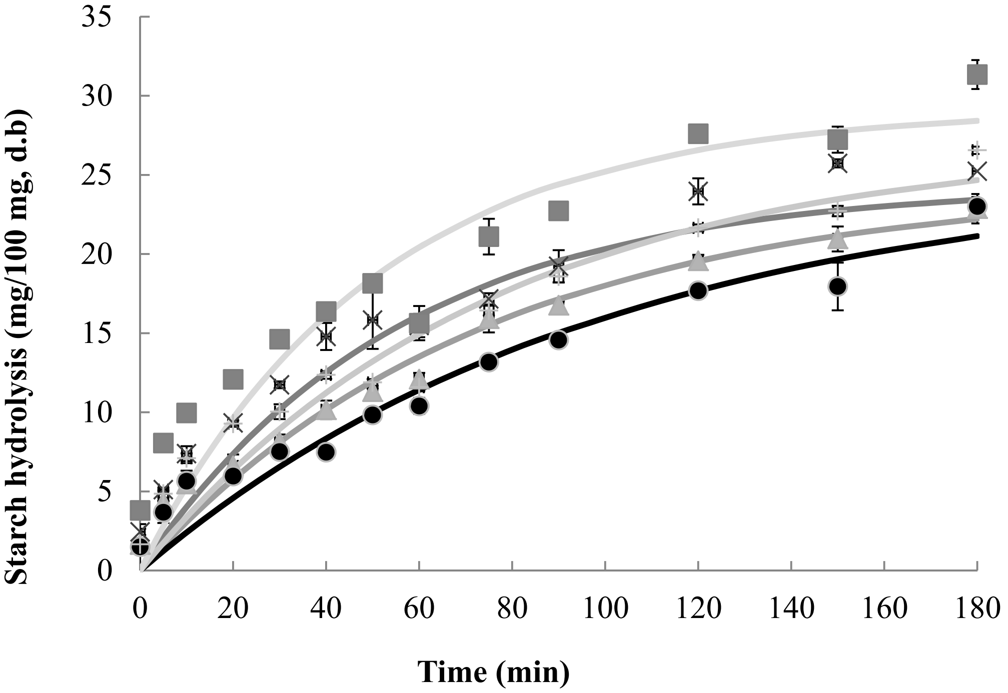

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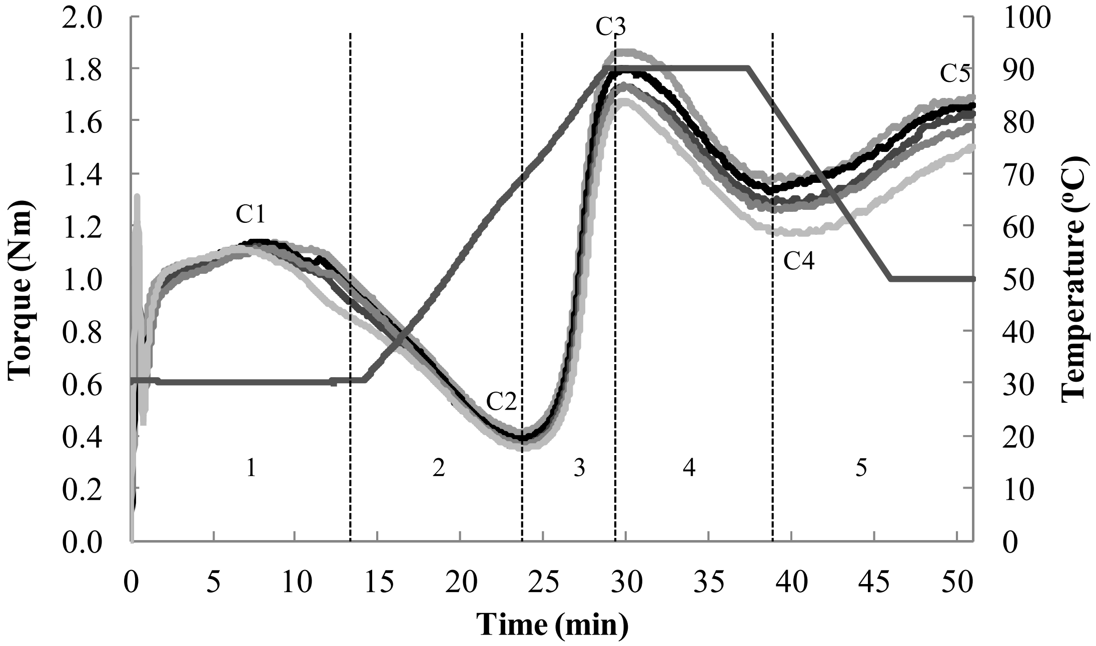
Fig.4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Temperature (ºC)
Time (min)
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1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
100
90
80
70
60
50
40
30
20
10
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10
15
20
25
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35
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45
50
C1
C2
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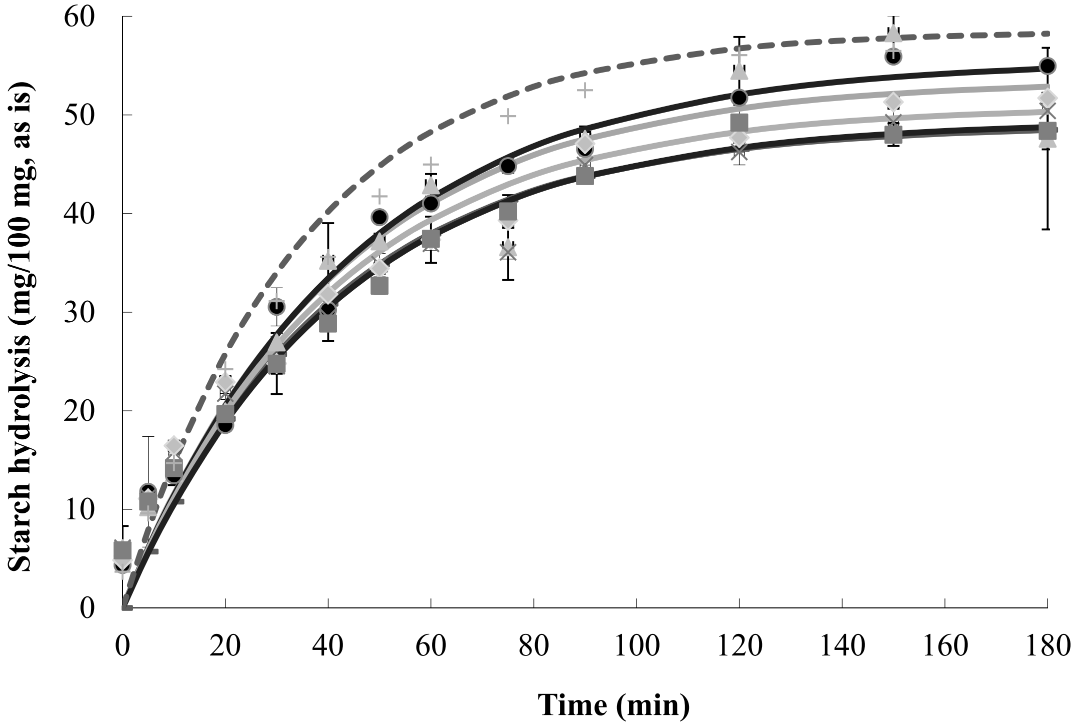
C4
C5
1
2
3
4
5

550 **Fig. 5**



Starch hydrolysis (mg/100 mg, as is)
0
10
20
30
40
50
60
0
20
40
60
80
100
120
140
160
180
Time (min)

551