Effect of different extrusion treatments and particle size distribution on the physico-chemical properties of rice flour

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Abstract

Rice flour is an interesting alternative for developing gluten free products, but its features do not always meet the process requirements. The objective of this study was to modify the functional properties of rice flour by combining extrusion and size fractionation. Different extrusion conditions (barrel temperature, feed moisture content and feed rate) were applied to vary the severity of the treatment on the flour constituents. Extrusion and mechanical fractionation of the rice flours modified their behavior affecting hydration, thermal and pasting features, besides their susceptibility to enzymatic hydrolysis. Specifically, onset and peak temperature increased and gelatinization enthalpy decreased when increasing barrel temperature of the extrusion. Fine flours with stronger extrusion (high temperature barrel) showed the highest susceptibility to enzymatic hydrolysis. Overall the combination of both physical treatments maybe an attractive alternative for obtaining clean label rice flours with modified features.

Keywords: extrusion, rice flour, particle size, thermal properties, hydration, enzymatic hydrolysis.

1. Introduction

Lately, there is an increasing interest for gluten free products that has prompted extensive research, which has been mainly focused on improving the quality of gluten free products. Nevertheless, the potential of flours from gluten free cereals has been scarcely exploited. Physical treatments have the benefit over the chemical ones of changing starch functionalities keeping the Green label (Jacobs & Delcour, 1998).

Rice flour functional properties are fully dependent on genotype and environmental conditions (Yeh, 2004) and besides that, postharvest treatments could be an alternative for modulating flour functional features. It is well known that rice grinding significantly affects rice flour properties, like water binding capacity and swelling power (Perdon et al., 2001). Recently, it has been shown that particle size fractionation of rice flour might be advisable for selecting specific physico-chemical properties like different hydration properties and enzymatic starch hydrolysis (de la Hera et al., 2013a); rheological properties (Moreira et al., 2013) or even oil barrier properties (Lee et al., 2013). Moreover, those fractionated flours showed different processing behaviour more suitable for bread or cake making depending on the particle size (de la Hera et al., 2013b,c).

Thermal treatments are highly attractive to modify the functional properties of the cereal flours. Extrusion cooking is considered high-temperature-short-time (HTST) during which flours are submitted to high temperatures and mechanical shearing at relatively low levels of moisture content (Camire et al., 1990). This treatment allows starch pregelatinization, denaturation of protein, enzyme (in)activation, and Maillard reactions, the extent of which are dependent on the severity of the extrusion. Those changes at the constituents' level modify the rheological behavior of flour (Hagenimana et al., 2006). During extrusion, the starch properties are

dependent on the temperature, initial moisture content and the screw speed (Wen et al., 1990). By arising the intensity of the treatment is possible to break down the amylopectin chains (Mercier & Feillet, 1975). In fact, Colonna et al. (1984) described that extruded wheat starches have amylose and amylopectin chains of lower molecular weight than the ones obtained by drum drying due to the shear effect, and that gave low thickening ability at low temperature (Doublier et al., 1986).

The extrusion also promotes important nutritional changes in the flours, like an increase in the soluble fiber content and a reduction in the lipid oxidation tendency, the content of antinutritional factors and the microbial population (Camire et al, 1990). Besides, it could increase the content of resistant starch in rice flours (Hagenimana et al, 2006), which is dependent on the treatment intensity (Alsaffar, 2011). Extrusion cooking is responsible for gelatinization and degradation of starch and also for changing the extent of molecular associations between components, e.g. the amylose– lipid complex that can affect the *in vitro* starch digestibility of the flours (Hagenimana et al, 2006).

Despite the impact of the extrusion on the molecular level, little attention has been paid to the variation of the functional properties of the flours by hydrothermal treatments (Clerici et al., 2009), even though physically modified flours are considered to be natural materials with high safety (Jacobs et al, 1998). In fact, Clerici et al. (2009) included 10% of extruded acid-modified rice flours for making gluten free breads. When using rice flours extruded in the presence of different amount of lactic acid, gluten free breads presented crust and crumb colour and texture values similar to those of wheat bread, although specific volume was rather low.

Considering the influence of the flour fractionation on the functional properties of the rice flours (de la Hera et al, 2013b), and the molecular changes induced by extrusion cooking, the

combination of both physical treatments could modify rice flour functional properties keeping the green label. The aim of this study was to modify the functional properties of rice flour by combining extrusion and size fractionation. With that purpose, different extrusion conditions were applied to vary the severity of the treatment on the flour constituents. The impact of processing on the flours was also followed by assessing the susceptibility of the flours to enzymatic hydrolysis.

2. Materials and methods

2.1 Materials

Rice flours were provided by Harinera Los Pisones (Zamora, Spain) that carried out the extrusion treatment in a single screw extruder Bühler Basf (Bühler S.A., Uzwil, Switzerland). Extrusion parameters were chosen according to the manufacturer advice in order to achieve flours with different properties. The length to diameter (L/D) ratio for the extruder was 20:1. Rice flour was subjected to different extrusion intensities (barrel temperature, moisture content of the mass feed and feed rate) yielding three types of extruded flours (1-3). Rice flour 1 and 2 were extruded at a maximum barrel temperature of 110°C with a feed rate of 700kg/h. For flours 1 and 2 feed moisture content and screw speed was 17% and 30%, and 453rpm and 397rpm, respectively. The diameter of the die hole and the number of holes used in those flours was 8mm and 18 holes, respectively. Rice flour 3 was extruded at a maximum barrel temperature of 140°C with a feed-rate of 500kg/h and feed moisture content of 25%. The screw speed was 340rpm, the diameter of the die hole was 6 mm and the number of holes was 9. The same rice flour (rice flour 0) without any treatment was used as a control.

Extruded product was dried by convection air and then ground with a compression roller till particle size was lower than 200 microns. Ground extrudates were sifted in a Bühler MLI 300B (Bühler AG, Uzwil, Switzerland) with screens of 132 and 200 microns to obtain fine (f) – lower than 132µm- and coarse (c) – 132µm-200 µm- extruded flours.

Flours were stored in air-tight plastic containers and held at 4°C until analysis.

Both the extruded and non-extruded flours were involved in the analytical measurements.

2.2 Methods

2.2.1. Flours characterization

Flours were analyzed following AACC method (AACC, 2012) for protein (AACC, 46-30.01) with a Leco TruSpec device (Leco, St. Joseph, MI, USA). The particle size distribution was measured using a particle size analyzer with laser diffraction Helos & Rodos (Sympatec, Clausthal-Zellerfeld, Germany) following AACC method (AACC, 55-40.01). Determinations were carried out in duplicate.

Free sugars

The glucose content was measured using a glucose oxidase-peroxidase (GOPOD) kit. The absorbance was measured using an Epoch microplate reader (BIOTEK EPOCH, Izasa, Barcelona, Spain) at 510 nm. In all cases four replicates were assayed for each experimental point.

Damage starch

The content of damaged starch was determined according to AACC 76-30A method (AACC, 2012). A fungal enzyme from *Aspergillus oryzae* (A6211, Sigma Chemical Co., St. Louis, MO, USA) was used in that analysis. Three determinations were made for each sample. Damaged starch was expressed as percentage of flour weight on dry basis.

127 Hydration properties

128 Hydration properties included swelling and water binding capacity (WBC) (Nelson, 2001).

129 Swelling volume or the volume occupied by a known weight of flour was evaluated by mixing

130 5g (± 0.1 mg) of flour with 100ml distilled water and allowing it to hydrate during 16h.

131 Water binding capacity defined as the amount of water retained by the flour after it has been

132 subjected to centrifugation was measured as described the method 56.30 (AACC, 2012).

133 Determinations were carried out in duplicate.

134 **Emulsifying properties**

135 Flour suspension (360 mL) of 0.5% (w/v) starch concentration was mixed with commercial

136 sunflower oil (Langosta, F. Faiges S.L, Daimiel, Ciudad Real, Spain) (36 mL). The content was

137 stirred for one min with a beater (Taurus Bapi 350 CP/CM, Taurus, Oliana, Lérida, Spain) to

138 disperse the sample in the oil. The suspensions were then centrifuged at 800xg for 10 min. The

139 emulsifying capacity (EC) was calculated as:

140 $EC=(ev/tv)*100$ (Eq. 2)

141 where ev is the emulsion volume and tv is total volume.

142 Emulsion stability (ES) against high temperatures, were determined in the emulsions that were

143 heated in a water bath at 80°C for 30 min, and centrifuged at 800xg for 10 min. ES was

144 calculated as:

$$ES = (f_{ev}/i_{ev}) * 100 \quad (\text{Eq. 3})$$

146

147 where f_{ev} is the final emulsion volume and i_{ev} is initial emulsion volume. Determinations were

148 carried out in duplicate.

149 Foaming properties

Aliquots (150mL) of 4% w/v suspension were whipped at moderate speed for one min using a beater (Taurus Bapi 350 CP/CM, Taurus, Oliana, Lérida, Spain). Foam volumes were recorded after 30 s. The foam capacity (FC) was calculated as follows:

$$FC=(ifv/tsv)*100 \quad (\text{Eq. 4})$$

where *ifv* is the initial foam volume and *tsv* is the total suspension volume.

The foam stability (FS) was calculated as the foam volume after 20 min.

$$FS=(ffv/tsv)*100 \quad (\text{Eq. 5})$$

where *ffv* is the foam volume after 20 min and *tsv* is total suspension volume. Results were the average of two determinations.

Pasting characteristics

Pasting properties of flours were analyzed using the standard method (AACC, 2012), (AACC, 61-02.01) with a Rapid Visco Analyser (RVA-4) (Newport Scientific Pty Ltd., Warriewood, Australia) controlled by Thermocline software (Newport Scientific Pty. Limited, Warriewood, Australia) for Windows.

Thermal properties

Analyses were performed in a differential scanning calorimeter DSC-7 (Perkin–Elmer, Waltham, MA, USA), using aluminum pans (PE 0219-0062). The equipment was calibrated with Indium and an empty pan was used as a reference. Flour (3 mg) was loaded into the aluminum pan and distilled water (10μL) was added with the help of a Hamilton micro syringe. Samples were hermetically sealed and allowed to stand for 1 h at room temperature before heating in the DSC. The calorimeter scan conditions were set as follows: samples were kept at 30°C for 2 min, heated from 30 to 110°C at 5°C/min. Onset temperature (T_o), peak temperature (T_p), gelatinization temperature range ($T_p - T_o$), peak height index ($\Delta H_g / T_p - T_o$) as well as the

173 enthalpy of starch gelatinization (ΔH_g) (expressed as mJ/mg of sample) were determined. All
174 samples were run in quadruplicate.

175 **Colour of flours**

176 Colour was measured using a Minolta CN-508i spectrophotometer (Minolta, Co.LTD, Tokyo,
177 Japan) with the D65 standard illuminant and the 2° standard observer. Results were expressed in
178 the CIEL a^*b^* colour space. Colour determinations were made 5×2 times on each sample of
179 flour.

180 **Enzymatic hydrolysis of starch**

181 Starch hydrolysis was measured following the method described by Gularte and Rosell (2011)
182 with minor modifications. Briefly, for free sugars removal, flour sample (100 mg) suspended in
183 two milliliters of 80% ethanol was kept in a shaking water bath at 85°C for five minutes, and
184 then centrifuged for 10 min at 1000×g. The pellet was incubated with porcine pancreatic α -
185 amylase (10 mg/ml) (Type VI-B, ≥ 10 units/mg solid, Sigma Chemical, St. Louis, MO, USA)
186 and amyloglucosidase (3300 U/ml) (Sigma Chemical, St. Louis, MO, USA) in 10 ml of 0.1M
187 sodium maleate buffer (pH 6.0) in a shaking water bath at 37 °C (0.25–16 h). Aliquots of 200 μ l
188 were withdrawn during the incubation period. Aliquots were mixed with 200 μ l of ethanol
189 (96%) to stop the enzymatic reaction and the sample was centrifuged for 5 min at 10000×g and
190 4 °C. The precipitate was washed twice with 50% ethanol (100 μ l) and the supernatants were
191 pooled together and kept at 4 °C for further glucose determination.

192 The remnant starch after 16 h hydrolysis was solubilized with 2ml of 2M KOH using a Polytron
193 ultraturrax homogenizer IKA-T18 (IKA works, Wilmington, NC, USA) during 1min at speed 3.
194 The homogenate was diluted with 8ml 1.2M sodium acetate pH 3.8 and incubated with 100 μ l

amyloglucosidase (3300 U) at 50 °C for 30 min in a shaking water bath. After centrifuging at 2000×g for 10 min, supernatant was kept for glucose determination.

The glucose content was measured using a glucose oxidase-peroxidase (GOPOD) kit. The absorbance was measured using an Epoch microplate reader (Biotek Instruments, Winooski, VT, USA) at 510 nm. Starch was calculated as glucose (mg)×0.9. Replicates (n=2-4) were carried out for each determination.

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

$$C_t = C_{\infty} (1 - e^{-kt}) \quad \text{Eq. 5}$$

Where C_t is the concentration of glucose released at time t , C_{∞} is the concentration at the end point, and k is the pseudo-first order rate constant. Although this equation requires the estimation of an accurate C_{∞} , it was useful because long reaction times were applied to determine resistant starch after complete enzymatic hydrolysis. The plot of $\ln [(C_{\infty} - C_t) / C_{\infty}] = -kt$ against t was used to estimate the slope that corresponded to $-k$.

However, as recently suggested Butterworth et al. (2012), the linear plot of $\ln (dC/dt)$ against t was also represented to calculate the slope ($-k$), and the intercept on the y axis was used for calculating the $\ln (k C_{\infty})$. This plot was used to demonstrate if the data were of logarithmic form and the rate constant remained unchanged along the whole hydrolysis reaction, as recommended Poulsen et al. (2003).

2.2.2. Statistical analysis

Multiple analyses of variance were used to determine the individual effects of thermal treatment and particle size of flours. Fisher's least significant differences test was used to calculate the means with their 95% confidence intervals. Several correlations were also run. The statistical

analysis was performed with the Statgraphics Plus Centurion XVI software (Statpoint Technologies, Inc., Warrenton, VA, USA).

3. Results and Discussion

Rice flour was subjected to different extrusion treatments that differed on the maximum barrel temperature and feed moisture content in order to obtain different extrusion intensities. de la Hera et al. (2013b,c) reported the functional properties and processing behavior of fractionated flours. Size fractionation allowed obtaining flours more suitable for bread or cake making depending on the particle size. In this study, extruded flours were ground and then separated in two fractions coarse extruded flour (132 μ m-200 μ m) and fine extruded flour (<132 μ m). Overall eight samples were obtained from each batch, which differed on the level of extrusion (identified as 0-3, higher values are related to higher intensity of extrusion conditions) and the particle size (coarse, fine). Flour 0 was not subjected to any extrusion treatment and was used as a reference.

3.1 Damage starch and free sugars

To get a complete picture of the effect of extrusion and particle size a multiple analysis of variance was applied to the experimental results (Table 1). The extrusion intensity (barrel temperature, moisture content) and particle size (fine and coarse) had a significant effect on the content of free sugars (Table 1), which increased with the extrusion temperature and moisture content and with the reduction of the particle size (18.74% in coarse flour vs 24.21% in fine flour). In fact, flour 3 (treated at higher barrel temperature) showed 49.91 % free sugars whereas no treated flour (or flour 0) had a sugar content of 6.92%. Nevertheless, no significant differences were observed between the free sugars content of the control (flour 0) and the mild

extrusion treatment (flour 1). Thus the hydrolysis responsible of the sugar release required a minimum barrel temperature and also sufficient feed moisture content, since flours 1 and 2 were extruded at the same temperature and with different moisture feeding. Extrusion induced a progressive increase of the damage starch content with the temperature raise in the extrusion treatment, likely due to damage produced by the shears force and the heat during extrusion (Camire et al, 1990). Conversely, damage starch decreased with the particle size, showing coarse flours the greatest amount of damage starch (19.98%), which agrees with the trend observed by de la Hera et al. (2013a), when studying the features of different particle size fractions of rice flours.

3.2 Hydration, emulsifying and foaming properties

Hydration, emulsifying and foaming properties were significantly affected by the extrusion process (0-3) and the particle size of the flours (coarse and fine) (Table 1). Hydrations properties (WBC and swelling) increased with the extrusion temperature and moisture content and also with the particle size of the flour. WBC increased from 1.26 g water /g solid (flour 0) to 4.94 g water /g solid when extrusion was carried out at the maximum temperature (flour 3). Those effects were partially attributed to the increase in the amount of damage starch since it was found a positive correlation between the amount of damage starch and WBC ($r=0.88$) and with the swelling ($r=0.88$). Moreover, the cooking produced during extrusion led to gelatinized starch that would have higher WBC and swelling, as occurred with the water absorption index (Hagenimana et al, 2006). Camire et al. (1990) proposed that the breakage of the starch granule integrity led to a poorly ordered molecular phase with hydroxyl groups prone to bind water molecules.

The extrusion significantly reduced the emulsifying capacity (EC) of the flours, with the exception of flour 3 (86.34), compared to the control flour (flour 0) (87.58), being the values for flour 1 and 2, 84.92 and 85.70, respectively. And an increase of the EC was observed with the severity of the extrusion, namely flour 3 (86.34) > flour 2 (85.70) > flour 1 (84.92). That effect must result from the protein and starch changes during extrusion process. Considering the proteins, extrusion forces the unfolding and aggregation due to protein crosslinking involving SH/SS interchange, oxidation and hydrophobic interactions (Rosell & Foegeding, 2007), which might result in a decrease of the EC of the flour. As the extrusion temperature increase, starch modification might partially mask the protein denaturation. Gelatinized starch has greater number of hydroxyl groups, which would be available to form hydrogen bonds with the proteins leading to better emulsion capacity.

Emulsion stabilities (ES) were higher in the flours obtained from lower extrusion temperature (flour 1 and 2), showing values of 113.20 and 113.23, respectively. Likely the denaturation of the rice proteins during extrusion increased the stability. The particle size (coarse or fine) of the rice flours did not affect significantly the EC, but the ES significantly increased with the particle size reduction, from 110.80 in coarse flours to 113.61 in fine flours. A reduction in the particle size of the flour improves the emulsifying properties of the flours (Aluko et al., 2009). In the present study, it was observed a significant positive relationship between the ES and the free sugars content ($r=0.93$, $P<0.001$), which could reduce the total charge of the proteins leading the formation of interfacial protein membranes that stabilize the emulsion (Aluko et al, 2009).

Extrusion improved the foaming capacity (FC) of the rice flours (Table 1), but there was no trend with the extrusion severity (temperature or moisture content). The foam stability (FS) could be only measured in flour 3 (50.00 for fine flour and 45.18 for coarse flour), because very

unstable foams were obtained with the other flours. The FC has been attributed to its microstructure, size and distribution of the gas cells and the interfacial properties (Zhang et al., 2011). Hydrothermal treatments can improve the foaming properties, like it has been reported with corn kernels (Boladea et al., 2002). Nevertheless, the minor effect observed in the extruded rice flours could be attributed to the low protein content of the rice flour. Protein isolates show great foaming capacity that improves with the hydrothermal treatments (Wang & Johnson, 2001). The effect of extrusion on foam formation followed an opposite trend to the one observed on the emulsion formation, which suggests that different mechanisms are involved during interfacial membrane formation at the air-water and oil-water interfaces. Concerning the particle size, the major FC was observed in fine flours (26.77) compared to coarse flours (22.92). This effect could be explained by the greater availability of lowering interfacial components in those flour fractions, as proposed Aluko et al. (2009).

3.3. Colour

Luminosity (L^*) of the flours significantly decreased with the extrusion temperature and moisture content (Table 1), from 90.55 in flour 0 to 88.62 in flour 3. Conversely, the a^* and b^* increased. Nevertheless, whereas a^* steadily increased with the extrusion temperature and moisture content (flours 0 to 3) was observed in the case of the luminosity and b^* , no trend was observed in the case of a^* . The extrusion process could lead to Maillard reactions and a reduction of the lipids oxidation due to enzymes inactivation that induces the formation of melanoidins and the pigments protection, which in turn produces the modification of the flours color (Camire et al, 1990). Moreover, higher L^* and lower a^* y b^* were obtained in the fine flours. It has been

proposed that fine flours had higher surface area that favors the contact of the constituents with the oxygen promoting the pigments oxidation (Atwell, 2001).

3.4. Pasting characteristics

Pasting plots of the extruded flours are displayed in Figure 1a (coarse flour) and in Figure 1b (fine flour). Concerning the extrusion treatment (flours 0 to 3), the viscosity during heating and cooling decreased with the extrusion intensity, obtaining minimum viscosity in flour 3 (with the highest intensity). Nevertheless, when particle size was taken into account, a progressive decrease of viscosity was observed in the coarse flours (Figure 1a), but fine flours (Figure 1 b) from treatment 1 and 2 did not show any difference in the pasting profile. Since flour 1 and 2 were treated at maximum barrel temperature of 110°C and the unique variation was the feed moisture content, it seems that the effect on pasting profiles was independent on the feed moisture content during extrusion when small particle size were subjected to this treatment.

Peak viscosity was significantly dependent on the damage starch content ($r=-0.79$), which agree with previous studies connecting peak viscosity with gelatinized and damage starch that was related to the polymerization degree of the starch granules (Barres et al., 1990). The reduction observed in the final viscosity and setback was displaying the extension of the effect on the amylose chains, which might lose the ability to retrograde during cooling due to their fragmentation during extrusion. This effect agrees with previous results of Doublier et al. (1986).

3.5. Differential Scanning Calorimetry (DSC)

The effect of extrusion treatment and particle size on the thermal properties of the starch is shown in Table 2. In the range of temperature tested (from 30 to 110°C), flours exhibited one endothermic peak corresponding to amylopectin gelatinization, with the exception of flour 3 (no peak detected). The absence of an endothermic peak for flours 3 indicated total gelatinization of amylopectin. Indeed, these results agree with those previously discussed regarding the very small pasting curve observed in the extruded flours 3 at 50°C. The extrusion treatment significantly modified the gelatinization temperatures (T_o , T_p , T_c) of the flours, and those temperatures were also dependent on the particle size of the flours (coarse and fine flours). Gelatinization temperatures (T_p) were sifted to higher values when flours were treated at increasing extrusion intensity (3 was the highest intensity), but the temperature range (T_p-T_o) was not affected. Higher gelatinization temperature indicated that more energy is required to initiate gelatinization of the starch suggesting that extrusion is affecting the outer and more amorphous part of the granule and is progressing to the core of the granule till no crystalline structure is left for gelatinization (flour 3).

When comparing extruded flours, the gelatinization enthalpy (ΔH) was significantly reduced in flour 2, where extrusion temperature and moisture content was sufficient to induce starch gelatinization, likely due to an increase of the damage starch content (Chiu & Solarek, 2009). Nevertheless, the mild extrusion treatment (flour 1) gave significantly higher gelatinization enthalpy (3.075 J/g) compared to the untreated flour (flour 0) (2.525 J/g). Taking into account that treatment 1 was applied using lower feed moisture content (insufficient to complete gelatinization), the higher enthalpy of this sample could be attributed to a reorientation of the structure of the amorphous region to resemble that of the crystalline region (Camire et al, 1990). The extrusion process modifies the crystalline structure of the starch granule affecting the

temperature at which swelling starts (Camire et al, 1990). Fine flours showed lower gelatinization temperatures than the corresponding coarse flours, but without affecting the peak high index and the gelatinization enthalpy (Table 2).

3.6. Starch hydrolysis

The susceptibility of the extruded flours to the enzymatic hydrolysis was analyzed. Figure 2 shows the kinetic plots of the extruded flours and the effect of the particle size. The enzymatic hydrolysis profiles were dependent on the particle size, and fine flours showed faster hydrolysis and reached higher asymptotic values than coarse flours. de la Hera et al. (2013a) observed lower hydrolysis rate in the coarse flours when studied the effect of particle size distribution on the rice flour functionality. This result could be attributed to the high surface area of the fine flours that increases the water diffusion and enzyme accessibility. The hydrolysis curves were fitted to a first order kinetics according to Goñi et al. (1997) and also to Butterworth et al. (2012) to obtain the kinetic parameters (Table 3). As it was observed in the plots, there was an increase in the equilibrium concentration (C_{∞}) parallel to the extrusion intensity (except flour 2f). Regarding the rate of the hydrolysis, k , there was no general trend with the extrusion intensity. There was great agreement with the equilibrium concentration estimated from both fitted methods, indicating that the kinetic parameters can be fitted to a logarithmic function and that the rate constant did not vary along the hydrolysis reaction (Poulsen et al., 2003).

Resistant starch was also quantified to determine the potential impact of the extrusion on the structural level of starch. Although there was no clear tendency about the resistant starch content, the highest extrusion intensity gave the flours with the lower level of RS (flour 3). This finding disagrees with previous observations of Hagenimana et al. (2006), who found an

increase in RS content with the treatment severity. Those authors attributed the increase in RS to the formation of amylose-lipid complexes during the extrusion, which retarded the enzymatic digestion. Therefore, results divergence might be explained because of the lower content of amylose of the flours in the present study compared with the reported ones. In addition Chinnaswamy & Hannah (1990) reported a change in the percentage of amylose/amylopectin ratio in extruded corn flours that was ascribed to both chains fragmentation, being more intense in the former. That fact could affect the starch hydrolysis rate. Moreover, Hagenimana et al. (2006) stated that the susceptibility of the extruded starches to be enzymatically hydrolyzed was directly related to the intensity of the extrusion treatment.

The particle size did not significantly affect the hydrolysis rate, but fine flours showed higher values of C_{∞} and lower amount in RS. Al-Rabadi et al. (2011) stated that fine extruded flours of barley and sorghum had major digestibility than the coarser ones. The most compact structure, besides the smaller surface area of coarser flours, could hinder the accessibility of the enzymes within the starch structure, since diffusion of the enzyme is the first stage in the enzymatic hydrolysis (Al-Rabadi et al. 2011; Ghaid et al., 2009).

4. Conclusion

Extrusion and mechanical fractionation of the rice flours modified their behavior affecting hydration, thermal and pasting features, besides their susceptibility to enzymatic hydrolysis. The severity of the extrusion treatment was accompanied by an increase in the amount of damage starch and free sugars content, the former contributing to the Maillard reaction, which affected the luminosity of the flours. In parallel, hydration ability increased with the extrusion intensity, leading higher viscosity in cold solution, which might be very interesting for some food

applications. Thermal properties (temperature and enthalpy) increased with the intensity of the extrusion and that effect was intensified with the greatest particle size of the flours. Fine flours with stronger extrusion showed the highest susceptibility to enzymatic hydrolysis and extrusion process increased that effect. Therefore, extrusion and fractionation can be an alternative to produce flours with different functional properties, which might be useful in gluten free breadmaking. Future studies will be undertaken to test these flours in breadmaking process.

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Table 1: Significant individual effects of extrusion treatment (1-3) and particle size (coarse, fine) on free sugars, damaged starch, emulsifying, foaming and colorimetric properties of rice flours.

	Overall Mean	Extrusion treatment				Particle size	
		0	1	2	3	c	f
Free Sugars (%)	21.47	6.92a(2.08)***	9.49a(0.88)	19.58b(0.64)	49.91c(10.52)	18.74a(15.08)**	24.21b(21.73)**
Damaged Starch (%)	18.08	6.66a(1.69)***	9.85b(1.38)	23.33c(5.95)	32.49d(4.66)	19.98b(13.71)**	16.18a(8.83)
WBC (g water/g solid)	2.42	1.26a(0.05)***	1.35b(0.06)	2.13c(0.25)	4.94d(0.05)	2.46b(1.60)*	2.39a(1.59)
Swelling (mL/g)	3.50	0.95a(0.25)***	1.55b(0.19)	2.90c(1.10)	8.60d(0.43)	3.85b(3.30)**	3.15a(3.22)
EC	86.21	87.58c(1.25)**	84.92a(0.53)	85.70ab(0.39)	86.34bc(1.35)	86.60a(1.71)	85.82a(0.80)
ES	112.21	111.49a(2.47)***	113.20b(0.55)	113.23b(0.72)	110.90a(2.93)	110.80a(2.12)***	113.61b(0.27)
FC	24.85	17.94a(1.17)***	29.97c(7.15)	21.44b(1.86)	30.04c(1.51)	22.92a(5.47)***	26.77b(7.12)
<i>L</i> *	91.0	94.36c(0.42)***	90.55b(2.01)	90.43b(1.42)	88.62a(2.56)	89.66a(3.05)***	92.31b(1.46)
<i>a</i> *	0.19	-0.17a(0.21)***	0.23c(0.19)	0.16b(0.04)	0.55d(0.14)	0.23b(0.40)***	0.15a(0.17)
<i>b</i> *	9.63	6.82a(0.46)***	10.22b(1.68)	10.37b(1.32)	11.13c(2.14)	10.83b(2.35)***	8.44a(1.23)

Particle size: coarse (c), fine (f). Extrusion treatment: control (0); barrel temperature 110°C, feed rate 700kg/h, moisture content 17% (1); barrel temperature 110°C, feed rate 700kg/h, moisture content 30% (2); barrel temperature 140°C, feed rate 500/h, moisture content 25% (3)

Values followed by different letters within each parameter for each factor (thermal treatment and particle size) indicate significant differences. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Average values followed by the standard deviation (in parenthesis).

WBC, water binding capacity; EC, emulsifying capacity; ES, emulsion stability; FC, foaming capacity.

502 **Table 2.** Significant individual effects (extrusion treatment and particle size) on thermal properties.

		Extrusion treatment				Particle size	
	Overall						
	Mean	0	1	2	3	c	f
T _o (°C)	68.0	65.5a(5.7)***	67.4b(1.7)	71.1c(0.9)	n.d	70.3b(1.4)***	65.7a(4.5)
T _p (°C)	74.0	71.2a(4.6)***	74.4b(0.7)	76.4c(0.7)	n.d	75.6b(1.0)***	72.4a(4.1)
T _c (°C)	80.6	77.9a(3.9)**	81.4b(1.5)	82.5b(1.18)	n.d	81.8b(1.0)**	79.3a(4.0)
T _p -T _o (°C)	6.0	5.8a(1.1)	7.0a(1.8)	5.3a(0.2)	n.d	5.3a(0.6)	6.7a(1.6)
ΔH (J/g)	2.383	2.525b(0.330)***	3.075c(0.754)	1.550a(0.191)	n.d	2.483a(1.049)	2.283a(0.507)
PHI (J/g*°C)	0.392	0.450b(0.057)*	0.450b(0.191)	0.275a(0.050)	n.d	0.433a(0.163)	0.350a(0.104)

503

504 Particle size: coarse (c), fine (f). Extrusion treatment: control (0); barrel temperature 110°C, feed rate 700kg/h, moisture content 17%
505 (1); barrel temperature 110°C, feed rate 700kg/h, moisture content 30% (2); barrel temperature 140°C, feed rate 500/h, moisture
506 content 25% (3).

507 Values followed by different letters within each parameter for each factor (thermal treatment and particle size) indicate significant
508 differences. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

509 n.d.: Not detected.

510 Average values followed by the standard deviation (in parenthesis).

511 T_o , gelatinization onset; T_p , peak temperature; T_c , conclusion temperature, T_p-T_o , gelatinization range, ΔH , enthalpy and PHI, peak
512 high index.

Table 3. Kinetic parameters extracted from first-order and LOS plots of different flours.

	k (min ⁻¹) by first order eq.	k (min ⁻¹) by LOS	C_{∞} (%)	C_{∞} (%) by LOS	Resistant starch (%)
0f	0.043(0.001)	0.044(0.001)	137.48(5.14)	150.63(5.10)	5.52(0.50)
0c	0.053(0.006)	0.051(0.005)	124.39(3.61)	130.72(3.55)	5.91(0.86)
1f	0.074(0.009)	0.071(0.007)	358.42(12.39)	388.85(13.45)	3.29(0.38)
2f	0.061(0.011)	0.059(0.010)	338.79(13.08)	364.30(14.08)	4.01(0.74)
3f	0.145(0.015)	0.143(0.014)	387.35(11.58)	727.46(12.10)	2.23(0.36)
1c	0.054(0.001)	0.053(0.001)	134.98(5.02)	143.25(4.74)	6.10(1.32)
2c	0.076(0.006)	0.073(0.008)	143.38(4.86)	156.65(4.20)	5.75(0.98)
3c	0.061(0.004)	0.059(0.006)	230.75(7.25)	243.55(7.56)	2.11(0.51)

k , kinetic constant; C_{∞} , equilibrium concentration

Numbers in sample codes are referred to extrusion intensity and letters are associated to coarse (c) or fine (f) flour.

Average values followed by the standard deviation (in parenthesis).

522 **FIGURE CAPTIONS**

523 **Figure 1a.** Effect of extrusion treatment on the pasting properties of coarse rice flours. Flour 0
524 (black line), flour 1 (clear grey line), flour 2 (intermediate tone grey line), flour 3 (dark grey
525 line). Temperature profile (discontinuous line).

526 **Figure 1b.** Effect of extrusion treatment on the pasting properties of fine rice flours. Flour 0
527 (black line), flour 1 (clear grey line), flour 2 (intermediate tone grey line), flour 3 (dark grey
528 line). Temperature profile (discontinuous line).

529 **Figure 2.** Effect of extrusion treatment on the enzymatic hydrolysis of rice flours with different
530 particle size. Flour 0 (●, black line), flour 1 (▼, clear grey line), flour 2 (■, intermediate tone
531 grey line), flour 3 (◆, dark grey line). Coarse flours (a), fine flours (b).