Modification of wheat flour functionality and digestibility through different extrusion conditions

Mario M. Martínez[1], Cristina M. Rosell[2], Manuel Gómez[1]*

[1] Food Technology Area, E.T.S. Ingenierías Agrarias. Valladolid University, Ed. La Yutera, Avd. Madrid 44, 34004 Palencia, Spain.

[2] Institute of Agrochemistry and Food Technology (IATA-CSIC). Avda Agustin Escardino, 7. Paterna-46980, Spain.

*Corresponding author e-mail: pallares@iaf.uva.es

Tel: +34 (9) 79-108495 Fax +34 (9) 79-108302

Abstract

Continuous innovation in foodstuff and their higher quality requirements force food industry to look for flours with new specific functionalities. The objective of this work was to modify wheat flour functionality by using extrusion. This treatment significantly affected hydration, emulsifying, thermal and pasting properties of wheat flours besides their susceptibility to enzymatic hydrolysis and their amount of resistant starch. Thermal enthalpy decreased as the extrusion severity increased, indicating a higher amount of gelatinized starch. Hydration properties significantly increased, specifically 5-fold water binding capacity and 9-fold swelling compared with untreated wheat flour. Emulsifying capacity and the free sugar content increased in parallel with the extrusion severity. The susceptibility to enzymatic hydrolysis increased and the amount of resistant starch (RS) decreased as the extrusion severity augmented. Overall, extrusion allows modifying wheat flour features but it is advisable to select adequate extrusion conditions to achieve the desirable functionality.

Keywords: extrusion, pasting properties, wheat flour, thermal properties, hydration, enzymatic hydrolysis.

1. Introduction

Native starches and flours are widely used as ingredients, due to their particular complex polymeric characteristics, which make them suitable for numerous food applications. However, continuous innovation in foodstuff and their higher quality requirements force to adapt those commodities to the emerging needs in terms of functionality. Chemical, enzymatic and/or physical modifications have been the alternatives to modulate the functionality and properties of the raw starches (Chiu and Solarek, 2009)**.**

Extrusion is a high-temperature-short-time (HTST) physical treatment during which flours or starches are subjected to high temperatures and mechanical shearing at relatively low levels of moisture content (Camire, Camire and Krumhar, 1990). Extrusion allows starch gelatinization, denaturation of protein, microbial reduction, enzyme (in)activation and color changes, the extent of which are dependent on the conditions of the extrusion (Wen, Rodis and Wasserman, 1990). Those changes at the constituents' level modify the rheological behavior of flour batters (Hagenimana, Ding and Fang, 2006). Extrusion cooking is also responsible 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. Besides, it could be obtained an increase in the content of resistant starch, which is dependent on the treatment intensity (Hagenimana et al., 2006). It must be remarked that extrusion allows changing functionality by keeping the Green label (Jacobs and Delcour, 1998).

Mason (2009) reported that starches and flours modified by extrusion could be used in food products as thickening and gelling agents. This same author indicated that spray and drum-dried starches provided a suitable thickening, creamy and smooth texture for instant dry mixes such as puddings, desserts, soups and gravy and sauce bases, and these characteristics could also be achieved by extrusion treatments. Wheat extruded flours have been also useful to increase the bread yield in bakery process (Martinez, Oliete and Gómez, 2013). Rheological and water absorption properties of these flours define their adequacy for different uses.

However, research carried out in extrusion processes has been focused on starch, owing to the important changes that are produced on starch functional properties, such as gelatinization temperature, cold viscosity of pastes, retrogradation and so on (Mason, 2009); without considering a possible flour treatment. Nevertheless, the presence of other flour constituents might also affect starch functionality. In fact, some interactions between starch and non-starch components of flours have been reported due to the heat-moisture treatment of sorghum (Sun, Han, Wang and Xiong, 2013) or rice flours (Puncha-arnon and Uttapap, 2013). For this reason it is possible that those interactions are also produced during the extrusion process.

The aim of this work was to modify wheat flour functionality by using physical treatments like extrusion. 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 functional properties of flours (damaged starch, hydration, emulsifying, foaming, pasting and thermal properties) and their susceptibility to enzymatic hydrolysis.

2. Materials and methods

2.1 Materials

Wheat flour was 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). The length to diameter (L/D) ratio for the extruder was 20:1. Wheat flour was subjected to different extrusion treatments, where barrel temperature and/or feed rate and moisture content of the mass feed were modified, as specified in Table 1. Five types of extruded flours numbered from 1 to 5 indicating the severity of the extrusion treatment (1 the mildest and 5 the strongest), were obtained. Wheat flour without any treatment (wheat flour 0) was used as a control. The first factor took into account to number flours according to their treatment intensity, was the barrel temperature. Then, within the same temperature, the lower feed rate and the higher feed moisture content, the more intense the extrusion treatment is, since not only the mean residence time increases and therefore the treatment time, but also the water amount available for starch gelatinization.

Extruded product was dried by convection air and then ground with a compression roller till particle size was lower than 200 microns. Flours were stored in air-tight plastic containers and held at 4°C until analysis.

2.2 Methods

2.2.1. 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.

2.2.2. Damage starch

The content of damaged starch was determined according to AACC 76-30A method (AACC, 2012). A fungal alfa amylase 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.

2.2.3. Hydration properties

Hydration properties included swelling and water binding capacity (WBC) (Nelson, 2001). Swelling volume or the volume occupied by a known weight of flour was evaluated by mixing 5g (±0.1mg) of flour with 100ml distilled water and allowing it to hydrate during 16h.

Water binding capacity defined as the amount of water retained by the flour after it has been subjected to centrifugation was measured as described in the method 56.30 (AACC, 2012). Determinations were carried out in duplicate.

2.2.4. Emulsifying properties

Flour suspension (360 mL) of 0.5% (w/v) starch concentration was mixed with commercial sunflower oil (Langosta, F. Faiges S.L, Daimiel, Ciudad Real) (36 mL). The content was stirred for one min with a beater (Taurus Bapi 350 CP/CM, Taurus, Oliana, Lérida) to disperse the sample in the oil. The suspensions were then centrifuged at 800x*g* for 10 min. The emulsifying capacity (*EC*) was calculated as:

$$EC=(ev/tv)*100 \qquad \text{(Eq. 1)}$$

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

Emulsion stability (*ES*) against high temperatures, were determined in the emulsions that were heated in a water bath at 80ºC for 30 min, and centrifuged at 800x*g* for 10 min. *ES* was calculated as:

$$ES=(fev/iev)*100 \qquad \text{(Eq. 2)}$$

where *fev* is the final emulsion volume and *iev* is initial emulsion volume. Determinations were carried out in duplicate.

2.2.5. 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). Foam volumes were recorded after 30 s. The foam capacity (*FC*) was calculated as follows:

$$FC = (ifv/tsv)*100 \qquad \text{(Eq. 3)}$$

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.

$$FS = (ffv/tsv)*100 \qquad \text{(Eq. 4)}$$

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

2.2.6. 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. The flour slurry was prepared by dispersing 3.5g (±0.1g) of the flour in 25g (±0.1g) of distilled water.

2.2.7. Thermal properties

Analyses were performed in a differential scanning calorimeter DSC-7 (Perkin–Elmer, 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 oven. The calorimeter scan conditions were set as follows: samples were kept at 30°C for 2 min, heated from 30 to 110ºC at 5ºC/min. Onset temperature (T_o), peak temperature (T_p), gelatinization temperature range (T_p- T_o), peak height index (ΔH_g/ T_p- T_o) as well as the enthalpy of starch gelatinization (ΔH_g) (expressed as mJ/mg of sample) were determined. All samples were run in quadruplicate.

2.2.8. Enzymatic hydrolysis of starch

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

The remnant starch after 16 h hydrolysis was solubilized with 2ml of 2M KOH using a Polytron ultraturrax homogenizer IKA-T18 (IKA works, Wilmington, USA) during 1min at speed 3. The homogenate was diluted with 8ml 1.2M sodium acetate pH 3.8 and incubated with 100µl amyloglucosidase (3300 U) at 50 ºC 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, 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, Garcia-Alonso and Saura-Calixto, 1997):

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

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. 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, Warren, Grassby, Patel and Ellis (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, Ruiter, Visser and Iversen, 2003).

2.2.9. Statistical analysis

Simple analyses of variance were used to determine the effects of thermal treatment. 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

Wheat flour was subjected to different extrusion treatments that differed on the maximum barrel temperature, screw speed and feed moisture content in order to obtain different extrusion intensities. Overall five flours subjected to extrusion treatments were obtained identified as 1 to 5 and the flour without any treatment (control) was named as flour 0 (Table 1).

3.1 Damage starch and free sugars

Extrusion intensity had a significant effect on free sugars content, although a minimum intensity was necessary for promoting changes (Table 2). No significant differences were observed between the free sugars content of the control (flour 0) and the mild extrusion treatment (flour 1). In the mild treatments (low barrel temperature, low feed moisture content and/or high feed rate), damages and changes of starch did not influence free sugars content, and thus no hydrolysis was produced. As extrusion intensity increases, higher starch gelatinization was produced, breaking the starch granules physically and opening their crystalline structure, thus the access of hydrolytic enzymes was made easier (Mu, Abegunde, Sun, Deng and Zhang, 2013). In order to produce the gelatinization of starch, a minimum barrel temperature and feed moisture content are necessary (Camire, et al., 1990; Chao-chi Chuang and Yeh, 2004), which are not reached in milder treatments. Feed rate have an influence on mean residence time and therefore would also have an influence on gelatinization process.

Extrusion is also able to promote enzyme inactivation, however, in light of the results, extrusion conditions used in this study did not produce enough enzyme inactivation to stop their activity.

Extrusion lead to a progressive increase of damage starch content with the increasing of extrusion treatment, probably due to the damage produced by shear stress and temperature on starch granules during extrusion (Camire et al., 1990). Nonetheless, no differences were observed between flour 1 and 2 in spite of barrel temperature differences. Moreover, considering differences observed in damaged starch content of flour 2 and 3, which were extruded at the same temperature, feed rate and feed moisture content significantly affected the damage produced on starch granules as Chao-Chi Chuang, et al. (2004) observed studying the effects of three types of screw elements on glutinous rice flour in a single screw extruder.

3.2 Hydration, emulsifying and foaming properties

Hydration, emulsifying and foaming properties were significantly affected by the extrusion process (Table 2). A progressive increase in the hydration properties (WBC and swelling) was observed as the extrusion intensity increased, but a minimum temperature besides feed rate and feed moisture content in the extrusion was needed to promote changes in hydration properties. No differences were appreciated between flour 0 and flour 1 in both hydration properties, neither among control (flour 0) and flour 1 and 2 in the case of swelling. Thus, not only was high barrel temperature necessary but also low feed rate and high feed moisture content were necessary to modify hydration properties of flours. Chao-Chi Chuang et al. (2004) observed that the degree of starch gelatinization in extrudates was dependent on the mean residence time during extrusion, which in turn was correlated with the feed rate, increasing the degree of gelatinization by decreasing the feed rate.

The effects on hydration properties could be partially attributed to the increase in damaged starch content since it showed a significant positive correlation with WBC (r=0.92) and swelling (r=0.92). On the other hand, 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. Moreover, the cooking produced during extrusion led to starch gelatinization that contributes to raise WBC and swelling, as Hagenimana et al. (2006) appreciated in their values of water absorption index.

The extrusion significantly increased the emulsifying capacity of the wheat flours when sufficient feeding water was available and adequate barrel temperature. To become evident changes in *EC* of the flours, 120ºC were necessary (flours 2, 3 and 4) and the greatest effect (flour 5) was observed when temperature of 160ºC was reached. Barrel temperature could produce protein and starch changes during extrusion process. Extrusion forces protein unfolding and aggregation due to protein crosslinking involving SH/SS interchange, oxidation and hydrophobic interactions (Rosell and Foegeding, 2007) along with starch gelatinization that increases the number of hydroxyl groups available to form hydrogen bonds with the proteins, leading to better emulsion capacity (Mason, 2009).

Emulsion stabilities did not follow a steady trend with the extrusion severity, having flour 3 the same *ES* than the control. Emulsion stability depends greatly on the oil globule size and its interfacial tension. As it has been commented previously, extrusion forces the protein unfolding and aggregation, which could minimize the barrier effect against oil droplet coalescence (Aluko, Mofolasayo and Watts, 2009). Thereby, extruded flours although had high capacity to form emulsions, those were not stable due to coalescence phenomena, as indicated the significant negative correlation between *EC* and *ES* (r =-0.84, P<0.001).

In general, extrusion worsened the foaming capacity (with the exception of flour 1 which did not have significant difference with control) and foaming stability of wheat flours, even though no clear trend was observed. Similar results were exposed by Bolade, Usman, Rasheed, Benson and Salifou (2002), who observed how corn flours subjected to intense hydrothermal treatments did not improve their foaming capacity. This diminution of foaming capacity of flours could be due to protein unfolding and aggregation induced by the extrusion process. The *FC* results from microstructure, size and distribution of the gas cells and the interfacial properties (Zhang, Bai and Zhang, 2011). The opposite trend observed in the emulsifying and foaming properties supports the different mechanisms involved during interfacial membrane formation at the air-water and oil-water interfaces (Stauffer, 1990).

3.3. Pasting characteristics

Pasting plots of the extruded flours are displayed in Figure 1. When wheat flours were suspended in water, the major value for the initial viscosity at 50ºC was observed in the flour 5, which suggested the presence of already pregelatinized starch. Chao-Chi Chuang et al. (2004) in a study with glutinous rice flour observed that with the increase of the mean residence time, an increase of temperature and starch gelatinization took place. This greater gelatinization in the most severe treatments can be the reason for the differences in the initial viscosity at 50ºC.

When flours were subjected to heating-cooling cycle in RVA, extrusion effect was readily evident on the plots. Pasting temperatures were shifted to lower values as the extrusion intensity increased, with exception of flour 5 that showed a completely different viscosity plot with low viscosity along the heating-cooling cycle. A minimum barrel temperature and moisture content was needed to induce changes in the flour viscosity, because of that minimum changes were observed in flour 1. As the extrusion treatment intensified on the flours, the viscosity during heating and cooling increased, but it seems that viscosity decreased when extrusion induced partial starch breakdown (flour 4), and that effect was even more dramatic when changes beyond gelatinization occurred (flour 5). The intermediate extrusion conditions applied to flour 2 and 3 seems to affect the starch granules increasing the viscosity in the cycle. Hoover and Vasanthan (1994) already observed this effect when conditions were no sufficient to induce starch gelatinization. Those authors attributed it to an increase of the granular rigidity resulting from an increase in the crystalline order and in the amylo-lipids complexes amount; and starch chain interactions within the amorphous region. Nonetheless, Biliaderis (2009) commented that cereals subjected to heat-moisture treatment, lose their crystallinity. In extrusion process, where the combination of heating and swelling of amorphous starch destabilizes crystalline regions, extrusion severity might be an alternative to control the degree of flours modification (Camire et al. 1990).

The extensive gelatinization that the wheat flours undergo when are subjected to severe extrusion intensity (flour 5) could promote the viscosity decrease during the heating-cooling cycle, as it was observed by Hagenimana et al., (2006) and Sharma, Singh and Subramanian, (2013).

Peak viscosity obtained during heating was significantly dependent on the damage starch content (r=-0.71), which might be explained by the changes in the polymerization degree of the starch granules after damaging (Barres, Verges, Tayeb and DellaValle, 1990).

The reduction observed in the final viscosity and setback (difference between the minimum viscosity during heating and the final viscosity after cooling) in flour subjected to harsh extrusion (flour 5), indicated the extension of the effect on the amylose chains, which might lose the ability to retrograde during cooling owing to their fragmentation during extrusion. This effect agrees with previous results of Doublier, Colonna and Mercier (1986).

3.4. Differential Scanning Calorimetry (DSC)

The effect of extrusion treatment on the thermal properties of the wheat flours is shown in Table 3. In the range of temperature tested, flours exhibited one endothermic peak, with the exception of flour 5, corresponding to amylopectin gelatinization. Therefore, the diverse extrusion conditions applied to wheat flours were sufficient to change some starch features but were not strongly enough to complete starch gelatinization. Only in the case of flour 5, the absence of an endothermic peak confirmed total gelatinization of amylopectin. Indeed, this result agrees with that previously discussed regarding the very small viscosity plot obtained for flour 5. The extrusion treatment significantly modified the gelatinization temperatures of the flours. Gelatinization temperatures were progressively sifted to higher values when flours were treated at increasing extrusion intensity, but the temperature range and the peak height index were 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 5). Those results agree with the viscosity plots recorded by the RVA. Additionally, pasting temperature was sifted to lower temperatures due to the effect of extrusion on the outer structure of the granules, without affecting the crystalline internal one, which will lead higher gelatinization temperatures.

When comparing extruded flours, the gelatinization enthalpy was significantly reduced due to the intensity of the extrusion, which was expected since extrusion induces starch gelatinization and an increase of the damage starch content (Chiu et al., 2009), leading to a reduction of the native starch granules able to gelatinize (Biliaderis, Page, Maurice and Juliano, 1986). The extrusion process modifies the crystalline structure of the starch granule affecting the temperature at which swelling starts (Camire et al., 1990).

3.5. Starch hydrolysis

The susceptibility of the extruded flours to the enzymatic hydrolysis was analyzed, following the kinetic plots (Figure 2). The hydrolysis curves were fitted to a first order kinetics according to Goni et al. (1997) and also to Butterworth et al. (2012) to obtain the kinetic parameters (Table 4). As it was observed in the plots, there was a slight increase in the equilibrium concentration reached after hydrolysis of the wheat flours extruded at 120ºC. Our study are consistet with the one presented by Hagenimana, et al., (2006), who stated that the susceptibility of the extruded starches to be enzymatically hydrolyzed was directly related to the intensity of the extrusion treatment. However, wheat flours extruded at lower temperature (flour 1) displayed much faster and extensive hydrolysis, and the same trend was observed at the most intense extrusion treatment (flour 5). Therefore, depending on extrusion parameters, starch modifications could be different. The lower barrel temperature and the lower feed moisture content of flour 1 (insufficient to gelatinize the starch), together with the longer residence time, would produce major structural rearrangements without gelatinization, increasing the contact between amorphous starch and enzymes and therefore the susceptibility to enzyme-catalyzed hydrolysis.

When the kinetic parameters were extracted from the hydrolysis time curves (Table 4), no general trend was observed between the rate of hydrolysis (*k)* and the extrusion intensity. There was great agreement with the equilibrium concentration estimated from both fitting 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. In general, a decrease in the amount of resistant starch present in the extruded flours was observed, with the exception of flour 1 that showed higher content of resistant starch. This finding disagrees with previous observations of Hagenimana et al. (2006) who found an increase in RS content with the treatment severity in high-amylose long rice. Those authors attributed the increase in RS to the formation of amylose-lipid complexes during the extrusion, which retarded the enzymatic digestion (Collier and O´Dea, 1983). Therefore, results divergence might be explained due to the high amylose content of rice flour and also its higher gelatinization temperature compared with the wheat flour. In addition, Chinnaswamy and 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 amount of RS. It is convenient to highlight the greater values of RS and starch hydrolysis of flour 1. de Mosqueda, Perez, Juliano, del Rosario and Bechtel (1986) observed that extrusion mild treatments did not modify the amylose chain length in rice flours, thus the creation of V-type structures would be higher protecting starch from degradation. Moreover, as it was commented by Biliaderis (2009), the mild treatment heat-moist also increase the susceptibility to enzyme-catalysed hydrolysis, thus the non-resistant starch in our flour 1 would be more easily hydrolysed.

4. Conclusion

Extrusion of wheat flours might be an alternative to obtain wheat flours with different technological functionality. Hydration, thermal, emulsifying and pasting properties of wheat flours besides their susceptibility to enzymatic hydrolysis can be modified by extrusion. Starch gelatinization increased with the extrusion severity, augmenting the hydration properties and the viscosity in cold solution of wheat flours. In parallel, extrusion also enhanced the emulsifying capacity and increased the free sugars of wheat flours, making them suitable for some foodstuff.

In general, the susceptibility to enzymatic hydrolysis increased and the amount of RS decreased as the extrusion severity increased.

Acknowledgements

Authors acknowledge the financial support of Junta de Castilla y León (VA054A12-2) and Generalitat Valenciana (Project Prometeo 2012/064). Authors are also grateful to Harinera Los Pisones, (Zamora, Spain) for supplying the wheat flours. Mario M. Martínez would like to thank predoctoral fellowship from University of Valladolid.

5. References

AACC, 2012. Approved methods of the American Association of Cereal Chemists, 76-30a (damaged starch), 56-30 (WBC), 61-02.01 (RVA) (11th ed.). St. Paul, Minnesota: American Association of Cereal Chemists.

Aluko, R., Mofolasayo, O., & Watts, B., 2009. Emulsifying and foaming properties of commercial yellow pea (Pisum sativum L.) seed flours. Journal of Agriculture and Food Chemistry 57, 9793-9800.

Barres, C., Verges, B., Tayeb, J., & Della Valle, G., 1990. Transformation of wheat flour by extrusion cooking. Influence of screw configuration and operating conditions. Cereal Chemistry 67, 427-433.

Biliaderis, C., Page, C., Maurice, T., & Juliano, B., 1986. Thermal characterization of rice starches: A polymeric approach to phase-transitions of antigranulocytes starch. Journal of Agricultural and Food Chemistry 34, 6-14.

Biliaderis, C., 2009. Structural transitions and related physical properties of starch, in: BeMiller, J., Whistler, R. (Eds.), Starch. Chemistry and Technology. Academic Press, New York. USA., pp. 293-372.

Bolade, M., Usman, M., Rasheed, A., Benson, E., & Salifou, I., 2002. Influence of hydrothermal treatment of maize grains on the quality and acceptability of Tuwon masara (traditional maize gel). Food Chemistry 79, 479-483.

Butterworth, P., Warren, F., Grassby, T., Patel, H., & Ellis, P., 2012. Analysis of starch amylolysis using plots for first-order kinetics. Carbohydrate Polymers 87, 2189-2197.

Camire , M.E., Camire , A., & Krumhar, K., 1990. Chemical and nutritional changes in foods during extrusion. Critical Reviews in Food Science and Nutrition 29, 35-57.

Chao-Chi Chuang, G., & Yeh, A.I., 2004. Effect of screw profile on residence time distribution and starch gelatinization of rice flour during single screw extrusion cooking. Journal of Food Engineering 63, 21-31.

Chinnaswamy, R., & Hannah, M.A., 1990. Macromolecular and functional properties of native and extruded corn starch. Cereal Chemistry 67, 490-499.

Chiu, C., & Solarek, D., 2009. Modification of starch, in: BeMiller, J., Whistler, R. (Eds.), Starch. Chemistry and Technology. Academic Press, New York. USA, pp. 629-656.

Collier, G., & O´Dea, K., 1983. The effect of coingestion of fat on the glucose, insulin and gastric inhibitory polypeptide responses to carbohydrate and protein. American Journal of Clinical Nutrition 37, 941-944.

de Mosqueda, M.B., Perez, C.M., Juliano, B.O., del Rosario, R.R., & Bechtel, D.B., 1986. Varietal differences in properties of extrusion-cooked rice flour. Food Chemistry 19, 173-187.

Doublier, J.L., Colonna, P., & Mercier, C., 1986. Extrusion cooking and drum drying of wheat-starch. 2. Rheological characterization of starch pastes. Cereal Chemistry 63, 240-246.

Goñi, I., Garcia-Alonso, A., & Saura-Calixto, F., 1997. A starch hydrolysis procedure to estimate glycemic index. Nutrition Research 17, 427-437.

Gularte, M.A., & Rosell, C.M., (2011). Physicochemical properties and enzymatic hydrolysis of different starches in the presence of hydrocolloids. Carbohydrate Polymers 85, 237–244.

Hagenimana, A., Ding, X., & Fang, T., 2006. Evaluation of rice flour modified by extrusion cooking. Journal of Cereal Science 43, 38-46.

Hoover, R., & Vasanthan, T., 1994. Effect of heat-moisture treatment on the structure and physicochemical properties of cereal, legume and tuber starches. Carbohydrate Research, 33-53.

Jacobs, H., & Delcour, J., 1998. Hydrothermal modifications of granular starch, with retention of the granular structure: A review. Journal of Agricultural and Food Chemistry 46, 2895-2905.

Martinez, M., Oliete, B., & Gomez, M., 2013. Effect of the addition of extruded wheat flours on dough rheology and bread quality. Journal of Cereal Science 57, 424-429.

Mason, W.R., 2009. Starch use in foods, in: BeMiller, J., Whistler, R. (Eds.), Starch. Chemistry and Technology. Academic Press, New York. USA., pp. 745-795.

Mu, T., Abegunde, O., Sun, H., Deng, F., & Zhang, M., 2013. Physicochemical characterization of enzymatically hydrolyzed heat treated granular starches, Starch-starke 65, 893-901.

Nelson, A.L., 2001. Properties of Hihg-fibre ingredientes. Cereal Foods World 46, 93-97.

Poulsen, B., Ruiter, G., Visser, J., & Iversen, J., 2003. Determination of first order rate constants by natural logarithm of the slope plot exemplified by analysis of Aspergillus niger in batch culture. Biotechnology Letters 25, 565-571.

Puncha-Arnon, S., & Uttapap, D., 2013. Rice starch vs. rice flour: Differences in their properties when modified by heat-moisture treatment. Carbohydrate Polymers 91, 85-91.

Rosell, C., & Foegeding, A., 2007. Interaction of hydroxypropylmethylcellulose with gluten proteins: Small deformation properties during thermal treatment. Food Hydrocolloids 21, 1092-1100.

Sharma, P., Singh, V., & Subramanian, R., 2013. Pasting, swelling, and solubility characteristics of rice batter prepared from different wet grinding systems. Starch-Starke 65, 374-381.

Stauffer, C., 1990. Functional additives for bakery products. Van Nostrand Reinhold. New York.

Sun, Q., Han, Z., Wang, L., & Xiong, L., 2013. Physicochemical differences between sorghum starch and sorghum flour modified by heat-moisture treatment.

Wen, L., Rodis, P., & Wasserman, B. (1990). Starch fragmentation and protein insolubilization during twin-screw extrusion of corn meal. Cereal Chemistry, 67, 268-275.

Zhang, M., Bai, X., & Zhang, Z. (2011). Extrusion process improves the functionality of soluble dietary fiber in oat bran. Journal of Cereal Science, 54, 98-113.

FIGURE CAPTIONS

Figure 1. Effect of extrusion treatment on the pasting properties of wheat flours. Flour 0 (open triangle), flour 1 (clear grey line), flour 2 (discontinuous clear grey line with +), flour 3 (discontinuous intense tone grey line), flour 4 (black grey line), flour 5 (discontinuous black grey line). Temperature profile (discontinuous points).

Figure 2. Effect of extrusion treatment on the enzymatic hydrolysis of wheat flours. Flour 0 (open triangle), flour 1 (clear grey line), flour 2 (discontinuous clear grey line), flour 3 (discontinuous intermediate tone grey line), flour 4 (dark grey line), flour 5 (discontinuous dark grey line).

Figure 1

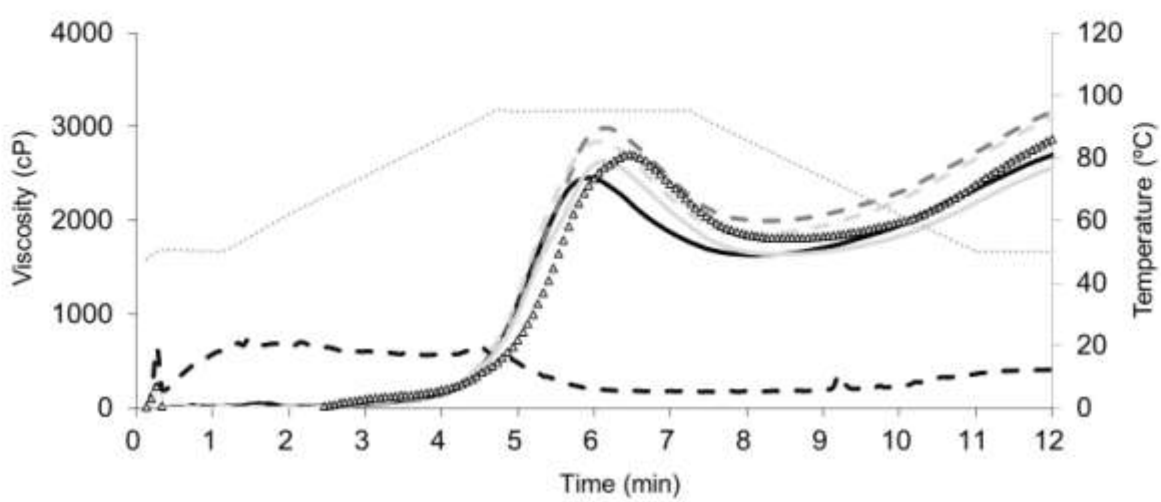
Viscosity (cP)
Temperature (ºC)
Time (min)
4000
3000
2000
1000
0
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12

Figure 2

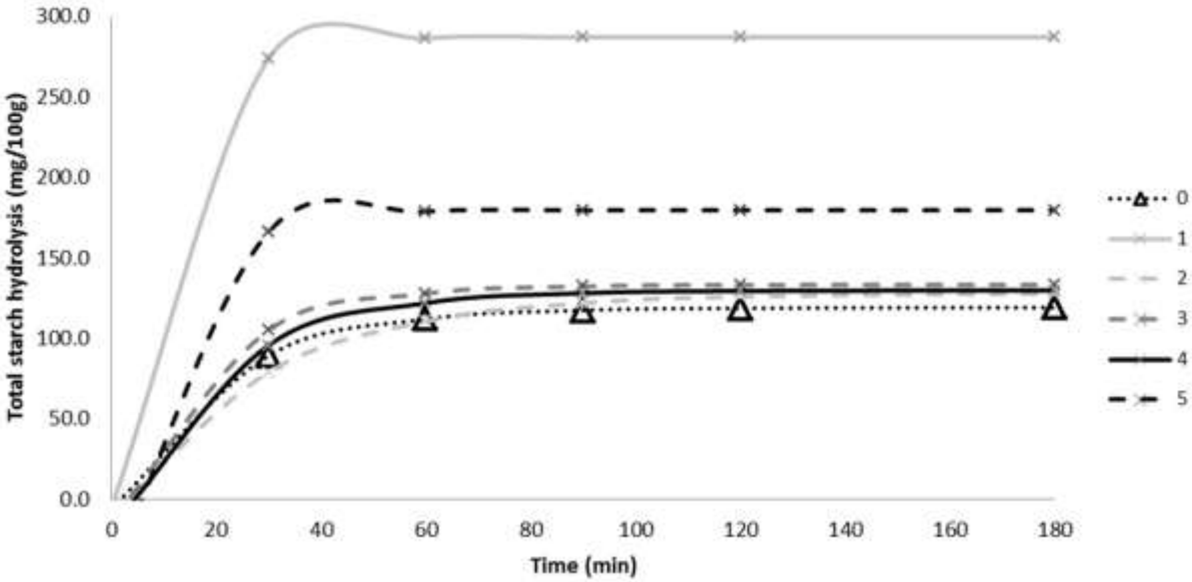
Total starch hydrolysis (mg/100g)
300.0
250.0
200.0
150.0
100.0
50.0
0.0
0
20
40
60
80
100
120
140
160
180
Time (min)
0
1
2
3
4
5

Table 1: Extrusion conditions applied to wheat flours.

Flour Code	Barrel Temperature (ºC)	Feed Rate (Kg/h)	Feed Moisture Content (%)
0	-	-	-
1	80	275	3.6
2	120	900	4.4
3	120	400	15
4	120	275	21.8
5	160	500	10

Table 2: Effect of extrusion treatment (0-5) on free sugars, damaged starch, hydration, emulsifying and foaming properties of wheat flours.

	Extrusion treatment					
	0	1	2	3	4	5
Free Sugars (%)	5.84a	5.42a	9.74b	13.80c	18.04d	44.22e
Damaged Starch (%)	4.97a	8.49b	9.14b	21.03c	26.08d	37.95e
WBC (g water/g solid)	0.78a	0,83a	1.28b	1.91c	2.19d	4.97e
Swelling (mL/g)	0.10a	0.40a	0.65a	2.12b	2.98c	9.50d
EC	82.97a	82.97a	85.00ab	85.16b	85.78b	90.78c
ES	115.18d	115.27d	106.8b	114.25d	110.16c	100.16a
FC	51.72c	51.99c	28.55b	19.59a	18.06a	30.58b
FS	86.33d	27.70b	69.64c	0.00a	0.00a	0.00a

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

Numbers in sample codes are referred to extrusion intensity, being number 0 ascribed to control sample.

Values followed by different letters within a row indicate significant differences ($P<0.05$).

Table 3. Effect of extrusion treatment (0-5) on the thermal properties of wheat flours.

	Extrusion treatment					
	0	1	2	3	4	5
T_o (ºC)	55.4a	55.8ab	58.8bc	59.2c	61.3c	n.d
T_p (ºC)	60.7a	61.6ab	63.1b	64.9c	65.2c	n.d
T_c (ºC)	68.7ab	66.7a	67.7a	70.1bc	71.6c	n.d
T_p-T_o (ºC)	5.3a	5.7a	4.3a	5.6a	3.9a	n.d
ΔH (J/g)	4.18b	2.86b	3.26b	2.85b	0.80a	n.d
PHI (J/g*ºC)	0.76b	0.49ab	0.77b	0.51ab	0.21a	n.d

n.d.: Not detected.

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

Numbers in sample codes are referred to extrusion intensity, being number 0 ascribed to control sample.

Values followed by different letters within a row indicate significant differences (P<0.05).Values followed by different letters within each parameter indicate significant differences.

Table 4. Kinetic parameters extracted from first-order and LOS plots of wheat flours subjected to different extrusion conditions.

	k (min^{-1}) by first order eq.	*k* (min^{-1}) by LOS	C_∞ (%)	C_∞ (%) by LOS	Resistant starch (%)
0	0.048	0.046	125.00	130.32	6.11
1	0.103	0.094	292.46	306.15	8.96
2	0.034	0.034	137.82	142.06	4.50
3	0.055	0.053	147.10	156.94	5.80
4	0.049	0.047	147.90	155.73	3.69
5	0.093	0.088	223.81	255.40	2.29

k, kinetic constant; C_∞, equilibrium concentration

Numbers in sample codes are referred to extrusion intensity, being number 0 ascribed to control sample.

Values followed by different letters within a row indicate significant differences (P<0.05).