Physicochemical modification of native and extruded wheat flours by enzymatic amylolysis

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Mario M. Martinez*, Joana Pico, Manuel Gómez.

Food Technology Area, College of Agricultural Engineering, University of Valladolid, 34004 Palencia, Spain.

* Corresponding author. E-mail: mario.martinez@iaf.uva.es

Tel: +34 (9) 79-108359. Fax +34 (9) 79-108302

Highlights

- Enzymatically hydrolysed native and extruded wheat flours were investigated.
- Hydrolysed extruded wheat flours showed a melt component joining the granules.
- Extruded flours had higher glucose, isomaltose, maltose, and maltotriose contents.
- Flours hydrolysed by amyloglucosidase showed a dark and reddish colour.

Abstract

Enzymatic hydrolysis could be an alternative way to modify flour functionality. The effect of two different enzymes, α-amylase and amyloglucosidase, and their combination on microstructure, oligosaccharide content, crystalline order, pasting, gel hydration, and colour properties of native and extruded wheat flours was investigated. Micrographs showed different mechanisms of actuation of the different enzymes on native and extruded flours, achieving greater than 300% and 500% increases of glucose and maltose contents, respectively, in extruded flours compared with their native counterparts. Native flours displayed higher values of water absorption capacity and swelling power than extruded flours. Flours treated by a combination of amylase and amyloglucosidase showed low swelling power. Regarding colour, native flours were darker and more reddish than extruded flours, whereas flours treated by amyloglucosidase, and therefore had a higher glucose content, were darker and more reddish.

Keywords: wheat flour; hydrolysis; amylase; amyloglucosidase; oligosaccharide

1 Introduction

Native starches and flours are widely used as raw materials, due to their particular polymeric characteristics, which make them suitable for numerous food applications. However, the new demands of the food industry are forcing manufacturers of starchy ingredients to find new functionalities. Starch modification by enzymatic hydrolysis could be an alternative way to control the functionality making the label cleaner unlike the chemically modified starches or syrups Starch hydrolysis generates products with different dextrose equivalents (DE), depending on the time of incubation and the amount and type of enzyme being used. Two major hydrolysis products are maltodextrins that consist of partly hydrolysed starch chains with a DE below 30, and glucose and maltose syrups with a DE above 40 that contain mono-, di-, and some higher saccharides (Baks, Kappen, Janssen, & Boom, 2008). Maltodextrins are nonsweet, cold water soluble, and have water-holding characteristics. They can be used as carrier or bulk agents, texture providers, spray-drying aids for the production of flavour enhancers, fat replacers, film formers, freeze-control agents to prevent crystallisation, or to supply nutritional value (Ba, Blecker, Danthine, Tine, Destain, & Thonart, 2013). Meanwhile, glucose and maltose syrups are employed in a variety of foods like soda water, sweets, baked products, ice-creams, sauces, baby food, conserves, and tined food.

Amylases, together with amyloglucosidases, are the enzymes most commonly used in starch hydrolysis. Alpha-amylase is an endoamylase that cleaves the α-1,4 glycosidic bonds of the amylose or amylopectin chain at internal positions (endo) to yield products (oligosaccharides with varying lengths and branched oligosaccharides called limit dextrins) with an α-configuration. Meanwhile, amyloglucosidase catalyses the hydrolysis of both α-1,4 and α-1,6 glycosidic bonds at the branching point to release β-

D-glucose residues of the polymer substrate (van der Maarel, van der Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2002). Because of these different mechanisms of amylolysis, selection of the type and amount of enzyme is important, since it will determine the physicochemical properties of the final flour or starch.

Native starch granules are semi-crystalline and resistant to enzyme hydrolysis. Native granular starch is hydrolysed very slowly by both amylases and amyloglucosidase, but disruption of the starch granular structure (gelatinisation) could enhance its chemical reactivity towards hydrolytic enzymes (Uthumporn, Shariffa, & Karim, 2012). Extrusion cooking is a hydrothermal treatment of high temperature and short duration, during which flours or starches are subjected to high temperatures and mechanical shearing at relatively low levels of moisture content (Camire , Camire , & Krumhar, 1990). By means of extrusion, it is possible to gelatinise the starch present in cereal flour (Martínez, Calviño, Rosell & Gómez, 2014). Several authors have used extruders to gelatinise native starch and hydrolyse it enzymatically (Govindasamy, Campanella, & Oates, 1997a, 1997b; Lee & Kim, 1990; Vasanthan, Yeung, & Hoover, 2001).

The vast majority of the studies about hydrolysis of cereal-based products focus on starch modification whereas flour modification has been scarcely investigated. Vasanthan, et al., (2001) studied the dextrinisation of barley flours with alpha-amylase by extrusion. Flours are fine, powdery materials obtained by grinding and sifting the starch-containing plant organelles. Components often found in flours include starch, non-starch polysaccharide, sugar, protein, lipid, and inorganic materials. Thereby, the interactions between starch and non-starch components of flour during hydrothermal and enzymatic treatments are possibly different from that of starch. Commercial wheat flour is produced by milling of wheat kernels, whereas wheat starch is generally obtained by gluten agglomeration. Such a treatment involves four major issues to

consider: raw materials, products, cost and operability (Maningat, Seib, Bassi, Woo & Lasater, 2009). Moreover, water consumption and effluent disposal demand careful operation of the plant (Maningat, Seib, Bassi, Woo & Lasater, 2009). Therefore the lower cost and environmental impact of subjecting wheat flour instead wheat starch to enzymatic hydrolysis could made flour modifications a better alternative for industrial processes..

Despite the particular physicochemical characteristics of extruded flours and their high susceptibility to enzymatic hydrolysis, the properties of their hydrolysed products have never been studied, nor have they been compared with hydrolysed products of native flours. The objective of the present study was to investigate the effect of a potential feasible industrial enzymatic hydrolysis (by alpha-amylase, amyloglucosidase, or a blend of both) on microstructure, oligosaccharide composition, crystallinity, pasting, colour, and hydration properties of native and extruded wheat flours.

2 Materials and methods

2.1 Materials

Native wheat flour (11.73% and 11.20% w/w of moisture and protein contents, respectively) was supplied by Harinera Castellana (Medina del Campo, Valladolid, Spain). Extruded modified wheat flour was provided by Harinera Los Pisones (Zamora, Spain), which performed the extrusion treatment using a Bühler Basf single screw extruder (Bühler S.A., Uzwil, Switzerland). The extrusion conditions were carried out based on preliminary experiences in order to ensure the starch gelatinization. The length-to-diameter (L/D) ratio for the extruder was 20:1. Wheat flour was extruded at a maximum barrel temperature of 160ºC and a feed moisture content of 50 L/h, with a feed rate of 500 kg/h and with a screw speed of 340 rpm. The extruded product was

dried by convection air till it reached 11.2% of moisture. Then it was ground with a compression roller to a particle size below 200 microns.

The amyloglucosidase from *Aspergillus niger* AMILASE™ AG 300L (300 AGU/mL) and the fungal alpha-amylase Fungamil® 800L from *Aspergullus oryzae* (800 FAU/g) were gently provided by Novozymes (Bagsvaerd, Denmark).

2.2 Methods

2.2.1 Flour hydrolysis

The quantity of enzymes was based on previous experiments, where the minimum amount of enzyme to produce changes in the viscosity of starch slurries was selected. Amylase and amyloglucosidase flour slurries with a 0.2% w/w of enzyme (flour basis) were made by dissolving 0.1 g (±0.001) of amylase or amyloglucosidase solution (20% w/w of enzyme) respectively into 40 mL (±0.01) of distilled water. In the case of using both enzymes simultaneously, 0.05 g (±0.001) of each enzyme was dissolved. The quantity of flour was also selected based on preliminary tests, in order to achieve suspensions easily dryable. Then, 10 g of flour were added to the enzyme solution previously prepared and mixed to achieve a homogenous paste. These pastes were covered by plastic film to avoid drying of the sample and then incubated at 50ºC for 2 hours. With the aim of bringing to an end the enzymatic activity, the pastes were heated at 105ºC for 4 hours. Afterwards, they were rested in a desiccator at room temperature for 3 minutes, before being milled in a Moulinex super junior s (Groupe Seb Iberica, S.A, Barcelona) for 20 seconds. Flours were stored in airtight plastic containers at 4°C until analysis. Thereby, the whole process of flour hydrolysis was performed considering the feasibility of further potential industrial processes in the food industry.

2.2.2 Environmental scanning electron microscopy (ESEM)

Flour photomicrographs were taken with a Quanta 200FEI (Hillsboro, Oregon, USA) ESEM. Photomicrographs were taken in beam deceleration mode (BDM) at 1.5 KeV in high vacuum mode with a backscattered electron detector (BSED).

2.2.3 Oligosaccharide content of flours by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD)

The aim of the HPAEC-PAD analysis was to determine the content of oligosaccharides in the extruded and non-extruded enzymatically treated flours. D-(+)-Glucose, maltose monohydrate, maltotetraose, and maltopentaose (Neat, Sigma-Aldrich, Steinheim, Germany), isomaltose (98%, Sigma-Aldrich, Steinheim, Germany), and maltotriose hydrate (95%, Sigma-Aldrich, Steinheim, Germany) were the standards employed to analyse these compounds in the flours studied.

Sample treatment consisted of solid-liquid extraction with MilliQ deionised water (Millipore, Molsheim, France) without derivatisation. Then, 0.5 g (±0.09) of the ground sample were weighed in a falcon tube, 15 mL of water were added, and the mixture was shaken for 5 minutes at 430 rpm in a shaker. Then, 2 mL of Carrez II reagent [potassium hexacyanoferrate (II) trihydrate, Panreac, Barcelona, Spain] were added and the mixture was shaken again for 5 minutes at 430 rpm. The mixture was centrifuged for 20 minutes at 12,000 rpm and 20ºC and immediately after, to avoid re-suspension of the precipitate, the supernatant was transferred to a flask. A second extraction was needed; therefore, another 15 mL of water were added to the solid phase obtained from the centrifugation, the mixture was again shaken for 5 minutes at 430 rpm and centrifuged for 20 minutes at 12,000 rpm and 20ºC. Afterwards, the supernatant was transferred to the flask containing the first extract. After making up to the volume with water, a suitable dilution was filtered with 0.45 μm nylon filters into the vial and then injected.

HPAEC-PAD analyses were carried out on a Metrohm system (Herisau, Switzerland) consisting of an 850 Professional IC with an isocratic pump, an automatic 858 Professional Sample Processor with ultrafiltration, an 872 extension module to provide another pump and the possibility of making gradients, and an IC Amperometric Detector working as a pulsed amperometric detector (PAD) with a gold electrode as the working electrode and a palladium electrode as the reference electrode. MagICnet software (Metrohm, Herisau, Switzerland) was used to analyse the chromatograms.

Separation was achieved on a Hamilton RCX-30 column and a Metrosep RP2 Guard precolumn from Metrohm (Herisau, Switzerland), with the same stationary phase as the column. Column and precolumn were thermostated at 30ºC and the PAD at 35ºC. The flow rate was 1.0 mL/min constantly and the volume injection was 20 µL. A binary gradient solvent system was used as mobile phase, consisting of (A) 50 mM NaOH (Panreac, Barcelona, Spain) and (B) a mixture of 500 mM NaAcO (Panreac, Barcelona, Spain) and 50 mM NaOH. The gradient was as follows: initial conditions of 95% A, then down linearly from the start to 15 minutes until 80% A, held from 15 to 25 minutes at 80% A, then returned to the initial conditions, rising linearly from 25 to 28 minutes to 95% A and finally, held at 95% A for 10 minutes. The total run was 38 minutes. The potentials and time periods for the pulsed amperometric detector were: E1, +100 mV (t1 = 300 ms); E2, +550 mV (t2 = 50 ms); and E3, –100 mV (t3 = 200 ms). Measurements were made in duplicate.

2.2.4 Flour crystallinity by X-ray diffraction

Crystallinity of samples was determined using a Bruker D8 Discover A25 X-ray diffractometer (Bruker AXS, Rheinfelden, Germany) equipped with a copper tube operating at 40 kV and 40 mA, producing CuKa radiation of 0.154 nm wavelength.

Diffractograms were obtained by scanning from 5 to 40º (2 theta), at the rate of 1.2º/min, a step size of 0.02º, a divergence slit width variable (DS) of 5 mm, and a scatter slit width (SS) of 2.92º.

2.2.5 Pasting properties

Pasting properties of the normal and extruded enzymatically treated flours were determined using a Rapid Visco Analyser (Model RVA-4C, Newport Scientific Pty. Ltd., Warriewood, Australia). The flour slurry was prepared by dispersing 3.5 g (±0.1) of the flour in 25 g (±0.1) of distilled water. The slurry was then poured into an aluminium canister and stirred manually using a plastic paddle for 20 seconds before being poured into the RVA machine. The heating and cooling cycles were programmed following general pasting method 61.02.01 (AACC, 2012). The test was run in duplicate.

2.2.6 Gel hydration properties

Water absorption index (WAI) or swelling capacity and water solubility index (WSI) of different rice flour fractions were determined following the method of Toyokawa, Rubenthaler, Powers, & Schanus (1989), with slight modification, as reported Rosell, Yokoyama, & Shoemaker (2011). Briefly, the flour (50.0 ± 0.1mg) sample was dispersed in 1.0 mL of distilled water in an eppendorf tube using a wire rod and cooked at 90ºC for 10 minutes in a water bath. The cooked paste was cooled in an ice water bath for 10 minutes, and then centrifuged at 3000 × *g* at 4ºC for 10 minutes. The supernatant was decanted into an evaporating dish and the dry solids were recovered by evaporating the supernatant at 105ºC till constant weight. Four replicates were made for each sample. Residues (Wr) and dried supernatants (Ws) were weighed and WAI, WSI, and swelling power (SP) were calculated as follows:

$$\text{WAI (g/g)} = Wr/Wi$$

$$WSI\ (g/100\ g) = (Ws/Wi) \times 100$$

$$SP\ (g/g) = Wr/(Wi - Ws)$$

where Wi is the sample weight (g, db). Values are expressed as the average of three four replicates.

2.2.7 Colour determination of flours

Colour was measured using a Minolta CN-508i spectrophotometer (Minolta, Co. Ltd. Tokyo, Japan) with the D65 standard illuminant and the 2° standard observer. The results are expressed in the CIE L*a*b* colour space. Colour determinations were made five times at two different points on each sample of flour.

2.3 Statistical analysis

Multiple analysis of variance was used to determine the individual effects of the type of enzyme and flour. Fisher's least significant difference (LSD) was used to describe means with 95% confidence intervals. Statistical analysis was performed with Statgraphics Centurion XVI software (Statpoint Technologies, Inc., Warrenton, VA, USA).

3 Results and discussion

3.1 Photomicrographs of flours

With the objective to observe the effect of extrusion on the susceptibility of flour particles to enzymatic hydrolysis by amylase and amyloglucosidase, the microstructure was analysed using ESEM (Figure 1). Native and extruded wheat flour particles not treated with enzymes (Figure 1a and Figure 1d) had a polygonal shape and small superficial irregularities. The fact that significant differences were scarcely observed between them would suggest that the drying process used to halt the enzymatic activity also produced partial gelatinisation of starch granules. However, there were some non-gelatinised starch granules in the native flour, whereas they were not found in the

244 extruded flour, where all starch granules were completely melted during the extrusion
245 treatment. Thus the observed differences cannot be explained only by the drying
246 process but also by the extrusion process.
247 Native flour particles treated by amylase (Figure 1b) appeared disaggregated, disrupted,
248 and pasted to each other, which could be explained by the leaching of some of the
249 amylose, which acted as a gluing material (Dura, Błaszczak & Rosell, 2014).
250 Meanwhile, extruded flour particles (Figure 1e) showed a more amassed structure, with
251 a melting component joining the granules. Chain fragmentation of polymers such as
252 amylose during the extrusion (Chinnaswamy & Hannah, 1990) and the enzymatic
253 processes could lead to leaching of amylose, resulting in greater abundance of gel in the
254 extruded samples.
255 Native flour particles treated by amyloglucosidase (Figure 1c) showed only a superficial
256 corrosion promoted by the enzyme, whereas extruded wheat flour (Figure 1f) was much
257 easier to hydrolase and the particles ended up almost completely disrupted, with a
258 melting component joining the granules. Therefore, a higher susceptibility of extruded
259 wheat flour to enzymatic hydrolysis was observed. Uthumporn, et al. (2012) has already
260 reported that starch molecules compacted inside the starch granules simply cannot be
261 readily accessed by enzymes. Thus, starch gelatinisation during extrusion enhances the
262 chemical reactivity of flour particles towards hydrolytic enzymes.
263 **3.2 Oligosaccharide content of flours**
264 The oligosaccharide content of native and extruded wheat flours was analysed by
265 HPAEC-PAD in order to evaluate the susceptibility of native and extruded wheat flours
266 to enzymatic hydrolysis (Figure 2). Maltotetraose and maltopentaose were not present
267 in any flour, or were under the limits of detection. We observed a three-fold increase of
268 maltose and maltotriose contents with amylase treatment compared with extruded

flours. Glucose content increased with the use of amyloglucosidase, especially in extruded wheat flours (six times higher than its native counterpart).

Vasanthan, et al. (2001) treated barley flours by simultaneous extrusion and hydrolysis and achieved a 25.5% of DP2 (oligosaccharides with a degree of polymerisation of 2). Nevertheless, they used 4% amylase (based on dry weight of flour). In our study, flours with 79.9% maltose were achieved with the use of only 0.2% amylase, and flours with 90.9% glucose were achieved with the use of only 0.2% amyloglucosidase. The starch gelatinisation achieved with the severe extrusion conditions used in our study could have increased the susceptibility of extruded flours to enzymatic hydrolysis, as Martínez et al. (2014) observed in rice flour, and therefore greater production of these oligosaccharides. As was also shown in the previous section, starch gelatinisation during extrusion enhanced the chemical reactivity of flour particles towards hydrolytic enzymes. Meanwhile, the combination of amylase and amyloglucosidase gave rise to flours with a high content of both glucose and maltose, which exceeded the amounts achieved with the use of those enzymes separately. No synergy between amylase and amyloglucosidase was found; only an additive effect was observed.

3.3 X-ray diffractometry (XRD)

The crystalline structures of enzymatically hydrolysed native and extruded wheat starches were observed using XRD. The diffractograms are shown in Figure 3. Less pronounced crystalline peaks were observed in all samples, indicating that the extrusion and especially the drying processes disrupted the native crystalline structures and produced a highly amorphous structure, as has already been reported by several authors (Rumruaytum, Borompichaichartkul, & Kongpensook, 2014; Van der Veen, Veelaert, Van der Goot, & Boom, 2006). All samples showed V-type crystalline peaks at 2θ of around 13 and 20º (Figure 3). The V-type crystalline structure originated from single

294 helical amylose, such as amylose-lipid complexes (Lopez-Rubio, Flanagan, Gilbert, &
295 Gidley, 2008), which could have been created during the drying process at the end of
296 the treatment, since no V-type crystal growth has been reported in wheat starch
297 subjected to annealing conditions (Biliaderis, 2009). The different shapes of the
298 diffractograms could be due to the different mechanisms of actuation of the enzymes
299 used. Nevertheless, when extruded flours were subjected to enzymatic hydrolysis, a B-
300 type crystalline peak at 2θ of around 21º was produced. The voids of this peak can
301 accommodate numerous water molecules (Perez, Baldwin & Gallant, 2009). The more
302 amassed structure seen in enzymatically hydrolysed extruded flours (Figure 1), which
303 was attributed to the leaching of amylose during the extrusion, could have produced this
304 B-type crystalline peak, which was even more intense with the use of amyloglucosidase.

305 **3.4 Pasting characteristics**

306 The effect of the enzymatic and extrusion treatments on the pasting properties of wheat
307 fours is shown in Figure 4. The low viscosities reached during heating and cooling in all
308 samples indicate that both extrusion (Martínez et al., 2014) and drying processes
309 (Rumruaytum, et al., 2014) gelatinised the starch. However, lower values of viscosity
310 were found for extruded wheat flours, which is consistent with previous studies where
311 the lower peak viscosity, the higher the amount of gelatinised and damaged starch
312 (Barres, Verges, Tayeb, & Della Valle, 1990).

313 Considering the effect of the enzymes, native flour treated by amyloglucosidase
314 displayed a higher viscosity than the rest of the enzymatically treated native flours,
315 whereas extruded flour treated by amyloglucosidase showed a lower viscosity than the
316 rest of the enzymatically treated extruded flours. The opposite effects indicate a strong
317 effect of the type of flour (extruded or non-extruded) on its susceptibility to enzymatic
318 hydrolysis by amyloglucosidases, as shown in the micrographs of Figure 1. The

319 reduction in the final viscosity observed for extruded wheat flours could be related to
320 the loss of the ability of the amylose chain to retrograde during cooling, due to its
321 fragmentation during extrusion, an effect that agrees with previous results of Doublier,
322 Colonna, & Mercier (1986). At the same time, this amylose fragmentation made it more
323 susceptible to attack by enzymes, thereby yielding greater oligosaccharide production.

324 **3.5 Gel hydration and colour properties**

325 The individual effects of the type of enzyme and the type of flour (native or extruded)
326 on gel hydration and colour properties of enzymatically treated wheat flours are shown
327 in Table 1. Native flours displayed higher WAI and SP than extruded flours; however,
328 no significant differences were found in WSI between them. Doublier, et al., (1986)
329 already reported a lower SP for extruded wheat starch compared with drum-dried wheat
330 starch, which was attributed to the compact structure of particles achieved during
331 extrusion that could diminish water accessibility. No significant differences were found
332 in WAI and WSI among the different enzymatic treatments. Nevertheless, flours treated
333 by a combination of amylase and amyloglucosidase showed lower values of SP, which
334 could be related to the higher disruption of flour particles, as a consequence of the
335 different mechanisms of actuation of amylase and amyloglucosidase.

336 Regarding colour properties, hydrolysed native flours showed lower luminosity, lower
337 hue, and higher chroma, indicating that after hydrolysis, these flours were darker, more
338 reddish, and had greater colour intensity than extruded flours. The Maillard reaction
339 takes place when reducing sugars such as glucose, amino acids, especially lysine, and
340 proteins are heated together (Camire , et al., 1990), whereas caramelisation is a term for
341 describing a complex group of reactions that occur due to direct heating of
342 carbohydrates, particularly reducing sugars (Pathare, Opara, & Al-Said, 2013). These
343 reactions produce high molecular weight coloured compounds, principally

hydroxymethylfurfural and melanoidins (Cho & Peterson, 2010; Purlis, 2010). Severe extrusion conditions can lead to a decrease in lysine content (Camire , et al., 1990). The more reactive the amino acid, the lower the production of hydroxymethylfurfural and melanoidins. Thus, extruded flours are clearer than native flours.

Meanwhile, flours submitted to enzymatic hydrolysis by amyloglucosidase and a combination of amylase and amyloglucosidase showed a dark, reddish, and intense colour. As shown in a previous section of this study and as reported by van der Maarel, et al. (2002), amyloglucosidase releases β-D-glucose residues. Glucose, besides being one of the main reactants that participate in Maillard and caramelisation thermal reactions that occur high-temperature processes such as baking, drying, and frying (Pathare, et al., 2013), is more reactive than other disaccharides such as maltose (Ameur, Mathieu, Lalanne, Trystram, & Birlouez-Aragon, 2007), producing darker flours.

4 Conclusions

The new demands of the food industry are forcing manufacturers of starchy ingredients to develop flours with different functionalities, such as higher sweetness and higher content of natural sugars making the label cleaner than chemically modified starches. This study showed changes in the microstructure, oligosaccharide profile, crystalline order, pasting, colour, and gel hydration properties of wheat flours subjected to extrusion and enzymatic treatment by α-amylase, amyloglucosidase, or a combination of both. The results suggest that starch gelatinisation of flour by extrusion increases its susceptibility to enzymatic hydrolysis, thereby achieving flours with a greater content of glucose and maltose, substrates for fermentative microflora and the main reactants that participate in Maillard and caramelisation thermal reactions. In general, enzymatic hydrolysis of extruded wheat flours offers an interesting way to achieve flours with

different functionalities, which could be of interest for different applications in the food industry, on which later works should deep.

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References

AACC. (2012). Approved methods of the American Association of Cereal Chemists, (11th ed.). St. Paul: American Association of Cereal Chemists.

Ameur, L., Mathieu, O., Lalanne, V., Trystram, G., & Birlouez-Aragon, I. (2007). Comparison of the effects of sucrose and hexose on furfural formation and browning in cookies baked at different temperatures. Food Chemistry, 101(4), 1407-1416.

Ba, K., Blecker, C., Danthine, S., Tine, E., Destain, J., & Thonart, P. (2013). Physicochemical characterization of dextrins prepared with amylases from sorghum malt. Starch-Starke, 65(11-12), 962-968.

Baks, T., Kappen, F., Janssen, A., & Boom, R. (2008). Towards an optimal process for gelatinisation and hydrolysis of highly concentrated starch-water mixtures with alpha-amylase from B. licheniformis. Journal of Cereal Science, 47(2), 214-225.

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. G. (2009). Structural transitions and related physical properties of starch. In J. BeMiller & R. Whistler (Eds.), Starch. Chemistry and Technology, (pp. 293-375). New York. USA: Academic Press.

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.

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 J. BeMiller & R. Whistler (Eds.), Starch. Chemistry and Technology, (pp. 629-656). New York. USA: Academic Press.

401 Cho, I., & Peterson, D. (2010). Chemistry of Bread Aroma: A Review. Food Science
402 and Biotechnology, 19(3), 575-582.
403 Doublier, J. L., Colonna, P., & Mercier, C. (1986). Extrusion cooking and drum drying
404 of wheat-starch. 2. Rheological characterization of starch pastes. Cereal Chemistry, 63,
405 240-246.
406 Dura, A., Błaszczak, W., & Rosell, C.M. (2014). Functionality of porous starch
407 obtained by amylase or amyloglucosidase treatments. Carbohydrate Polymers, 101,
408 837-845.
409 Govindasamy, S., Campanella, O., & Oates, C. (1997a). Enzymatic hydrolysis of sago
410 starch in a twin-screw extruder. Journal of Food Engineering, 32(4), 403-426.
411 Govindasamy, S., Campanella, O., & Oates, C. (1997b). The single screw extruder as a
412 bioreactor for sago starch hydrolysis. Food Chemistry, 60(1), 1-11.
413 Lee, Y., & Kim, K. (1990). Gelatinization and liquefaction of starch with a heat-stable
414 alpha-amylase. Journal of Food Science, 55(5), 1365-1366.
415 Lopez-Rubio, A., Flanagan, B., Gilbert, E., & Gidley, M. (2008). A novel approach for
416 calculating starch crystallinity and its correlation with double helix content: A combined
417 XRD and NMR study. Biopolymers, 89(9), 761-768.
418 Maningat, C. C., Seib, P. A., Bassi, S. D., Woo, K. S. & Lasater, G. D. (2009). Wheat
419 starch: Production, Properties, Modification and uses. in: BeMiller, J., Whistler, R.
420 (Eds.), Starch. Chemistry and Technology. Academic Press, New York. USA., pp. 441-
421 510.
422 Martínez, M. M., Calviño, A., Rosell, C. M., & Gómez, M. (2014). Effect of different
423 extrusion treatments and particle size distribution on the physicochemical properties of
424 rice flour. Food and Bioprocess Technology, DOI: 10.1007/s11947-014-1252-7.

425 Pathare, P., Opara, U., & Al-Said, F. (2013). Colour Measurement and Analysis in
426 Fresh and Processed Foods: A Review. Food and Bioprocess Technology, 6(1), 36-60.
427 Perez, S., Baldwin, P. M., & Gallant, D. J. (2009). Structural features of starch granules
428 I. In J. BeMiller, & R. Whistler (Eds.), Starch: Chemistry and technology. (pp 149-188).
429 New York. USA: Academic Press.
430 Purlis, E. (2010). Browning development in bakery products - A review. Journal of
431 Food Engineering, 99(3), 239-249.
432 Rosell, C., Yokoyama, W., & Shoemaker, C. (2011). Rheology of different
433 hydrocolloids-rice starch blends. Effect of successive heating-cooling cycles.
434 Carbohydrate Polymers, 84(1), 373-382.
435 Rumruaytum, P., Borompichaichartkul, C., & Kongpensook, V. (2014). Effect of drying
436 involving fluidisation in superheated steam on physicochemical and antioxidant
437 properties of Thai native rice cultivars. Journal of Food Engineering, 123, 143-147.
438 Toyokawa, H., Rubenthaler, G., Powers, J., & Schanus, E. (1989). Japanese noodle
439 qualities .1. Flour components. Cereal Chemistry, 66(5), 382-386.
440 Uthumporn, U., Shariffa, Y., & Karim, A. (2012). Hydrolysis of Native and Heat-
441 Treated Starches at Sub-Gelatinization Temperature Using Granular Starch Hydrolyzing
442 Enzyme. Applied Biochemistry and Biotechnology, 166(5), 1167-1182.
443 van der Maarel, M., van der Veen, B., Uitdehaag, J., Leemhuis, H., & Dijkhuizen, L.
444 (2002). Properties and applications of starch-converting enzymes of the alpha-amylase
445 family. Journal of Biotechnology, 94(2), 137-155.
446 Van der Veen, M., Veelaert, S., Van der Goot, A., & Boom, R. (2006). Starch
447 hydrolysis under low water conditions: A conceptual process design. Journal of Food
448 Engineering, 75(2), 178-186.

449 Vasanthan, T., Yeung, J., & Hoover, R. (2001). Dextrinization of starch in barley flours
450 with thermostable alpha-amylase by extrusion cooking. Starch-Starke, 53(12), 616-622.
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Figure captions

Figure 1. Scanning electron micrographs (2000× magnification) of native and extruded flours. Native wheat flour without enzyme treatment (a), native wheat flour treated with amylase (b), native wheat flour treated with amyloglucosidase (c), extruded wheat flour without enzyme treatment (d), extruded wheat flour treated with amylase (e), and extruded wheat flour treated with amyloglucosidase (f). The white circle indicates non-gelatinised starch granules.

Figure 2. Oligosaccharide content (g/kg) of enzymatically treated native and extruded flours, measured by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD). Glucose (black column), isomaltose (dark grey column), maltose (clear grey column), and maltotreose (white column). AM, Amylase; AMG, amyloglucosidase.

Figure 3. X-ray diffractograms of native (A) and extruded (B) wheat flours. Native wheat flour without enzyme treatment (a), native wheat flour treated with amylase (b), native wheat flour treated with amyloglucosidase (c), and native wheat flour treated with amylase and amyloglucosidase (d). Extruded wheat flour without enzyme treatment (e), native wheat flour treated with amylase (f), native wheat flour treated with amyloglucosidase (g), and native wheat flour treated with amylase and amyloglucosidase (h).

Figure 4. Effect of extrusion and enzymatic treatments on the pasting properties of native (a) and extruded (b) wheat flours. Flour without enzyme treatment (black line), flour treated with amylase (discontinuous black line), flour treated with amyloglucosidase (dark grey line), and flour treated with amylase and amyloglucosidase (discontinuous dark grey line). Temperature profile (discontinuous points).

479 Table 1: Effects of the type of enzyme and flour on hydration and colour properties

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			Enzyme			Flour	
	Media	SE	AM and AMG	Amyloglucosidase	Amylase	Native	Extruded
WAI (g/g)	4.14	1.51	3.64a	4.46a	4.30a	5.42b	2.85a
WSI (g/100 g)	4149	78	4140a	4121a	4185a	4104a	4194a
SP (g/g)	6.30	1.01	5.68a	6.83b	6.39b	7.05b	5.54a
OAC (g/g)	1.78	0.11	1.74a	1.83a	1.77a	1.76a	1.80a
*L**	73.60	5.77	70.32a	70.81a	79.68b	70.86a	76.35b
Hue	1.25	0.09	1.23b	1.19a	1.35c	1.23a	1.28b
Chroma	22.25	3.74	25.06c	23.41b	18.29a	24.34b	20.16a

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482 Values followed by different letters within each parameter for each factor (enzyme and
483 flour) indicate significant differences.

484 WAI, water absortion index, WSI, water solubility index, SP, swelling power, OAC, oil
485 absortion capacity, *L**, luminosity, SE, Standard deviation, AM, Amylase, AMG,
486 Amyloglucosidase

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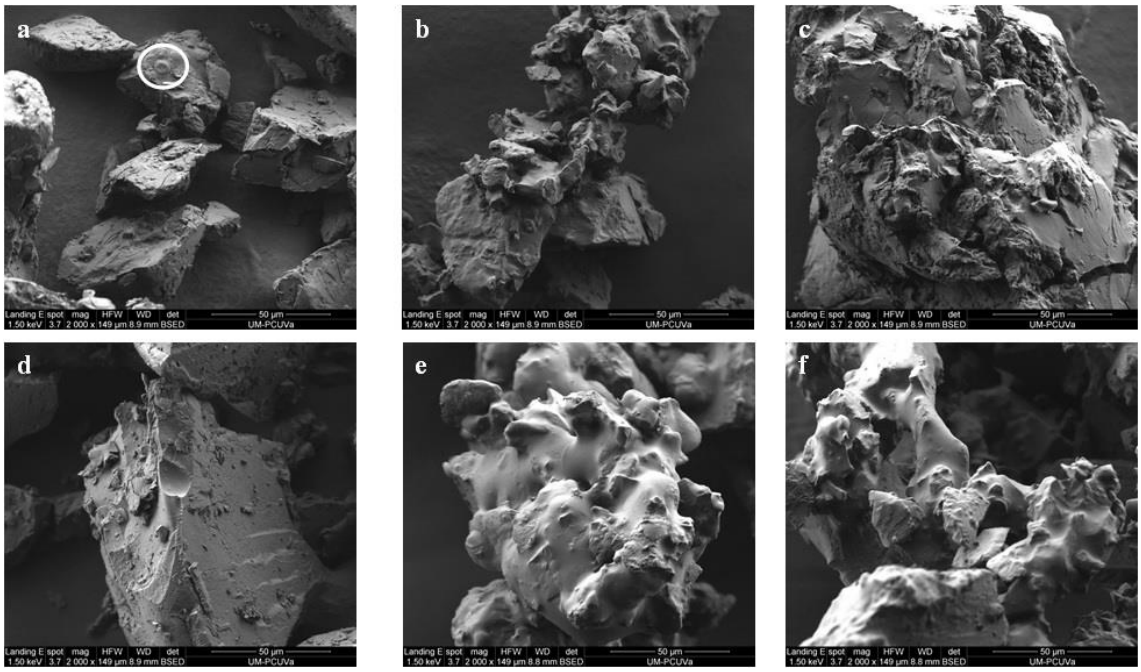
488 Figure 1



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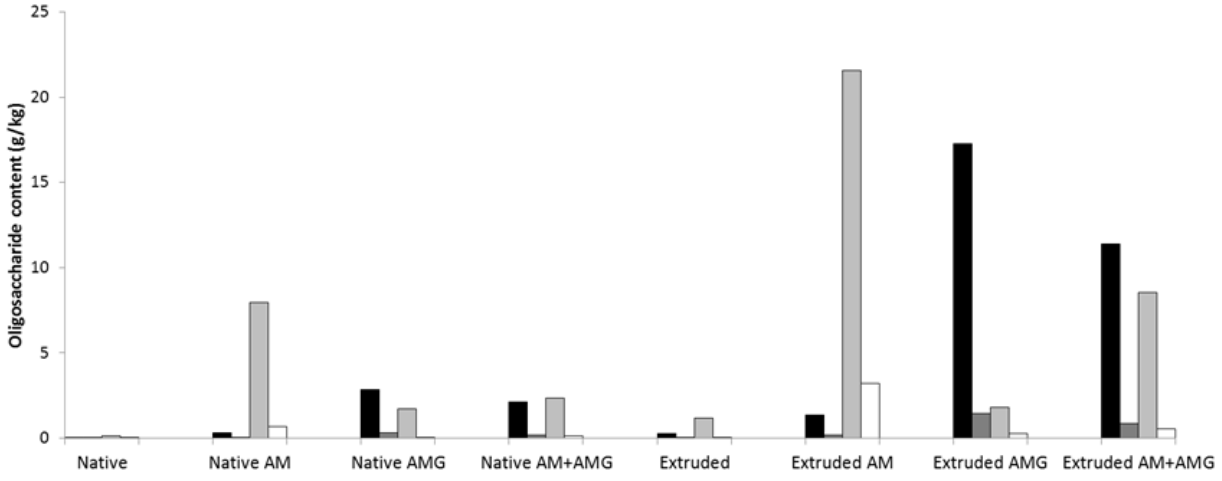
491 Figure 2



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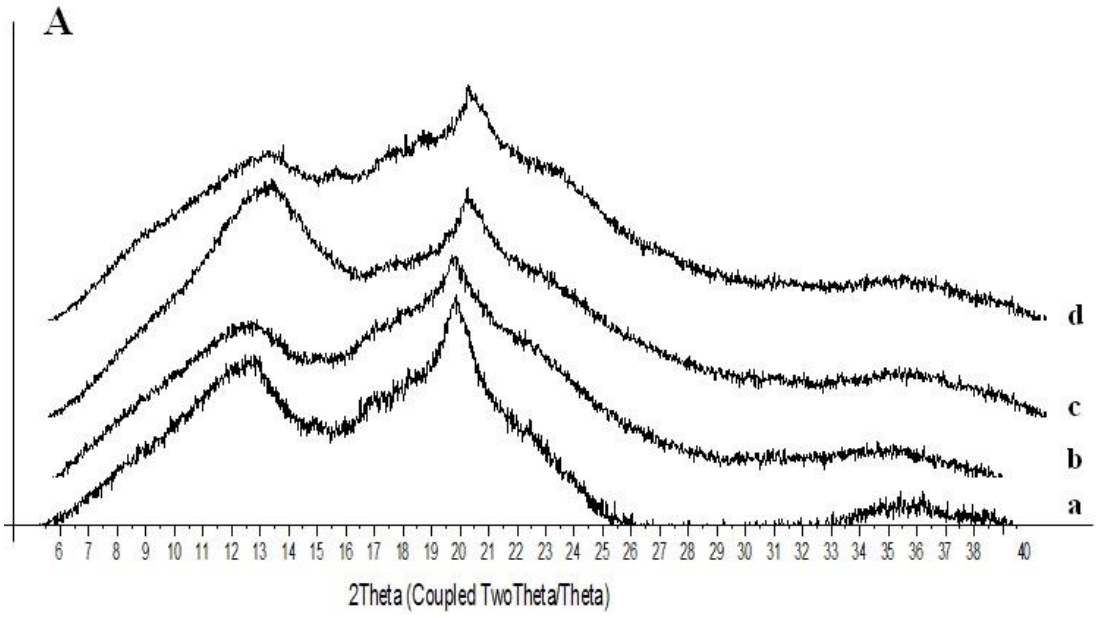
494 Figure 3



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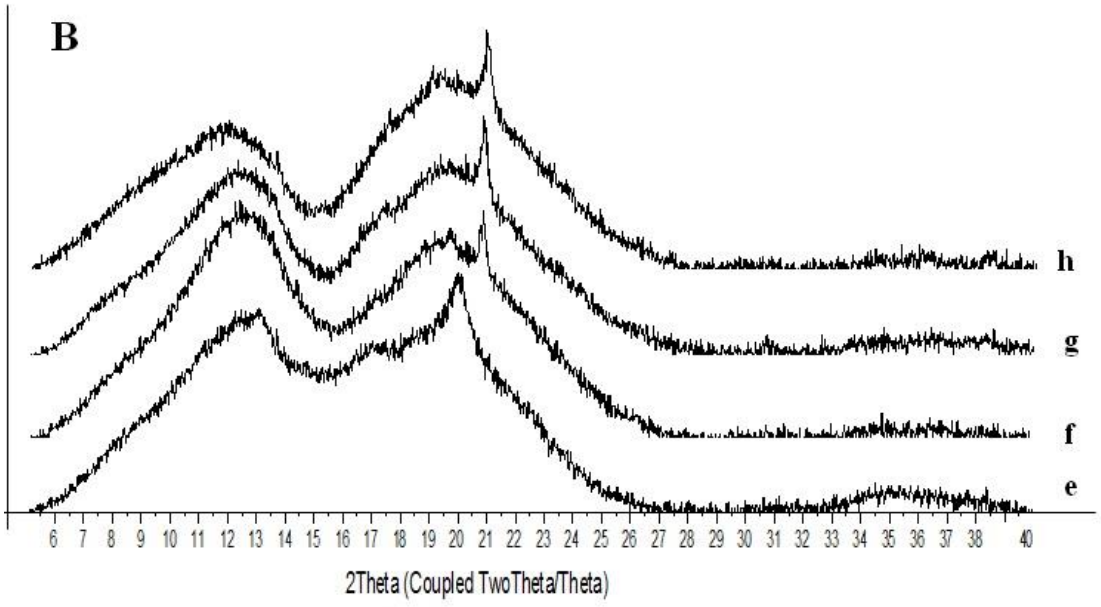

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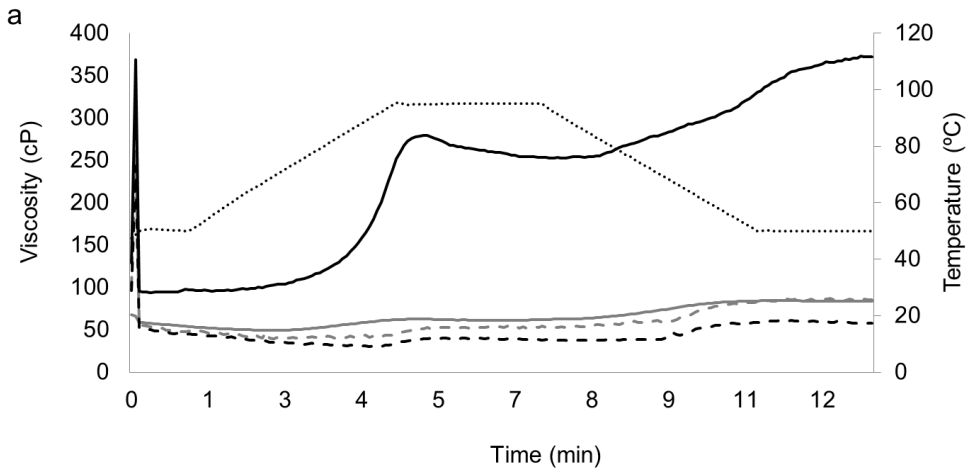
498 Figure 4



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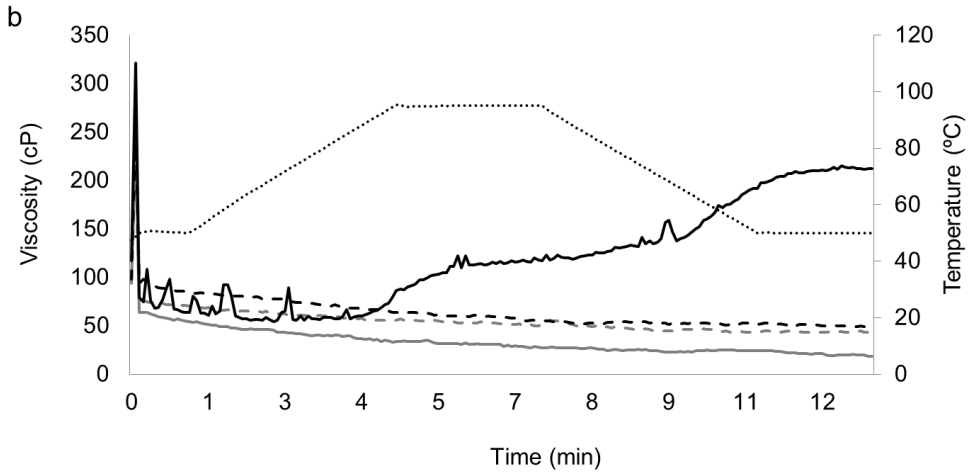

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