FUNCIONALITY OF POROUS STARCH OBTAINED BY AMYLASE OR AMYLOGLUCOSIDASE TREATMENTS

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Running title: Enzymatically treated corn starches

Abstract

Porous starch is attracting very much attention for its absorption and shielding ability in many food applications. The effect of two different enzymes, fungal α-amylase (AM) or amyloglucosidase (AMG), on corn starch at sub-gelatinization temperature was studied as an alternative to obtain porous starch. Biochemical features, thermal and structural analyses of treated starches were studied. Microscopic analysis of the granules confirmed the enzymatic modification of the starches obtaining porous structures with more agglomerates in the case of AMG treated starches. Several changes in thermal properties and hydrolysis kinetics were observed in enzymatically modified starches. Hydration properties were significantly affected by enzymatic modification being greater influenced by AMG activity, and the opposite trend was observed in the pasting properties. Overall, results showed that enzymatic modification at sub-gelatinization temperatures really offer an attractive alternative for obtaining porous starch granules to be used in a variety of foods applications.

Highlights

- Enzymatic treatment at sub-gelatinization temperature led to porous starch
- α-amylase or amyloglucosidase action on corn starch was studied
- Pasting and thermal properties of porous starch depended on the enzyme used
- Porous starch was more susceptible to enzymatic digestion

Keywords: corn starch; enzymatic modification; hydration properties; SEM; DSC; hydrolysis kinetics.

1. Introduction

Starch is widely used in food and industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent and water retention agent (Singh, Kaur & McCarthy, 2007). In general, native starches produce weak-bodied, cohesive, rubbery pastes when heated and undesirable gels when the pastes are cooled (Abbas, Khalil & Hussin, 2010). In order to meet its intended function, physical, chemical or enzymatic modifications are applied to achieve the functional properties not found in native starches (Jayakody & Hoover, 2002; Lacerda et al., 2008). The use of enzymatic modification has a number of advantages comprising fewer by-products, more specific hydrolysis products and high yield, besides better control of the process and end products with particular properties. There are many enzymes used to alter starch structure and to achieve desired functionality (Rosell & Collar, 2008). Enzymes hydrolyze (1→4) or (1→6) linkages between α-D glucopyranosyl residues. The most common enzymes for starch modification include α-amylase, β-amylase, glucoamylase, pullulanase, and isoamylase. α-Amylase can hydrolyze the (1→4)- α-glucosidic bonds of starch in an endo-action. Hydrolysis occurs in a random fashion at any (1→4)-linkage within the starch chain to rapidly reduce the molecular size of starch and the viscosity of the starch solution during pasting. Amyloglucosidase is an exo-acting enzyme that catalyzes the hydrolysis of both α-D-(1→4) and α-D-(1→6)-linkages from the non-reducing ends of the starch chain. Numerous researchers have investigated enzymatic hydrolysis of starches from cereals, roots, tubers, and legumes in terms of enzyme adsorption, action pattern, extent of hydrolysis, degree of crystallinity and hydrolysis products (Colonna, Leloup, & Buleon, 1992; Gallant, Bouchet, Buleon, & Perez, 1992; Hoover, 2001; Kimura & Robyt, 1996; Li, Vasanthan, Hoover, & Rossnagel, 2004). Taking into particular account that corn starch makes up more than 80% of the world market for starch, many researchers have been focused

on the hydrolysis products released from the enzymatic reaction (Huang et al., 2010; Khatoon, Sreerama, Raghavendra, Bhattacharya, & Bhat, 2009; Li & Ma, 2011; Miao, Zhang, Mu, & Jiang, 2011). Therefore, kinetics mechanism of the enzymatic reaction and hydrolysis products have been studied by other researchers but the features of the modified starches have been the focus of a few studies. Simultaneous hydrolysis of waxy corn starch with amylase and amyloglucosidase have been reported for modifying the digestion of the starch (Miao et al., 2011), and reducing the digestibility of corn starch by partial α-amylase treatment (Han, Ao, Janaswamy, Jane, Chandrasekaran, & Hamaker, 2006), or through the action of β-amylase or maltogenic α-amylase with transglucosidase (Ao et al., 2007). In addition, Lacerda et al. (2008) studied the thermal properties of corn starch treated with fungal α-amylase showing higher action on the amorphous region of the granule.

Lately, there is an increasing interest for the developing corn porous starch due to it has interesting properties for being used in the areas of food, medicine, chemical industry, cosmetics, agriculture and other fields. In fact, porous starch might be used in foods to ensure a steady release of spices, sweeteners, acid condiments, flavorings, or even to protect from light or oxygen highly oxidized compounds (Zhang, Cui, Liu, Gong, Huang, & Han, 2012). Porous starch is a modified starch that contains microsized pores on the surface and could be extended to the inner part of the granule. Previously, it has been reported its production by glucoamylase catalysis combined with ultrasonic treatment (Wu, Du, Ge, & Lv, 2011) and more recently Zhang et al. (2012) proposed the combination of α-amylase and glucoamylase optimising the kinetic reaction for increasing the yield. Nevertheless, there is no information about the contribution of each enzyme to the starch changes. Because of modified starch is widely used in food formulations, it is of particular interest to determine biochemical features of starch and how they affect its functional properties. The aim of this research was to

determine the independent effect of fungal α-amylase (AM) or amyloglucosidase (AMG) on corn starch at sub-gelatinization temperature, with special emphasis on biochemical features, thermal and structural analyses of treated starches.

2. Materials and methods

2.1. Materials

Corn starch samples were generously supplied by Huici Leidan (Navarra, Spain). The enzymes used were of food grade. Fungal α-amylase (AM) (Fungamyl 2500 SG) and amyloglucosidase (AMG) (Amyloglucosidase 1100L) were provided by Novozymes (Bagsværd, Denmark). Chemical reagents from Sigma-Aldrich (Madrid, Spain) were of analytical grade.

2.2. Methods

2.2.1. Sample preparation

Preliminary assays were carried out for optimizing enzymatic reactions (starch quantity and pH), and pH 4.0 was selected for AMG reaction and pH 6.0 in the case of AM modification. The quantity of enzymes was based on previous experiments, where the amount of enzyme required to hydrolyze 50% of the starch (15%, w/v) at 95ºC for 10 min was selected (Dura, Calviño, Blaszczak & Rosell, 2013).

Corn starch (5.0 g) was suspended in 25 mL of 20mM NaH_2PO_4 buffer at pH 6.0 or in sodium acetate buffer at pH 4.0, those starch samples were referred as control-6 or control-4, respectively. For obtaining the enzymatic treated starches, enzymes (4 U of AMG /g starch and 5 U of AM /g starch) were added to the starch suspension. Samples were kept in a shaking water bath (50 rpm) at 50 ºC for 24 hours. Then, 50 mL of water were added and suspensions were homogenized with a Polytron Ultraturrax homogenizer IKA-T18 (IKA

works, Wilmington, USA) during 1min at speed 3. Samples were centrifuged for 15 min at 7,000×*g* and 4 ºC. Starches were washed again and centrifuged at the same conditions as before. Supernatants were pooled together and boiled in a water bath for 10 min to inactivate the enzymes before any further analyses (hydration properties and iodine binding values). Sediments containing starch were freeze-dried and kept at -25 ºC for further thermal, biochemical and microstructural analyses. Four batches were prepared for each treatment.

2.2.2. Scanning electron microscopy (SEM)

The structural properties of the samples were studied using a JSM 5200 scanning electron microscope (JEOL, Tokyo, Japan). The corn starch powders were stick on a specimen holder using cuprum tape, and then coated with gold in a vacuum evaporator (JEE 400, JEOL, Tokyo, Japan). The obtained specimens were examined at an accelerating voltage of 10 kV.

The size of the holes induced by enzymatic action was measured using the image analysis program (Image J, UTHSCSA Image Tool software). Value was the average of 10 independent measurements. Eccentricity (e) was calculated for starch granules according to the equation 1:

$e = [1-(b^2/a^2)] \times 1/2$ Eq. (1)

where *a* corresponded to the larger diameter (length) and *b* to the shorter diameter (width) (Rojas, Rosell, Benedito de Barber, Pérez-Munuera & Lluchet, 2000). Values of eccentricity approaching 0 indicate very rounded structures, while values approaching 1 describe very elongated structures.

2.2.3. Starch content

Remaining free sugars on the treated starches were determined. Treated starch (0.1 g) placed in 10 mL Pyrex tubes was suspended in 2 mL of ethanol (80%) and incubated at 85 ºC in a shaking water bath (50 rpm) for five min and then centrifuged (1,000×*g*, 10 min, at room temperature). Supernatant was separated to measured free sugars released. This was performed for two times.

Damage starch was also determined by enzymatic method following the International Association for Cereal Chemistry standard method (ICC, 1996). The absorbance was measured using an Epoch microplate reader (Biotek Instruments, Winooski, USA) at 510 nm. Starch was calculated as glucose (mg) ×0.9. Replicates (n=4) were carried out for each determination.

2.2.4. Starch hydration properties and iodine binding values

Swelling parameters and water soluble compounds of modified corn starch samples were determined following the method reported by (Toyokawa, Rubenthaler, Powers, & Schanus, 1989), with slight modification as reported (Rosell, Yokoyama, & Shoemaker, 2011). Briefly, the supernatant was decanted into an evaporating dish and the weight of dry solids was recovered by evaporating the supernatant at 70 ºC till constant weight. Four replicates were made for each sample. Residues (Wr) and dried supernatants (Ws) were weighed. Swelling power (SP), solubility index (SI) and swelling capacity (SC) were calculated as follows:

Swelling Capacity (g/g)=*Wr*/*Wi* Eq. (2)

Solubility Index (%)=(*Ws*/*Wi*) x 100 Eq. (3)

Swelling Power (g/g)=*Wr/Wi* (100-*SI*) Eq. (4)

where *Wi* was the sample weight (g, db).

Iodine binding values are indicative of amylose complex formation. The iodine binding value was determined in the soluble supernatant. The soluble supernatant (40 µL) was mixed with 2 mL of an aqueous solution of 0.2% KI and 0.65% I_2. The absorbance at 690 nm was measured using a spectrophotometer (UV mini-1240, Shimadzu Corporation, Kyoto, Japan). Paste Clarity was directly measured in the supernatant as the absorbance at 650 nm using a spectrophotometer (UV mini-1240, Shimadzu Corporation, Kyoto, Japan). Values were the average from four replicates.

2.2.5. Pasting properties

The pasting properties were determined with rapid visco analyser (RVA) (Newport Scientific, model 4-SA, Warriewood, Australia) by following the American Association of Cereal Chemists Approved Method (AACC International, 1997), with some minor modifications. Distilled water (25 mL) was added to two grams of corn starches placed into the aluminium RVA canister. RVA settings during assessment were: heating from 50 to 95 ºC in 282 s, holding at 95 ºC for 150 s and then cooling to 50 ºC. Each cycle was initiated by a 10 s, 960 rpm paddle speed for mixing followed by a 160 rpm paddle speed for the rest of the assay. Viscosity was recorded during a heating-cooling cycle using Thermocline software for Windows (Newport Scientific Pty. Limited, Warriewood, Australia).

2.2.6. Thermal Properties

Thermal behavior from starch samples were determined using a differential scanning calorimeter (DSC) from Perkin–Elmer (DSC 7, Perkin–Elmer Instruments, Norwalk, CT), equipped with a thermal analysis data station (Pyris software, Perkin–Elmer Instruments, Norwalk, CT). For the study, corn starch samples were accurately weighed into aluminum

DSC pans, and de-ionized water was added by micropipette to achieve a water–sample ratio of 2:1. The sample pans were sealed and equilibrated at room temperature for one hour before analysis. Nitrogen was used to purge analyses cells. Instruments were calibrated with indium, using an empty pan as reference. Thermal analysis consisted on heating from 30 to 120 °C at a rate of 10 °C/min. The onset temperature T_o, peak temperature T_p, and conclusion temperature T_c, were determined from the heating DSC curves. Gelatinization enthalpy (ΔH) was evaluated based on the area of the main endothermic peak, and peak height index (PHI) was calculated as PHI= $\Delta H/ T_p - T_o$. All DSC experiments were replicated two times.

2.2.7. Starch hydrolysis kinetics

Starch hydrolysis was measured following the method described by (Gularte & Rosell, 2011) with minor modifications. Briefly, for free sugars removal, corn starch sample (0.1 g) suspended in two milliliters of 80% ethanol was kept in a shaking water bath at 85 ºC for five minutes, and then centrifuged for 10 min at 1000×*g*. The remaining pellet was incubated with porcine pancreatic α-amylase (6 U/ml) (Type VI-B, ≥10 units/mg solid, Sigma Chemical, St. Louis, USA) in 10 mL of 0.1M sodium maleate buffer (pH 6.9) in a shaking water bath at 37 ºC. Aliquots of 200 µL were withdrawn during the incubation period and mixed with 200 µL of ethanol (96%, w/w) to stop the enzymatic reaction and the sample was centrifuged at 10,000×*g* for 5 min at 4 ºC. The precipitate was washed twice with 50% ethanol (200 µl) and the supernatants were pooled together and kept at 4 ºC for further glucose enzymatic release. Supernatant (100 µL) was diluted with 850 µl of 0.1M sodium acetate buffer (pH 4.5) and incubated with 50 µL amyloglucosidase (33 U/mL) at 50 ºC for 30 min in a shaking water bath. After centrifuging at 2,000×*g* for 10 min, supernatant was kept for glucose determination.

213 The glucose content was measured using a glucose oxidase-peroxidase (GOPOD) kit
214 (Megazyme, Dublin, Ireland). The absorbance was measured using an Epoch microplate
215 reader (Biotek Instruments, Winooski, USA) at 510 nm. Starch was calculated as glucose
216 (mg) ×0.9. Replicates (n=4) were carried out for each determination.
217
218 Experimental data were fitted to a first-order equation (Goñi et al., 1997):
219 $C_t = C_\infty (1\text{-}e^{-kt})$ Eq. 5
220 Where C_t is the concentration of product at time t, C_∞ is the concentration at the end point,
221 and k is the pseudo-first order rate constant.
222
223 *2.2.8. Statistical analysis*
224 Experimental data were statistically analyzed for analysis of variance (ANOVA) using
225 Statgraphics Centurion XV software (Bitstream, Cambridge, N). When analysis of variance
226 indicated significant *F* values, multiple sample comparisons were also performed by Fisher's
227 least significant differences (LSD) test to differentiate means with 95% confidence. The
228 correlation matrix was also performed using Statgraphics Centurion XV software.
229
230 **3. Results and discussion**
231 Enzymatic modification of the corn starch was carried out independently with α-amylase or
232 amyloglucosidase under optimal pH conditions 6.0 for α-amylase and 4.0 for
233 amyloglucosidase. The action of each enzyme was compared with their specific control,
234 which was submitted to the same treatment in the absence of enzymes. Results will reflect the
235 effect of the pH and the enzymes on the starch features after being treated at sub-
236 gelatinization temperature (50 ºC). Zhang et al. (2012) found that 50 ºC was the most suitable
237 temperature for obtaining porous starch combining the action of AM and AMG. Also that

238 temperature was useful for keeping the hydrolysis degree under control impeding the collapse
239 and breakage of the granules that is produced during gelatinization.
240
241 *3.1. Microstructure of the starch*
242 To confirm the enzymatic action on the corn starch granules treated with α-amylase or
243 amyloglucosidase, the microstructure was analyzed using SEM at two different
244 magnifications 2000x (Figure 1) and 3500x (Figure 2) times. Corn starches without enzymatic
245 treatment (Figure 1 a,c; Figure 2 a,c) showed that pH did not affect the morphology of starch
246 granules. Granules appeared with round and polygonal granular shape, and their surface was
247 relatively smooth with some hollows, attributed to the fingerprints of the native protein
248 bodies. Huber & BeMiller (2000) explained that the crater-like impression resulted from
249 pressing of protein bodies into the soft endosperm of the growing kernel. At high
250 magnification some tiny cracks were visible. No swelling was observed in the native starch
251 and the starch soaked at 50 ºC for 24 hours (results not shown). At high magnification (Figure
252 2) it was observed that certain granules appeared pasted to each other, which was explained
253 by the effect of some amylose leached out that acted as gluing material. No holes were visible
254 on the control starches, indicating the absence of endogenous enzymes during the treatment
255 period. Rocha, Felizardo, Jane & Franco (2012) found that some holes could appear on the
256 starch surface due to endogenous enzymes action during annealing.
257
258 The effect of enzymatic treatment was readily visible in the starches microstructure (Figure 1
259 b, d; Figure 2 b, d). Enzymatically treated starches showed changes in the surface but the
260 shape of the granule hardly change. The microstructure of the starch granules was greatly
261 dependent on the enzymatic treatment, but in both cases porous starch granules were
262 obtained. α-Amylase produced some holes on the starch granules and its action was not

dependent on the granule size. Amyloglucosidase was greatly active on the starch granules obtaining very perforated granules. At high magnification (Figure 2 b) different concentric layers could be distinguished within the holes. Huber et al. (2000) described that in corn and sorghum starch granules, AMG produced openings to channels for providing access to the granule interior, and the surface pores enlarge through channels from hilum region toward the outsides. In the present study, it seems that both enzymes attacked the starch granules on the fingerprint of protein bodies, which seems to be the weaker points more susceptible to enzymatic hydrolysis. In addition, AMG treated starches displayed a more stacked structure, with a kind of gel joining the granules. Likely, the polymers' chains leached out more easily during AMG enzymatic incubation and resulted in more abundant gel in the treated starch granules. Alternatively, it could be that the hydrolyzed amylose chains that leached out to the periphery of the granules were readily able to crystallize after cooling yielding the jelly structure.

The analysis of the size of the holes revealed that α-amylase treatment led to small holes of 0.15μm ± 0.04μm, whereas amyloglucosidase action resulted in bigger holes (1.72μm ± 0.18μm) and the eccentricity ranged from 0.10 to 0.13, indicating very rounded pores in both cases.

It is generally accepted that granules contain amorphous and crystalline domains arranged in alternating concentric rings that create a semicrystalline environment within the granule (Ratnayake & Jackson, 2008), which were only evident on the AMG treated starch granules. Considering that the crystalline domains are mainly composed of amylopectin while bulk amorphous domains are made up of amylose traversed by non-crystalline regions of amylopectin, it might be expected that treatment at 50 ºC promotes changes in the amorphous areas of granules (Ratnayake et al., 2008), leading to more structured internal structure.

3.2. Assessing the enzymatic treatment of the corn starch

After the enzymatic treatment, some information about the released compounds was gathered to confirm again that the enzymatic modifications occurred on the starch granules. Because of that, paste clarity, solubility index, swelling power, and apparent amylose content were measured (Table 1). Considering the values obtained for the control at pH 4.0 and 6.0, it was observed that pH did not significantly affect those parameters. Paste clarity, related to the compounds leached out during enzymatic treatment, was only increased with the AMG treatment, which can be explained by the excision activity of this enzyme that hydrolyze α-1,4 and α-1,6 glycosidic linkages from non-reduced extremes releasing free sugars. Therefore, it was expected relatively higher amount of residual free sugars. In fact, the solubility index value was significantly (P<0.05) enhanced by AMG, followed by AM. Solubility index represents the amount of water soluble products obtained from the treated starch. AMG acted breaking the degree of association between intermolecular bonds (Dura et al., 2013), and more soluble compounds were leached. In opposition, it seems that the hydrolysis products released from α-amylase action were less soluble in water. Amylose that leached to the supernatant during incubation and enzymatic treatment was evaluated, but neither the pH nor the enzymatic modification of corn starch did cause changes in the apparent amylose content of the starches. These results indicated that the amount of leached amylose in the supernatant after thermal treatment at 50 ºC was insignificant, and that gelatinization of the starch granules did not occur during the process, which agrees with previous results (Rocha et al., 2012). Taking into account that no differences were observed on the apparent amylose content, thus water soluble compounds but no amylose chains were released during enzymatic treatment.

Swelling power slightly increased in enzymatically modified samples from control samples. Swelling power and solubility can provide evidence in assessing the extent of interaction

313 between starch chains within the amorphous and crystalline domains of the starch granule (Li,
314 Shu, Zhang, & Shen, 2011; Ratnayake, Hoover, & Warkentin, 2002).
315
316 The enzymatically treated starches were washed to remove the remnant of any hydrolysis
317 product and the swelling capacity of the porous starch granules as well as the amount of
318 damage starch was determined (Table 1). The amount of free sugars was also quantified in the
319 starch granule samples in order to verify the absence of hydrolysis products. The level of free
320 sugars in the samples was very low (<1%). Thus, the washing carried out to remove the
321 released products after hydrolysis was effective, although AMG treated starch had more
322 residual sugars than its respective control. Damage starch content did not differ among
323 samples. Presumably, enzyme treatment modified the structure of corn starch enough to
324 permit the water intake but no the rapid enzyme absorption and penetration into the starch
325 structure, required in the measurement procedure of damage starch. According to (Biliaderis,
326 1991), the crystalline areas of starch maintain the structure of the granule, control its behavior
327 in the presence of water and make it more or less resistant to chemical and enzymatic attack.
328 The amorphous zone of starch granules is the least dense, is more susceptible to enzymatic
329 attacks and absorbs more water at temperatures below the gelatinization temperature
330 (Zavareze & Dias, 2011).
331 Starch granule swelling is known to begin in the bulk relatively mobile amorphous fraction
332 and, in the more restrained amorphous regions immediately adjacent to the crystalline region
333 (Donovan, 1979). Factors such as temperature, pH and type of enzyme can greatly influence
334 the starch structure. When starch granules are heated in the presence of water, the starch
335 granules absorb water and swell but since the enzymatic reaction occurs at 50ºC, below
336 gelatinization temperature, the water absorption was rather low. Nonetheless, the porous
337 structures obtained by enzymatic treatments showed opposite behavior pertaining swelling

capacity, AMG reduced the swelling capacity of the starch granules, in contrast with the increase obtained in the AM treated starches. During annealing, molecules in the amorphous regions of the granule hydrate and increase the mobility of amorphous area, and cause a limited but reversible swelling of the granule (Perry & Donald, 2000). The annealing results in a more perfectly ordered structure and in an increase in the granule stability by improving the arrangement of double helices, as well as the perfection of starch crystallites (Jayakody & Hoover, 2008). Plausible explanations could be either the enzyme action on the granule surface seems to release the more accessible compounds that bind water molecules or the agglomerates structure reduces the surface area that might be in contact with water molecules.

3.3. Pasting properties

The pasting properties of the porous starches were determined following a heating-cooling cycle (Figure 3). Plots of the pasting portraits showed minor dissimilarities than expected taking into account the great microstructure divergences earlier mentioned. The viscosities of the control starches subjected to different pHs were initially overlapped indicating similar absorption. This agrees with reported results obtained for swelling capacity, but also alike swelling and gelatinization rate (Sandhu & Singh, 2007). The maximum viscosity reached after heating was higher at pH 6.0, and then higher viscosity was observed along the cooling stage. Therefore, pH during incubation affected the annealing process that might take place during incubation at 50 ºC, and lower pH caused larger changes than pH 6.0. Regarding the enzymes action, AMG treated starch showed a delayed pasting formation, likely water absorption and swelling ability of this starch was somewhat hindered after the enzymatic treatment, which was also observed when the hydration ability of the starch was determined. After gelatinization was completed, the AMG treated starch showed higher maximum viscosity, higher cooking stability and viscosity after cooling. Conversely, the AM treated

starch showed similar swelling and hydration than its control counterpart, but displayed lower maximum viscosity after cooking and cooling, which has been related to the reduction of the molecular size of starch (Miao et al., 2011).

Parameters recorded from the pasting curves are shown in Table 2. The onset temperature, the temperature where viscosity first increased, was rather similar in the starches kept at different pHs. The trend observed was the same as the one followed by the swelling capacity, which might be expected since the initial stage of the gelatinization is the water absorption and in consequence the starch swelling. Values of the onset temperature for the enzymatically treated starches confirmed that AMG treated starch swelled slowly compared to its counterpart control. Hydrolysis percentage induced by each enzyme was calculated referred to the maximum viscosity given by a known amount of starch obtained after heating and cooling. AM promoted a 11% hydrolysis, but AMG did not decrease the viscosity, despite the porous structure of the modified starch. The reduction in the viscosity led by AM was kept beyond heating and cooling. Therefore, although microstructure appearance of AM treated starch seems to be more intact than the one of AMG treated starch, pasting behavior suggested that AM affected the loosely packed internal region of the granules and in lesser extent the densely packed periphery. Miao et al. (2011) studying the digestion of waxy corn starch with a combination of pancreatic α- amylase and amyloglucosidase found that the end distant from the hilum of native cereal starch was more susceptible to amylolysis, and empty shells were obtained when the hydrolytic reaction progressed in major extent. Nevertheless, although these authors proposed that hydrolytic enzymes act primarily through surface erosion of the starch granules, the results obtained with AM in the present study seem to indicate that AM exerts its major action in the inner core and only small pinholes are necessary for entering. The opposite trend was observed with AMG that increased the viscosity of starch granules, compared to its control at pH 4.0, and gave lower breakdown related to the cooking stability

388 of the starch granules during heating (Rojas, Rosell, & Benedito de Barber, 2001). In
389 addition, this porous starch showed higher setback, related to amylose chains recrystallization,
390 which suggested that higher amount of amylose chains leached out of the treated granule and
391 they were rapidly able to form helical structures responsible of the gel formation.
392
393 *3.4. Thermal properties*
394 Thermal properties of the enzymatically modified corn starches were determined by dif-
395 ferential scanning calorimetry (DSC) to detect possible changes in physical states of the
396 starch structures (Table 3). The transition temperatures (T_o, T_p and T_c), gelatinization
397 temperature range (T_c–T_o), enthalpies of gelatinization (ΔH) and peak height index (PHI)
398 significantly (P<0.05) differed among samples. Gelatinization temperature (T_p) of the corn
399 starch was 71.4 ºC, which was higher than the 64-69 ºC previously described (Zhang et al.,
400 2012; Rocha et al., 2012). Nevertheless, the increase could be ascribed to the annealing
401 process that took place during incubation at 50ºC that produces an increase in the peak
402 temperature (Rocha et al., 2012). Differences were observed in the thermal parameters
403 derived from the pH effect; gelatinization temperatures were sifted to higher values, with
404 exception of the T_p, when increasing the reaction pH. The effect was even more accentuated
405 when compared the gelatinization temperature range at both pHs; pH 6.0 led to narrow
406 gelatinization range, that has been related to major proportion of crystalline region (Zhang et
407 al., 2012). It is well known that during the endothermic transition, namely gelatinization,
408 primarily water molecules freely diffuse into the amorphous region of starch and secondly
409 they penetrate the crystalline region (Biliaderis, 1991). Since differences were mainly
410 observed on the T_o, it might indicate that amorphous region was more affected at higher pH
411 during incubation.

412 Enzymatic treatment significantly modified the thermal properties of corn starch, delaying the
413 gelatinization process, which started at higher onset temperature (Table 3). Starch modified
414 with AMG showed the highest value of T_o (67.48 °C) compared to its control (61.43 °C),
415 therefore higher gelatinization temperature is required to initiate gelatinization of the starch,
416 which agrees with previous observations described in the pasting behavior. The overall effect
417 of enzymes on the gelatinization temperatures resulted in significantly lower gelatinization
418 temperature range, mainly in the case of AM. That result was due to a delay in the start of
419 gelatinization more than a shift of the conclusion temperature. α-Amylase is a very common
420 endoenzyme that cannot hydrolyze either the α-1,6 glycosidic bonds that form the branch
421 points in amylopectin, or the α-1,4 glycosidic bonds that are in close proximity to a branch
422 point. Higher transition temperatures in the AMG samples have been explained due to higher
423 degrees of crystallinity, which provide structural stability and make the granules more
424 resistant to gelatinization (Kaur, Singh, Ezekiel & Sodhi, 2009). Despite the different catalytic
425 mechanism of both enzymes, it seems that the modification of starch induced by enzymes
426 mainly affected the swelling behavior, which initially governs the gelatinization.
427 The higher ΔH of AM enzymatically modified corn starch compared to its respective control
428 suggested that the state of the crystalline and amorphous regions differed from those of the
429 AMG corn starch, in which the gelatinization enthalpy decreased in comparison with the
430 control sample at pH 4. The lower ΔH values indicated that hydrolyzed starch by AMG
431 required less thermal energy for gelatinization compared to the other samples. Gelatinization
432 involves the uncoiling and melting of the external chains of amylopectin that are packed
433 together as double helices in clusters (Cooke & Gidley, 1992). Crystallinity of starch is
434 caused essentially by amylopectin polymer interactions, with the outer branches of
435 amylopectin molecules interacting to arrange themselves into ‘‘crystallites’’ forming
436 crystalline lamellae within the granule (Tester, Karkalas, & Qi, 2004). Studies carried out on

437 granular starch and model crystallites confirmed that ΔH is mainly due to the disruption of the
438 double helices rather than the long range disruption of crystallinity (Cooke et al., 1992).
439 Therefore, AMG action affected the double helical conformation of the granule, both
440 amorphous and crystalline domains, and likely acting on the amylopectin side chains, due to
441 amylopectin plays a major role in starch granule crystallinity and the presence of amylose
442 lowers the melting point of crystalline regions and the energy for starting gelatinization
443 (Sodhi & Singh, 2003). Conversely, α-amylase acted mainly on the amorphous regions
444 leading to a more crystalline structure that requires higher gelatinization enthalpy.
445
446 Values of peak height index (PHI), a measure of uniformity in gelatinization, were clearly
447 influenced by the pH used during the incubation of starch. Again the lower pH (4.0) was more
448 aggressive on the starch granules, giving lower PHI, which suggests narrowed transition range
449 for gelatinization (Kruger, Walker, Knutson & Inglett, 1987). Compared with their respective
450 control starches, enzymatically modified corn samples showed higher PHI values, indicating
451 differences in the granule structure.
452 Overall, the low transition temperatures of the enzymatically treated starches indicates the
453 reduction in the amorphous domain of the granules, which was accompanied by changes in
454 the crystallite regions following a cooperative process in the case of AMG, but led to more
455 crystalline structures in the AM treated starches.
456
457 *3.5. Starch hydrolysis kinetics*
458 Even though corn starches are not consumed directly but after being subjected to different
459 processes, the enzymatic *in vitro* hydrolysis was carried out with the aim to determine the
460 susceptibility of those enzymatically treated starches, with a more porous structure, to the
461 enzymatic digestion. The digestibility curves of the enzymatically treated starches besides

462 their respective controls are displayed in Figure 4. No differences could be envisaged due to
463 the pH difference between the control starches; only at the very end of the digestion reaction
464 was observed that starch kept at pH 4.0 showed higher hydrolysis. In fact, the reaction rate
465 calculated as the slope of the very early hydrolysis stage (0-30 min), showed similar values
466 (0.036 mg $100g^{-1}$ s^{-1}). Again, low pH seems to affect in greater extent the granule structure. It
467 is clearly evident that enzymatically modified corn starches showed greater susceptibility to
468 be digested. Non treated starch granules offer great resistance to enzymatic hydrolysis, thus
469 the possible damage suffered by starch granules due to enzymatically treatment was clearly
470 visible in the digestion. The increase in the enzymatic hydrolysis suggested that the starch
471 granules became more accessible to enzyme hydrolysis. It has been reported that the presence
472 of pores on the surface of annealed granules favored the action of endogenous amylases
473 during annealing, contributing to a greater exposure of the amorphous areas to the exogenous
474 enzymes (Rocha et al., 2012; Zavareze et al., 2011). In the present study, the enzymes used
475 for obtaining the enzymatically treated starches originated porous granules with diverse
476 openings size, which seems to accelerate the enzymatic digestion, and that effect was even
477 greater in the case of AM treated starches. In fact, the reaction rate at early stage was for
478 AMG-starch and AM-starch 0.054 mg $100g^{-1}$ s^{-1} and 0.067 mg $100g^{-1}$ s^{-1}, respectively. The
479 pancreatic α-amylase affinity for digesting starches is dependent on the particle size of starch,
480 due to the enzyme feasibility for binding/absorption and the degree of order of starch has
481 important influence on the initial rate at which native starch is digested by amylase (Tahir,
482 Ellis, & Butterworth, 2010).
483
484 Starch hydrolysis curves were plotted to obtain the rate constant fitting the values to a first-
485 order equation. Table 4 shows that the pseudo-first order rate constant estimated by the first
486 order model described by Goni, Garcia Alonso, & Saura Calixto (1997). Significant (P<0.05)

differences were found on the digestibility constant (*k*) due to the pH, showing higher rate constant the starch kept at pH 6.0. It has been described that significant differences in *k* are indicative of structural differences (Butterworth , Warren, Grassby, Patel & Ellis, 2012), which agrees with previous observation encountered on pasting and thermal properties. Regarding the enzymatic treatment, porous starches showed lower digestibility constant compared to their respective controls and no differences were observed pertaining the enzyme hydrolytic action. It has been proposed that low *k* values are related to a slow diffusion of pancreatic amylase into the starch granule as digestion proceeds (Dhital, Shrestha & Gidley, 2010), although considering the microstructure of the porous granules the most plausible explanation to those values of rate constant seems to be the substrate exhaustion (Butterworth,Warren & Ellis, 2011).

The percentage of enzymatic hydrolysis from the samples increased progressively with time during incubation. As described Butterworth et al., (2012), the rate of reaction decreases as the time is extended and plots of the concentration of product formed (or quantity of starch digested) against time are logarithmic. The plots approach an end point where no further reaction is measurable no matter how much longer incubation times are prolonged. This response is predictable based on the assumption that the concentration of available starch substrate decreases with time as starch is converted to products.

The end point values (C_∞) obtained in the hydrolyzed process reflected the concentration at the equilibrium point (Table 4). Results were significantly different, being affected by pH and enzyme treatment. Starch kept at pH 4.0 reached higher C_∞ than that at pH 6.0, indicating again that low pH during annealing affected microstructure of the granule. Enzymatically modified corn starches resulted in higher C_∞ values compared to their respective controls. Higher concentration of final product reflected increased digestibility of starch granules. AM

512 treated starch showed higher C_∞ than AMG treated starch. Therefore, AM action led to more
513 accessible granules that were easily digested.
514
515 **4. Conclusion**
516 Demands of modified starches are increasing in parallel to the rapid development of food
517 industry. This study showed that enzymatic modification of corn starch by α-amylase or
518 amyloglucosidase at sub-gelatinization temperatures led to porous starch granules that
519 differed in both, the microstructure surface and the internal morphology. Results confirmed
520 that the loss of granular structural order and changes in both amorphous and crystalline
521 domains during sub-gelatinization temperatures can be more influenced by the
522 amyloglucosidase than α-amylase, and changes increased the susceptibility of starches to be
523 digested.
524 Overall, results showed that enzymatic modification at sub-gelatinization temperatures really
525 offer an attractive alternative for obtaining porous starch granules to be used in a variety of
526 foods applications. The degree of porosity could be controlled by using different enzymes or
527 a combination of enzymes might be considered as well.
528
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534
535 **References**

536 Abbas K.A., Khalil Sahar K., & Meor Hussin A.S (2010). Modified Starches and Their
537 Usages in Selected Food Products: A Review Study. *Journal of Agricultural Science*,
538 *2*, 2.
539 AACC International. Approved Methods of Analysis, 11th Ed. Method 61-02. Determination
540 of pasting properties of rice with the rapid viscoanalyzer. Approved October 15, 1997.
541 AACC International, St. Paul, MN, U.S.A.
542 Ao, Z., Simsek, S., Zhang, G., Venkatachalam, M., Reuhs, B. L., & Hamaker, B. R. (2007).
543 Starch with a slow digestion property produced by altering its chain length, branch
544 density, and crystalline structure. *Journal of Agricultural and Food Chemistry*, 55,
545 4540–4547.
546 Biliaderis, C. G. (1991). The structure and interactions of starch with food constituents.
547 *Canadian Journal of Physiology and Pharmacology, 69*, 60-78.
548 Butterworth, P. J., Warren, F. J., & Ellis, P. R. (2011). Human a-amylase and starch digestion:
549 An interesting marriage. *Starch-Stärke, 63*, 395–405.
550 Butterworth, P. J., Warren, F. J., Grassby, T., Patel, H., & Ellis, P. R. (2012). Analysis of
551 starch amylolysis using plots for first-order kinetics. *Carbohydrate Polymers, 87*,
552 2189-2197.
553 Colonna, P., Leloup, V., & Buleon, A. (1992). Limiting factors of starch hydrolysis.
554 *European Journal of Clinical Nutrition, 46*, S17-S32.
555 Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch
556 gelatinization - origin of the enthalpic transition. *Carbohydrate Research, 227*, 103-
557 112.
558 Dhital, S., Shrestha, A. K., & Gidley, M. J. (2010). Relationship between granule size and in
559 vitro digestibility of maize and potato starches. *Carbohydrate Polymers, 82*, 480–488.
560 Donovan, J. W. (1979). Phase-transitions of starch-water system. *Biopolymers, 18*, 263-275.

561 Dura, A., Calviño A., Blaszczak W., & Rosell C.M. (2013). Funcionality of enzymatically
562 treated corn starches above gelatinization temperature. Proceedings of the 7th
563 International Congress "Flour-Bread '13" & 9th Croatian Congress of Cereal
564 Technologists "Brašno–Kruh '13. Submitted.
565 Gallant, D. J., Bouchet, B., Buleon, A., & Perez, S. (1992). Physical characteristics of starch
566 granules and susceptibility to enzymatic degradation. *European Journal of Clinical*
567 *Nutrition, 46,* S3-S16.
568 Goni, I., Garcia Alonso, A., & Saura Calixto, F. (1997). A starch hydrolysis procedure to
569 estimate glycemic index. *Nutrition Research, 17,* 427-437.
570 Gularte, M. A., & Rosell, C. M. (2011). Physicochemical properties and enzymatic hydrolysis
571 of different starches in the presence of hydrocolloids. *Carbohydrate Polymers, 85,*
572 237-244.
573 Han, X.-Z., Ao, Z., Janaswamy, S., Jane, J.-L., Chandrasekaran, R., & Hamaker, B. R. (2006).
574 Development of a low glycemic maize starch: preparation and characterization.
575 *Biomacromolecules, 7,* 1162–1168.
576 Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of
577 tuber and root starches: a review. *Carbohydrate Polymers, 45,* 253-267.
578 Huang, Q., Xiong F.,, Xiao-Wei H., Fa-Xing, L., Shu-Juan Y., & Li, L. (2010). The effect of
579 enzymatic pretreatments on subsequent octenyl succinic anhydride modifications of
580 cornstarch. *Food Hydrocolloids, 24,* 60-65.
581 Huber,K.C., &BeMiller,J.N. (2000). Channels of maize and sorghum starch granules.
582 *Carbohydrate Polymers*. *41*, 269–276.
583 International Association for Cereal Chemistry. ICC (1996). Official Methods of Analysis,
584 standard method No. 164. Vienna.

585 Jayakody, L., & Hoover, R. (2002). The effect of lintnerization on cereal starch granules.
586 *Food Research International, 35,* 665–680
587 Jayakody, L., & Hoover, R. (2008). Effect of annealing on the molecular structure and
588 physicochemical properties of starches from different botanical origins – A review.
589 *Carbohydrate Polymers, 74*, 691–703.
590 Kaur, A., Singh, N., Ezekiel, R., & Sodhi, S. (2009). Properties of starches separated from
591 potatoes stored under different conditions. *Food Chemistry, 114,* 1396–1404.
592 Kaur B., Ariffin, F., Bhat, R., & Karim A. (2012). Progress in starch modification in the last
593 decade. *Food Hydrocolloids, 26,* 398-404.
594 Khatoon, S., Sreerama, Y. N., Raghavendra, D., Bhattacharya, S., & Bhat, K. K. (2009).
595 Properties of enzyme modified corn, rice and tapioca starches. *Food Research*
596 *International, 42*, 1426-1433.
597 Kimura, A., & Robyt, J. F. (1996). Reaction of enzymes with starch granules: Enhanced
598 reaction of glucoamylase with gelatinized starch granules. *Carbohydrate Research,*
599 *288*, 233-240.
600 Krueger, B. R., Walker, C. E., Knutson, C. A., & Inglett, G. E. (1987). Differential Scanning
601 Calorimetry of Raw and Annealed Starch Isolated from Normal and Mutant Maize
602 Genotypes. *Cereal Chemistry, 64*, 187-190.
603 Lacerda, L.G., Menegassi Azevedo, J.A., da Silva Carvalho Filho, M.A., Demiate, I.M.,
604 Schnitzler, E., Porto de Souza Vandenberghe, L., & Soccol, C.R. (2008). Thermal
605 characterization of partially hydrolyzed cassava (Manihot esculenta) starch granules.
606 *Brazilian Archives of Biology and Technology, 51,* 1209-1215.
607 Li, D. H., & Ma, Y. (2011). Impact of enzymatic hydrolysis on the crystal structure, and
608 thermal and textural properties of corn starch. *African Journal of Biotechnology, 10*,
609 3430-3435.

610 Li, J. H., Vasanthana, T., Hoover, R., & Rossnagel, B. G. (2004). Starch from hull-less
611 barley: V. In-vitro susceptibility of waxy, normal, and high-amylose starches towards
612 hydrolysis by alpha-amylases and amyloglucosidase. *Food Chemistry, 84*, 621-632.
613 Li, W.,Shu C.,, Zhang, P., & Shen, Q. (2011). Properties of Starch Separated From Ten Mung
614 Bean Varieties and Seeds Processing Characteristics. *Food and Bioprocess*
615 *Technology, 4*, 814-821.
616 Miao, M.,Zhang, T.,Mu, W., & Jiang, B. (2011). Structural characterizations of waxy maize
617 starch residue following in vitro pancreatin and amyloglucosidase synergistic
618 hydrolysis. *Food Hydrocolloids, 25*, 214-220.
619 Perry, P.A., & Donald, A.M. (2000). SANS study of the distribution of water within starch
620 granules. *International Journal of Biological Macromolecules, 28*, 31–39.
621 Ratnayake, W. S., Hoover, R., & Warkentin, T. (2002). Pea starch: Composition, structure
622 and properties - A review. *Starch-Starke, 54*, 217-234.
623 Ratnayake, W. S., & Jackson, D. S. (2008). Advances in Food and Nutrition Research. *Starch*
624 *Gelatinization.* (pp. 221-268). Elsevier Inc.
625 Rocha, T. S., Felizardo, S. G., Jane, J., & Franco, C. M. L. (2012). Effect of annealing on the
626 semicrystalline structure of normal and waxy corn starches. *Food Hydrocolloids*, *29*,
627 93-99.
628 Rojas, J.A., Rosell, C.M., Benedito, C., Pérez-Munuera, I., & Lluch, M.A. (2000). The baking
629 process of wheat rolls followed by cryo scanning electron microscopy. *European Food*
630 *Research and Technology*, *212*, 57–63.
631 Rojas, J.A., Rosell, C.M., & Benedito, C. (2001). Role of maltodextrins in the staling of
632 starch gels. *European Food Research and Technology*, *212*, 364–368
633 Rosell, C.M. & Collar, C. (2008). Effect of various enzymes on dough rheology and bread
634 quality. In: Recent Research Developments in Food Biotechnology. Enzymes as

635 Additives or Processing Aids. Ed R. Porta, P. Di Pierro and L. Mariniello. Research
636 Signpost, Kerala, India. Pp 165-183.
637 Rosell, C. M., Yokoyama, W., & Shoemaker, C. (2011). Rheology of different hydrocolloids–
638 rice starch blends. Effect of successive heating–cooling cycles. *Carbohydrate*
639 *Polymers, 84*, 373-382.
640 Sandhu, K. S., & Singh N. (2007). Some properties of corn starches II: Physicochemical,
641 gelatinization, retrogradation, pasting and gel textural properties. *Food Chemistry,*
642 *101*, 1499–1507.
643 Singh, J., Kaur, L., & McCarthy, O.J. (2007). Factors influencing the physico-chemical,
644 morphological, thermal and rheological properties of some chemically modified
645 starches for food applications-A review. *Food Hydrocolloids, 21*, 1–22.
646 Sodhi, N. S., & Singh, N. (2003). Morphological, thermal and rheological properties of
647 starches separated from rice cultivar's grown in India. *Food Chemistry, 80*, 99-108.
648 Tahir, R., Ellis P.R., & Butterworth, P.J. (2010). The relation of physical properties of native
649 starch granules to the kinetics of amylolysis catalysed by porcine pancreatic a-
650 amylase. *Carbohydrate Polymers, 81*, 57–62.
651 Tester, R. F., Karkalas, J., & Qi, X. (2004). Starch - composition, fine structure and
652 architecture. *Journal of Cereal Science, 39*, 151-165.
653 Toyokawa, H., Rubenthaler, G. L., Powers, J. R., & Schanus, E. G. (1989). Japanese noodle
654 qualities. 1. Flour components. *Cereal Chemistry, 66*, 382-386.
655 Wu, Y., Du, X.F., Ge, H.H., & Lv, Z. (2011). Preparation of microporous starch by
656 glucoamylase and ultrasound. *Starch-Stärke, 63*, 217-225 .
657 Zavareze, E., & Dias, A. (2011). Impact of heat-moisture treatment and annealing in starches:
658 A review. *Carbohydrate Polymers, 83*, 317-328.

Zhang, B., Cui, D., Liu, M., Gong, H., Huang, Y., & Han, F. (2012) Corn porous starch: Preparation, characterization and adsorption property. *International Journal of Biological Macromolecules, 50,* 250–256.

664

665 **Figure captions**

666 **Figure 1.** Scanning electron micrograph of corn starch samples treated enzymatically (b, d)
667 and their counterparts controls (a, c). Magnification 2000x. Control pH 4 (a); AMG (b);
668 Control pH 6 (c); AM (d).

669

670 **Figure 2.** Scanning electron micrograph of corn starch samples treated enzymatically (b, d)
671 and their counterparts controls (a, c). Magnification 3500x. Control pH 4 (a); AMG (b);
672 Control pH 6 (c); AM (d).

673

674 **Figure 3.** RVA profiles of the corn starches treated with amylase (◊) or amyloglucosidase (□)
675 compared with their respective controls (without enzymatic treatment) in closed symbols (pH
676 4.0, ■ ; and pH 6.0, ♦).

677

678 **Figure 4.** Enzymatic starch hydrolysis of the corn starches treated with amylase (◊) or
679 amyloglucosidase (□) compared with their respective controls (without enzymatic treatment)
680 in closed symbols (pH 4.0, ■ ; and pH 6.0, ♦).

681

682

683 **Table 1.** Effect of enzymatic treatment on the hydration properties and chemical composition

684 of the resulting porous starches.

	Control-4	AMG	Control-6	AM
Paste clarity (Abs 650nm)	0.01±0.01a	0.26±0.03a	0.04±0.01a	0.03±0.00a
Solubility index (%)	1.13±0.01a	20.08±2.16c	1.27±0.13a	4.3±0.08b
Swelling power (g/g)	1.96±0.12a	1.93±0.00a	1.82±0.01a	2.68±0.15b
Amylose content (Abs 690nm)	0.03±0.01a	0.01±0.01a	0.04±0.02a	0.02±0.01a
Swelling capacity (g/g)	1.94±0.11b	1.54±0.04a	1.80±0.01b	2.56±0.14c
Free sugars (mg/100mg)	0.10±0.01a	0.63±0.15b	0.11±0.03a	0.18±0.09a
Damaged starch (mg/100mg)	3.16±0.24a	2.49±1.22a	3.40±0.24a	3.03±0.97a

685 Mean ± standard deviation values followed by different letters within a column denote

686 significantly different levels ($P < 0.05$) (n = 4).

687

688

689

690 **Table 2.** Effect of enzymatic treatment on the pasting parameters of corn starch.

691

	Control-4	AMG	Control-6	AM
Onset Temp (ºC)	83.2b	86.4c	82.5a	83.2b
Peak Time (min)	5.6a	6.5b	5.5a	5.7a
Peak viscosity (cP)	788a	809b	838c	747a
Trough (cP)	650a	681b	715c	633a
Breakdown (cP)	138b	128a	123a	114a
Final Viscosity (cP)	773a	853b	861b	767a
Setback (cP)	123a	172c	146b	134ab
Hydrolysis 95°C (%)	0	0	0	11
Hydrolysis 50°C (%)	0	0	0	11

692

693

Table 3. Thermal properties of enzymatically modified corn starches determined by DSC.

	Control-4	AMG	Control-6	AM
T_o (°C)	61.43a	67.48d	64.71b	66.54c
T_p (ºC)	71.37b	72.18c	71.36b	70.43a
T_c (ºC)	75.00a	77.00c	76.00b	75.00a
T_p-T_o	9.94d	4.70b	6.65c	3.89a
ΔH (J/g)	28.24b	20.40a	28.04b	29.87c
PHI (J/g ºC)	2.84a	4.34b	4.22b	7.68c

T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, ΔH = enthalpy change, PHI = Peak High Index.

Values followed by different letters within a column denote significantly different levels ($P < 0.05$) (n = 4).

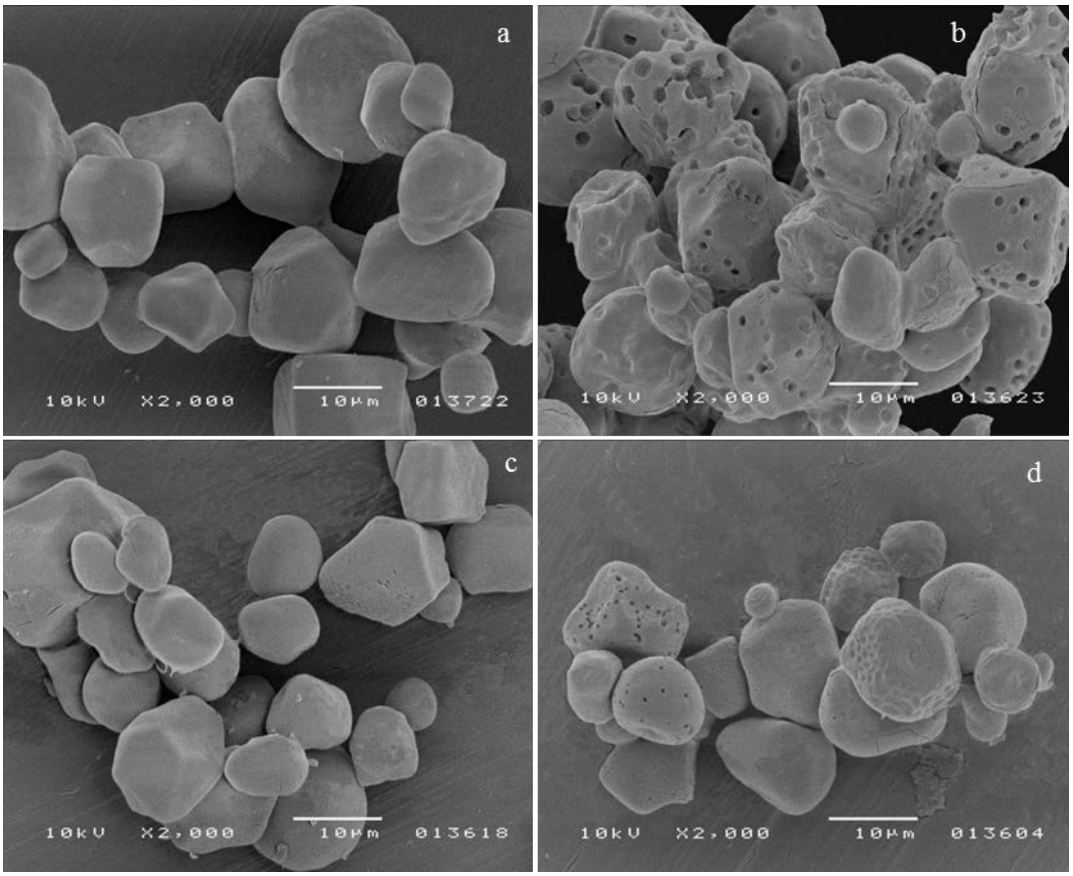
Table 4. Kinetic parameters extracted from first-order fitting of the experimental enzymatic hydrolysis of modified corn starches.

	Control-4	AMG	Control-6	AM
k (min^{-1})	0.027±0.009	0.017±0.001	0.039±0.002	0.017±0.003
C_∞ (%)	1.939±0.150	4.114±0.219	1.607±0.065	4.592±0.195

Mean±standard deviation values (n = 4).

707

708 **Figure 1.**



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712 **Figure 2.**

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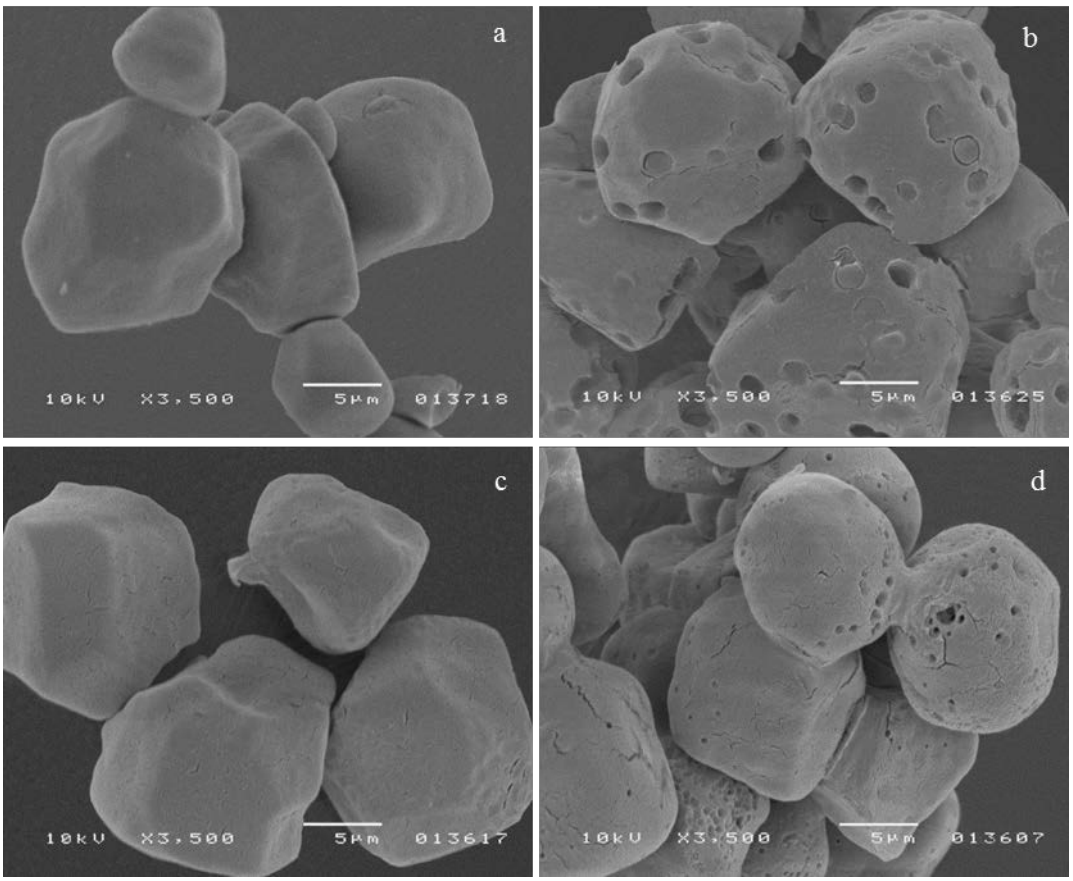

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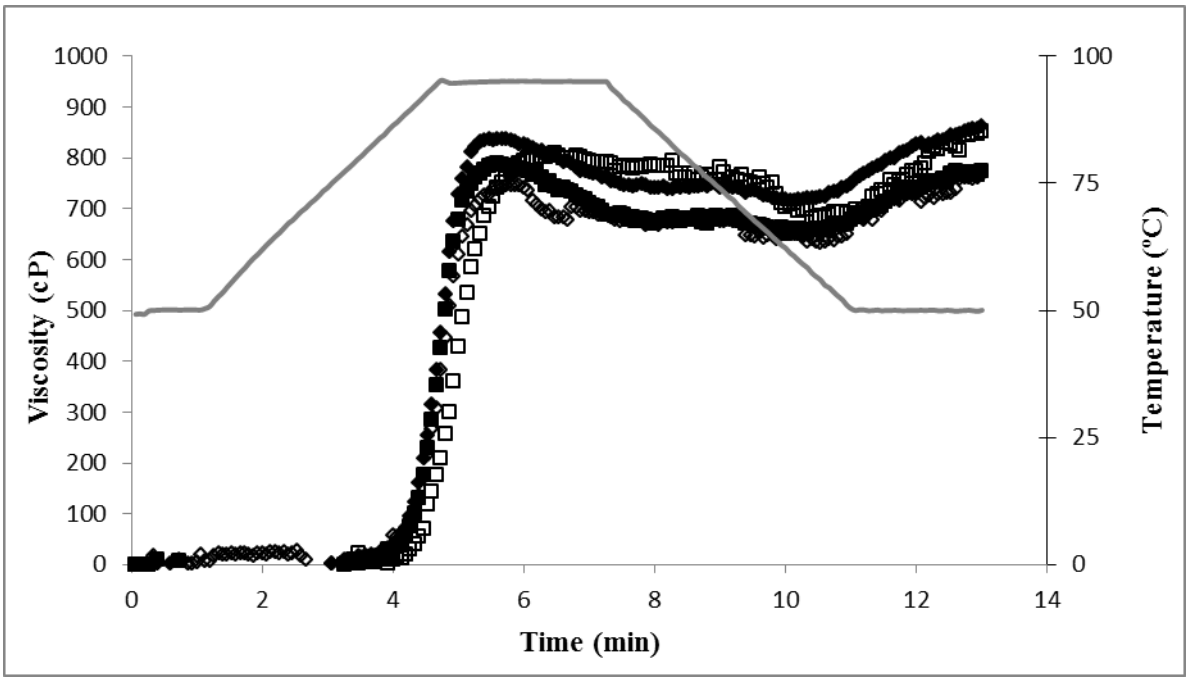
716 **Figure 3**.



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720 **Figure 4.**

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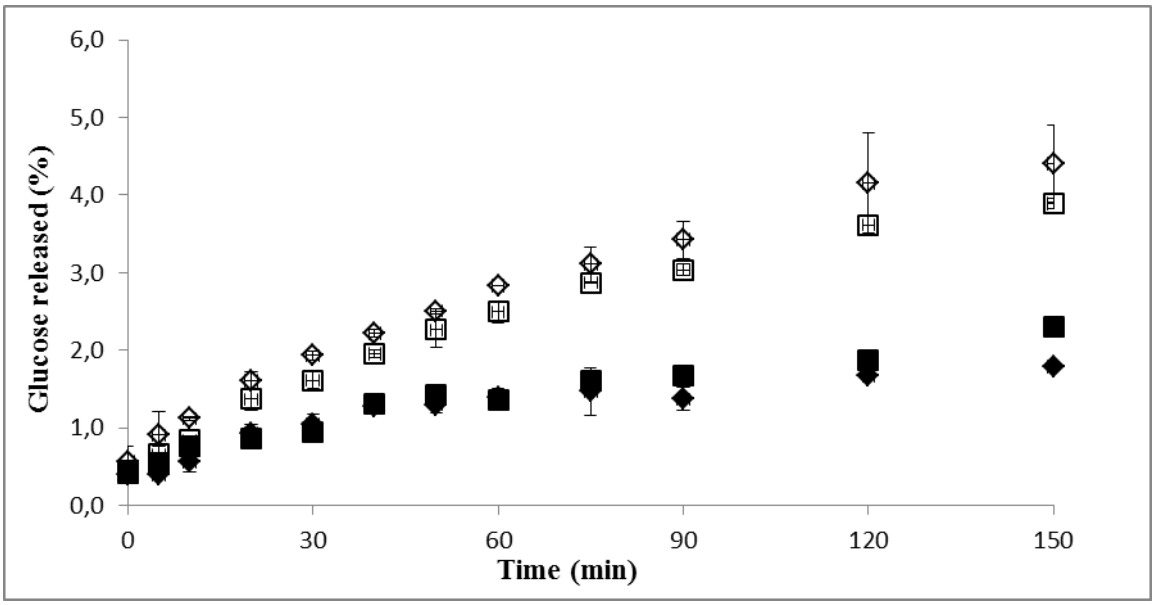

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