

1 **Comparison of porous starches obtained from different enzyme types and levels**

2 Yaiza Benavent-Gil and Cristina M. Rosell\*

3 Institute of Agrochemistry and Food Technology (IATA-CSIC), C/ Agustin Escardino,  
4 7, Paterna 46980, Valencia, Spain.

5 \*Corresponding author e-mail: crosell@iata.csic.es. Phone number +34 963900022. Fax  
6 number: +34 963636301

7

8 **Abstract**

9 The objective was to compare the action of different hydrolases for producing porous  
10 corn starches. Amyloglucosidase (AMG),  $\alpha$ -amylase (AM), cyclodextrin-  
11 glycosyltransferase (CGTase) and branching enzyme (BE) were tested using a range of  
12 concentrations. Microstructure, adsorptive capacity, pasting and thermal properties were  
13 assessed on the porous starches. SEM micrographs showed porous structures with  
14 diverse pore size distribution and pore area depending on the enzyme type and its level;  
15 AMG promoted the largest holes. Adsorptive capacity was significantly affected by  
16 enzymatic modification being greater influenced by AMG activity. Unexpectedly,  
17 amylose content increased in the starch treated with AMG and BE, and the opposite  
18 trend was observed in AM and CGTase treated samples, suggesting different mode of  
19 action. A heatmap illustrated the diverse pasting properties of the different porous  
20 starches, which also showed significant different thermal properties, with lower  $T_0$  and  
21  $T_p$ . Porous starch properties could be modulated by using different enzymes and  
22 concentrations.

23 **Keywords:** Porous starch, amyloglucosidase,  $\alpha$ -amylase, CGTase, branching enzyme,  
24 microstructure.

25

## 26        **1. Introduction**

27    Porous starches are now attracting much attention due to their great adsorption ability  
28    (Zhang, Cui, Liu, Gong, Huang & Han, 2012). Those starches contain abundant pores  
29    from the surface to the center of the granules, which increase the specific surface area,  
30    acting as excellent natural absorbents. In fact, there is a growing interest in exploiting  
31    their properties in different food and non-food areas. In food industry, porous starches  
32    are used as colorants, spices, flavorings, sweeteners carriers and also for protection of  
33    sensitive elements such as oils, minerals, vitamins, bioactive lipids, food pigments such  
34    as  $\beta$ -carotene and lycopene which are sensitive to light, oxidation or high temperature  
35    (Belingheri, Giussani, Rodriguez-Estrada, Ferrillo & Vittadini, 2015; Luo et al., 2013;  
36    Majzoobi, Hedayati & Farahnaky, 2015).

37    Enzymatic treatments have been performed for obtaining porous starches, mainly  
38    applying glucoamylases and  $\alpha$ -amylases (Sujka & Jamroz, 2007). Cassava starch  
39    granules were treated with  $\alpha$ -amylase from *Bacillus amyloliquefaciens* without altering  
40    the size or morphology of the granules but significantly changing their properties  
41    (Ichihara, Fukuda, Takaha, Yuguchi & Kitamura, 2013). The combination of  
42    glucoamylase and  $\alpha$ -amylase has been also proposed due to their synergistic action to  
43    hydrolyze raw starch completely very rapidly (Sun et al., 2010). In fact, porous starch  
44    was obtained using a combination of  $\alpha$ -amylase and glucoamylases activity after  
45    optimizing the kinetic reaction to increase the reaction yield (Zhang, Cui, Liu, Gong,  
46    Huang & Han, 2012). Later, Dura, Błaszczak and Rosell (2014) compared the  $\alpha$ -  
47    amylase and glucoamylase individual action to determine their effect on biochemical  
48    features, thermal and structural properties of corn starch. Researchers concluded that  $\alpha$ -  
49    amylase or amyloglucosidase when acting on corn starch at sub-gelatinization  
50    temperatures for 24 or 48 hours led to porous starch granules that differed in both, the

51 microstructure surface and the internal morphology. Similarly, Chen (2012) hydrolyzed  
52 native corn starch granules using glucoamylase at 50 °C for 1-8 h studying the impact of  
53 enzyme/granule ratio and hydrolysis time on the microstructure of porous starch.  
54 Research carried out on enzymatic treatments of starches has been accomplished using  
55 diverse enzymes and experimental conditions (Sorndech et al., 2016; Uthumporn,  
56 Zaidul & Karim, 2010), which complicates results comparison and a real understanding  
57 of the enzymes action on the structure and functionality of the starches.  
58 In addition, other starch acting enzymes like  $\alpha$ -glucanotransferases have received  
59 considerable attention to remodel parts of the amylose and amylopectin molecules by  
60 cleaving and reforming  $\alpha$ -1,4- and  $\alpha$ -1,6-glycosidic bond (van der Maarel & Leemhuis,  
61 2013) or in the case of cycloamylose glucanotransferase for producing cyclodextrins  
62 (CDs) (Yamamoto, Zhang & Kobayashi, 2000). Nevertheless,  $\alpha$ -glucanotransferase  
63 such as branching enzyme or the cycloamylose glucanotransferase have been not tested  
64 for obtaining porous starches.  
65 The aim of this study was to compare the effect of different enzymes on corn starch  
66 properties, taking also into account the impact of enzyme level. Amyloglucosidase  
67 (AMG), fungal  $\alpha$ -amylase (AM), cyclodextrin-glycosyltransferase (CGTase) and  
68 branching enzyme (BE) were used to trigger particular starch functionalities.

## 69 **2. Materials and methods**

### 70 *2.1. Materials*

71 Corn starch was purchased from Miwon (Seoul, Korea). Amyloglucosidase (EC  
72 3.2.1.3), fungal  $\alpha$ -amylase (EC 3.2.1.1), cyclodextrin-glycosyltransferase (EC 2.4.1.19)  
73 and branching enzyme (EC 2.4.1.18) activities were provided by commercial food grade  
74 preparations (Amyloglucosidase 1100, Fungamyl 2500 SG, Toruzyme® 3.0 L and  
75 Branchzyme) supplied by Novozymes (Bagsværd, Denmark). AMG activity was 1100

76 AGU/g (amyloglucosidase activity defined as the amount of enzyme that cleaves 1  
77  $\mu\text{mol}$  of maltose per min at 37 °C); AM activity was 2500 FAU/g (fungal amylase  
78 activity); CGTase activity was 3 KNU/mL (kilo novo alpha amylase unit); BE activity  
79 was 50000 BEU/mL (branching enzyme units). All the other chemicals were analytical  
80 reagent grade and used without further purification. All solutions and standards were  
81 prepared by using deionized water.

## 82 2.2. *Preparation of porous starch*

83 The preparation of porous starch was based on the method of Dura *et al.* (2014; 2016)  
84 with minor modifications. Corn starch (20 g) was suspended in 100 mL of 20 mM  
85 sodium acetate buffer at pH 4.0 (AMG) or sodium phosphate buffer at pH 6.0 (AM,  
86 CGTase, BE). Then, different enzyme concentrations, expressed in units of enzyme  
87 stock solutions per grams of starch (U/g starch), were added to the starch suspensions,  
88 separately. The lowest enzyme concentration was the minimum recommended by the  
89 manufacturer (5.5 AMG U/g, 5.5 AM U/g, 0.1 CGTase U/g and 500 BE U/g),  
90 increasing concentrations (2, 3, 6 and 10 times the initial level) were also tested.  
91 Samples were kept in a shaking water bath (50 rpm) at 50 °C for 2 h. Then samples  
92 were centrifuged for 15 min at 7000  $\times g$  at 4 °C. Supernatants were boiled in a water  
93 bath for 10 min to inactivate the enzymes before any further analyses. Sediments were  
94 washed twice with 50 mL of water, homogenized with a Polytron Ultraturrax  
95 homogenizer IKA-T18 (IKA works, Wilmington, USA) for 1 min at speed 3, and then  
96 centrifuged at the same conditions as before. Washed sediments were freeze-dried and  
97 kept at 4 °C for subsequent analyses. Starch samples were subjected to the same  
98 procedure, without adding enzyme, at pH 4.0 (A-0) and pH 6.0 (P-0), and used as  
99 references. Two batches were prepared for each treatment.

## 100 2.3. *Scanning electron microscopy (SEM)*

101 The granule morphology of native, controls and treated starches was observed using a  
102 JSM 5200 scanning electron microscope (SEM) (JEOL, Tokyo, Japan). Samples were  
103 coated with gold in a vacuum evaporator (JEE 400, JEOL, Tokyo, Japan) prior to  
104 observation. The obtained samples were examined at an accelerating voltage of 10 kV  
105 and magnified 3,500x times.

106 The microstructure analysis was carried out using the image analysis program (ImageJ,  
107 UTHSCSA Image Tool software). The SEM images were saved as 8-bit tiff format.  
108 Scale was initially set using the relationship between pixels and known distance.  
109 Threshold was assessed applying the default algorithm and then particle analysis was  
110 carried out. The following parameters were measured: granule size and the pore size.  
111 The area occupied by pores in a starch granule was calculated as the sum of the areas of  
112 all the pores of a starch granule divided by granule pore. Values were the average of 20  
113 independent measurements.

114 *2.4. High performance anion exchange chromatography (HPAEC)*

115 The hydrolysis compounds (oligosaccharides and CDs) lixiviated during enzymatic  
116 treatment were quantified according to Dura and Rosell (2016). Samples were filtered  
117 through a 0.45 µm pore size membrane (Millex-HV) and then injected (10 µL) into  
118 HPAEC through a CarboPac PA-100 column (250 mm × 4 mm) at flow rate 1.0  
119 mL/min, coupled to a pulsed amperometric detector (Dionex). Solutions included: A  
120 (water), B (1 mol/L NaOH) and C (1 mol/L C<sub>2</sub>H<sub>3</sub>NaO<sub>2</sub>). Running profile applied was:  
121 time zero, 92.5% A, 5% B, 2.5% C; 25 min, 85% A, 5% B, 10% C; 1 min, 70% A, 15%  
122 B, 15% C; 3 min, 66% A, 15% B, 19% C; 5 min, 57% A, 15% B, 28% C; 1.5 min, 37%  
123 A, 15% B, 48% C. Standards of known concentrations were previously analyzed.

124 *2.5. Amylose content of enzymatically treated starches*

125 The amount of amylose of the starches was analyzed in triplicate using a commercial  
126 assay kit (Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland) based on  
127 the concanavalin A method (Gibson, Solah & McCleary, 1997).

#### 128 2.6. *Damage starch*

129 Damaged starch levels were estimated at least in duplicate following the American  
130 Association of Cereal Chemists, method 76-31.01 (2000).

#### 131 2.7. *Adsorption of water and sunflower oil*

132 Adsorptive capacity of starches for water and sunflower oil were determined according  
133 to the method described by Yousif, Gadallah and Sorour (2012) with a slight  
134 modification. Starch (0.1 g) and solvent (1 mL, water or oil) were mixed and vortexed  
135 for 30 min at room temperature. Slurries were centrifuged 10 minutes at 3,000 x g and  
136 decanted. When no more water or sunflower oil was dropped off onto the filter paper,  
137 weight of the sediment was measured. The adsorption capacity was calculated as the  
138 weight of the wetted sediment divided by the dry weight of sample (g/g).

#### 139 2.8. *Viscosity measurement*

140 The pasting properties of native and enzymatically modified starches were measured  
141 with the Rapid Visco Analyzer (RVA-4500, Perten Instruments, Hägersten, Sweden).  
142 Starch (2 g based on 14% moisture content) was added to 20 mL of water placed into  
143 the aluminum RVA canister. Slurries underwent a controlled heating and cooling cycle,  
144 from 50 to 95 °C in 282 s, holding at 95 °C for 150 s and then cooling to 50 °C. The  
145 initial speed for mixing was 960 rpm for 10 s, followed by a 160 rpm paddle speed that  
146 was maintained for the rest of assay. Pasting parameters such as pasting temperature,  
147 peak viscosity, breakdown (peak viscosity-hot paste viscosity), final viscosity, setback  
148 (cold paste viscosity-peak viscosity) were recorded using Thermocline software for  
149 Windows (Perten Instruments, Hägersten, Sweden).

150 2.9. *DSC thermal analysis*

151 The gelatinization characteristics of modified starches were determined using a  
152 differential scanning calorimetry (DSC) from Perkin–Elmer (DSC 7, Perkin–Elmer  
153 Instruments, Norwalk, CT). The slurry of starch and water (3:1) was placed into stainless  
154 steel capsules. Capsules were hermetically sealed and equilibrated at room temperature  
155 for one hour before analysis. The samples were scanned from 30 to 120 °C at a heating  
156 rate of 10 °C/min under nitrogen atmosphere, using an empty stainless steel capsule as  
157 reference. The temperature values obtained were the onset temperature ( $T_o$ ), peak  
158 temperature ( $T_p$ ), and conclusion temperature ( $T_c$ ). The enthalpy of gelatinization ( $\Delta H$ )  
159 was estimated based on the area of the main endothermic peak, expressed as joule per  
160 gram sample (J/g).

161 2.10. *Statistical analysis*

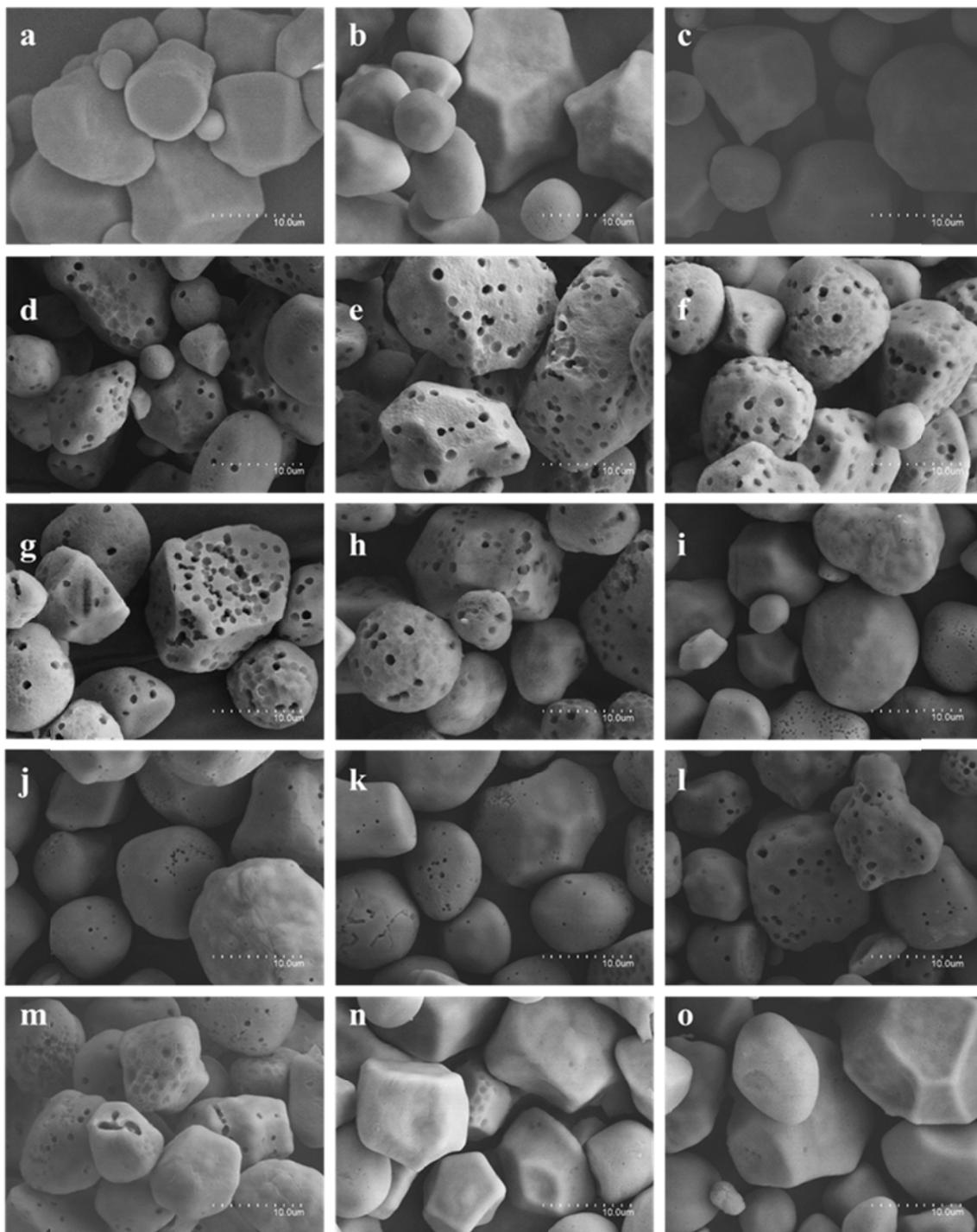
162 All experiments were repeated at least in duplicate. Experimental data were statistically  
163 analyzed using an analysis of variance (ANOVA) and values were expressed as a mean  
164  $\pm$  standard deviation. Fisher’s least significant differences test was used for assessment  
165 of significant differences among experimental mean values with 95% confidence.  
166 Statistical computations and analyses were conducted using Statgraphics Centurion XV  
167 software (Bitstream, Cambridge, N).

168 **3. Results**

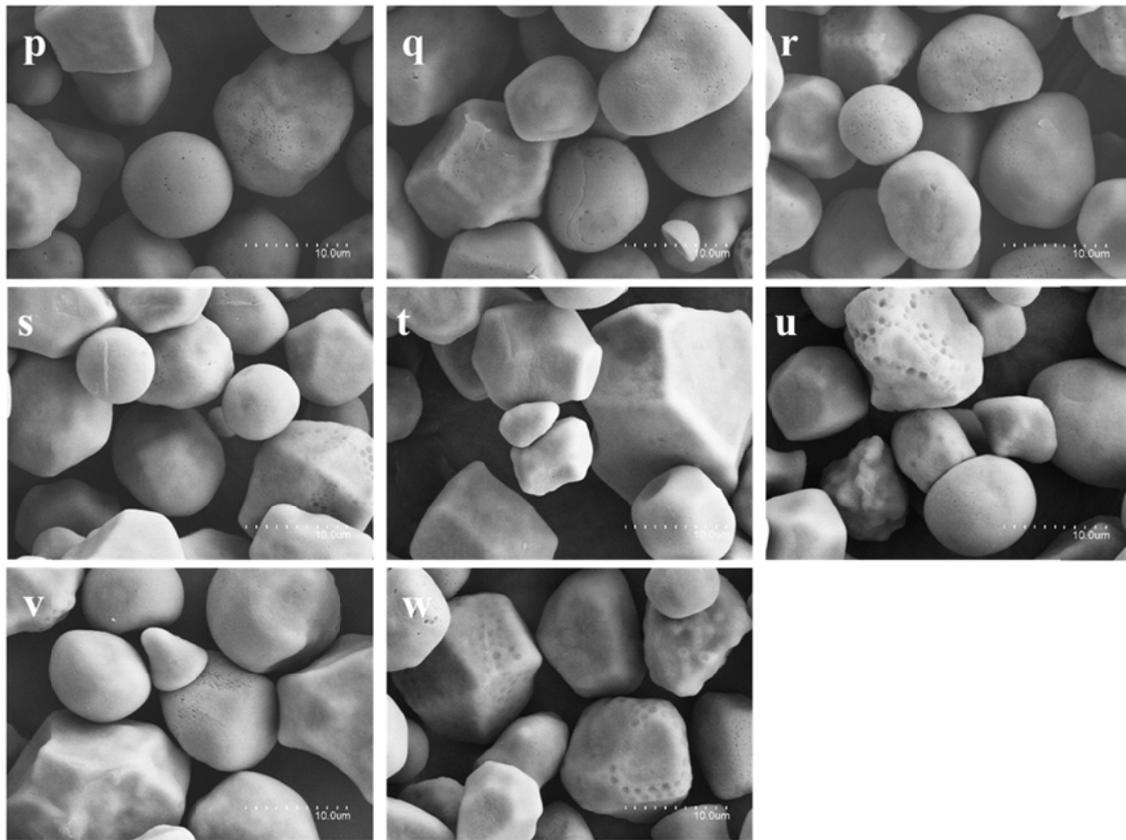
169 3.1. *Microstructure analysis*

170 The shape, size, structure and surface characteristics of corn starch granules tested  
171 (native, references and treated starches) were investigated using SEM (Figure 1). Native  
172 starch granules displayed an irregular and mostly polygonal shape with relatively  
173 smooth surface (Figure 1a). Reference starches (Figure 1 b,c) had similar appearance to  
174 native starch, showing no evidence of rupture, breakage or pores due to the incubation

179 with buffer; results that were analogous to those reported previously (Dura, Błaszczak  
180 & Rosell, 2014; Dura & Rosell, 2016). The effect of enzymatic treatment was readily  
181 visible in the modified starches microstructure, obtaining in all cases porous starch  
182 granules, without affecting the shape of the granule (Figure 1 d-w).



180



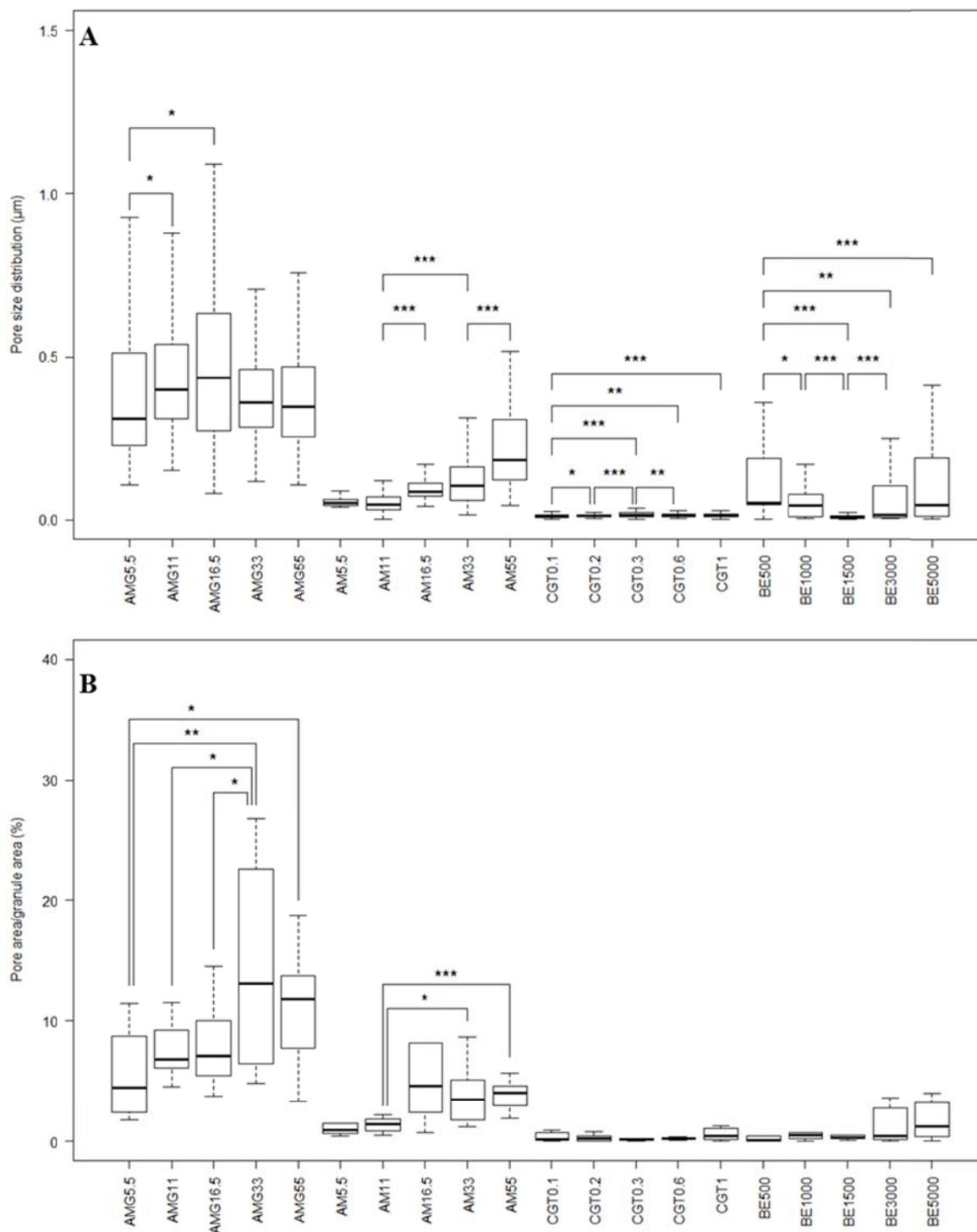
181

186 Figure 1: Scanning electron micrograph of and native corn starch (a), samples treated  
 187 enzymatically (d-w) and their counterparts controls (b and c). Magnification 3500×. Reference  
 188 A-0 (b); Reference P-0 (c); AMG 5.5, 11, 16.5, 33 and 55 (d-h); AM 5.5, 11, 16.5, 33 and 55 (i-  
 189 m); CGTase 0.1, 0.2, 0.3, 0.6 and 1 (n-r); BE 500, 1000, 1500, 3000 and 5000 (s-w). Numbers  
 190 following enzyme abbreviations are referred to the enzyme activity applied.

197 To give some objective results about the action of the enzymes, the pore size and the  
 198 ratio pore area to starch granule area (related to the abundance of pore per granule) were  
 199 quantified using image analysis (Figure 2). The pore size as well as pore area  
 200 distribution was significantly affected by the type of enzyme and also their level. AMG  
 201 action resulted in starch granules with larger pores and wider size distribution (Figure 2  
 202 A). In opposition, CGTase led to the lowest pore size. As the concentration of AMG  
 203 increased, the size of the pores progressively augmented until 16.5 U of AMG were  
 204 added; at higher enzyme level no further pore size increase was observed, although a  
 205 significant increase in the ratio pore area to granule area was observed (Figure 2 B)  
 206 indicating more pores per granule. Nevertheless, it was noted that at higher AMG  
 207 concentrations appeared some depressions in the granules, which resulted from the

197 eroding action of the enzyme onto the granule surface. Aggarwal and Dollimore (2000)  
198 also observed a visible increase in the size of the pores when augmented the AMG  
199 concentrations, till enzyme activity (800 U/g starch) was so pronounced that walls  
200 around pinholes were broken, leading to large irregular holes and broken structure.  
201 Similarly, pore size increased with the amount of AM or CGTase added, although both  
202 treatments resulted in smaller pore size than AMG treatment. The ratio pore to granule  
203 area of AM treated starches also maintained a similar pattern to the AMG samples,  
204 while it remained constant when CGTase enzyme was used. The BE enzyme produced  
205 very irregular pore sizes without any trend with the level of enzyme. It should be noted  
206 that the pore size was bigger when lower concentrations of enzymes were used, but in  
207 those cases pores resembled wide craters instead of deep holes. At higher enzyme  
208 concentration, smaller and deeper pinholes appeared, leading a mixture of  
209 heterogeneous sizes.

210 When starch granules are incubated with amylolytic enzymes, the enzymes migrate  
211 through the channels and initiate hydrolysis leading to an inside out pattern of digestion  
212 (Chen & Zhang, 2012). Nevertheless, the present study reveals that different porous  
213 starches could be obtained depending on the type, thus it is possible to modulate the  
214 number and size of pores by using either different amylolytic enzyme or level of  
215 enzyme.



217

220 Figure 2: Image analysis from SEM photographs. A) Pore size and B) pore surface area  
 221 distribution for each enzyme by boxplot. Numbers following enzyme abbreviations are referred  
 222 to the enzyme activity applied.

221 3.2. *CDs and oligosaccharides released during enzymatic treatment*

221 To understand the action of the enzymes on the starch granules, the released compounds  
222 after the incubation were analyzed. Table 1 listed the oligosaccharides and cyclodextrins  
223 contents released per starch ( $\text{mg } 100 \text{ g}^{-1}$ ). As expected, neither oligosaccharides nor  
224 cyclodextrins (CDs) were released from the reference samples (data not shown), neither  
225 from AMG treatment. No oligosaccharides (from DP1 to DP5) were released when corn  
226 starches were subjected to BE hydrolysis. BE cleaves  $\alpha$ -(1  $\rightarrow$  4)-O-glycosidic bonds  
227 and transfers the cleaved-glucan to  $\alpha$ -(1  $\rightarrow$  6) position leading to branched glucan  
228 mixtures (Roussel et al., 2013).  
229

230 Table 1: Oligosaccharides and cyclodextrins released after corn starch hydrolysis by AMG, AM and CGTase. Results are expressed in mg 100 g<sup>-1</sup> of starch.

| Enzyme type   | Enzyme (U/g starch) | Glucose      | Maltose      | Maltotriose | Maltotetraose | Maltopentaose | $\alpha$ -CD | $\beta$ -CD |
|---------------|---------------------|--------------|--------------|-------------|---------------|---------------|--------------|-------------|
| <b>AMG</b>    | 5.5                 | 16.19 ± 1.31 | n.d          | n.d         | n.d           | n.d           | n.d          | n.d         |
|               | 11                  | 15.64 ± 1.39 | n.d          | n.d         | n.d           | n.d           | n.d          | n.d         |
|               | 16.5                | 16.16 ± 1.17 | n.d          | n.d         | n.d           | n.d           | n.d          | n.d         |
|               | 33                  | 15.57 ± 1.08 | n.d          | n.d         | n.d           | n.d           | n.d          | n.d         |
|               | 55                  | 15.49 ± 1.01 | n.d          | n.d         | n.d           | n.d           | n.d          | n.d         |
| <b>AM</b>     | 5.5                 | 9.76 ± 0.04  | 10.81 ± 0.20 | 7.68 ± 0.13 | 2.05 ± 0.02   | n.d           | n.d          | n.d         |
|               | 11                  | 11.60 ± 0.27 | 8.82 ± 0.22  | 3.23 ± 0.40 | 1.90 ± 0.14   | 0.18 ± 0.00   | n.d          | n.d         |
|               | 16.5                | 12.42 ± 0.06 | 9.48 ± 0.39  | 2.38 ± 0.17 | 1.57 ± 0.38   | n.d           | n.d          | n.d         |
|               | 33                  | 13.94 ± 0.41 | 9.70 ± 0.13  | 0.55 ± 0.05 | 1.18 ± 0.01   | n.d           | n.d          | n.d         |
|               | 55                  | 15.23 ± 0.16 | 10.49 ± 0.20 | 0.27 ± 0.09 | 0.42 ± 0.08   | n.d           | n.d          | n.d         |
| <b>CGTase</b> | 0.1                 | 1.23 ± 0.03  | 0.54 ± 0.05  | 0.51 ± 0.09 | 0.50 ± 0.13   | 0.01 ± 0.00   | 2.25 ± 0.09  | n.d         |
|               | 0.2                 | 1.37 ± 0.03  | 1.07 ± 0.00  | 0.85 ± 0.04 | 0.96 ± 0.06   | 0.02 ± 0.00   | 2.33 ± 0.06  | n.d         |
|               | 0.3                 | 0.83 ± 0.04  | 1.07 ± 0.05  | 0.93 ± 0.04 | 1.22 ± 0.09   | 0.02 ± 0.00   | 2.73 ± 0.24  | n.d         |
|               | 0.6                 | 0.70 ± 0.08  | 1.19 ± 0.17  | 0.97 ± 0.13 | 1.00 ± 0.13   | 0.01 ± 0.00   | 1.73 ± 0.02  | n.d         |
|               | 1                   | 1.27 ± 0.02  | 1.78 ± 0.00  | 1.37 ± 0.02 | 1.60 ± 0.05   | 0.03 ± 0.00   | 2.09 ± 0.14  | n.d         |

231 n.d. non detected

232

233 Regarding the other amylolytic enzymes, starch-converting enzymes have been  
234 classified into exo-amylases and endo-amylases owing to their cleavage action, and  
235 results displayed that difference (Table 1). AMG treatment released exclusively glucose,  
236 and the amount remained constant independently on the enzyme concentration.

237 Amyloglucosidase is a well-known exo-amylase, releasing only glucose residues from  
238 amylose or amylopectin chains (Bouchet-Spinelli, Coche-Guérente, Armand, Lenouvel,  
239 Labbé & Fort, 2013). However, saturation of the non-reducing-ends of starch chains has  
240 been reported when enough glucoamylase is present (Chen & Zhang, 2012), which  
241 would explain the steady glucose level.

242 In addition, the endo-amylases, AM and CGTase, are able to cleave  $\alpha$ -1–4 glycosidic  
243 bonds existing in the internal part (endo-) of a polysaccharide chain. As expected, AM  
244 majorly converted starch to glucose followed by maltose. Moreover, the amount of  
245 short chain oligosaccharides, ranging from DP1 to DP2 increased with the amount of  
246 AM added, whereas DP3, DP4 and  $\alpha$ -CD chains decreased. Conversely, the amount of  
247 short chain oligosaccharides ranging from DP1 to DP5 decreased as increasing the level  
248 of CGTase added, with a simultaneous increase in  $\alpha$ -CD. Overall, CGTases convert  
249 amylose or amylopectin into a mixture of  $\alpha$ -,  $\beta$ - and  $\gamma$ -CD and some dextrans, and the  
250 proportion was dependent on the enzyme specificity (Terada, Yanase, Takata, Takaha &  
251 Okada, 1997), but also on the substrate, complexing agents and reaction conditions  
252 (Blackwood & Bucke, 2000).

### 253 3.3. *Amylose, damaged starch content and adsorptive capacity*

254 Amylose and damaged starch contents were determined in the treated starches (Table  
255 2). The statistical analysis indicated that the enzymatic treatment significantly modified  
256 the amylose content, the amount of damage starch and the adsorption properties of the  
257 starches; but the enzyme level only prompted significant effect on the amount of

258 damage starch and adsorptive water capacity. Amylose content showed a significant  
259 moderate correlation with the damaged starch content ( $r=0.6684$ ,  $P<0.0000$ ), mainly  
260 ascribed to the action of AMG and BE. Concerning the specific action of each enzyme,  
261 a significant reduction in amylose content, with the subsequent increase in amylopectin,  
262 was observed after AM and CGTase treatments, without observing any trend with the  
263 level of enzyme applied. These results are in agreement with the inverse relationship  
264 reported between the amylose content and the amount of hydrolyzed starch (Tester, Qi  
265 & Karkalas, 2006), and also with the trend reported for CGTase modified starches  
266 (Dura & Rosell, 2016). Nevertheless, previous results with AM and AMG indicated  
267 that at lower concentrations than the one of the present study no change in the amylose  
268 content was observed even when increasing the enzymatic treatment to 24 or 48 hours  
269 (Dura, Błaszczak & Rosell, 2014).

270 Damaged starch was hardly affected by the action of AM and CGTase, although a  
271 tendency to decrease it was observed in the case of CGTase. Considering that  
272 microstructure analysis confirmed the impairment of the granule, it seems that the  
273 experimental assay for quantifying damage starch was not sensible or reliable enough to  
274 distinguish the degree of damage. Conversely, AMG and BE treatment promoted the  
275 opposite trend, the amylose content appeared to increase but not always significantly,  
276 and the amount of damage starch significantly augmented, particularly in the case of  
277 BE. Regarding the level of BE applied, a clear decrease of damage starch content was  
278 observed when increasing the enzyme concentration. Starch granules have a unique  
279 semi-crystalline supramolecular structure with concentric layers of amorphous and  
280 crystalline regions radiating from the hilum (Ratnayake & Jackson, 2008). Taking into  
281 account that the amylopectin side chains form the framework of the crystalline lamellae,  
282 with branching points located in the amorphous domains, where the majority of the

283 amylose is located (Copeland, Blazek, Salman & Tang, 2009), it seems that depending  
284 on the enzymatic treatment amylose or amylopectin are preferentially hydrolyzed.  
285 Results on amylose content suggested that AM and CGTase attacked more proportion  
286 of amylose, leading an increase in the amount of amylopectin, suggesting deeper  
287 pinholes and the attack of amorphous and crystalline structure. In opposition, AMG and  
288 BE seem to hydrolyze preferentially the amylopectin chains, increasing the proportion  
289 of amylose in the surface of starch granule, thus bigger and less deep holes, which  
290 agrees with microstructure results.

291 Table 2: Effect of enzymatic treatment on the water and oil adsorption capacity and chemical composition (amylose content and damaged starch) of the  
 292 resulting porous starches

| Enzyme type   | Enzyme (U/g starch) | Amylose content (%) |        |     | Damaged starch (%) |        |     | Adsorptive water capacity (g/g) |        |     | Adsorptive oil capacity (g/g) |        |     |
|---------------|---------------------|---------------------|--------|-----|--------------------|--------|-----|---------------------------------|--------|-----|-------------------------------|--------|-----|
| <b>Native</b> | 0                   | 25.76               | ± 0.82 | de  | 15.41              | ± 0.19 | cd  | 0.74                            | ± 0.02 | a   | 1.14                          | ± 0.05 | g-h |
| <b>AMG</b>    | 5.5                 | 23.47               | ± 0.35 | cd  | 21.30              | ± 0.05 | e   | 1.12                            | ± 0.03 | hi  | 1.10                          | ± 0.05 | e-h |
|               | 11                  | 27.36               | ± 1.31 | e-g | 22.77              | ± 0.17 | f   | 1.25                            | ± 0.04 | j   | 1.27                          | ± 0.00 | h-j |
|               | 16.5                | 26.97               | ± 0.31 | e-g | 23.64              | ± 0.15 | f   | 1.45                            | ± 0.08 | k   | 1.41                          | ± 0.02 | j   |
|               | 33                  | 28.01               | ± 4.76 | e-g | 21.51              | ± 0.07 | e   | 1.44                            | ± 0.08 | k   | 1.35                          | ± 0.02 | j   |
|               | 55                  | 26.91               | ± 0.16 | g   | 20.66              | ± 0.05 | e   | 1.46                            | ± 0.06 | k   | 1.32                          | ± 0.03 | ij  |
| <b>AM</b>     | 5.5                 | 19.53               | ± 1.82 | ab  | 14.97              | ± 0.05 | a-d | 1.16                            | ± 0.06 | ij  | 0.85                          | ± 0.28 | a-d |
|               | 11                  | 18.56               | ± 0.46 | ab  | 15.01              | ± 0.63 | a-d | 1.07                            | ± 0.01 | g-i | 0.96                          | ± 0.08 | c-f |
|               | 16.5                | 18.95               | ± 0.38 | ab  | 15.40              | ± 0.22 | cd  | 0.85                            | ± 0.03 | b-e | 0.76                          | ± 0.08 | a-c |
|               | 33                  | 21.24               | ± 0.41 | a-c | 15.13              | ± 0.37 | b-d | 0.71                            | ± 0.06 | a   | 0.86                          | ± 0.08 | a-d |
|               | 55                  | 19.17               | ± 0.82 | ab  | 16.03              | ± 0.73 | d   | 0.93                            | ± 0.03 | d-f | 0.71                          | ± 0.01 | ab  |
| <b>CGTase</b> | 0.1                 | 21.26               | ± 0.19 | a-c | 14.38              | ± 0.05 | a-c | 0.90                            | ± 0.07 | c-f | 0.86                          | ± 0.05 | a-d |
|               | 0.2                 | 19.45               | ± 1.07 | ab  | 14.37              | ± 0.19 | a-c | 0.89                            | ± 0.04 | f-h | 1.09                          | ± 0.10 | e-h |
|               | 0.3                 | 19.58               | ± 2.39 | ab  | 13.68              | ± 0.07 | a   | 0.97                            | ± 0.07 | c-f | 0.98                          | ± 0.17 | d-g |
|               | 0.6                 | 21.91               | ± 0.14 | bc  | 13.05              | ± 0.91 | a-b | 0.80                            | ± 0.03 | e-g | 1.13                          | ± 0.33 | f-i |
|               | 1                   | 21.66               | ± 0.64 | bc  | 14.41              | ± 0.10 | a-c | 0.93                            | ± 0.04 | a-c | 0.96                          | ± 0.27 | c-g |
| <b>BE</b>     | 500                 | 28.96               | ± 0.15 | fg  | 30.66              | ± 0.11 | ij  | 0.75                            | ± 0.07 | ab  | 0.85                          | ± 0.01 | a-d |
|               | 1000                | 18.90               | ± 0.84 | d-f | 31.18              | ± 0.63 | j   | 0.79                            | ± 0.04 | a-c | 0.66                          | ± 0.09 | a   |

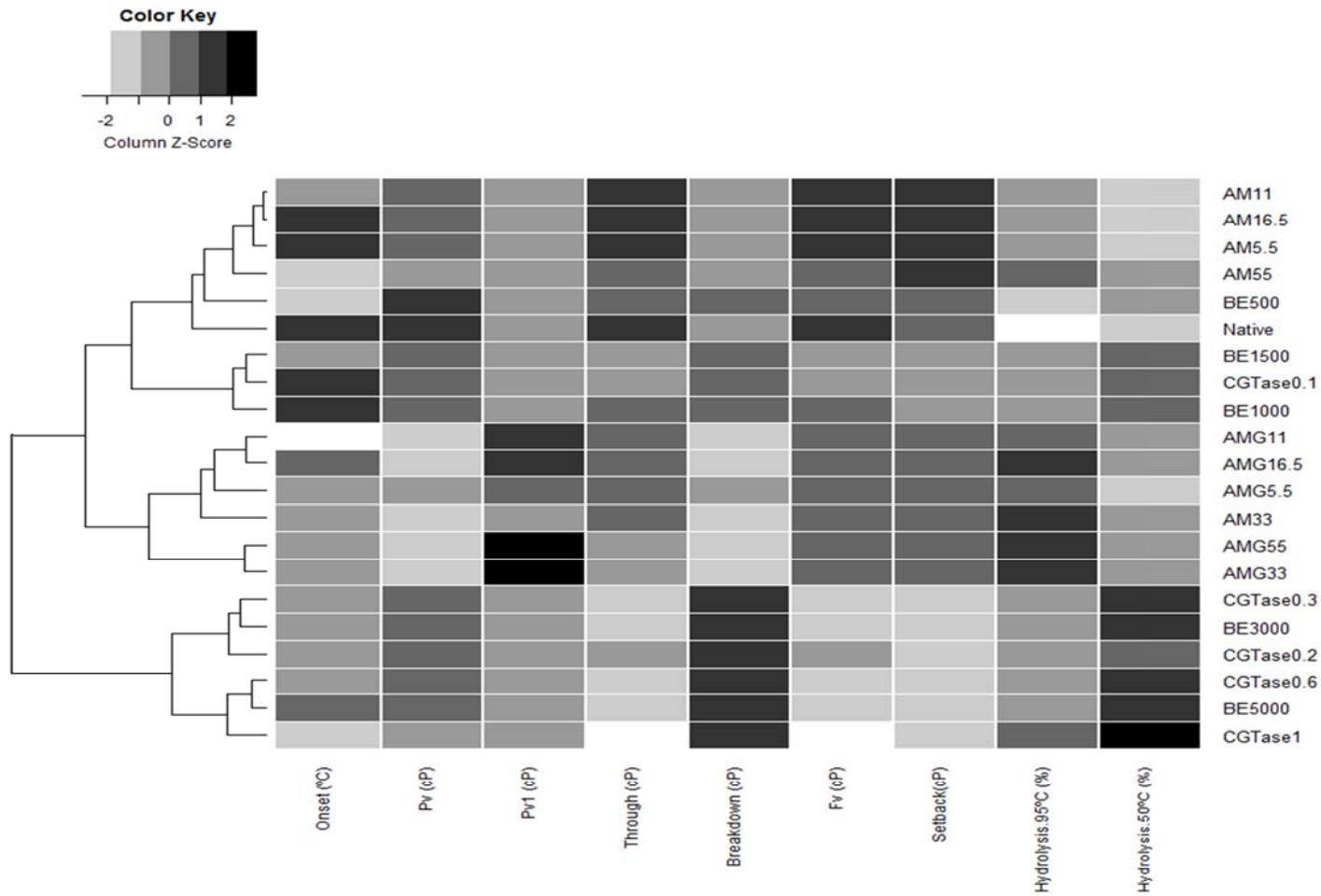
|                       |                     |       |        |                |       |        |               |      |        |                |      |        |                |
|-----------------------|---------------------|-------|--------|----------------|-------|--------|---------------|------|--------|----------------|------|--------|----------------|
|                       | 1500                | 28.54 | ± 1.36 | <sup>e-g</sup> | 29.61 | ± 1.39 | <sup>hi</sup> | 0.88 | ± 0.02 | <sup>c-f</sup> | 0.90 | ± 0.00 | <sup>b-e</sup> |
|                       | 3000                | 27.11 | ± 1.65 | <sup>d-f</sup> | 29.06 | ± 1.22 | <sup>h</sup>  | 0.82 | ± 0.05 | <sup>a-d</sup> | 0.85 | ± 0.11 | <sup>a-d</sup> |
|                       | 5000                | 27.25 | ± 0.65 | <sup>e-g</sup> | 27.76 | ± 2.06 | <sup>g</sup>  | 0.87 | ± 0.12 | <sup>b-f</sup> | 0.84 | ± 0.12 | <sup>a-d</sup> |
| <b><i>P-value</i></b> | <b>Enzyme type</b>  |       | 0.00   |                |       | 0.00   |               |      | 0.00   |                |      | 0.00   |                |
|                       | <b>Enzyme (U/g)</b> |       | 0.11   |                |       | 0.00   |               |      | 0.02   |                |      | 0.17   |                |

293

294 The adsorptive capacity of modified starches for water and sunflower oil are also  
295 summarized in Table 2. The hydrophilic nature was significantly dependent on both  
296 enzyme type and concentration, while hydrophobic nature depended only on the enzyme  
297 type. In general, all enzymatic treatments increased the water adsorption capacity of the  
298 starches; among them, AMG showed the greatest effect, followed by AM, CGTase and  
299 BE treatment. Likely, the size of the pores originated by AMG was responsible of this  
300 behavior due to the increase of the surface area. The adsorptive oil capacity of starch  
301 was only significantly modified when treated with AMG. Chen and Zhang (2012)  
302 obtained an increase in both solvents retention ability respect to native starch, due to the  
303 increase in the surface area promoted by the starch treatment with AMG (11 U/g  
304 starch), which agrees with results of the present study. Therefore, it seems that the pore  
305 size plays a fundamental role for oil adsorption, which was only sufficient in the case of  
306 AMG hydrolysis.

#### 307 *3.4. Enzymatic modification effects on pasting and thermal starch properties*

308 To illustrate the pasting characteristics of the porous starches obtained from different  
309 type of enzymes a heatmap was constructed with the pasting properties (Figure 3). The  
310 heatmap of the hierarchical clustering of the RVA properties for the modified samples  
311 was analyzed on the basis of similarities and differences in starch pasting properties,  
312 including onset, peak viscosity, through, breakdown, final viscosity, setback, hydrolysis  
313 percentage at 95 °C and 50 °C (Figure 3). The dendrogram consisted of three major  
314 clusters. One cluster contained native, AM treated samples and the minor concentration  
315 of CGTase and BE treatments, up to 1500 U/g starch. Another cluster essentially  
316 included AMG treated starches and one AM treated sample. The last cluster comprised  
317 CGTase and BE treated starches using high enzyme levels.



318

319 Figure 3: Hierarchical clustering of RVA profiles. A heat map representing the hierarchical clustering of the Z scores of the enzyme activities related to viscoelastic  
 320 properties, when compared AMG, AM, CGTase and BE enzyme treatment. The Z scores represent the dispersion around the overall mean of the viscoelastic properties and  
 321 weighted by their standard errors. The scale of the intensity is shown in the top corner. Rows represent samples and column viscoelastic properties. Numbers following  
 322 enzyme abbreviations are referred to the enzyme activity applied. Pv: peak viscosity; Pv1: additional peak viscosity; Fv: final viscosity

323 It was evident from the heatmap that enzymes changed the pasting performance of  
324 starch suspensions and the effect was also dependent on their concentrations,  
325 particularly in the case of CGTase and BE. The onset temperature, indicative of the  
326 initial viscosity increase, was significantly decreased by all enzyme studied,  
327 independently of the concentration used. Therefore, lower cooking temperature was  
328 required for the gelatinization of porous starches, likely due to faster water absorption  
329 on the starch granules, since a negative correlation was observed between onset  
330 temperature and pore size ( $r = -0.4581$ ,  $P < 0.001$ ). AM treated samples showed similar  
331 pasting behavior to native starch, unless the maximum viscosity that decreased after  
332 treatment. AM acts on the starch molecules breaking  $\alpha$ -(1-4) linkages and providing  
333 dextrans, which present lower swelling during gelatinization (Rocha, Carneiro &  
334 Franco, 2010). Porous starches had significantly lower peak viscosity, through, final  
335 viscosity and setback compared to native, which agree with previous results (Dura,  
336 Błaszczak & Rosell, 2014). In the case of AMG treated samples they were grouped due  
337 to their lower peak viscosity and breakdown and higher final viscosity and setback,  
338 besides the presence of an additional peak viscosity (Pv1) during heating, prior to the  
339 common peak viscosity at 95 °C. This additional peak was negatively correlated with  
340 peak viscosity, showing a progressive increase in the first peak in parallel to the  
341 reduction of peak viscosity. The decrease of peak viscosity due to the joint action of  $\alpha$ -  
342 amylase and glucoamylase has been explained by the disintegration of fragile granules  
343 owing to their porous structure, leading to less viscous slurries (Uthumporn, Zaidul &  
344 Karim, 2010). In this regard, pore size, ratio of pore area to granule area and water  
345 adsorptive capacity was negatively correlated with peak viscosity, confirming this  
346 hypothesis.

347 Porous starches obtained with very high levels of CGTase or BE were mainly  
348 characterized by very low values of final viscosity and setback, and high breakdown  
349 values. Those effects have been reported when wheat starch was treated by CGTase  
350 Gujral and Rosell (2004).

351 The values for the thermal properties of native starch (Table 3) agrees with previous  
352 reported results for corn (Jane et al., 1999). In modified starches,  $T_o$ ,  $T_p$  and  $\Delta H$   
353 significantly ( $P < 0.05$ ) varied owing to the type of enzyme used and its level, but  $T_c$   
354 was only significantly affected by the type of enzyme. Porous starches showed lower  $T_o$   
355 and  $T_c$  than native starch. In the case of AMG treated starches those temperatures  
356 decreased when increasing the level of enzyme during treatment. Moreover, lower  
357 energy ( $\Delta H$ ) was required to promote starch gelatinization, likely due to less energy was  
358 needed to unravel and melt the unstable double helices during gelatinization (Chung,  
359 Liu & Hoover, 2009).

360 On the other hand, BE enzyme produced starches with lower  $T_o$  and  $T_p$ , but similar  $T_c$   
361 to native starch. Conclusion temperature ( $T_c$ ) was only significantly reduced by AM.  
362 Correlation analysis indicated that all gelatinization parameters evaluated except  
363 enthalpy were positively correlated ( $P < 0.05$ ) with amylose content, but not with  
364 damaged starch, pore size or pore area to starch granule, which are in agreement with  
365 previous observations (Stevenson, Doorenbos, Jane & Inglett, 2006). In addition,  
366 enthalpy was negatively correlated with water ( $r = -0.3555$ ,  $P < 0.05$ ) and oil adsorption  
367 capacity ( $r = -0.4078$ ,  $P < 0.01$ ).

368

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

| Enzyme type    | Enzyme (U/g starch) | T <sub>0</sub> (°C) |        |     | T <sub>p</sub> (°C) |        |     | T <sub>c</sub> (°C) |        |    | ΔH (J/g)    |        |     |
|----------------|---------------------|---------------------|--------|-----|---------------------|--------|-----|---------------------|--------|----|-------------|--------|-----|
| Native         | 0                   | 63.28               | ± 0.14 | i   | 68.20               | ± 0.00 | h   | 74.71               | ± 0.17 | b  | 20.66       | ± 1.27 | c-e |
| AMG            | 5.5                 | 62.96               | ± 0.21 | g-i | 66.70               | ± 0.24 | a-e | 74.32               | ± 0.68 | b  | 20.26       | ± 1.08 | b-e |
|                | 11                  | 63.26               | ± 0.10 | hi  | 67.53               | ± 0.00 | g   | 74.86               | ± 0.08 | b  | 19.18       | ± 1.70 | bc  |
|                | 16.5                | 63.26               | ± 0.15 | hi  | 67.37               | ± 0.47 | fg  | 74.65               | ± 0.11 | b  | 16.64       | ± 0.14 | aa  |
|                | 33                  | 62.80               | ± 0.57 | f-h | 67.03               | ± 0.71 | c-g | 74.45               | ± 0.92 | b  | 19.64       | ± 1.75 | b-d |
|                | 55                  | 62.65               | ± 0.47 | d-g | 66.95               | ± 1.06 | b-g | 73.88               | ± 1.43 | b  | 19.06       | ± 0.38 | bc  |
| AM             | 5.5                 | 62.00               | ± 0.36 | a-c | 66.45               | ± 0.12 | a-c | 73.93               | ± 0.04 | a  | 20.77       | ± 0.18 | c-e |
|                | 11                  | 61.86               | ± 0.50 | a   | 66.28               | ± 0.35 | a   | 73.81               | ± 0.62 | a  | 23.37       | ± 1.13 | f   |
|                | 16.5                | 61.93               | ± 0.20 | a   | 66.37               | ± 0.00 | ab  | 73.86               | ± 0.06 | a  | 19.43       | ± 0.49 | b-d |
|                | 33                  | 62.24               | ± 0.22 | a-e | 66.70               | ± 0.24 | a-e | 73.12               | ± 0.40 | a  | 19.82       | ± 2.70 | b-e |
|                | 55                  | 61.98               | ± 0.11 | ab  | 66.37               | ± 0.24 | ab  | 73.62               | ± 0.13 | a  | 21.67       | ± 0.94 | d-f |
| CGTase         | 0.1                 | 62.49               | ± 0.12 | c-g | 67.28               | ± 0.12 | c-g | 73.98               | ± 0.12 | ab | 19.35       | ± 1.39 | bc  |
|                | 0.2                 | 61.99               | ± 0.01 | a-c | 66.37               | ± 0.24 | ab  | 73.27               | ± 0.46 | ab | 20.99       | ± 0.87 | c-e |
|                | 0.3                 | 62.01               | ± 0.12 | a-c | 66.37               | ± 0.00 | ab  | 73.34               | ± 0.18 | ab | 18.15       | ± 0.56 | ab  |
|                | 0.6                 | 62.20               | ± 0.08 | a-d | 66.62               | ± 0.12 | a-d | 73.68               | ± 0.09 | ab | 19.47       | ± 1.02 | b-d |
|                | 1                   | 62.46               | ± 0.10 | b-g | 67.03               | ± 0.24 | c-g | 73.67               | ± 0.26 | ab | 19.11       | ± 0.58 | bc  |
| BE             | 500                 | 62.81               | ± 0.28 | f-h | 67.28               | ± 0.12 | c-g | 74.25               | ± 0.46 | ab | 23.72       | ± 1.00 | f   |
|                | 1000                | 62.73               | ± 0.40 | e-g | 67.03               | ± 0.24 | c-g | 74.18               | ± 0.96 | ab | 21.95       | ± 1.43 | ef  |
|                | 1500                | 62.30               | ± 0.05 | a-f | 66.78               | ± 0.12 | a-f | 73.30               | ± 0.24 | ab | 20.31       | ± 0.84 | b-e |
|                | 3000                | 62.48               | ± 0.28 | b-g | 67.12               | ± 0.35 | d-g | 74.04               | ± 0.77 | ab | 20.94       | ± 1.39 | c-e |
|                | 5000                | 62.47               | ± 0.32 | b-g | 66.87               | ± 0.24 | a-f | 73.76               | ± 0.19 | ab | 20.02       | ± 0.70 | b-e |
| <i>P-value</i> | Enzyme type         | <b>0.00</b>         |        |     | <b>0.01</b>         |        |     | <b>0.04</b>         |        |    | <b>0.03</b> |        |     |
|                | Enzyme (U/g)        | <b>0.00</b>         |        |     | <b>0.00</b>         |        |     | <b>0.06</b>         |        |    | <b>0.03</b> |        |     |

370

371 To = onset temperature, Tp = peak temperature, Tc = conclusion temperature,  $\Delta H$  = enthalpy change. Values followed by different letters within a column  
372 denote significantly different levels ( $P < 0.05$ ) ( $n = 3$ ).

373 **4. Conclusions**

374 Porous starches could be obtained by enzymatic treatment of corn starch at sub-  
375 gelatinization temperature. The size distribution of the pores and their area were  
376 dependent on the type of enzyme used for the starch treatment, but also the level of  
377 enzyme. AMG led to porous starches with larger holes, whereas the smallest were  
378 obtained with CGTase. Porous starches differed in their pasting performance and  
379 thermal properties, besides adsorptive water or oil capacities. By selecting the type of  
380 enzyme and its level it could be modulated the degree of porosity.  
381 Enzymatic treatment of native starch granules reveals as a powerful tool to modify the  
382 properties of starch. The added value and feasibility of this methodology on different  
383 sources of starch should be examined.

384 **Acknowledgements**

385 Authors acknowledge the financial support of the Spanish Ministry of Economy and  
386 Competitiveness (Project AGL2014-52928-C2-1-R) and the European Regional  
387 Development Fund (FEDER). Y. Benavent-Gil would like to thank predoctoral  
388 fellowship from Spanish Ministry of Economy and Competitiveness.

389 **References**

- 390 Aggarwal, P., & Dollimore, D. (2000). Degradation of starchy food material by thermal  
391 analysis. *Thermochimica Acta*, 357–358, 57-63.
- 392 American Association of Cereal Chemists, A. (2000). Approved methods of AACC  
393 (10th ed.). St. Paul, MN: American Association of Cereal Chemists. Method 76-31.
- 394 Belingheri, C., Giussani, B., Rodriguez-Estrada, M. T., Ferrillo, A., & Vittadini, E.  
395 (2015). Oxidative stability of high-oleic sunflower oil in a porous starch carrier. *Food*  
396 *Chemistry*, 166, 346-351.
- 397 Blackwood, A. D., & Bucke, C. (2000). Addition of polar organic solvents can improve  
398 the product selectivity of cyclodextrin glycosyltransferase: Solvent effects on cgtase.  
399 *Enzyme and Microbial Technology*, 27(9), 704-708.
- 400 Bouchet-Spinelli, A., Coche-Guérente, L., Armand, S., Lenouvel, F., Labbé, P., & Fort,  
401 S. (2013). Functional characterization of starch-degrading enzymes using quartz crystal  
402 microbalance with dissipation monitoring (QCM-D). *Sensors and Actuators B:*  
403 *Chemical*, 176, 1038-1043.
- 404 Copeland, L., Blazek, J., Salman, H., & Tang, M. C. (2009). Form and functionality of  
405 starch. *Food Hydrocolloids*, 23(6), 1527-1534.

406 Chen, G., & Zhang, B. (2012). Hydrolysis of granular corn starch with controlled pore  
407 size. *Journal of Cereal Science*, 56(2), 316-320.

408 Chung, H.-J., Liu, Q., & Hoover, R. (2009). Impact of annealing and heat-moisture  
409 treatment on rapidly digestible, slowly digestible and resistant starch levels in native  
410 and gelatinized corn, pea and lentil starches. *Carbohydrate Polymers*, 75(3), 436-447.

411 Dura, A., Błaszczak, W., & Rosell, C. M. (2014). Functionality of porous starch  
412 obtained by amylase or amyloglucosidase treatments. *Carbohydrate Polymers*, 101,  
413 837-845.

414 Dura, A., & Rosell, C. M. (2016). Physico-chemical properties of corn starch modified  
415 with cyclodextrin glycosyltransferase. *International Journal of Biological*  
416 *Macromolecules*, 87, 466-472.

417 Gibson, T. S., Solah, V. A., & McCleary, B. V. (1997). A Procedure to Measure  
418 Amylose in Cereal Starches and Flours with Concanavalin A. *Journal of Cereal*  
419 *Science*, 25(2), 111-119.

420 Gujral, H. S., & Rosell, C. M. (2004). Modification of pasting properties of wheat  
421 starch by cyclodextrin glycosyltransferase. *Journal of the Science of Food and*  
422 *Agriculture*, 84(13), 1685-1690.

423 Ichihara, T., Fukuda, J., Takaha, T., Yuguchi, Y., & Kitamura, S. (2013). Limited  
424 Hydrolysis of Insoluble Cassava Starch Granules Results in Enhanced Gelling  
425 Properties. *Journal of Applied Glycoscience*, 61(1), 15-20.

426 Jane, J., Chen, Y., Lee, L., McPherson, A., Wong, K., Radosavljevic, M., &  
427 Kasemsuwan, T. (1999). Effects of amylopectin branch chain length and amylose  
428 content on the gelatinization and pasting properties of starch 1. *Cereal chemistry*, 76(5),  
429 629-637.

430 Luo, Z., Cheng, W., Chen, H., Fu, X., Peng, X., Luo, F., & Nie, L. (2013). Preparation  
431 and properties of enzyme-modified cassava starch-zinc complexes. *Journal of*  
432 *agricultural and food chemistry*, 61(19), 4631-4638.

433 Majzoobi, M., Hedayati, S., & Farahnaky, A. (2015). Functional properties of  
434 microporous wheat starch produced by  $\alpha$ -amylase and sonication. *Food Bioscience*, 11,  
435 79-84.

436 Ratnayake, W. S., & Jackson, D. S. (2008). Chapter 5 Starch Gelatinization. *Advances*  
437 *in Food and Nutrition Research* (Vol. Volume 55, pp. 221-268): Academic Press.

438 Rocha, T. d. S., Carneiro, A. P. d. A., & Franco, C. M. L. (2010). Effect of enzymatic  
439 hydrolysis on some physicochemical properties of root and tuber granular starches.  
440 *Food Science and Technology (Campinas)*, 30(2), 544-551.

441 Roussel, X., Lancelon-Pin, C., Viksø-Nielsen, A., Rolland-Sabaté, A., Grimaud, F.,  
442 Potocki-Véronèse, G., Buléon, A., Putaux, J.-L., & D'Hulst, C. (2013). Characterization  
443 of substrate and product specificity of the purified recombinant glycogen branching  
444 enzyme of *Rhodothermus obamensis*. *Biochimica et Biophysica Acta (BBA) - General*  
445 *Subjects*, 1830(1), 2167-2177.

446 Sorndech, W., Sagnelli, D., Meier, S., Jansson, A. M., Lee, B.-H., Hamaker, B. R.,  
447 Rolland-Sabaté, A., Hebelstrup, K. H., Tongta, S., & Blennow, A. (2016). Structure of  
448 branching enzyme- and amyloamylase modified starch produced from well-defined  
449 amylose to amylopectin substrates. *Carbohydrate Polymers*, 152, 51-61.

450 Stevenson, D. G., Doorenbos, R. K., Jane, J. I., & Inglett, G. E. (2006). Structures and  
451 functional properties of starch from seeds of three soybean (*Glycine max* (L.) Merr.)  
452 varieties. *Starch □ Stärke*, 58(10), 509-519.

453 Sujka, M., & Jamroz, J. (2007). Starch granule porosity and its changes by means of  
454 amylolysis. *International agrophysics*, 21(1), 107.

455 Sun, H., Zhao, P., Ge, X., Xia, Y., Hao, Z., Liu, J., & Peng, M. (2010). Recent advances  
456 in microbial raw starch degrading enzymes. *Applied biochemistry and biotechnology*,  
457 *160*(4), 988-1003.

458 Terada, Y., Yanase, M., Takata, H., Takaha, T., & Okada, S. (1997). Cyclodextrins Are  
459 Not the Major Cyclic  $\alpha$ -1,4-Glucans Produced by the Initial Action of Cyclodextrin  
460 Glucanotransferase on Amylose. *Journal of Biological Chemistry*, *272*(25), 15729-  
461 15733.

462 Tester, R. F., Qi, X., & Karkalas, J. (2006). Hydrolysis of native starches with amylases.  
463 *Animal Feed Science and Technology*, *130*(1-2), 39-54.

464 Uthumporn, U., Zaidul, I. S. M., & Karim, A. A. (2010). Hydrolysis of granular starch  
465 at sub-gelatinization temperature using a mixture of amylolytic enzymes. *Food and*  
466 *Bioproducts Processing*, *88*(1), 47-54.

467 van der Maarel, M. J. E. C., & Leemhuis, H. (2013). Starch modification with microbial  
468 alpha-glucanotransferase enzymes. *Carbohydrate Polymers*, *93*(1), 116-121.

469 Yamamoto, K., Zhang, Z. Z., & Kobayashi, S. (2000). Cycloamylose (cyclodextrin)  
470 glucanotransferase degrades intact granules of potato raw starch. *Journal of agricultural*  
471 *and food chemistry*, *48*(3), 962-966.

472 Yousif, E. I., Gadallah, M. G. E., & Sorour, A. M. (2012). Physico-chemical and  
473 rheological properties of modified corn starches and its effect on noodle quality. *Annals*  
474 *of Agricultural Sciences*, *57*(1), 19-27.

475 Zhang, B., Cui, D., Liu, M., Gong, H., Huang, Y., & Han, F. (2012). Corn porous  
476 starch: Preparation, characterization and adsorption property. *International Journal of*  
477 *Biological Macromolecules*, *50*(1), 250-256.

478