PHYSICO-CHEMICAL PROPERTIES OF CORN STARCH MODIFIED WITH CYCLODEXTRIN GLYCOSYLTRANSFERASE

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Abstract

Cyclodextrin glycosyltransferase (CGTase) has been used to produce cyclodextrins (CDs) from starches, but their ability to modify starches has been barely explored. The effect of CGTase on corn starch at sub-gelatinization temperature (50 ºC) and at different pH conditions, pH 4.0 and pH 6.0, was evaluated. Biochemical features, thermal and structural analysis, oligosaccharides and CDs content were studied. Microscopic analysis of the granules confirmed the enzymatic modification of the starches obtaining structures with irregular surface and small pinholes. The extent of the starch modification was largely dependent on the pHs, being higher at pH 6.0. This was also confirmed by the low viscosity of the resulting pastes during a heating and cooling cycle. Thermal parameters were not affected due to enzymatic treatment. Modified starches were less susceptible to undergo α-amylase hydrolysis. CDs released were higher for samples treated at pH 4.0. Therefore, CGTase modification of corn starches at sub-gelatinization temperature offers an attractive alternative for obtaining porous starches with different properties depending on the pH conditions.

Key words: starch, enzymatic modification, pasting properties, Cyclodextrin

Glycosyltransferase.

1. Introduction

Starch is a valuable ingredient being widely used in the food industry owing to its unique thermal, structural and functional properties. However, starch use in the food industry is limited by their weak-bodied, cohesive, poor thermal, shear and acid stability [1]. Because of that physical, chemical and enzymatic modifications have been proposed for modulating the functional properties of native starches [2]. Beyond all types of modifications, enzymatic modification has a number of advantages comprising replacement of synthetic chemicals, lowering energy consumption levels and fewer by-products. Nowadays, the increasing interest for clean labeled modified starches has prompted the enzymatic modification of starches. Particularly, those catalyzed by amylases and amyloglucosidases [3, 4], looking for understanding starch modification and the released products. Cyclodextrin glycosyltransferase (CGTase) is an endoenzyme, member of the α-amylase family. This enzyme catalyzes four kinds of reactions by cleaving α-1,4 glycosidic bonds present in the inner part of a polysaccharide chain [5]. CGTase usually has minor hydrolysis activity and mainly catalyzes three transglycosylation reactions: cyclization, coupling and disproportionation. The production of CDs is the specific reaction of CGTase [6]. The most common CDs are α-, β-, and γ-CD consisting of six, seven, and eight glucose monomers in cycles, respectively. Extensive research has been carried out to optimize catalysis conditions for increasing the CDs yields [7-9]. Gujral et al. [10] proposed CGTase as antistaling agent in gluten free breads owing its action on rice starch and CDs production and their effect on wheat starch pasting behavior and the dynamic rheology was evaluated by Gujral and Rosell [11]. More recently, Han et al. [12]

reviewed the heterologous expression strategies used for enhancing the production of CGTases and discuss the molecular engineering approaches used to improve the production, secretion, and properties of CGTase. In spite of previous research on the CDs production for many industrial/pharmaceutical applications, there is no information about the contribution of the enzyme to the starch changes. Therefore, the aim of this study was to explore the enzymatic modification of corn starch granules with CGTase under sub-gelatinization conditions, which might open the possibility of obtaining enzymatically modified corn starch with diverse functionality. Special emphasis was placed on understanding the biochemical features, thermal and structural modifications promoted by the enzyme.

2. Materials and methods

2.1. Materials and reagents

Corn starch samples were purchased by Daesang Corporation (Korea). Cyclodextrin-glycosyltransferase (CGTase, EC 2.4.1.19) from *Thermoanaerobacter* sp (Toruzyme® 3.0 L) of food grade was provided by Novozymes (Bagsværd, Denmark). Chemical reagents from Sigma-Aldrich (Madrid, Spain) were of analytical grade.

2.2. Methods

2.2.1. Preliminary test to determine the conditions for the enzymatic reaction

Preliminary assays were carried out to determine the impact of pH on the enzymatic reaction of CGTase. 25 mL of 20 mM sodium phosphate buffer (for the range pH 6.0 to pH 8.0) or 20 mM sodium acetate buffer (for the range pH 3.0 to pH 5.0) were added to two grams of corn starch placed into the aluminum canister and then the enzyme (0.32 U of CGTase /10 g starch) was added. A heating-cooling cycle was applied using the rapid visco analyzer 4500 (RVA) (Perten Instruments SA, Stockholm, Sweden) (RVA),

heating from 50 to 95 ºC in 282 s and then cooling to 50 ºC in 282 s. Viscosity was recorded using Thermocline software for Windows provided by Perten Instruments SA. The level of hydrolysis at 95 ºC and 50 ºC was defined as the %-change in paste viscosity recorded in the RVA at 50 ºC and 95 ºC.

2.2.2. Sample preparation

Corn starch (10.0 g) was suspended in 50 mL of 20 mM sodium phosphate buffer at pH 6.0 or in 20 mM sodium acetate buffer at pH 4.0. Starch samples were referred as C6 or C4, respectively. Enzyme (0.32 U of CGTase /10 g starch) was added to the starch suspension. Samples were kept in a shaking water bath (50 rpm) at 50 ºC for 24 and 48 hours. Starch suspensions without the addition of enzyme were used as reference. Distilled water (50 mL) was added for washing and suspensions were centrifuged for 15 min at 7,000×*g* and 4 ºC. Starches were washed twice to remove residual enzyme. No further release of sugars was produced, confirming the complete removal of the enzyme. Supernatants were pooled together and boiled in a water bath for 10 min to inactivate the enzyme before any further analyses. Sediments containing starch were freeze-dried and kept at -25 ºC.

2.2.3. Scanning electron microscopy (SEM)

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). Structural properties of the samples were assessed at 10 kV accelerating voltage with a SEM (S-4800, Hitachi, Ibaraki, Japan). The microstructure analysis was carried out using image analysis software (Image-Pro Plus 7.0, Media Cybernetics, USA) in the Central Service for Experimental Research of the Universidad de Valencia.

2.2.4. High Performance Anion Exchange Chromatography (HPAEC)

100 Supernatants containing released hydrolysis compounds were freeze-dried and
101 oligosaccharides and CDs were detected by HPAEC through a CarboPac PA-100
102 column (250 mm × 4 mm), coupled to a pulsed amperometric detector (Dionex). The
103 flow rate was 1.0 mL/min and the volume injection 10 μL. Using solutions A (water), B
104 (1 mol/L NaOH), C (1 mol/L $C_2H_3NaO_2$), and D (water), the following running profile
105 was applied: time zero, 46.25% A, 5% B, 2.5% C, 46.25% D; 25 min, 42.5% A, 5% B,
106 10% C, 42.5% D; 1 min, 35% A, 15% B, 15% C, 35% D; 3 min, 33% A, 15% B, 19%
107 C, 33% D; 5 min, 28.5% A, 15% B, 28% C, 28.5% D; 1.5 min, 18.5% A, 15% B, 48%
108 C, 18.5% D. For the identification and quantification of each compound, standards of
109 known concentrations were previously analyzed. Analysis was carried out at least in
110 duplicate.

111 *2.2.5. Starch hydration properties*

112 Swelling parameters and water soluble released compounds of modified corn starch
113 samples were determined following the method reported by Rosell et al. [13]. The
114 iodine binding, indicative of amylose complex formation, was determined in the soluble
115 supernatant. The soluble supernatant (40 μL) was mixed with 2 mL of an aqueous
116 solution of 0.2% KI and 0.65% I_2. The absorbance at 690 nm was measured using a
117 spectrophotometer (UV mini-1240, Shimadzu Corporation, Kyoto, Japan). Paste clarity
118 (PC) was directly measured in the supernatant as the absorbance at 650 nm using a
119 spectrophotometer. Values were the average from four replicates.

120 *2.2.6. Starch hydrolysis kinetics*

121 Starch hydrolysis was measured following the method described by Dura et al. [14] for
122 gelatinized and non-gelatinized samples. To obtain gelatinized samples previous to
123 starch hydrolysis, corn starch sample (0.1 g) was suspended in 2 mL of 0.1 M sodium
124 maleate buffer (pH 6.9) and incubated 15 min at 100 ºC. Samples were then placed in

125 water bath at 37 ºC. When temperature was reached, porcine pancreatic α-amylase
126 (Type VI-B, ≥10 units/mg solid, Sigma Chemical, St. Louis, USA) 40 CU/g $_{starch}$ and
127 240 CU/g $_{starch}$ (CU, Ceralpha Units) was added for gelatinized and non-gelatinized
128 samples, respectively.
129 *2.2.7. Pasting properties*
130 The pasting properties were determined with RVA by following the American
131 Association of Cereal Chemists Approved Method [15] Again, the level of hydrolysis at
132 95 ºC and 50 ºC was defined as the %-change in paste viscosity recorded in the RVA at
133 50 ºC and 95 ºC.
134 *2.2.8. Thermal Analysis of starch*
135 Thermal behavior of starch samples was determined using a DSC from Perkin–Elmer
136 (DSC 7, Perkin–Elmer Instruments, Norwalk, CT). Corn starch samples were accurately
137 weighed into aluminum DSC pans and de-ionized water was added by micropipette to
138 achieve a water–sample ratio of 3:1. Pans were sealed and equilibrated at room
139 temperature for one hour before analysis. Instrument was calibrated with indium, using
140 an empty pan as reference. Thermal analysis consisted on heating from 30 to 120 °C at a
141 rate of 5 °C/min. The onset temperature T_o, peak temperature T_p, and conclusion
142 temperature T_c, were determined from the heating DSC curves. ΔH was evaluated based
143 on the area of the main endothermic peak, and peak height index (PHI) was calculated
144 as PHI= $\Delta H/ T_p - T_o$. All DSC experiments were replicated three times.
145 *2.2.9. Statistical analysis*
146 Experimental data were statistically analyzed for analysis of variance (ANOVA) using
147 Statgraphics Centurion XV software (Bitstream, Cambridge, N). When analysis of
148 variance indicated significant *F* values, multiple sample comparisons were also
149 performed by Fisher's least significant differences (LSD) test to differentiate means

150 with 95% confidence. The correlation matrix was also performed using Statgraphics
151 Centurion XV software.
152
153 **3. Results and discussion**
154 Previous analysis were performed to investigate the impact of pH and temperature on
155 the enzymatic reaction and to select suitable reaction conditions for the enzymatic
156 modification of corn starch. The level of hydrolysis obtained at 95 ºC was rather low, as
157 indicated the %-change in paste viscosity recorded in the RVA at 95 ºC (Fig. 1).
158 Therefore, despite CGTase belongs to the α-amylase family, it showed rather low
159 hydrolysis activity, which agrees with previous findings [5]. Enzymatic activity was
160 mainly revealed during the cooling stage of the RVA analysis, resulting in high starch
161 hydrolysis (50 ºC) in the pHs range of 4.0 to 7.0, with two maxima observed at pHs 4.0
162 and 6.0. Those pHs were selected to perform further enzymatic modification of corn
163 starch.
164 *3.1 Microstructure of the starch*
165 Samples were examined by SEM. No changes in granule size and shape and no holes
166 were visible in C4 and C6 (Fig. 2a and b), and surfaces appeared smooth without any
167 evidence of fissures. When samples were subjected to 50 ºC for 24 and 48 hours (Fig.
168 2c, d, g and h), changes were observed only after 48 hours treatment, starch granules
169 showed shapeless structures, losing its smooth appearance, presumably due to
170 annealing. Jayakody et al. [16] reviewed the effect of annealing in granules morphology
171 of different tubers and root starches reporting changes on the surface of the granules
172 after treatment. These results were in accordance with Rocha et al. [17], who found that
173 the structural characteristics of normal and waxy starch granules were affected by
174 annealing.

175

176 The effect of enzymatic treatment was readily visible in the starches microstructure as
177 pinholes (Figs. 2e, f, i and j). CGTase was greatly active on the starch granules,
178 resulting in uneven superficial porous that augmented as the time of incubation
179 increased. The surface of the granules was extensively eroded with numerous fissures
180 after 48 hours incubation, being less pronounced in samples treated at pH 4.0. It is
181 generally accepted that starch granules have a unique semi-crystalline supramolecular
182 structure with concentric layers of amorphous and crystalline regions radiating from the
183 hilum [18]. Considering that the crystalline domains are mainly composed of
184 amylopectin while bulk amorphous domains are made up of amylose traversed by non-
185 crystalline regions of amylopectin, it might be expected that treatment with CGTase
186 promotes changes in the amorphous areas of granules, leading to an internally structured
187 morphology. When starch granules are incubated with amylolytic enzymes, the enzymes
188 migrate through the channels [19] and initiate hydrolysis leading to an inside out pattern
189 of digestion [20]. Surface pores were irregularly distributed, either absent, present in
190 clusters or scattered over the surface [21]. Micrographs confirmed that CGTase also led
191 to porous corn starch but having smaller and randomly distributed holes.

192 *3.2. Enzymatic treatment of corn starch*

193 Hydrothermal properties and amylose content were significantly affected (Table 1). The
194 ANOVA indicated a significant effect of the pH on the paste clarity, related to the
195 compounds leached out, which was higher when treated at pH 6.0. The iodine binding
196 value, thus the amount of amylose leached to the supernatant, after thermal treatment at
197 50 ºC was greater for samples soaked at pH 4.0 for some time. The solubility index (SI)
198 value was significantly ($P < 0.05$) enhanced due to enzymatic treatment and the time of
199 treatment. SI determines the amount of solid compounds leached when breaking

intermolecular bonds between amylose and amylopectin. Despite the enzymatic activity at both pH, the amylose released was low, likely due to interaction between amylose-amylose and/or amylose-amylopectin [22] or reduction in granular swelling [23]. In fact, enzymatically treated corn starches showed significantly lower swelling capacity (SC), thus less amount of water was absorbed by these starches. It seems that either some physical impediment for bounding water molecules or the hydrophobic nature of the internal wall of the pinholes should be responsible of that behavior. Therefore, the enzymatic treatment modified the granular integrity of the starch affecting its swelling capacity.

Overall, CGTase was greatly active breaking the degree of association between molecular bonds for longer time of incubation comparing with the control samples at both pH and more soluble compounds were leached, as was previously reported for amylase and amyloglucosidase [24].

3.3. CDs and oligosaccharides released during enzymatic treatment

Contents of glucose, maltose, maltotriose, maltotetraose, maltopentaose, α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin in mg 100 g^{-1} of starch are presented in Table 2. As it was expected, non-enzymatically treated samples did show neither oligosaccharides nor CDs, with the exception of the sample soaked at pH 4.0 for 48 hours that presented a small amount of glucose. Oligosaccharides and CDs were released from the corn starches when subjected to CGTase hydrolysis at pH 4.0 or 6.0. CGTase is an endo-amylase that cleaves α-1,4-glycosidic bonds present in the inner part of a polysaccharide chain [25]. Results showed that the pattern of released compounds was dependent on the pH. At pH 6.0 the CGTase released mainly oligosaccharides and the production of CDs required longer incubation times (48 hours). Conversely, the treatment carried out at pH 4.0 released major amount of CDs, predominantly ß-cyclodextrin.

225 At pH 6.0 the primarily cyclodextrin was the α-CD, which agree with Yamamoto et al.'s
226 [26] findings, when similar conditions were applied to heat treated potato starch at pH
227 5.5. Additionally, Kim et al. [27] reported the production of a small amount of
228 cyclodextrin. It must be stressed that being the CGTase a member of α-amylase family
229 usually reactions are carried out at pH 6.0, but present results showed that specificity
230 was pH-dependent.

231 *3.4. Starch hydrolysis kinetics*

232 Gelatinized samples (Fig 3 C, D) showed faster hydrolysis kinetic than non-gelatinized
233 samples (Fig 3 A, B). Structural changes occurred during gelatinization of starch, losing
234 its original granular structure and crystalline order and leading to more susceptible to
235 enzymatic hydrolysis towards α-amylase [28].

236 Non-enzymatically treated starches showed higher susceptibility to be hydrolyzed, with
237 the exception of samples soaked for 48 hours. Likely, annealing after 48 hours-soaking
238 induces structural changes that made the granule more resistant to enzymatic attack.
239 Enzymatically treated starches were less susceptible to hydrolysis, exhibiting slower
240 hydrolysis and reaching lower maximum. Despite that the presence of surface porous in
241 the starch granules could suggest an increase in the susceptibility to be hydrolyzed, as
242 occurs with amylase or amyloglucosidase attack [14], results indicated the opposite
243 behavior. Enzymatic treatment of the starch by CGTase seems to affect the amorphous
244 zone, releasing sugars from those accessible chains, but leading to a more crystalline
245 structure that was more resistant to the amylase hydrolysis [29]. It must be remark at
246 this point that CGTase catalyze mainly transglycosylation reactions, which can lead to
247 starch structures less susceptible to amylase hydrolysis. The annealing after 48 hours led
248 to starches with hydrolysis patterns close to those obtained with enzymatically treated
249 starches.

Faster hydrolysis (0-40 min) was observed in all the gelatinized starches (Fig. 3 C, D), rapidly achieving a plateau at higher glucose percentage than the one obtained in non-gelatinized starches. During the gelatinization of starch the molecular order and thus birefringence disappears, the starch granule loses crystallinity, swelling of the granule is followed by leaching of mainly amylose, and when further heated, starch granules are disrupted and partial solubilization is achieved. Gelatinization is a process that transforms the native crystalline structure of the starch granules into more amorphous structure, losing their physical integrity, favoring enzymes access to the starch chains. In consequence, enzymatically treated samples after gelatinization showed similar hydrolysis plots to their counterparts without treatment, and that effect was even more pronounced at pH 4.0 (Fig. 3 C) than at pH 6.0 (Fig. 3 D). Therefore, it seems that when starches loss their crystalline structure, due to gelatinization; structural changes promoted by CGTase were hardly evident.

3.5. Pasting properties

Typical pasting curves were observed when starch suspensions were subjected to a heating and cooling cycle (Fig.4). Enzymatic treatment at different pH promoted differences in the viscosity pattern of the starches especially during cooling, which was lower for the starches treated at pH 6.0. Therefore, pH affected the annealing process that might take place during soaking at 50 ºC. The effect of enzymatic treatment was manifested as a decrease in the maximum peak viscosity. After reaching the maximum viscosity, the swollen starch granules were easily broken and disintegrated by stirring, which was related to the starch stability during heating. Enzymatically treated starches at pH 6.0 showed lower stability than those performed at pH 4.0. PH significantly affected the peak viscosity, breakdown and final viscosity (Table 3). Enzymatic

275 treatment resulted in significant changes on peak viscosity, trough, final viscosity and
276 setback. Enzymatic modification of rice starch with pullulan also affected peak viscosity
277 values, trough and final viscosity [30]. In addition, enzymatic treatment exerted
278 significant effects on hydrolysis percentage at 95 ºC and 50 ºC. Therefore, the activity
279 of CGTase led to starches with lower maximum peak viscosity and when performed at
280 pH 6.0 also lower viscosity was obtained after cooling. Enzymatically modified samples
281 at pH 6.0 showed lower setback and final viscosity than samples treated at pH 4.0,
282 which suggested that lower amount of amylose chains were able to form helical
283 structures responsible of the gel formation. Presumably, CGTase at pH 6.0 induced
284 greater hydrolysis of the amylose chains, confirming the different action of this enzyme
285 at both pH, which also agree with micrograph observations that showed erosion and
286 pinholes in the surface of starch granules.

287 *3.6. Thermal parameters*

288 Transition temperatures (T_o, T_p and T_c), gelatinization temperature range (T_p-T_o), ΔH
289 and the PHI are presented in Table 4. The pH during treatment significantly affected all
290 thermal parameters. Unexpectedly, enzymatic treatment did not significantly modified
291 thermal behavior of the starches, despite differences observed in the pasting parameters.
292 It seems that changes promoted by CGTase were at the granule surface, whereas pH
293 soaking during prolonged periods led to inner granule changes. Gelatinization
294 temperature (T_p) of the corn starch samples range from 65.01 to 74.11 ºC, being slightly
295 higher at pH 6.0 after the treatment, likely due to the annealing process that took place
296 during soaking at 50 ºC that rose the peak temperature and narrowed the gelatinization
297 temperature range [14]. However, contradictory results have been reported regarding the
298 gelatinization behavior of starches after annealing. Krueger et al. [31] observed

299 enhanced enthalpies for wild corn starches and mutant genotypes, while Tester et al.
300 [23] reported that the enthalpy for corn starch remained unaltered after annealing.
301 ΔH values were significantly affected by pH and time of the treatment, being lower for
302 samples treated at pH 6.0 for longer period. Considering that the ΔH reflects the
303 disruption of double helices and crystalline order, partial solubilization of amylose and
304 the development of glucan chain–water complexes and chain–chain interactions [32], it
305 seems that treatment at pH 6.0 was more aggressive. The decrease observed at pH 6.0
306 suggests that some of the double helices present in crystalline and in non-crystalline
307 regions of the granule may have disrupted under the conditions treatment, which
308 suggested that annealing was pH dependent. Values of PHI were clearly influenced by
309 the pH used during the incubation of starch. Again the pH 6.0 was more aggressive on
310 the starch granules, giving lower PHI, which suggests narrowed transition range for
311 gelatinization [31].

312 **4. Conclusions**

313 CGTase offers an alternative for modulating the corn starch properties at sub-
314 gelatinization temperature and it encompasses the applications of this enzyme not only
315 to be used for producing CDs. The extent of starch modification was pH dependent,
316 being more aggressive at pH 6.0, where oligosaccharides were majorly released.
317 Conversely, milder modification could be obtained at pH 4.0, at which greater amount
318 of cyclodextrins were released. CGTase seems to hydrolyze the amorphous part of the
319 starch leading to starches less susceptible to amylase hydrolysis. Porous starches could
320 be obtained with this enzymatic treatment, which keep their thermal characteristics but
321 show different pasting behavior, again dependent on the pHs of the enzymatic
322 treatment.

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380

381 **Table 1.** Effect of enzymatic treatment on the paste clarity, solubility index, amylose
382 content and hydration properties (swelling power and swelling capacity) of the resulting
383 starches.

Sample	Time (h)	Paste clarity (Abs 650nm)	Solubility index (%)	Swelling power (g/g)	Iodine binding (Abs 690 nm)	Swelling capacity (g/g)
C4	0	0.01±0.00	1.04±0.00	0.87±0.00	0.000±0.000	0.86±0.00
C4	24	0.01±0.00	2.80±0.05	0.86±0.01	0.043±0.003	0.84±0.01
C4	48	0.01±0.01	1.58±0.32	0.87±0.00	0.033±0.004	0.85±0.00
CGT4	24	0.00±0.00	3.35±0.07	0.87±0.00	0.000±0.000	0.84±0.00
CGT4	48	0.01±0.00	4.47±0.02	0.86±0.00	0.000±0.024	0.83±0.00
C6	0	0.01±0.00	0.72±0.00	0.86±0.00	0.000±0.009	0.85±0.00
C6	24	0.02±0.01	1.65±0.08	0.86±0.00	0.048±0.003	0.85±0.00
C6	48	0.01±0.00	4.58±0.18	0.90±0.00	0.000±0.005	0.86±0.00
CGT6	24	0.01±0.00	3.87±0.00	0.86±0.00	0.000±0.000	0.83±0.00
CGT6	48	0.02±0.01	6.14±2.00	0.89±0.02	0.006±0.016	0.83±0.00
P value	Time	0.23	0.04	0.05	0.443	0,24
	pH	0.02	0.12	0.09	0.002	0.64
	Enzyme	0.58	0.01	0.79	0.416	0.00

384 Mean ± standard deviation values (n = 3).

385

386 **Table 2.** Oligosaccharides and cyclodextrins released after corn starch hydrolysis by

387 CGTase. Results are expressed in mg 100 g^{-1} of starch.

Sample	Time (h)	Glucose	Maltose	Maltotetriose	Maltotetraose	Maltopentaose	α-CD	β-CD	γ-CD
C4	0	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
C4	24	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
C4	48	0.783	<dl	<dl	<dl	<dl	<dl	<dl	<dl
CGT4	24	3.171	4.204	2.600	1.691	0.461	2.233	3.759	0.300
CGT4	48	5.519	6.967	4.549	4.007	1.234	3.162	4.950	<dl
C6	0	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
C6	24	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
C6	48	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
CGT6	24	5.240	7.075	4.814	0.725	0.041	0.004	<dl	<dl
CGT6	48	5.007	4.933	2.884	0.086	0.383	1.142	1.014	<dl

388 <dl means under detection limit.

389

390 **Table 3.** Effect of enzymatic treatment on the pasting parameters of corn starch.

Sample	Time (h)	Onset temp (ºC)	Peak time (min)	Peak viscosity (cP)	Trough (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Hydrolysis 95°C (%)	Hydrolysis 50°C (%)
C4	0	76±0	5±0	4499±0	2711±0	1788±0	4320±0	1609±0	0±0	0±0
C4	24	76±0	5±0	4433±192	2719±95	1715±96	4430±122	1712±26	1±4	-3±3
C4	48	72±7	5±0	4263±3	2638±5	1626±2	4346±1	1708±4	5±0	-1±0
CGT4	24	65±16	5±0	4076±66	2639±5	1438±71	4301±44	1663±49	9±1	0±1
CGT4	48	67±14	5±0	3843±57	2527±50	1317±6	4195±44	1669±6	15±1	3±1
C6	0	75±0	5±0	4749±0	2751±0	1998±0	4625±0	1874±0	0±0	0±0
C6	24	77±0	4±0	4530±129	2724±40	1807±89	4576±47	1852±7	5±3	1±1
C6	48	64±17	4±0	4760±107	2791±192	1969±85	4762±187	1971±5	0±2	-3±4
CGT6	24	57±0	4±0	4270±0	2203±0	2067±0	3456±0	1253±0	10±0	25±0
CGT6	48	72±5	4±0	4441±158	2363±133	2078±25	3846±175	1483±42	6±3	17±4
P value	Time	0.90	0.02	0.58	0.95	0.68	0.95	0.69	0.63	0.94
	pH	0.81	0.00	0.00	0.34	0.00	0.72	0.81	0.25	0.02
	Enzyme	0.31	0.93	0.00	0.00	0.39	0.00	0.01	0.00	0.00

391 Values followed by different letters within a column denote significantly different

392 levels ($P < 0.05$) (n = 3).

393

394 **Table 4.** Thermal properties of enzymatically modified corn starches determined by

395 DSC.

Sample	Time (h)	T_o (ºC)	T_p (ºC)	T_c (ºC)	T_p-T_o	Area (mJ)	ΔH (J/g)	PHI (J/g ◦C)
C4	0	65.11±0.30	68.54±0.54	73.46±1.39	3.46±0.26	103.19±10.11	11.00±0.79	3.21±0.40
C4	24	65.66±0.27	68.70±0.50	73.63±0.17	3.19±0.28	117.25±8.01	12.19±0.76	3.89±0.29
C4	48	66.28±0.37	69.45±0.33	73.34±0.53	3.17±0.10	113.48±4.85	12.33±0.73	3.86±0.24
CGT4	24	65.01±0.91	68.62±0.79	71.73±1.87	3.73±0.36	108.28±11.75	12.05±1.80	3.20±0.52
CGT4	48	66.34±0.50	69.58±0.57	73.22±1.03	3.27±0.24	120.83±8.74	12.98±1.18	3.87±0.60
C6	0	65.40±0.21	68.75±0.22	74.04±1.23	3.39±0.06	93.21±4.27	10.17±0.27	3.01±0.06
C6	24	65.88±0.91	69.29±1.22	73.00±2.16	3.41±0.32	113.06±7.15	11.75±0.99	3.70±0.51
C6	48	66.39±0.14	69.50±0.27	73.48±0.31	3.05±0.26	107.18±12.95	10.68±0.99	3.49±0.56
CGT6	24	66.14±0.34	69.29±0.28	72.40±0.51	3.19±0.12	115.18±8.26	12.52±0.91	3.92±0.17
CGT6	48	66.78±0.25	69.62±0.28	74.11±0.24	2.8±0.10	97.71±11.17	10.01±0.69	3.53±0.33
P value	Time	0.87	0.44	0.70	0.87	0.70	0.01	0.05
	pH	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Enzyme	0.56	0.61	0.48	0.64	0.41	0.66	0.39

396 T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, ΔH =

397 enthalpy change, PHI = peak height index. Values followed by different letters within a

398 column denote significantly different levels ($P < 0.05$) (n = 4).

399

400 **Figure captions**

401 **Figure 1.** Hydrolysis activity of CGTase at different pHs. The level of hydrolysis at 95
402 ºC and 50 ºC was defined as the %-change in paste viscosity recorded in the RVA at 50
403 ºC and 95 ºC.

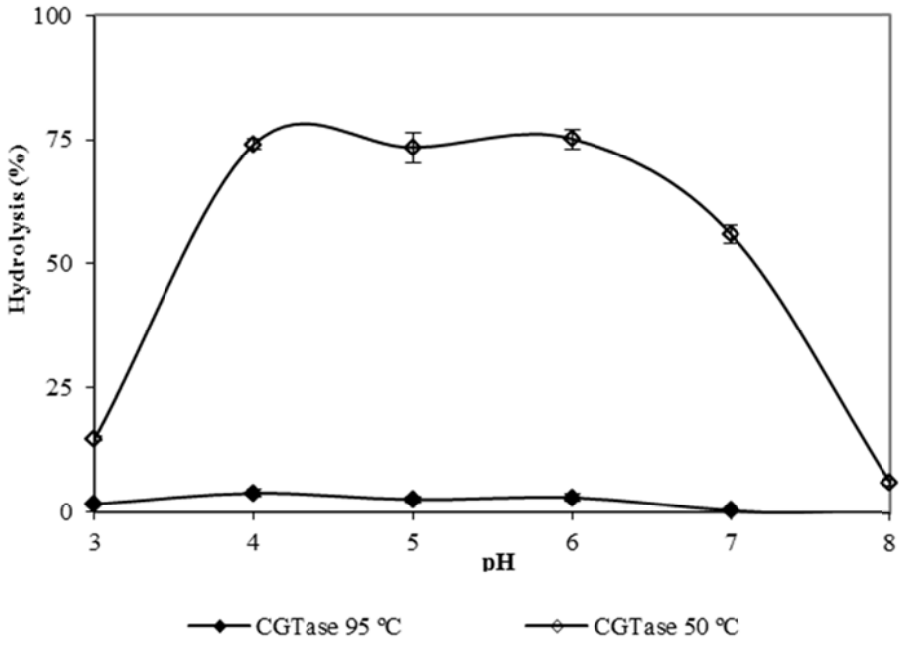
404 **Figure 2.** Scanning electron micrograph of corn starch samples (a, b, c, d, g and h) and
405 their counterparts treated enzymatically (e, f, i and j) at pH 4.0, after 24 h (c, e) or 48 h
406 (g, i) and at pH 6.0 after 24 h (d, f) or 48 h (i, j). Magnification 3500×. Control pH 4.0
407 (a); Control pH 6 (b); Control pH 4.0, 24 h (c); Control pH 6, 24 h (d); CGTase pH 4.0,
408 24 h (e); CGTase pH 6.0, 24 h (f); Control pH 4.0, 48 h (g); Control pH 6.0, 48 h (h);
409 CGTase pH 4.0, 48 h (i); CGTase pH 6.0, 48 h (j).

410 **Fig. 3.** Enzymatic starch hydrolysis profiles of non-gelatinized samples (A, B) and
411 gelatinized corn starches (C, D). Corn starches treated with CGTase at pH 4.0 (A, C)
412 and pH 6.0 (B, D) for 24 hours (■) and 48 hours (♦) were compared with their
413 counterparts without enzymatic treatment after 24 hours (□) or 48 hours (◊). Native
414 starch was included (+).

415 **Fig. 4.** RVA profiles of the corn starches at pH 4.0 (A) and pH 6.0 (B) treated with
416 CGTase at 24 hours, (■) and 48 hours, (♦) compared with their respective controls
417 (without enzymatic treatment) at 24 hours, (□); and 48 hours, (◊). Control sample
418 without any treatment at pH 4.0 and pH 6.0, enclosed symbols, (+).

419

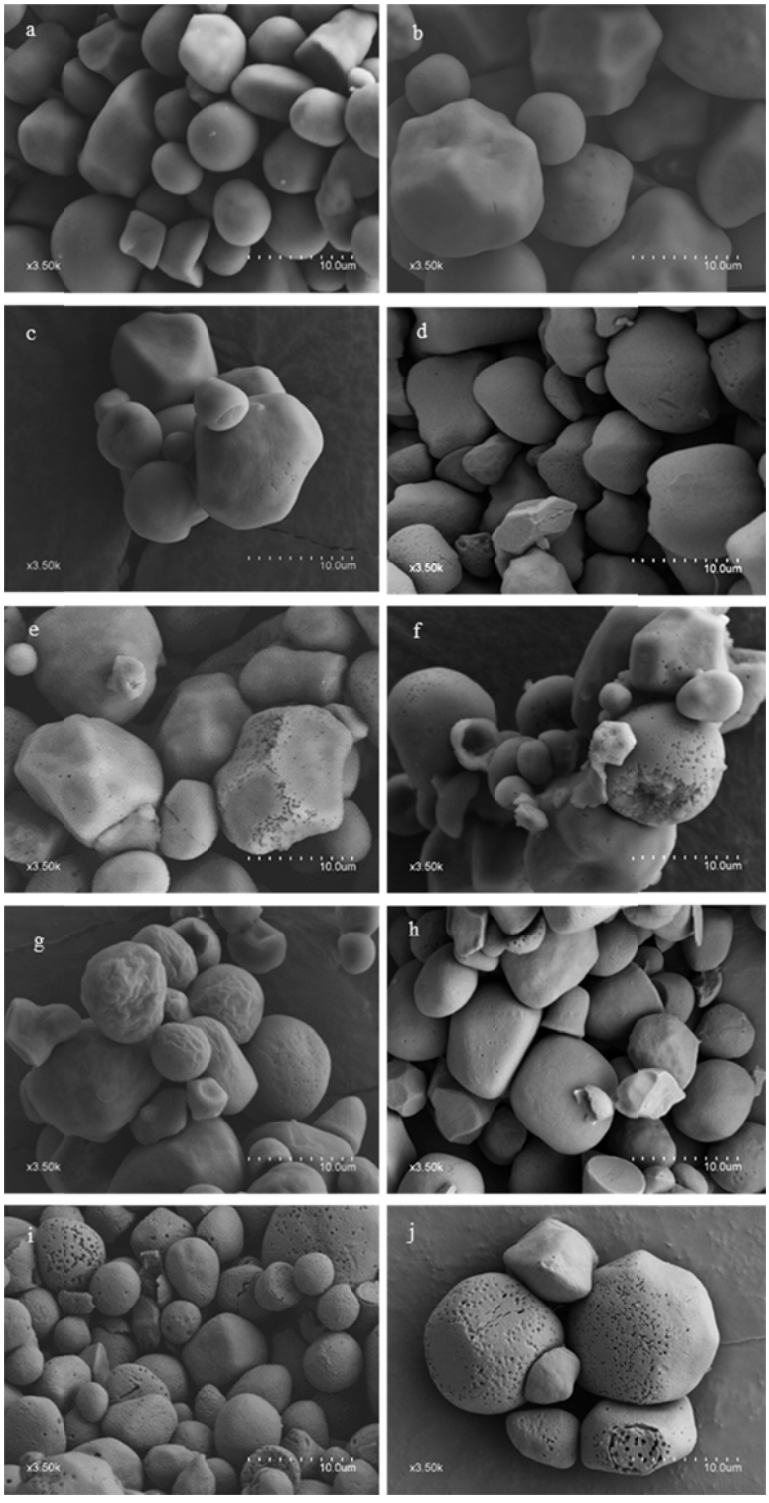
421 **Figure 1**



422

423

Figure 2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10.0um
b
x3.50k
10.0um
c
x3.50k
10.0um
d
x3.50k
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e
x3.50k
10.0um
f
x3.50k
10.0um
g
x3.50k
10.0um
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10.0um
j
x3.50k
10.0um

427 **Figure 3**

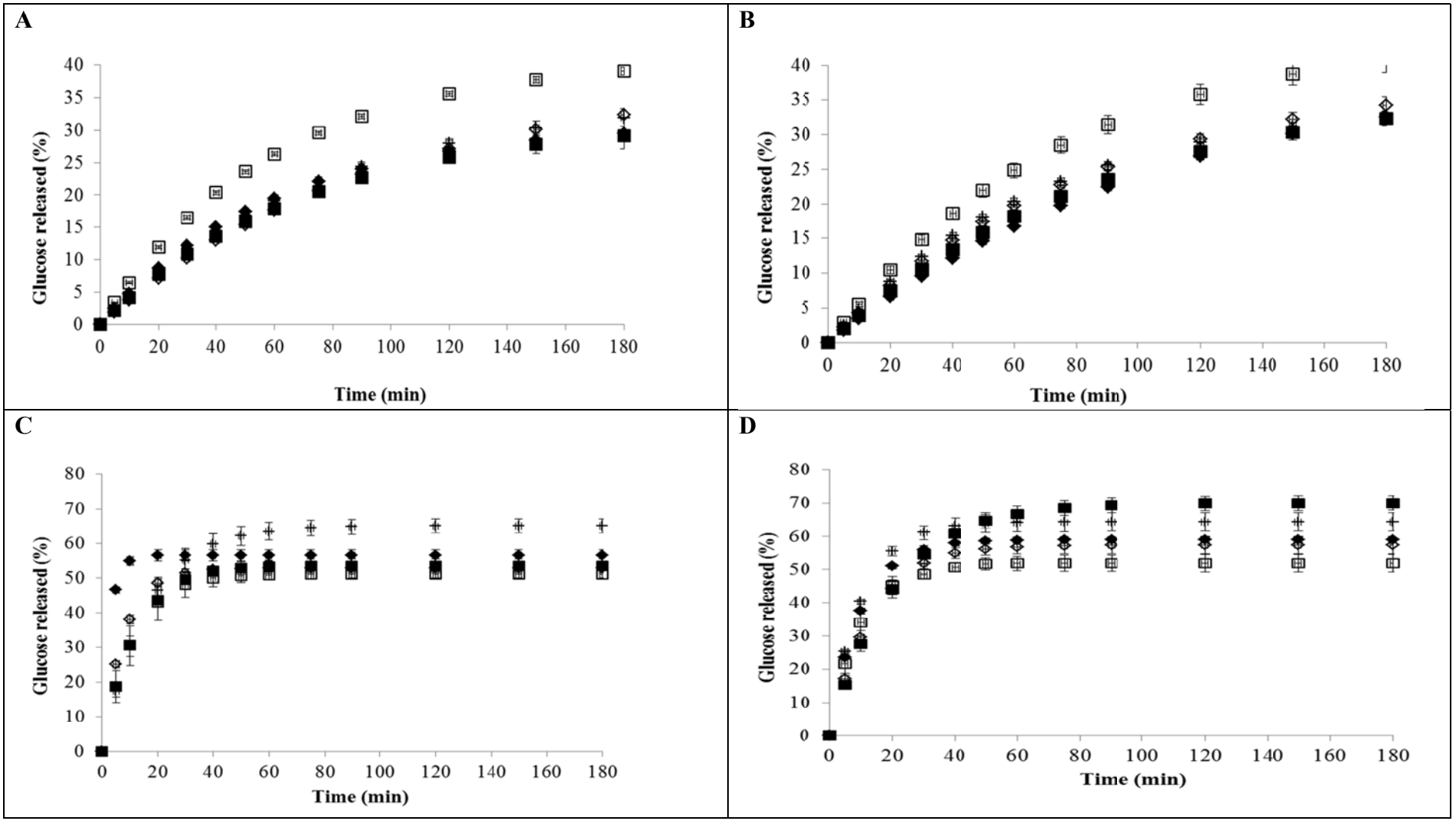
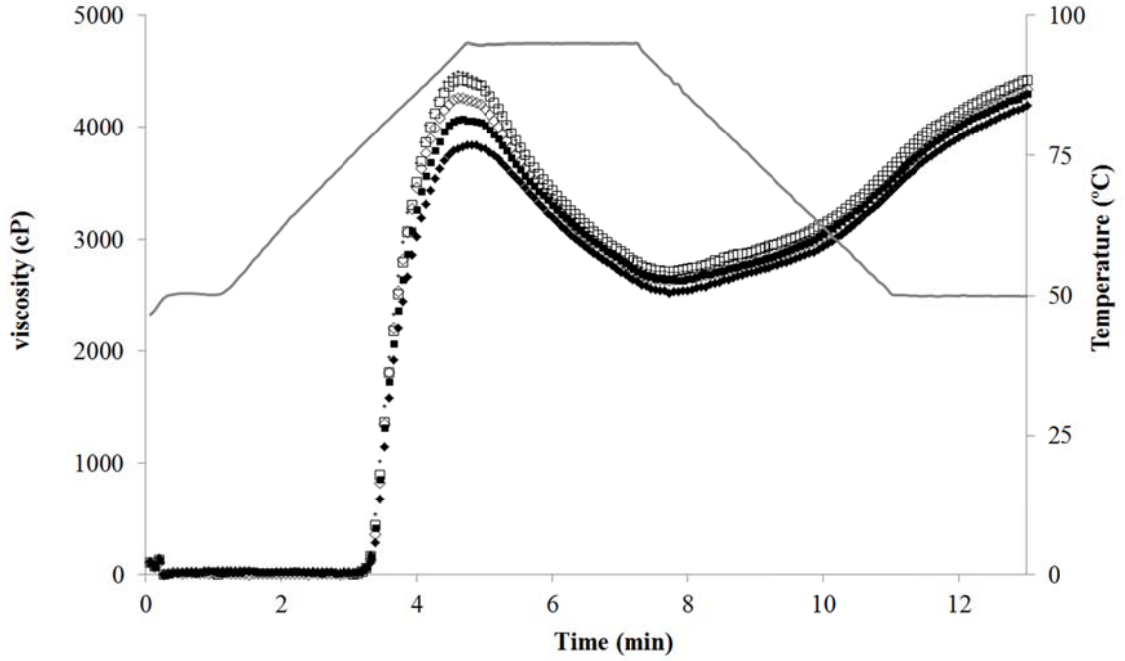

A
Glucose released (%)
Time (min)
B
Glucose released (%)
Time (min)
C
Glucose released (%)
Time (min)
D
Glucose released (%)
Time (min)

Figure 4

A


viscosity (cP)
Temperature (°C)
Time (min)
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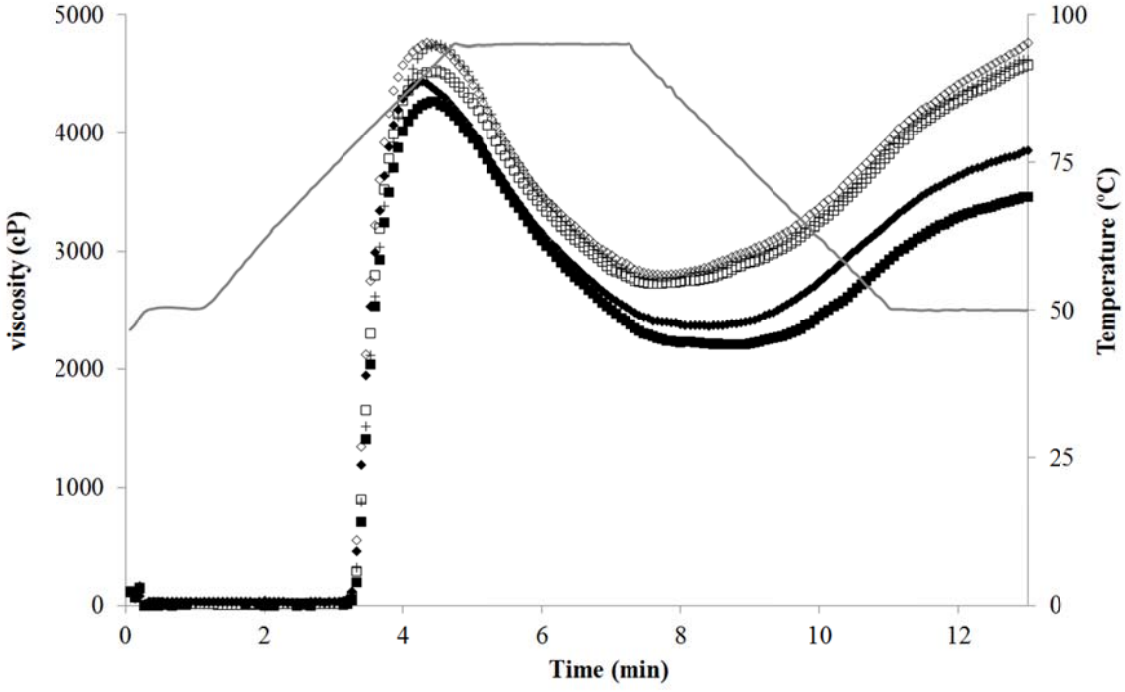
3000
4000
5000
0
25
50
75
100
0
2
4
6
8
10
12

B


viscosity (cP)
Temperature (°C)
Time (min)
0
1000
2000
3000
4000
5000
0
25
50
75
100
0
2
4
6
8
10
12