

1 EFFECT OF HYDROTHERMAL TREATMENT ON PHYSICOCHEMICAL  
2 PROPERTIES OF WHEAT, WAXY AND STANDARD MAIZE STARCHES

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## 18 ABSTRACT

19 Standard maize (SMS), waxy maize (WMS) and wheat (WTS) starches were  
20 hydrothermally treated at three pressure levels. Effects of D.I.C. processing conditions  
21 on thermal characteristics, enzyme susceptibility, pasting and rheological properties  
22 were investigated. For treated starches an increase of transition temperatures ( $T_o$ ,  $T_p$ ), a  
23 narrowing of width of gelatinization endotherms and a decrease of gelatinization  
24 enthalpies ( $\Delta H$ ), were observed. At 3 bar/10 min  $\Delta H$  decreased from 11.4 J.g<sup>-1</sup> to 1.7  
25 J.g<sup>-1</sup>, from 15.5 J.g<sup>-1</sup> to 2.1 J.g<sup>-1</sup> and from 9.4 J.g<sup>-1</sup> to 0 J.g<sup>-1</sup> for SMS, WMS and WTS,  
26 respectively. At same conditions, starches showed a significant increase in enzymatic  
27 hydrolysis, yield of saccharification increased from 19% to 44%, from 21% to 59% and  
28 from 55% to 79% for SMS, WMS and WTS, respectively. The study suggested that  
29 structural modifications influence in-vitro hydrolysis and the access to WMS  
30 ultrastructure by enzymes seems to be easier than that of SMS. The rheological  
31 behaviour was modified for treated starches: a decrease in the peak viscosity, measured  
32 with a viscoamylograph Brabender, and in the apparent viscosity, measured with a  
33 controlled stress rheometer, was observed.

34 **Keywords:** Starch; D.I.C. hydrothermal treatment; functional properties; Enzyme  
35 hydrolysis

## 36 1. INTRODUCTION

37 Starch has many applications in food and non-food industries. For this reason,  
38 modifications are often made to native starches to give them specific properties for  
39 particular uses. Starch properties can be modified through controlled application of heat  
40 and moisture which produces physical modifications within the granules. Annealing and  
41 heat-moisture treatment (HMT) cause a physical modification of starches with respect  
42 to size, shape or birefringence via controlled application of heat-moisture (Stute, 1992).

43 In the annealing, starch is suspended in excess water and heated below the gelatinisation  
44 temperature, at relatively low values, (40–55 °C) (Stute, 1992). Annealing results in  
45 perfection of the crystalline properties that narrow the gelatinization temperature  
46 interval by shifting them towards higher values (Hublin, 1994). Annealing does not  
47 increase or only moderately the enthalpy of gelatinization (Lawal, 2005).

48 In the HMT, starch is exposed to higher temperatures ( $\approx 120$  °C), commonly above  
49 gelatinization temperature, at very restricted moisture content (18-30%) during 16 h in  
50 some cases (Hoover and Manuel, 1996) and shorter in others (Lim et al., 2001). HMT  
51 has been shown to alter structure and physicochemical properties of standard maize,  
52 waxy maize, amylo maize, potato, wheat (Stute, 1992; Hoover and Manuel, 1996; Lim et  
53 al., 2001). The general effects of HMT on starch are the loss of the birefringence, the  
54 increase of the gelatinization temperatures and the broadening of the gelatinization  
55 temperature range, the increase of water bonding capacities and enzymes  
56 susceptibilities, the decrease of swelling and the increase of solubility. These  
57 modifications have consequences on functionality of treated starches: the viscograph hot  
58 paste consistencies decrease and starch paste became short and shear-stable (Lorenz and  
59 Kulp, 1982; Hoover and Manuel, 1996; Gunarante and Hoover, 2002). Hoover and

60 Vasanthan (1994) and Hoover and Manuel (1996) have shown that the extent of  
61 starch chain associations within amorphous regions and the degree of crystalline  
62 order are altered during HMT of wheat, oat, normal maize, waxy maize, high amylose  
63 maize, potato and lentil starches. The physical properties of heat moisture treated  
64 starches depend on the starch origin and treatment conditions used (Hoover and  
65 Vasanthan, 1994). Many authors showed that HMT increased the susceptibility of starch  
66 to hydrolysis by  $\alpha$ -amylases (Kulp and Lorenz, 1982; Maruta et al., 1994; Gunaratne  
67 and Hoover, 2002) and the extent of the susceptibility varies with botanical source  
68 (Colonna and Buléon, 1992; Li et al., 2004; Srichuwong et al., 2005). According to  
69 Lorenz and Kulp (1982) the considerable reduction in relative crystallinity of wheat  
70 starch caused by HMT resulted in increased enzyme susceptibility. Planchot et al.  
71 (1997) cited by Gunaratne and Hoover (2002), have postulated that the fraction of total  
72 crystalline material is an important factor defining the rate and extent of  $\alpha$ -amylase  
73 hydrolysis.

74 The D.I.C. (*Détente Instantanée Contôlée*: “Instantaneous Controlled pressure Drop”)   
75 hydrothermal process, developed in our laboratory some years ago (Zarguili, 2006) is  
76 more close to HMT. The major effects observed are almost similar except the  
77 gelatinization range temperature where the D.I.C. treatment caused its narrowing  
78 (Zarguili, 2006) as observed with annealing (Hublin, 1994). Preliminary studies on  
79 SMS and WMS starches (Loisel et al., 2006, Zarguili et al., 2006) showed a partial or  
80 total gelatinization of by D.I.C. treated starch, according to processing conditions. The  
81 increase of processing time and pressure level induced the narrowing of the  
82 gelatinization temperature range, the shift of the characteristics temperatures to higher  
83 values and the decrease of gelatinization enthalpy. The occurrence of gelatinization of

85 treated starches was clearly attested by the increase of median volume diameter in cold  
86 water and a loss of birefringence under polarized light. Maruta et al. (1994) observed for  
87 HMT treatment that the pressure is often required to ensure a sufficient heating, but it is  
88 not easy to achieve a uniform distribution and penetration of heat into the starch layer.  
89 These authors improved the conventional method by the introduction of reduced  
90 pressure in order to satisfy practical requirements for industrial production. They  
91 observed that the combination of reduced pressure during HMT of starch allows  
92 homogeneous diffusion of steam and an effective heat transfer to the starch granules.  
93 During D.I.C. treatment, an initial vacuum of 50 mbar was established before  
94 introducing steam in processing vessel. As demonstrated by Zarguili et al. (2006), this  
95 initial vacuum allows to reduce the resistance of the air and thus to facilitate the  
96 diffusion of steam into the product. Consequently, the time necessary to reach the steam  
97 equilibrium temperature is reduced (Zarguili, 2006). The originality of D.I.C. method  
98 compared to other physical treatments is that the starches are treated at residual  
99 moisture content of 13% (dry basis) no hydration step is then used. The conventional  
100 methods require previous hydration of starch before the physical treatment. During the  
101 D.I.C. treatment, the starch heating is obtained by the absorption of latent heat of steam  
102 condensation which causes an increase in the moisture content as the processing time  
103 and pressure level increase. Changes of the moisture distribution were measured during  
104 the treatment and modelled by Zarguili et al. (2007).

105 The objective of this study is to understand the physicochemical changes produced on  
106 native starches after D.I.C. hydrothermal treatment. Under identical conditions  
107 (processing pressure and time), the effects of D.I.C. process on the changes of thermal

108

- 109 transition characteristics, enzyme digestibility, pasting and rheological properties were  
110 evaluated on SMS, WMS and WTS starches.

## 112 2. MATERIALS AND METHODS

### 113 2.1. MATERIALS

114 SMS, WMS (Waxilys 200) and WTS starches were supplied by Roquette Frères  
115 (Lestrem, France). The amylose content was of 27-28% for SMS and WTS and lower  
116 than 1% for WMS. The moisture content of these starches was about 12% wet basis.

117 Fungamyl 800 L, a  $\alpha$ -amylase from *Aspergillus oryzae* was used for starch liquefaction.

118 The enzyme activity was 800 FAU/g (1 Fungal  $\alpha$ -amylase FAU is the amount of  
119 enzyme which breaks down 5.26 g of starch per hour according Novozyme's standard  
120 method for the determination of  $\alpha$ -amylase). AMG 300 L, a glucoamylase from  
121 *Aspergillus niger* was used for saccharification. The enzyme activity was 300 AGU/ml  
122 (1 Novo Amyloglucosidase Unit (AGU) is defined as the amount of enzyme which  
123 hydrolyzes 1 micromoles maltose per minute under standardized conditions according  
124 Novozymes. The enzymes were gift from Novozymes, Denmark.

### 125 2.2. METHODS

#### 126 2.2.1. Moisture content

127 The starch moisture content was determined by air oven at 105 °C during 24 h,  
128 according to the A.F.N.OR standard method.

#### 129 2.2.2. D.I.C. hydrothermal treatment

130 The equipment and procedure of D.I.C. hydrothermal treatment were largely described  
131 in previous studies (Loisel et al., 2006; Zarguili et al., 2006). During the treatment, 22 g  
132 of starch (13% dry basis) disposed in circular containers (diameter: 20 cm; height: 5

134 cm) were placed in the treatment reactor. An initial vacuum of 50 mbar was established.  
135 Saturated steam was introduced into the vessel at fixed pressure and maintained for a  
136 predetermined time. In this study the processing pressure was fixed at 1 bar (100 °C), 2  
137 bar (122 °C) and 3 bar (135 °C). The pressurisation is followed by an abrupt  
138 decompression towards vacuum (50 mbar). After the vacuum phase, atmospheric air is  
139 injected to return to atmospheric pressure for sample recovery. During the treatment,  
140 starch is heated by the absorption of latent heat of vapour condensation that causes an  
141 increase in the moisture content.

#### 142 2.2.3. *Differential Scanning Calorimetry (DSC)*

143 Thermal characteristics of treated starch were studied by using a Micro DSC III  
144 (SETERAM, France). Starch samples (800 mg) were placed in a stainless pan; distilled  
145 water was added to get a ratio of 1:9 (w/w) starch:water, mix, and then the sample pan  
146 was sealed. Sample pan was heated at a rate of 1.2 °C/min from 30 to 110 °C. Thermal  
147 transition of starch samples were defined as  $T_o$  (onset temperature),  $T_p$  (peak  
148 temperature) and  $\Delta H$  was referred to as the gelatinization enthalpy. The gelatinization  
149 temperature range (R) was calculated as  $2(T_p - T_o)$  as described by Krueger et al.  
150 (1987). The degree of gelatinization of treated starch was calculated by the following

151 equation (Marshall et al., 1993).  $DG(\%) = \left(1 - \frac{\Delta H_t}{\Delta H_{raw}}\right) \times 100$ , where DG is the degree

152 of gelatinization of D.I.C. treated starch,  $\Delta H_t$  and  $\Delta H_{raw}$  the gelatinization enthalpy of  
153 treated and native starch, respectively.

155 2.2.4. *Enzyme hydrolysis*

156 The dried samples (300 mg) were mixed with 3 ml of citrate phosphate buffer (pH 4.6)  
157 in a 5 ml tube. The mixture was treated with enzymes in two steps (a), liquefaction and  
158 saccharification, or only in one step of saccharification (b). The first step, liquefaction,  
159 was performed at 50 °C with 32 U/l (10 g/l) of Fungamyl 800 L for 120 min. The  
160 second step, saccharification, was performed at 65 °C with 1.5 U/l (7 ml/l) of AMG 300  
161 L for 60 min. The saccharification was performed at 65 °C with 1.5 U/l (7 ml/l) of  
162 AMG 300 L for 120 min.

163 2.2.5. *Determination of reducing equivalents*

164 During the starch hydrolysis samples were withdrawn for analysis of reducing sugars  
165 content using 3, 5-dinitrosalicylic acid method (DNS). In all samples, the reaction was  
166 stopped by adding 1 volume of water and by heating at 100 °C for 5 min. After  
167 centrifugation of the solution at 3000 rpm for 10 min, a 0.5 ml of a diluted supernatant  
168 was transferred to a test tube and 0.5 ml of DNS reagent (10 g dinitrosalicylic acid + 200  
169 ml NaOH 2M + 300 g potassium sodium tartrate + qsp 1l distilled water) was added. The  
170 tubes were allowed to stand for 10 min in boiling water and cooled to room temperature  
171 in ice water. The absorbance of the samples was measured at 550 nm using a  
172 spectrophotometer (Shimadzu mini UV 1240). Amount of reducing equivalents was  
173 calculated using a glucose standard curve. The starch hydrolysis yield of samples was  
174 calculated by:

$$175 \text{ Starch hydrolyse (\%)} = \left( \frac{\text{Reducing sugar expressed as glucose in sample g/l}}{\text{weight of dried starch in sample g/l}} \right) \times 100$$

177 *2.2.6. Pasting properties using Viscograph Brabender*

178 The processed samples are powdery products that have to be rehydrated for analytical  
179 purpose. This was performed using the Brabender Viscograph to obtain a starch paste  
180 under repeatable conditions. The starch concentrations were chosen in order to lie  
181 within the sensitivity range of the Viscograph: i.e. 6% (w/w) for SMS, 4% (w/w) for  
182 WMS and 7% (w/w) for WTS. Starch was slurried in demineralized water at room  
183 temperature, and then submitted to gradual heating (1.5 °C/min) from 30 to 96 °C; this  
184 temperature was maintained for 10 min and was followed by a cooling step (1.5 °C/min)  
185 down to 70 °C before sampling. The moisture content was determined directly after the  
186 pasting procedure, to check the starch concentration before rheological measurements.  
187 The relevant values obtained from the pasting profile were: onset of the pasting  
188 temperature ( $T_o$ ), temperature of peak viscosity ( $T_p$ ) and peak viscosity (PV) in  
189 Brabender units (100 BU for 25 cmg).

190 *2.2.7. Rheological measurements*

191 Flow behaviour of starch pastes were measured using a controlled stress rheometer (TA  
192 Instrument AR1000) with the cone/plate geometry (6 cm/2°). An aliquot of the starch  
193 dispersion pasted at 60 °C in the Viscograph Brabender was poured onto the plate of the  
194 rheometer preheated at 60 °C, then covered by a layer of paraffin oil to avoid  
195 evaporation. For flow measurements, two up-down shear scans from 0.01 to 660 s<sup>-1</sup> (4  
196 min each) were linearly applied, followed by a logarithmic stepwise decrease from 660  
197 to 0.01 s<sup>-1</sup>, after equilibrium for each shear rate, as described by Nayouf et al., 2003.  
198 Rheological properties were obtained using Herschel-Bulkley model, according to

199 equation  $\tau = \tau_0 + k\dot{\gamma}^n$ , where  $\tau_0$  is the yield stress (Pa), k the consistency index  
200 ( $\text{Pa}\cdot\text{s}^n$ ) and n the flow behaviour index (dimensionless). The Herschel-Bulkley model  
201 fitted the data satisfactorily ( $R^2 = 0.98$ ).

#### 202 2.2.8. *Polarised light microscopy*

203 Dilute native and D.I.C. treated standard starch suspensions (1:20) were viewed under  
204 polarised light (magnifying 400 X) using a phase contrast microscope equipped with a  
205 CCD camera.

## 206 3. RESULTS AND DISCUSSION

### 207 3.1. Thermal properties

208 The effect of D.I.C. hydrothermal treatment on gelatinization temperatures [onset ( $T_o$ ),  
209 peak ( $T_p$ ), and temperature range (R)] and gelatinization enthalpy ( $\Delta H$ ) of treated  
210 starches (SMS, WMS and WTS) were studied. The obtained values were compared with  
211 those of native starches. The native starches displayed differences in gelatinization  
212 temperatures and enthalpy changes, as indicated by DSC analysis (Table 1).  $T_o$ ,  $T_p$ , R  
213 and  $\Delta H$  of SMS and SWS were higher than corresponding values for wheat.  $T_o$ ,  $T_p$  and  
214  $\Delta H$  of the native starches followed the order: WMS>SMS>WTS.  $T_o$  and  $T_p$  were of  
215 65.4 and 71.3.4 °C for WMS, of 63.1 and 69.6 °C for SMS, of 50.4 and 56.6 °C for  
216 WTS. According to Gunaratne and Hoover (2002), the differences in gelatinization  
217 temperatures among starches can be attributed to the interplay of three factors:  
218 molecular structure of amylopectin (unit chain length, extent of branching), starch  
219 composition (amylose to amylopectin ratio, amount of lipid complexed, amylose chains,  
220 phosphorous content) and granular architecture (crystalline to amorphous ratio).  
221 Amylopectin plays a major role in starch granule crystallinity, the presence of amylose  
222 lowers the melting temperature of crystalline regions and the energy for starting  
223 gelatinization (Flipse et al., 1996). That explains the high gelatinization temperatures  
224 and enthalpy values of WMS, which contains mainly amylopectin. Whereas Noda et al.  
225 (1998) attributed the influenced of DSC parameters to the molecular architecture of  
226 crystalline region, which corresponds to the distribution of amylopectin short chains and  
227 not to the proportion of crystalline region which corresponds to the amylose to  
228 amylopectin ratio. The gelatinization temperatures ranges (R) of native starches (Table  
229 1), calculated as  $2(T_p - T_o)$  were 13 °C, 11.8 °C and 6.2 °C for SMS, WMS and WTS,

230 respectively. Fredriksson et al. (1998) reported that wide temperature range  
231 implied a large amount of crystals with varied stability. Cooke and Gidley (1992)  
232 have shown that  $\Delta H$  is due mainly to the disruption of the double helices rather than the  
233 longer range disruption of crystallinity. Gunaratne and Hoover (2002) postulated that  
234 the higher  $\Delta H$  values could be attributed to the presence of higher number of double  
235 helices and/or weaker interaction between adjacent amylopectin double helices within  
236 the crystalline domains of the native granules. The higher gelatinization parameters of  
237 WMS suggest that the chains forming the double helices are longer with greater  
238 interchain association than those of WTS.

239 For all starches, D.I.C. hydrothermal treatment increased  $T_o$  and  $T_p$ , but decreased  $R$  and  
240  $\Delta H$  (Table 1).  $T_o$  and  $T_p$  increased with increasing pressure level and processing time  
241 and shifted to higher values (Fig. 1). At 2 bar (122 °C) and processing time of 90 min,  
242  $T_o - T_p$  shifted from 63.1 - 69.6 °C (native SMS) to 72.9 - 77.5 °C, from 65.4 - 71.3 °C  
243 (native WMS) to 73.1 - 77.8 °C and from 50.4 - 56.6 °C (native WTS) to 65.2 - 68.0 °C.  
244 At the same D.I.C. conditions (1 bar/90 min), the increase in  $T_o$  (6.5 °C) and  $T_p$  (5.8 °C)  
245 for SMS was equivalent to that observed for WTS [ $T_o$  (7.2 °C) and  $T_p$  (4.9 °C)],  
246 whereas this increase was lower for WMS [ $T_o$  (2.6 °C) and  $T_p$  (2.4 °C)]. Similar  
247 increases have been reported on HMT of cereal starches (Lim et al., 2001; Hoover and  
248 Manuel, 1996; Hoover and Vasanthan, 1994). Lim et al. (2001) observed an increase in  
249  $T_o$  and  $T_p$  from 62.6 and 66.9 °C for native maize to 63.4 and 76.7 °C, after HMT  
250 (120°C during 1 hour and 30% of water content). Hoover and Manuel (1996) observed a  
251 large increase in  $T_o$ ,  $T_p$  and  $T_c$  for standard maize and amylo maize V starches, but a  
252 small increase in waxy and Dull waxy maize starches. According to these authors, the  
253 change in transition temperature following HMT is probably influenced by the decrease

254 in the destabilization effect of the amorphous regions when the starch crystallites  
255 melt, due to the formation of amylose-lipid complexes and interactions between  
256 and among amylose chains.

257 As discussed previously, the gelatinization temperature range (R) gives an indication of  
258 the quality and heterogeneity of crystallites (Fredriksson et al., 1998; Gunaratne and  
259 Hoover, 2002), R reflects the size and crystallites perfection. Many researchers have  
260 already showed the broadening of the gelatinization temperature of starches after HMT  
261 treatment (Hoover and Manuel, 1996; Lim et al., 2001; Adebowal et al., 2005), and this  
262 broadening depends on botanical source. An inverse tendency was observed with D.I.C.  
263 treated starches, a narrowing of the gelatinization temperature range for the three  
264 starches, as observed with annealed starches (Hublin, 1994). A greater decrease in  
265 gelatinization range was observed in SMS and WTS compared to WMS (Table 1).  
266 According to Hublin (1994), the temperature range reflects the degree of cohesion  
267 between crystallites, when R decreases a stronger cohesion exists between crystallites.  
268 The narrower temperature range exhibited by D.I.C. treated starches suggests the  
269 presence of crystallites of homogenous stability. We suppose that the D.I.C. treatment  
270 allowed first the fusion of crystallites of low cohesion, which required less energy to  
271 melt and a reinforcement of the interactions between the remaining crystallites chains.  
272 Therefore, the residual structure after treatment contains a greater homogeneity of  
273 crystallites.

274 The gelatinization enthalpies of treated starches (Tables 1) showed that the  $\Delta H$  values  
275 depend on the intensity of D.I.C. conditions. The gelatinization enthalpies decreased  
276 progressively for the three types of starches with increasing pressure level and  
277 processing time. The extent of the decrease after treatment followed the order :

278 WMS>SMS>WTS. At processing pressure of 2 bar and 90 min, the gelatinization  
279 enthalpies decrease for WTS from 9.4 (native) to 0.1 J.g<sup>-1</sup>, for SMS from 11.4  
280 (native) to 7.1 J.g<sup>-1</sup> and for WMS from 15.5 (native) to 11.2 J.g<sup>-1</sup>. By considering  
281 gelatinization degree, we noted after D.I.C. treatment (2 bar and 90 min) a partial  
282 gelatinization of 98.9%, 37.7% and 27.7%, for WTS, SMS and WMS, respectively.  
283 Vermeulen et al., 2006 observed on potato starch the decrease of gelatinisation enthalpy  
284 with more severe HMT conditions, but effects of moisture content are considerable only  
285 at higher temperature, between 90 and 120 °C. According to Gunaratne and Hoover  
286 (2002), the decrease in  $\Delta H$  on HMT suggest that some of the double helices present in  
287 crystalline and non crystalline regions of the granule could be disrupted under the  
288 conditions prevailing during treatment. Thus, few double helices would unravel and  
289 melt during gelatinization of HMT treated starches.

290 We already showed on standard maize starch (Zarguili et al., 2007) that during D.I.C.  
291 treatment the moisture content of starch granules increases as pressure and processing  
292 time increase, due to the absorption of accumulated steam. The initial moisture content  
293 of starch (13%, base dries) is different from the real moisture content present during the  
294 treatment. This increase reaches an equilibrium value after a given time which depends  
295 on the level of processing pressure. At the pressure of 1 and 2 bar, the equilibrium  
296 moisture content was 17% and 26% (dry basis) and reached after 30 and 60 min of  
297 D.I.C. treatment, respectively. However, for pressure level of 3 bar the measure of  
298 equilibrium moisture content (beyond 60 min) could not be taken because of the  
299 formation of a compact lump making measurement difficult. From this study, at 3 bar  
300 and 10 min, the moisture content uptake was 18% (dry basis).

301 3.2. ENZYME DIGESTIBILITY

302 In a first reaction, the enzyme digestibility of native WMS was compared to the  
303 digestibility of two WMS treated by D.I.C. This digestibility was carried out in two  
304 steps. The first step, liquefaction, was performed with Fungamyl 800 L for 120 min.  
305 The second step, saccharification, was performed with AMG 300 L for 60 min. The  
306 kinetics of liquefaction and saccharification (not showed) of WMS treated by D.I.C. at 1  
307 bar with a processing time of 90 min are very similar to those obtained from native  
308 WMS. These results suggest that the low D.I.C. conditions (1 bar/90 min) do not have a  
309 significant effect on the enzymatic hydrolysis. It is important to note that the yield of  
310 liquefaction is very low, only 10% obtained after 2 hours.

311 On the other hand, we observe that kinetics of liquefaction and saccharification but also  
312 hydrolysis yields of WMS hydrotreated at 3 bar with a processing time of 10 min is  
313 strongly improved. The liquefaction with Fungamyl involved the production of maltose  
314 syrup with a little production of glucose syrup. Very fast is the rate, with a hydrolysis  
315 yield around of 40% obtained in less of 10 min. The saccharification with AMG  
316 involved the production of glucose syrup, is observed by TLC analysis (Fig 2). After 1  
317 hour of saccharification, a yield of hydrolysis of 80% is obtained. These first  
318 experimentations demonstrate that the high D.I.C. conditions (3 bar/10 min) have a big  
319 effect on rates and yields of liquefaction and saccharification.

320 A similar effect was observed with saccharification without preliminary liquefaction  
321 (results not showed.). A hydrolysis yield of 70% is obtained after 60 min using WMS  
322 treated by D.I.C. at 3 bar/10 min, while a hydrolysis yield near of 55% is obtained after  
323 130 min with WMS native.

324 The effects of D.I.C. process on the enzymatic hydrolysis of WMS were compared  
325 to those obtained on SMS and WTS starches. Saccharification alone with AMG  
326 was carried out (Fig. 3), we observe that in all starches the kinetics of saccharification is  
327 more efficient from treated starches than native starches. The more pronounced increase  
328 in hydrolysis was observed for all starches when severe D.I.C. conditions were applied  
329 (pressure level  $\geq 3$  bar, table 1). The hydrolysis yield after 30 min of the three native  
330 starches was increased after D.I.C. treatment at 3 bar for 10 min following the order:  
331 WMS (39%), SMS (24%) and WTS (21%).

332 The increase in the susceptibility towards enzymatic hydrolysis after treatment suggests  
333 a strong link with the structural modifications induced by the heat treatment. The  
334 increase of starch hydrolysis is concomitant with the decrease of the gelatinization  
335 enthalpy. That was observed in precedent experimentations (Table 1). In a recent work  
336 we showed that the D.I.C. treatment decreased the relative crystallinity of  
337 hydrothermally treated starches as the severity of processing conditions increased  
338 (Zarguili, 2006). The increase in the accessibility of starches to enzyme attack after  
339 HMT has been also reported by several researchers (Gunaratne and Hoover, 2002).

### 340 *3.3. Pasting properties*

341 Typical Brabender Viscograph curves of native and D.I.C. treated starches are shown in  
342 Fig. 2, of SMS, WMS and WTS. The pasting properties of analysed starches are  
343 summarized in table 2. It is difficult to compare the pasting values of starches because  
344 of the differences in starch concentrations used during measurements. The starch  
345 concentrations were chosen in order to lie within the sensitivity range of the  
346 Viscograph: i.e. 6% for SMS, 4% (w/w) for WMS and 7% for WTS.

347 No change in pasting temperature was observed for D.I.C. SMS at 1 bar. The peak  
348 occurring at 97.5 °C and the onset of the pasting temperature at 82.5 °C for native  
349 and SMS treated at 1 bar for 90 min (Table 2). The PV increased from 835 UB (native)  
350 to 870 UB for SMS treated for 90 min and 1 bar. At severe D.I.C. conditions (2 bar for  
351 90 min and 3 bar for 10 min), starch produced a very soft gel which was not measurable  
352 under the experimental conditions. While the pasting temperature of WMS increased  
353 following D.I.C. treatment. The PV decreases with increase of the intensity of D.I.C.  
354 conditions (processing pressure and time). However, at same pressure, processing time  
355 seems to reduce viscosity (result not given). D.I.C. treatment decreased  $T_o$  of WTS,  
356 from 80 °C for native starch to 70 °C for D.I.C. WTS at 3 bar for 10 min. The  $T_p$   
357 remained unchanged after D.I.C. treatment. At the same D.I.C. condition (1 bar and 90  
358 min) like SMS, PV of D.I.C. WTS was higher than native starch. WTS treated at 3 bar  
359 for 10 min showed a cold swelling behaviour with a rapid rehydration traduced by a  
360 Brabender viscosity of about 180 UB (Fig. 4). This cold viscosity is explained by a  
361 partial solubilization of WTS already pregelatinized following D.I.C. treatment.  
362 Compared to SMS and WTS starches, the final viscosity of WMS, that is virtually free  
363 of amylose, is lower. This is probably related to the differences of amylose leaching and  
364 its contribution to the setback viscosity. It well known that when gelatinized starch  
365 paste is subject to cooling the extent of viscosity increase is mainly governed by the  
366 rapid reassociation of linear amylose chains by formation of gel matrix.

367 Many authors have been observed that HMT increased pasting temperature  
368 characteristics and decreased of Brabender pasting viscosity at 95 °C after 30 min  
369 holding time for standard maize, amylo maize (Hoover and Manuel, 1996), potato  
370 (Hoover and Vasanthan, 1994) and lentil, oat (Hoover and Vasanthan, 1994). However,

371 for waxy maize starch heated in conventional oven, pasting properties seemed to be  
372 unaffected (Hoover and Manuel, 1996). According these authors, the decrease of  
373 viscosity could be explained by the increase of inter and intramolecular hydrogen bonds  
374 due to the association of the amylose chains and the formation of the complex amylose-  
375 lipid after hydrothermal treatment. Recent work showed by the X-ray diffraction pattern  
376 of SMS D.I.C. treated at 2 bar for 60 min and 3 bar for 0.5 and 15 min the partial loss  
377 (treatment at 2 bar) or total (treatment at 3 bar) of the crystalline structure and the  
378 presence of a crystalline amylose-lipid complex formed during D.I.C. treatment  
379 (Zarguili, 2006). Eliasson (1985) reported that amylose-lipid complex inhibits the  
380 swelling of starch. Tester and Morrison (1990) reported also that amylopectin  
381 contributes to swelling, whereas the amylose and lipids inhibits swelling.

#### 382 *3.4. Flow properties*

383 The flow properties of native and D.I.C. SMS, WMS and WTS starch dispersions were  
384 studied in the shear rate range of 0.01-660 s<sup>-1</sup> and the results are presented in Table 2.  
385 All the curves (not shown) exhibited a non-newtonian shear thinning behaviour with or  
386 without a yield stress. The shear-thinning behaviour appear clearly ( $n < 1$ ), except for the  
387 SMS suspensions treated at 2 bar for 90 min and 3 bar for 10 min, whose rheological  
388 behaviour is rather newtonian ( $n = 1$ ,  $\tau_o = 0$ ). For all treated starches, one observed the  
389 decrease of yield stress ( $\tau_o$ ), consistency index ( $k$ ), and apparent viscosity ( $\eta$ ) with the  
390 increase in processing pressure and time. For SMS treated at 2 and 3 bar, no yield stress  
391 was measured (Table 2). A drastic reduction in the apparent viscosity was observed,  
392 with values close to those of water. These results confirm those obtained by the  
393 Viscograph Brabender at the same D.I.C. conditions. Contrary to the SMS, the flow

394 behaviour index of WMS remained unchanged after D.I.C. treatment, n was almost  
395 constant and its value is approximately equal to that of the native starch (0.53).

396 The variation of viscosity deduced using the Herschel-Bulkley model for the three  
397 starches at various D.I.C. conditions, agrees overall with pasting viscosity values  
398 measured by Brabender. The reduction in viscosity after D.I.C. treatment is mainly  
399 allotted to the reduction in swelling capacity of treated starches. The rheological  
400 behavior of starch suspensions is known to be the result of a combination of two major  
401 factors: the continuous phase and the volume fraction of dispersed phase. In the range of  
402 concentrations used in this work (Loisel et al., 2006), the volume fraction appears to be  
403 close to unity: the suspension can then be described as a packing of swollen starch  
404 granules, the overall behavior being governed by the dispersed phase (Doublier et al.,  
405 1987).

#### 406 3.5. MICROSCOPIC OBSERVATIONS

407 The polarized light microscope images (not shown) of D.I.C. treated SMS, WMS and  
408 WTS starches showed that the starch granules size did not appear to have changed. At 1  
409 and 2 bar for 90 min of processing time, about half of the starch granules exhibited  
410 birefringence with a few swollen granules. The gradual loss of birefringence observed  
411 using microscopy is also reflected in the result obtained by DSC measurements (Table  
412 1). We observed that the cross polarization is still clear on a significant number of  
413 granules of treated SMS and WMS. The degree of gelatinization calculated for these  
414 starches were of 23.7 - 37.7% and 2.6 - 27.7%, respectively. For WTS treated at  
415 pressure of 2 bar and 90 min a few starch granules exhibited birefringence with little  
416 swelling. The DSC measurements indicated that the starch granules were almost  
417 completely gelatinized (98.9%). At 3 bar and 10 min, all starch granules of WST lost

418 their birefringence. Whereas for SMS and WMS some intact granules are visible,  
419 this confirms the DSC results, where the gelatinization degrees of the residual  
420 structure at this condition, were of 85.1 and 86.5%, respectively.

#### 421 **4. CONCLUSIONS**

422 This study has shown that the D.I.C. treated starches gelatinise at higher temperatures  
423 and over narrower temperature ranges than native starches. Moreover, gelatinization  
424 enthalpies decreased progressively for the three treated starches with increasing pressure  
425 level and processing time. The extent of the decrease followed this order:  
426 WMS>SMS>WTS. The similar enzymatic behaviour was observed on D.I.C. treated  
427 starches. For all starches the kinetics of saccharification was more efficient from treated  
428 than native starches. The enzymatic susceptibility of starches is directly related to the  
429 structural modifications produced by the hydrotraitement. The considerable reduction in  
430 relative crystallinity caused by the D.I.C. treatment had as consequence the increase of  
431 the enzymes susceptibility.

## 432 References

- 433 Adebawal, K.O., Afolabi, T.A., Olu-Owolabi, B.I., 2005. Hydrothermal treatments of  
434 finger millet (*Eleusine coracana*) starch. *Food Hydrocolloids* 19 (6), 974-983.
- 435 Colonna, P., Buléon, A., 1992. New insight on structure and properties. Page 25 in:  
436 *Cereal Chemistry and Technology, a long Past and a Bright Future*. 9th International  
437 Cereal and Bread Congress, Paris.
- 438 Cooke, D., Gidley, M.J., 1992. Loss of crystalline and molecular order during starch  
439 gelatinization: origin of the enthalpic transition. *Carbohydrate Research*, 227, 13-112.
- 440 Donovan, J.W., 1979. Phase transitions of the starch-water system. *Biopolymes*, 18,  
441 263-275.
- 442 Doublier, J. L., Llamas, G., Le Meur, M., 1987. A rheological investigation of cereal  
443 starch pastes and gels. Effect of pasting procedures. *Carbohydrate Polymers*, 7, 251-  
444 275.
- 445 Eliasson, A.C., 1985. Retrogradation of starch as measured by differential scanning  
446 calorimetry. Pages 93 in: *New Approaches to research on cereal Carbohydrates*. R. D.  
447 Hill, and L. Munck, eds. Elsevier Applied science: London.
- 448 Flipse, E., Keeteld, C.J.A.M., Jacobson, E., Visser, R.G.F., 1996. The dosage effect of  
449 the wiltytype GBSS allele is linear for GBSS activity but not for amylose content:  
450 Absence of amylose has a distinct influence on the physico-chemical properties of  
451 starch. *Theoretical and Applied Genetics*, 92, 121-127.
- 452 Fredriksson, H., Silverio, J., Andersson, R., Eliasson, A. -C., Aman, P., 1998. The  
453 influence of amylose and amylopectin characteristics on gelatinization and

454 retrogradation properties of different starches. *Carbohydrate Polymers*, 35, 119-  
455 134.

456 Gunaratne, A., Hoover, R., 2002. Effect of heat-moisture treatment on the structure and  
457 physical properties of tuber and root starches. *Carbohydrate Polymers*, 49, 425-437.

458 Hoover, R., Manuel, H., 1996. Effect of heat-moisture treatment on the structure and  
459 physicochemical properties of normal maize, waxy maize, dull waxy maize and  
460 amylo maize V starches. *Journal of Cereal Science*, 23, 153-162.

461 Hoover, R., Vansanthan, T., 1994. Effect of heat-moisture treatment on the structure  
462 and physicochemical properties of cereal, tuber, and legume starches. *Carbohydrate*  
463 *Research*, 252, 33-53.

464 Hublin, L., 1994. Influence des caractéristiques structurales des amidons natifs sur leur  
465 réactivité chimique. PhD dissertation, Université de Nantes. France.

466 Imberty, A., Buléon, A., Tran, V., Perez, S., 1991. Recent advances in knowledge of  
467 starch structure. *Starch/ Stärke*, 43, 375-384.

468 Krueger, B.R., Knutson, C.A., Inglett, G. E., Walke, C.E., 1987. A differential scanning  
469 calorimetry study on the effect of annealing on gelatinization behavior of corn starch.  
470 *Journal of Food Science*, 52, 715-718.

471 Lawal, O.S., 2005. Studies on the hydrothermal modifications of new cocoyam  
472 (*Xanthosoma sagittifolium*) starch. *International journal of biological macromolecules*,  
473 37 (5), 268-277.

474 Li, J.H., Vasanthan, T., Hoover, R., Rosnagel, B.G., 2004. Starch hull-less barley: IV.  
475 Morphological and structural changes in waxy, normal and high-amylose starch

- 476 granules during heating. *Food Research International*, 37, 417-428.
- 477 Lim, S.T., Chang, E.H., Chung, H.J., 2001. Thermal transition characteristics of heat-  
478 moisture treated corn and potato starches. *Carbohydrate Polymers*, 46, 107-115.
- 479 Loisel, C., Maache-Rezzoug, Z., Esneault, C., Doublier, J.L., 2006. Effect of  
480 hydrothermal treatment on the physical and rheological properties of maize starches.  
481 *Journal of Food Engineering*, 73, 45-54.
- 482 Lorenz, K., Kulp, K., 1982. Cereal and root starch modification by heat-moisture  
483 treatment. I. physicochemical properties. *Starch/ Stärke*, 34, 50-54.
- 484 Marshall, W.E., Wadsworth, JI., Verma, L.R., Velupillai, L., 1993. Determining the  
485 degree of gelatinization in parboiled rice: comparison of subjective and an objective  
486 method. *Cereal Chemistry*, 70, 226-230.
- 487 Maruta, I., Kurahashi, Y., Takayano, R., Hayashi, K., Yoshino, Z., Komaki, T., Hara,  
488 S., 1994. Reduced-pressurized heat-moisture treatment. A new method for heat-  
489 moisture treatment of starch. *Starch/Stärke*, 46, 177-181.
- 490 Noda, T., Takahata, Y., Sato, T., Suda, I., Morishita, T., Ishiguro, K., 1998.  
491 Relationships between chain length distribution of amylopectinand gelatinization  
492 properties within the same botanical origin for sweet potato and buckwheat.  
493 *Carbohydrate Polymers*, 37, 153-158.
- 494 Planchot, V., Colonna, P., Buléon, A. Gallant, D., 1997. Amylosis of starch granules and  
495 a-glucan crystallites. In R. J. Frazier, A. M. Donald & P. Richmond, *Starch structure  
496 and functionality* (PP. 141-152). Cambridge, UK : Royal Society of Chemistry.
- 497 Srichuwong, S., Sunarti, T.C., Mishima, T., Isono, N., Hisamatsu, M., 2005. Starches

498 from different botanical sources II: Contribution of starch structure to swelling and  
499 pasting properties. *Carbohydrate Polymers*, 62, 25-34.

500 Stute, H., 1992. Hydrothermal modification of starches: the difference between  
501 annealing and heat-moisture treatment, *Starch/Stärke*, 44, 205-214.

502 Tester, R.F., Morrison, W.R., 1990. Swelling and gelatinization of cereal starches. I.  
503 Effects of amylopectin, amylase and lipids. *Cereal chemistry*, 67, 551-557.

504 Vermeulen, R., Goderis, B., Delcour, J.A., 2006. An X-ray study of hydrothermally  
505 treated potato starch. *Carbohydrate Polymer*, 64, 364-375.

506 Zarguili, 2006. Etude de l'effet de l'hydrotraitement D.I.C. sur les propriétés  
507 structurales et fonctionnelles des amidons de différentes origines botaniques. PhD  
508 dissertation, Université de La Rochelle. France

509 Zarguili, I., Maache-Rezzoug, Z., Loisel, C., Doublier, J.-L., 2006. Influence of D.I.C.  
510 hydrothermal process conditions on the gelatinization properties of standard maize  
511 starch. *Journal of Food Engineering*, 77 (3), 454-461.

512 Zarguili, I., Maache-Rezzoug, Z., Loisel, C., Doublier, J.-L., 2007. A mathematical  
513 model to describe the change of moisture distribution in maize starch during D.I.C.  
514 hydrothermal treatment. *International Journal of Food Science and Technology*, in  
515 press.

Table 1. DSC characteristics of native and D.I.C. treated starches, of SMS, WMS and WTS starches, at various steam pressures level and processing time. Hydrolysis yield after 30 min of saccharification with AMG.

Starch source	D.I.C. treatment	Gelatinization parameters					Hydrolysis yield (%)
		T <sub>o</sub> (°C)	T <sub>p</sub> (°C)	R (°C)	ΔH (J/g)	DG (%)	
SMS	Native	63.1±0.4	69.6±0.5	13.0	11.4	0.0	19
	1 bar/90 min	69.6±0.2	75.4±0.4	11.6	8.7	23.7	nd
	2 bar/90 min	72.9±0.6	77.5±0.8	9.2	7.1	37.7	nd
	3 bar/10 min	74.8±0.9	79.8±0.7	10.0	1.7	85.1	1.7
WMS	Native	65.4±0.9	71.3±0.9	11.8	15.5	0.0	15.5
	1bar/90 min	68.0±0.7	73.7±0.6	11.4	15.1	2.6	nd
	2 bar/90 min	73.1±0.9	77.8±0.8	9.5	11.2	27.7	nd
	3 bar/10 min	75.2±0.2	77.9±0.3	5.4	2.1	86.5	2.1
WTS	Native	50.4±0.2	56.6±0.2	6.2	9.4	0.0	9.4
	1 bar/90 min	57.6±0.5	61.5±0.5	3.9	8.0	14.9	nd
	2 bar/90 min	65.2±0.3	68.0±0.3	2.8	0.1	98.9	nd
	3 bar/10 min	-	-	-	0.0	100	0.0

T<sub>o</sub>: onset temperature; T<sub>p</sub>: peak temperature. Temperature values are means of triplicate determinations ± standard deviation. R: temperature range calculated as 2 (T<sub>p</sub> – T<sub>o</sub>). ΔH: enthalpy of gelatinization with the mean absolute error (three repetitions) of 0.5 J/g. DG: degree of gelatinization. nd: not determined.

Table 2. Pasting and rheological characteristics of native and D.I.C. treated starches at pressure of 1, 2 and 3 bar for two processing time.

Starch source	D.I.C. treatment	Pasting properties			Rheological properties			
		T <sub>o</sub> (°C)	T <sub>p</sub> (°C)	PV <sup>d</sup> (UB) <sup>e</sup>	τ <sub>o</sub> (Pa)	K (Pa.s <sup>n</sup> )	n	η (Pa.s)
SMS <sup>a</sup>	native	82.5	97.5	835	2.28	1.39	0.57	3.68
	1 bar/90 min	82.5	97.5	870	1.25	0.82	0.57	2.07
	2 bar/90 min	-	-	0	0.00	0.00	1.00	0.00
	3 bar/10 min	-	-	0	0.00	0.00	0.99	0.00
WMS <sup>b</sup>	native	72.5	83.0	640	0.70	0.88	0.53	1.58
	1 bar/90 min	77.5	88.5	625	0.18	0.33	0.57	0.52
	2 bar/90 min	82.0	90.0	400	0.16	0.18	0.63	0.35
	3 bar/10 min	81.0	88.5	490	0.17	0.27	0.59	0.45
WTS <sup>c</sup>	native	80.0	97.5	562	0.95	0.44	0.78	1.39
	1 bar/90 min	77.5	97.5	640	0.96	0.45	0.82	1.42
	2 bar/90 min	75.0	97.5	400	0.55	1.90	0.59	2.46
	3 bar/10 min	70.0	97.5	385	0.00	0.03	0.89	0.03

<sup>a</sup> 6% (w/w) aqueous standard maize starch suspension; <sup>b</sup> 4% (w/w) aqueous waxy maize starch suspension; <sup>c</sup> 7% (w/w) aqueous wheat starch suspension; T<sub>o</sub>: onset of the pasting temperature <sup>d</sup> PV, peak viscosity. <sup>e</sup> UB, Units Brabender. τ<sub>o</sub>: yield stress; K: consistency index; n: flow behaviour index (K and n were determined from Herschel-Bulkley model); η: apparent viscosity measured for shear rate of 1s<sup>-1</sup>.

### Figure captions

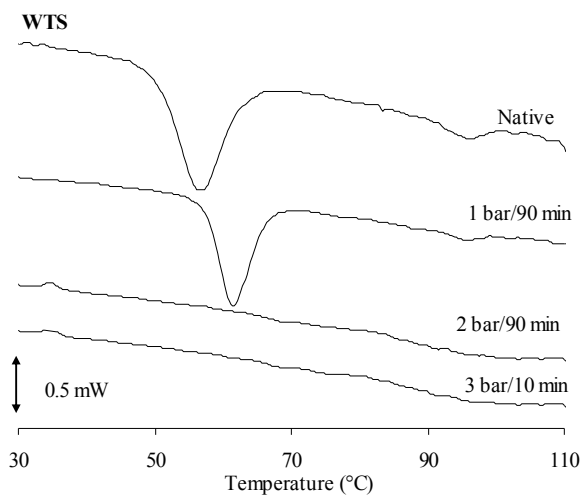
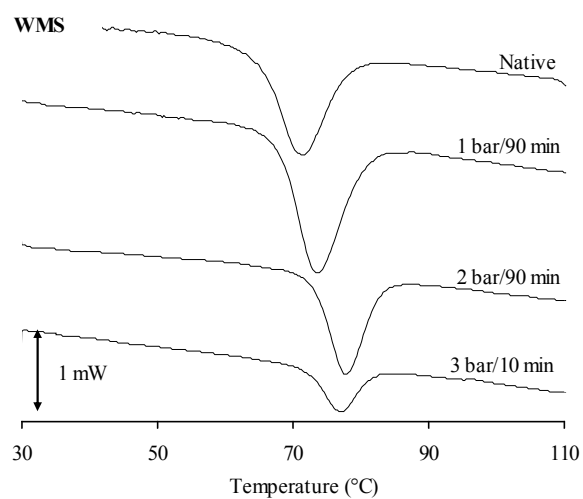
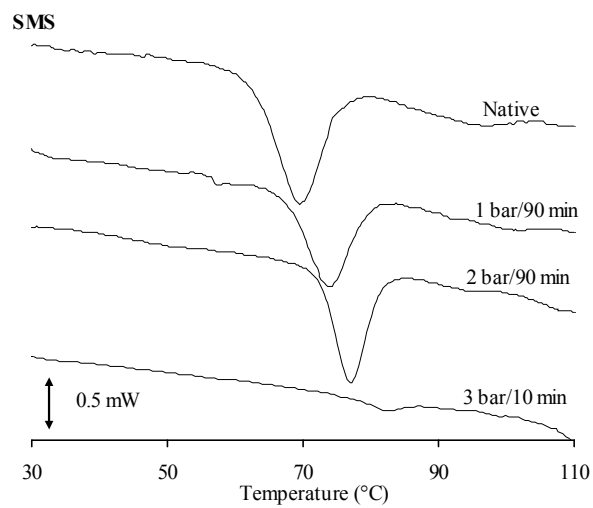
Fig. 1. Differential scanning calorimetry curves of native and DIC treated standard maize (SMS), waxy maize(WMS) and wheat(WTS) starches at processing time of 90 and 10 min and pressure level of 1 (100°C), 2 (122°C)and 3 bar (135°C).

Fig. 2. TLC analysis of WMS hydrolysis. Native WMS after 20 min of Liquefaction (A), treated WMS at 3 bar/10 min after 20 min of Liquefaction (B), native WMS after 120 min of Liquefaction and 60 min of saccharification (C) treated WMS at 3 bar/10 min after 120 min of Liquefaction and 60 min of saccharification (D).

Fig. 3. Time course of several starches hydrolysis by AMG. WMS native (●), WMS treated by DIC at 3 bar/10 min (○), WTS native (■), WTS treated by DIC at 3 bar/10 min (□), SMS native (▲) and SMS treated by DIC at 3 bar/10 min (△).

Fig. 4. Brabender curves of native and DIC treated SMS, WMS and WTS.

**Fig. 1**



**Fig. 2**

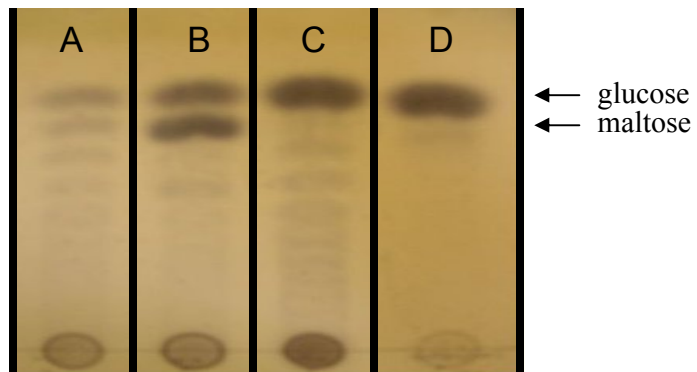


Fig. 3

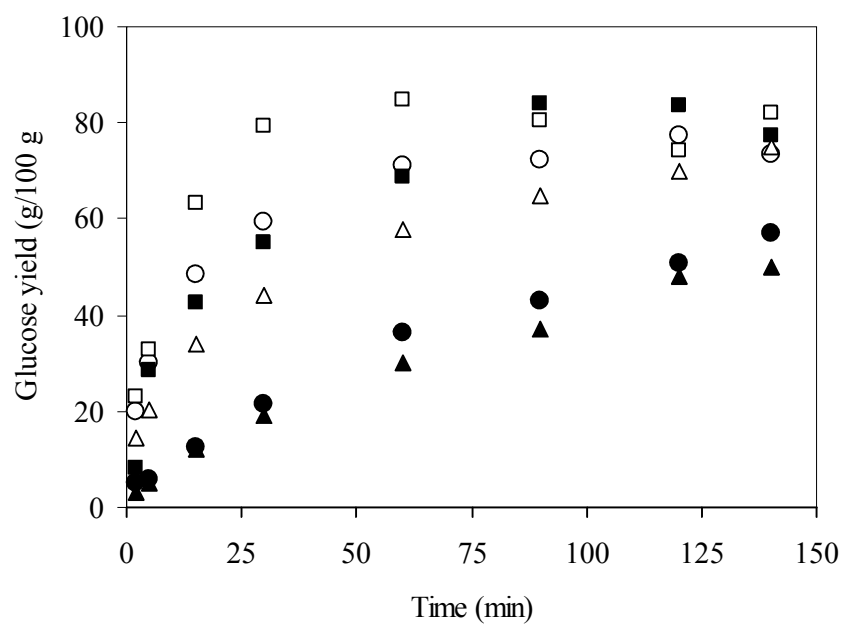


Fig. 4

