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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 (ΔH), were observed. At 3 bar/10 min ΔH decreased from 11.4 J.g⁻¹ to 1.7
25 J.g⁻¹, from 15.5 J.g⁻¹ to 2.1 J.g⁻¹ and from 9.4 J.g⁻¹ to 0 J.g⁻¹ 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 (≈ 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 α -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 α -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 α -amylase from *Aspergillus oryzae* was used for starch liquefaction.

118 The enzyme activity was 800 FAU/g (1 Fungal α -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 α -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 ΔH 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, ΔH_t and ΔH_{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⁻¹ (4
196 min each) were linearly applied, followed by a logarithmic stepwise decrease from 660
197 to 0.01 s⁻¹, 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 τ_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 (ΔH) 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 ΔH of SMS and SWS were higher than corresponding values for wheat. T_o , T_p and
214 ΔH 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 ΔH 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 ΔH 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 ΔH (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 ΔH 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⁻¹, for SMS from 11.4
280 (native) to 7.1 J.g⁻¹ and for WMS from 15.5 (native) to 11.2 J.g⁻¹. 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 ΔH 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 ≥ 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 $0.01\text{-}660\text{ s}^{-1}$ 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 (τ_o), consistency index (k), and apparent viscosity (η) 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.

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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 _o (°C)	T _p (°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_o: onset temperature; T_p: peak temperature. Temperature values are means of triplicate determinations ± standard deviation. R: temperature range calculated as 2 (T_p – T_o). 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 _o (°C)	T _p (°C)	PV ^d (UB) ^e	τ _o (Pa)	K (Pa.s ⁿ)	n	η (Pa.s)
SMS ^a	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 ^b	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 ^c	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

^a 6% (w/w) aqueous standard maize starch suspension; ^b 4% (w/w) aqueous waxy maize starch suspension; ^c 7% (w/w) aqueous wheat starch suspension; T_o: onset of the pasting temperature ^d PV, peak viscosity. ^e UB, Units Brabender. τ_o: 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⁻¹.

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

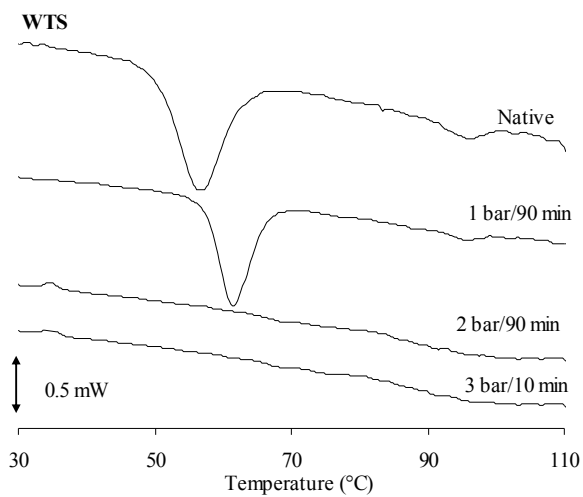
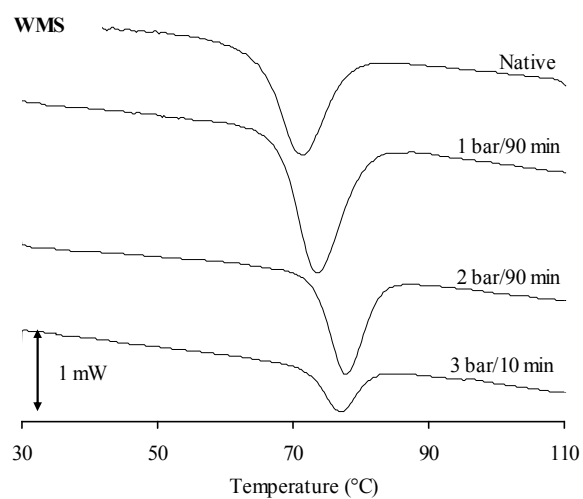
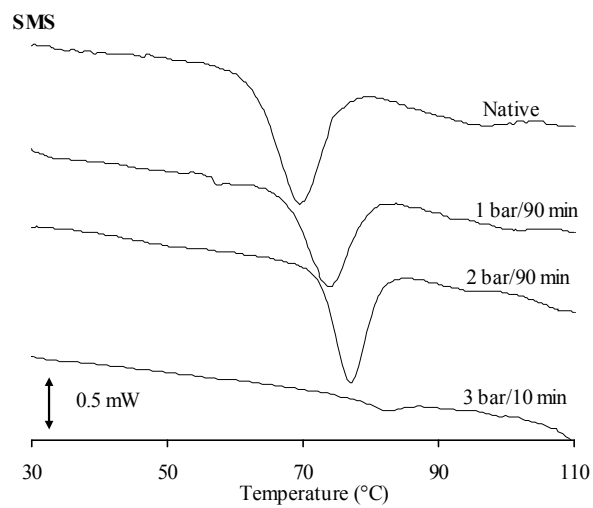


Fig. 2

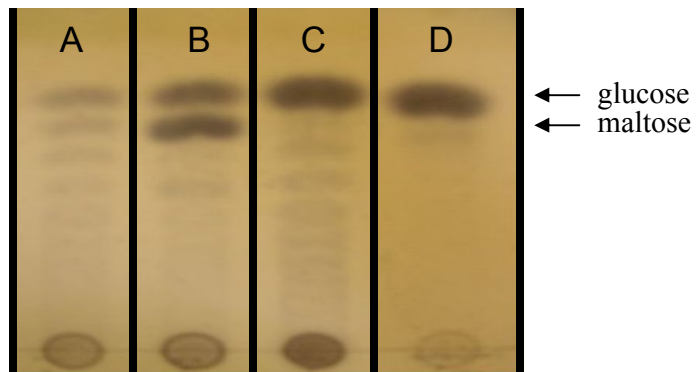


Fig. 3

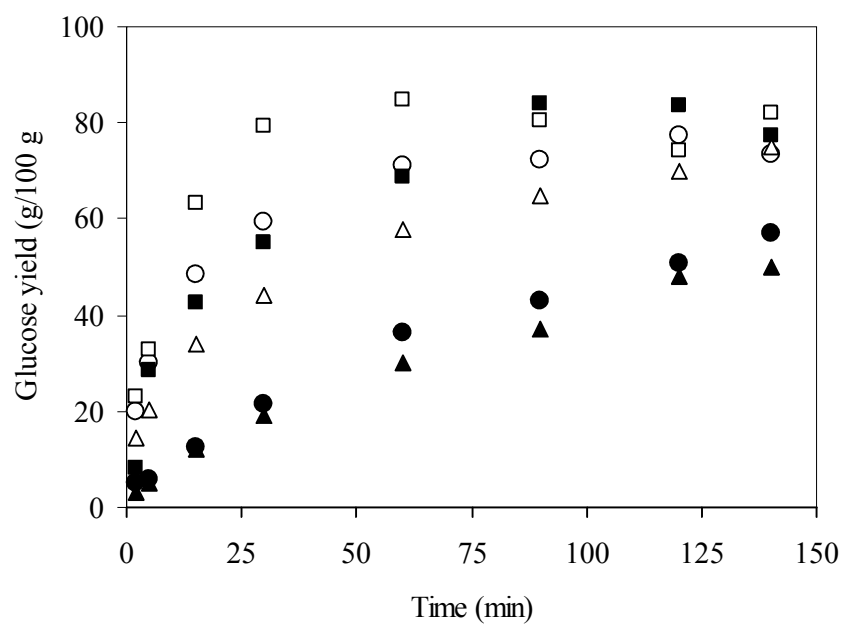


Fig. 4

