

1 **CHANGES IN GELATINISATION AND PASTING PROPERTIES OF**
2 **VARIOUS STARCHES (WHEAT, MAIZE AND WAXY MAIZE) BY THE**
3 **ADDITION OF BACTERIAL CELLULOSE FIBRILS.**

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33 **ABSTRACT**

34 The aim of this work was to analyse the effect of bacterial cellulose fibrils (BCF)
35 on the gelatinization profile and pasting properties of starches from different
36 sources (wheat, maize and waxy maize) and amylose contents. Blends of 8%
37 starch with different BCF levels (0, 0.5, 2, 6 and 10% based on the dry weight
38 of starch) were prepared and tested by Rapid Visco-Analysis (RVA), Differential
39 Scanning Calorimetry (DSC) and both Optical and Polarized Light Microscopy.
40 Results showed that BCF produce a significant modification of pasting properties.
41 The pasting temperature was reduced but viscosities (peak, final, trough,
42 breakdown and final) increased. The reduction in pasting temperature at the
43 highest BCF addition was 20^oC higher for maize and wheat starches but only 2^oC
44 higher for waxy maize starch. In contrast to the pasting temperature, the
45 gelatinisation temperature by DSC for all three starches slightly varied upon BCF
46 addition, but the gelatinisation enthalpy was reduced to a greater extent than
47 values reported for the addition of other hydrocolloids to starch blends. Optical
48 and polarized light microscopy showed the presence of domains rich in starch
49 and highly aggregated BCF in all three starches evaluated. The increase in
50 viscosity and decrease in pasting temperature are discussed in terms of changes
51 in starch concentrations in the starch rich domain. These results open interesting
52 perspectives in the use of bacterial cellulose and plant cell walls to design novel
53 bio-composites to structure foods.

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55 Keywords: bacterial cellulose, starch, gelatinisation, pasting properties,
56 advanced materials.

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65 **1. INTRODUCTION**

66 Cellulose is the most abundant biopolymer in nature. It is mainly produced by
67 plants, trees and bacteria by condensation of glucose units during the
68 photosynthesis process in plants and trees. Long chains of anhydro-glucose
69 units, joined via β -1,4-glycosidic linkages (C–O–C), are formed during this
70 process (Eichhorn, 2011). Cellulosic materials consist of both crystalline and
71 amorphous domains, in different proportions depending of cellulose source
72 (Ciolacu et al., 2011). The presence of para-crystalline or amorphous cellulose
73 is often significant, although it varies from species to species (Eichhorn, 2011).
74 The physical properties of cellulose, as well as their chemical behaviour and
75 reactivity, are strongly influenced by the arrangement of the cellulose molecules
76 with respect to each other and to the fibre axis (Ciolacu et al., 2011). One type
77 of cellulosic material that has received much recent attention as potential new
78 functional material for industrial applications is bacterial cellulose.

79 Bacterial cellulose is a biopolymer formed by nanofibrils, which is synthesized
80 mainly by *Acetobacter xylinus* and *Acetobacter hansenii* (Shah et al., 2013).
81 These microorganisms are able to create in their extracellular matrix a complex
82 network of cellulose fibres by a highly regular intra- and inter-molecular
83 hydrogen bonds network resulting in a weak gel structure. This is the basis of
84 Nata de Coco a traditional sweet candy desert originating in the Philippines
85 (Tabuchi, 2007). Bacterial cellulose has a unique structure, composed by
86 nanofibrils forming a nanostructured network characterized by high purity (free
87 of components such as lignin and hemicellulose) Because of its nanostructure
88 bacterial cellulose shows a high mechanical stability, high water absorption
89 capacity in the wet state and full biocompatibility making this material feasible
90 to be used in wide variety of applications (Picheth et al., 2017; Lee, Buldum,
91 Mantalaris, & Bismarck, 2014; Shah et al., 2013). More recently the production,
92 structure and applications of bacterial cellulose has been reviewed in the context
93 of food use (Grishkewich, Mohammed, Tang, & Tam, 2017; Shi, Zhang, Phillips,
94 & Yang, 2014; Ullah, Santos, & Khan, 2016). Incorporation of hydrocolloids,
95 particularly pectin and mannans during the synthesis of bacterial cellulose has
96 frequently been used to prepare models of the plant cell wall structure (Lopez-

97 sanchez et al., 2017; Whitney, Brigham, Darke, Reid, & Gidley, 1998). Remnants
98 of plant cell walls can be used to replace soluble hydrocolloids in structuring
99 foods and may give health benefits (Foster, 2011; Padayachee, Day, Howell, &
100 Gidley, 2017).

101 For many years there has been extensive interest in hydrocolloid:starch blends
102 because of their inclusion in a wide range of food products. A review by Bemiller
103 (2011) identified a large number of starch hydrocolloid blends, however we are
104 not aware of any studies where bacterial cellulose has been added to starch to
105 modify pasting behaviour. This paper describes a preliminary study to determine
106 how bacterial cellulose fibrils modify the gelatinisation profile and pasting
107 properties of starch. Starch gelatinisation is a physical transition that takes place
108 in a starch granule and modifies the functional properties (e.g. solubility,
109 viscosity, water holding capacity) as a response to high temperature and water.
110 Although there is not a formal definition, gelatinisation has been described as
111 "the collapse of molecular order inside the starch granule which produce
112 irreversible structural changes related with an increase in granule volume,
113 melting of crystalline form, loss of birefringence and increasing in starch
114 solubility due to effect of temperature in an environment of high moisture"
115 (Belitz, Grosch, & Schieberle, 2009; J. N. BeMiller & Huber, 2008). Normally the
116 gelatinisation is measured by microscopy, differential calorimetry, X-Ray
117 diffraction among other techniques. The modification of the gelatinization profile
118 of starches by other biostructures is important for a number of reasons, including
119 its potential effect on the extent of retrogradation on cooling and presumably on
120 generation of low-digestive and resistant starch (Mishra, Hardacre, & Monro,
121 2012). Appelqvist et al (1995) also described an application of freeze-thaw
122 resistance in starch sauces when mixed with hydrocolloids. In the case of starch
123 pasting, it is regarded as a consequence of gelatinisation and is generally
124 followed by viscosity changes. Indeed, as a result of starch gelatinisation, a
125 viscoelastic mass is obtained (called paste), which consists of a continuous phase
126 that is a molecular dispersion of suspended starch polymer molecules forming a
127 network and a discontinuous phase of swollen granules, granules ghosts and
128 granule fragments (Bemiller, 2011b). A common technique used to follow starch

129 pasting is the Rapid-Visco Analysis (RVA) which was developed from the well-
130 known Bravender curves of starch viscosity used in the industry.
131 Studies looking at starch hydrocolloid interactions have generally involved only
132 one starch source and several hydrocolloids. In this study maize, wheat and
133 waxy maize starches were selected because of their industrial importance, but
134 also due to some structural differences between them. For instance, the waxy
135 maize starch contains only traces of amylose whereas the amylose content of
136 maize and wheat starches is ~25-29% but this could vary with source and
137 extraction method (Bertoft, 2017). Swelling of granules on heating will be
138 influenced by the presence of amylose-lipid complexes, which could be more
139 present in high amylose cereal starches than do normal and waxy starches
140 (Debet & Gidley, 2006; Pérez, Baldwin, & Gallant, 2009). In terms of starch
141 granule size, they have been well characterised. 5-20 μm (diameter) in maize
142 and waxy maize and 2-36 μm (diameter) in wheat. However, wheat starch shows
143 a bimodal distribution in size. Considering the typical X Ray diffraction pattern
144 all these starches correspond to type-A starch (Buléon, 1998; Jane, 2009).
145 The objective of this work was to determine how the addition of bacterial
146 cellulose fibrils modify the gelatinisation profile and pasting properties of starch
147 from different sources (wheat, maize and waxy maize).

148

149 **2. MATERIALS AND METHODS**

150 2.1. Materials

151 Native wheat, maize and waxy maize starches were purchased from Sigma
152 Aldrich (Germany) in powder form. Dried sheets of bacterial cellulose fibrils
153 (BCF) were kindly provided by Membracel (Brazil). The starches and bacterial
154 cellulose were used as received without further purification and stored at room
155 temperature until further use.

156

157 2.2. Preparation of starch-BCF suspensions

158 BCF was added to each starch in a concentration of 0, 0.5, 2, 6 and 10%
159 w/weight dry starch (Equation 1), using distilled water as solvent. BCF dried
160 sheets were processed prior mixing following the protocol proposed by Quero et

161 al. (2015). In the first step a well defined amount of BCF was held overnight in
162 excess of distilled water in order to promote full hydration. In the next step, the
163 BCF suspension was homogenized using a high power kitchen blender (Thomas
164 "Premium", Germany) for 20 minutes, then followed by vacuum filtration using
165 8 µm diameter filter papers (Whatman 541, USA). At the same time, starch
166 water suspensions at 8% (w/v) were prepared for each starch type. In the final
167 step, the filtered BCF was added to each starch suspension and stirred for 15
168 min at room temperature in order to get homogeneous suspensions.

169

$$170 \quad BCF \text{ concentration } (\%, w/w) = \left(\frac{BCF \text{ weight}}{BCF \text{ weight} + \text{Starch weight}} \right) \times 100$$

171 (Equation 1)

172

173 A control sample, in this case BCF in the absence of starch, was prepared
174 following the same protocol.

175

176 2.3 Measurement Viscoelasticity of BC Suspensions

177 A preliminary characterization of the viscoelasticity of BCF suspensions in water
178 in the absence of starch was carried out using a rheometer (Physica MCR 301,
179 Anton Paar, Germany) equipped with parallel plate geometry. BCF suspensions
180 were prepared at concentration of 0.05, 0.1 and 0.2% (w/v). Measurements
181 were made in the linear viscoelastic region at a frequency of 1Hz and strain of
182 0.5%. The temperature was scanned from 10°C to 40°C at a rate of 5°C/min.

183

184 2.4 Measurements of Pasting Properties

185 Pasting properties of starch-BCF blend were analysed by Rapid-Visco-Analysis
186 (RVA super 4, Newport Scientific, Australia) in accordance with the methodology
187 proposed by Sullo & Foster (2010) with minor modifications. 25-28 g of each
188 suspension was weighed in aluminium canisters and inserted into the
189 instrument. Pasting profiles were obtained as a function of temperature as
190 follow: holding at 25°C during 5 min, heating between 25-95°C at 5°C/min,
191 holding at 95°C during 5 min, cooling to 25°C at 5°C/min and holding at 25°C
192 during 5 min. The analysis was performed under constant stirring (160 RPM).

193 The pasting properties measured were: 1) pasting temperature (temperature at
194 which starch granules begin to swell and gelatinise due to water uptake, which
195 is recorded from the onset of the viscosity peak); 2) peak viscosity (maximum
196 paste viscosity achieved in the heating stage of the profile); 3) trough viscosity
197 (minimum paste viscosity achieved after holding at the maximum temperature);
198 4) breakdown viscosity (difference between peak and trough viscosities); 5) final
199 viscosity (viscosity at the end of the run); 6) setback viscosity (difference
200 between final viscosity and trough viscosity). All the measurements were carried
201 out at least in quadruplicate.

202

203 2.5 Differential Scanning Calorimetry (DSC)

204 Gelatinization temperature ($^{\circ}\text{C}$) and enthalpy (ΔH , $\text{J/g}_{\text{starch}}$) were measured by
205 differential scanning calorimetry (DSC 1, Mettler-Toledo, Switzerland) following
206 the protocol reported by Karlsson & Eliasson (2003) with minor modifications.
207 In order to improve the resolution signal a higher of the starch weight
208 suspensions were use, keeping the BCF weight fractions indicated previously (0,
209 0.5, 2, 6 and 10% w/w). The starch concentration was 20% w/v. ~ 20 μL of
210 starch-BCF blend suspensions were loaded into 40 μL aluminium pans and then
211 hermetically sealed. The DSC was calibrated using indium (melting temperature
212 and enthalpy of $156.5 \pm 1.56^{\circ}\text{C}$, $\Delta H = 28.6 \pm 1$ J/g), and an empty pan was
213 used as a reference. Thermal properties of the suspensions were measured as
214 follow: holding temperature at 5°C during 3 min, heating from 5°C to 85°C at
215 $10^{\circ}\text{C}/\text{min}$, and holding at 85°C during 3 min. Gelatinization temperature ($^{\circ}\text{C}$)
216 was recorded from the onset of endothermic peak associated to starch granule
217 swelling and structural changes (Biliaderis, 2009), while gelatinisation enthalpy
218 was considered as the area under the endothermic peak. Gelatinisation enthalpy
219 was normalised in terms of starch dry mass and was expressed in $\text{J/g}_{\text{starch}}$. All
220 measurements were performed in triplicate.

221

222 2.6 Polarized Light Microscopy

223 Structural features of the starch-BCF blends after starch complete gelatinisation
224 were analysed by light and polarized light microscopy (DIAPLAN, Leitz,

225 Germany). One aliquot was transferred from each fully gelatinised starch-BCF
226 from RVA canister immediately after analysis and deposited directly on a clean
227 dry glass surface and covered by a clean coverslip. Light and polarized light
228 images were obtained using a magnification 10X. Pictures were taken by a digital
229 camera connected to the microscope (PixelINK PL-A662, Canada). A set of
230 pictures were captured and those most representative of each blend were
231 selected for analysis.

232

233 2.7 Statistical Analysis

234 Where appropriate, the statistical significance was assessed by a paired t-test
235 (same variances) and ANOVA using the Solver tool in Excel (Office 2016,
236 Microsoft Corp.).

237

238 **3 RESULTS**

239 3.1 Viscoelasticity of BCF suspensions

240 The viscoelasticity of BCF suspensions in the absence of starch was only weakly
241 dependent on temperature. Values of G' of 0.74, 5.10 and 34.6 Pa were obtained
242 at BCF concentrations of 0.05, 0.1 and 0.2 w/v respectively (Supplementary
243 data, Figure S-1). The corresponding values of $\tan\delta$ (G''/G') were 0.17, 0.16 and
244 0.14 respectively. These low values of $\tan\delta$ support the description of a weak
245 gel structure at low concentrations in the temperature range studied (Ikeda &
246 Nishinari, 2001).

247

248 3.2 Pasting Properties of BCF starch blends

249 Changes in viscosity and pasting parameters of starch-BCF blends produced by
250 RVA as a function of temperature and BCF weight fraction are presented in Figure
251 1 and Figure 2 respectively. Figure 1 shows the viscosity profile for the different
252 starches (wheat, maize and waxy maize) at different concentrations of BCF. It
253 is evident that BCF has a significant effect ($p < 0.05$) on the viscosity of all three
254 starches during gelatinisation and re-association (retrogradation) stages. The
255 presence of BCF generates a significant increase in viscosity, which is observed
256 even with the lowest BCF concentration (0.5% w/w), indicating that very low

257 concentrations of BCF can produce an increment in starch viscosity. This
258 behaviour was observed in all three starches considered in this study (wheat,
259 maize and waxy maize), although the magnitude of change in viscosity observed
260 in waxy maize starch was lower than the one observed in wheat and maize
261 starches (Figure 1). Figure 2 shows effect of BCF concentrations on the pasting
262 temperature and the viscosity-pasting parameters (Peak, Trough, Breakdown,
263 Final and Setback). These parameters were derived from the results shown in
264 Figure 1. Most interesting is the pasting temperature which can be defined as
265 the temperature at which the increase in viscosity (onset) is observed and can
266 be related with the starting point of starch gelatinisation (Phimolsiripol,
267 Siripatrawan, & Henry, 2011). A marked shift to a lower pasting temperature
268 was observed for wheat and maize starch when the concentration of BCF was
269 increased (Figure 1a and 1b, Figure 2a). However, in the case of waxy maize
270 starch the presence of BCF produced a much lower effect on the pasting
271 temperature. These results are in agreement with those shown in Sullo & Foster
272 (2010), and Sullo (2012), who identify that when starch is embedded in a
273 hydrocolloid solution the composite viscosity would be affected by changes in
274 the two phases, and the way the two phases interact with each other. They also
275 highlight that an increase in effective concentration of starch in the starch phase
276 would promote interactions between starch granules, and that the higher
277 viscosity of the continuous phase might enable the detection of the early stages
278 of granule swelling which would be undetected when starch is dispersed in water.
279 Sullo (2012) also showed that waxy maize behaved differently from native maize
280 starch, upon gelatinisation in the presence of guar gum, methylcellulose or
281 hydroxypropylmethylcellulose. Abdulmola, Hember, Richardson, & Morris (1996)
282 explained an unexpected increase in moduli in a xanthan:starch system in terms
283 of xanthan promoting an interaction between gelatinised starch granules due to
284 a depletion flocculation mechanism, by which low concentration of xanthan
285 promotes the attraction of gelatinised starch granules and therefore the bringing
286 of them together.

287

288 3.3 Thermal Properties

289 Both gelatinisation temperature ($^{\circ}\text{C}$) and gelatinisation enthalpy (ΔH , $\text{J/g}_{\text{starch}}$) of
290 starch-BCF blends are shown in Figure 3. The presence of BCF significantly
291 reduces ($p < 0.05$) the gelatinisation enthalpy in all three starches studied,
292 however BCF addition had little effect on the gelatinization temperature recorded
293 from the onset of endothermic peak associated to starch granule swelling
294 (Section 2.5).

295

296 3.4 Morphological Analysis

297 Morphological analysis of starch (wheat, maize and waxy maize) blended with
298 10% BCF after complete gelatinisation by RVA are presented in Figure 4. Results
299 using polarised and non-polarised light are shown. These images show the
300 presence of two material domains in the blend. The BCF can be seen as
301 birefringent bundles with dimensions up to 500 microns. No birefringence
302 ascribed to the starch domain is observed.

303

304 **DISCUSSION**

305 4.1 Viscoelasticity of Bacterial Cellulose Suspensions

306 Early work on the rheology of a series of celluloses ranging from
307 microcrystalline cellulose to bacterial cellulose was interpreted in terms of the
308 length to diameter ratio (L:D) of the rod shaped particle (Tatsumi, Shioka, &
309 Oto, 2002). The dynamic viscosity of the bacterial cellulose used in this previous
310 work was higher than found here. This could reflect differences in the L:D ratio
311 of cellulose but could also be due to differences in the degree of dispersibility.
312 Although interpretation in terms of the length to diameter L:D was successful in
313 predicting rheology particularly the yield stress, microscopic examination by SEM
314 and AFM of bacterial cellulose blends has repeatedly shown the presence of a
315 continuous network of ultrafine fibres (Evans, Clarck, & Morrison, 1998; Shi et
316 al., 2014; Tatsumi et al., 2002). The weak gel structure for BCF has some
317 analogy to xanthan gum though values of G' in this current work are higher than
318 reported for xanthan gum. For example the relatively recent study of Choi,
319 Mitchell, Gaddipati, Hill & Wolf (2014) gave a value of G' of $\sim 4\text{Pa}$ for a 0.4%
320 w/w solution xanthan. Biopolymers which have a stiff rod shaped structure have

321 a strong tendency to associate partly because the entropy decrease (which
322 prevents association) is lower than for more flexible polymers. This will not only
323 promote weak gel structures but also make dispersibility more difficult. In these
324 respects, there are some similarities between xanthan and bacterial cellulose
325 though the analogy should not be taken too far. Xanthan is a water soluble
326 polyelectrolyte whereas bacterial cellulose is not water soluble. However, Foster
327 (2010) and Lad, Samanci, Mitchell & Foster (2010) hypothesise that xanthan is
328 driven into a nematic liquid crystalline state when competing for water with
329 swollen starch granules, affecting the viscosity of the composite in different ways
330 to that expected, and seen for more random coil polysaccharides. Similar
331 differences in xanthan and guar gum have also been reported more recently by
332 Heyman, Vos, Depypere & Meeren (2014). Therefore, xanthan as a more rigid
333 hydrocolloid could be seen to be more similar to cellulose, given those
334 observations and the ones reported here, and rigid rods would be more effective
335 at promoting depletion flocculation as described by Abdulmola et al. (1996).

336

337 4.2 Starch Bacterial Cellulose Blends

338 When compared with other hydrocolloids, addition of bacterial cellulose has a
339 greater effect on the gelatinisation and pasting properties of starch. This can be
340 most clearly seen by the large reduction in enthalpy (Figure 3).

341 It is appropriate to consider the system as a dispersion of BCF in the starch
342 phase in a similar way to the structure of BCF gelatine blends (Quero et al.,
343 2015). The degree of dispersion will be governed by the volume fraction occupied
344 by the BCF network. In our case, the volume occupied by the BCF network will
345 increase as it is observed. If starch is partly excluded from this network then
346 even low amounts of BCF will increase significantly the concentration of starch
347 in its own domain. The gelatinisation temperature of starch measured by
348 viscosity changes will correspond to the temperature when the starch
349 concentration of swollen starch granules approaches a critical concentration
350 given approximately by the equation $cS = 1$, where S is the extent of swelling
351 and c the concentration of starch (Steeneken, 1989). The rheology of phase
352 separated mixtures of biopolymers has been extensively studied in terms of the

353 behaviour of mixed gels. Increasing the concentration in both domains will
354 increase the overall modulus and the viscosity though the exact model will
355 depend on nature of the deformation and interactions between the two domains.
356 Although this hypothesis has attractive features, a large number of questions
357 remain, some of which could be resolved by determining the phase diagram for
358 the blend. The assumption we have is that there is less starch in the cellulose
359 region of the BCF fibre-network. The microscopy images support this but it has
360 not been quantified.

361 Therefore, the questions of interest for discussion are: Why should waxy maize
362 starch behave differently from the other two starches? and what is the origin of
363 the large decrease in gelatinisation enthalpy?.

364 These two questions will be briefly discussed in turn. On gelatinisation of wheat
365 and maize starches, amylose will be released from the swollen starch granule.
366 This released amylose would be expected to interact with the bacterial cellulose,
367 as reported by Lin, Lopez-sanchez & Gidley (2015) where it was shown that
368 there was extensive binding of dextrans extracted from potato during
369 fermentation of bacterial cellulose. This interaction was non-specific and
370 reversible compared with the well-known interactions between mannan based
371 polysaccharides and bacterial cellulose (Whitney et al., 1998). However waxy
372 maize starch does not contain the linear amylose fraction, and therefore the
373 absence of interaction with amylose occurring outside the swollen starch granule
374 may explain the very small change in the pasting temperature with BCF
375 concentration observed in waxy maize starch compared with the large change
376 observed for the two amylose containing starches. Indeed, the viscosity
377 differences between maize and wheat starch are small reflecting the similar
378 amylose contents. Interestingly, when comparing these observations with Sullo
379 & Foster (2010) and Sullo (2012), who saw similar effects, the explanation of
380 'binding' of amylose to other hydrocolloids would need to be assumed also. This
381 is not a phenomenon that has been studied extensively, and may open up a new
382 area of research. An alternative explanation is that the previously described
383 'depletion flocculation effect' (Abdulmola et al., 1996) would indicate that the
384 waxy maize granules are inherently softer than the wheat or maize starches,

385 upon gelatinisation, and that the viscous continuous phase does not promote the
386 detection of the early onset of gelatinisation of these 'softer' granules, or that
387 the effect would only be noticeable at much higher granule packing fractions for
388 waxy maize starch.

389 The lower peak viscosity for the blend containing waxy maize may also reflect
390 the lower rigidity and integrity of the gelatinized waxy maize starch granule. A
391 consequence of this will be a weaker concentration dependence of the viscosity
392 in the concentrated starch phase (Steeneken, 1989). This could explain the
393 lower viscosity observed in the blends containing waxy starch. This lack of
394 integrity because of the lack amylose network may allow some of the waxy maize
395 starch to penetrate the cellulose network, in other words it is suggested that the
396 phase diagram for the system containing waxy maize starch would be different
397 to the other two starches.

398 Decreases in enthalpy on hydrocolloid addition to starches has been reported
399 before, and it is important to appreciate the role of water. Interesting results
400 have been reported by Khanna & Tester (2006) and Torres, Moreira, Chenlo &
401 Morel (2013) on starch konjac mannan and guar gum blends, respectively, but
402 these studies have been carried out at lower water contents than used in our
403 study. At these lower water contents of below $1.5 \text{ g}_{\text{H}_2\text{O}}/\text{g}_{\text{starch}}$ two endotherms
404 are generally observed (Donovan, 1979). The high temperature endotherm,
405 which is dependent on water content, is often interpreted in terms of the Flory
406 relationship for the melting of polymer crystallites. The level of water in the
407 current system is much higher than this limiting value and single endotherms
408 are observed in all cases (data not showed). When excess water is present
409 ($>2\text{g}_{\text{H}_2\text{O}}/\text{g}_{\text{starch}}$) the value of enthalpy will be independent of the water content
410 (Donovan, 1979), therefore a dilution effect is not to be expected under our
411 conditions.

412 In the work of Cooke & Gidley (1992), where NMR was used to determine the
413 starch double helical content, it was showed that the loss in enthalpy can be well
414 explained by a change in the double helical content. On the other hand, the
415 decrease in enthalpy has been also interpreted as due to water binding by the
416 cellulose. We are a bit uncomfortable in terms of such a non-specific

417 interpretation, however if there is starch in the cellulose domain we may suggest
418 that if starch is protected for losing the double helical structure of amylose during
419 heating because of water binding in the BCF network, or by changes in mobility
420 and possibly interactions with the cellulose surface, this could help to explain the
421 decrease in enthalpy observed in this study. Hence, in the bacterial cellulose
422 system it would be of interest to measure the starch double helical content (e.g.
423 by NMR), as a function of temperature, to confirm this hypothesis. Other possible
424 explanation could be related with as starch is dispersed and phase concentrated
425 in a cellulose continuous phase, the water available to the starch in the starch
426 phase is insufficient for helix melting and solvation which may therefore occur
427 at a much higher temperature.

428

429

430 **CONCLUSIONS**

431 The presence of low levels of BCF modifies the pasting properties of starch
432 granules (wheat, maize and waxy maize) during gelatinisation in RVA testing.
433 All three starches show a large increase in peak viscosity on BCF incorporation
434 but the reduction of pasting temperature is much lower for waxy maize than the
435 two native cereal starches. In contrast, the gelatinisation temperature measured
436 by DSC did not show significant differences on incorporation of BCF, but the
437 gelatinisation enthalpy was reduced, which can be explained by the failure of
438 starch within the cellulose domain to lose double helical structure on heating
439 because of water binding in the BCF network. Following pasting in the RVA,
440 morphological analysis by optical and polarised light microscopy showed the
441 presence of two clearly defined domains, one of them containing fully gelatinised
442 starch granules and the other rich in a complex structure of highly aggregated
443 bacterial cellulose fibrils. The viscosity increase on heating may be due to
444 increase in the starch concentration in the starch domain upon the addition of
445 BCF, and possibly as a result of previously reported interactions between
446 amylose and cellulose. The lack of this second mechanism for waxy maize starch
447 could be used to explain the lower decrease in pasting temperature when

448 compared with maize and wheat starches. As reported previously when
449 dispersed in water, BCF forms weak, elastic gel structure at low concentrations.
450 These results open interesting perspectives in the use of bacterial cellulose in
451 the presence of starch for designing bio-composites with advanced properties
452 and tailored structures to be used in food structure design and for enhanced or
453 personalised nutrition, as well as in other related biopolymer applications.
454 Specifically, the results could also be relevant for the structuring of foods by
455 plant cell wall fractions rather than soluble hydrocolloids, which is an area of
456 current interest.

457

458

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640 **Figure Captions**

641

642 **Figure 1.** RVA profiles in starch from different sources: a) wheat, b) maize, c)
643 waxy maize, as a function of BCF concentration. From bottom to top: 0, 0.5, 2,
644 6 and 10% db. Green line correspond to bacterial cellulose RVA profile. Red line
645 correspond to temperature scan used during analysis.

646

647 **Figure 2.** Pasting properties of wheat, maize and waxy maize starch modified
648 by BCF: a) pasting temperature, b) peak viscosity, c) trough viscosity, d)
649 breakdown viscosity, e) final viscosity, and f) setback viscosity. Continuous lines
650 correspond only to guide to eye.

651

652 **Figure 3.** Gelatinisation enthalpy and temperature of starch from different
653 sources as a function of BCF concentration: a) wheat, b) maize, and c) waxy
654 maize. Black circles correspond to temperature while empty circles to enthalpy.
655 Continuous lines correspond only to guide to eye.

656

657 **Figure 4.** Images taken by Optical Microscopy (top) and Polarized Light
658 Microscopy (bottom) in starch blended with 10% BCF after complete
659 gelatinisation by RVA: wheat (A1 and A2 respectively), maize (B1 and B2
660 respect.), and waxy maize (C1 and C2 respect). White bar represents 100
661 microns.