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1 **Characteristics of starch-based films plasticised by glycerol and by the ionic liquid**
2 **1-ethyl-3-methylimidazolium acetate: a comparative study**

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19 **ABSTRACT**

20 This paper reports the plasticisation effect of the ionic liquid, 1-ethyl-3-methylimidazolium acetate
21 ([Emim][OAc]), as compared with the traditionally used plasticiser, glycerol, on the characteristics of
22 starch-based films. For minimising the additional effect of processing, a simple compression moulding
23 process (which involves minimal shear) was used for preparation of starch-based films. The results
24 show that [Emim][OAc] was favourable for plasticisation, i.e., disruption of starch granules (by
25 scanning electron microscopy), and could result in a more amorphous structure in the starch-based
26 materials (by X-ray diffraction and dynamic mechanical analysis). ¹³C CP/MAS and SPE/MAS NMR
27 spectroscopy revealed that not only was the crystallinity reduced by [Emim][OAc], but also the
28 amorphous starch present was plasticised to a more mobile form as indicated by the appearance of
29 amorphous starch in the SPE/MAS spectrum. Mechanical results illustrate that, when either glycerol or
30 [Emim][OAc] was used, a higher plasticiser content could contribute to higher flexibility. In spite of the
31 accelerated thermal degradation of starch by [Emim][OAc] as shown by thermogravimetric analysis, the
32 biodegradation study revealed the antimicrobial effect of [Emim][OAc] on the starch-based materials.
33 Considering the high-amylose starch used here which is typically difficult to gelatinise in a traditional
34 plasticiser (water and/or glycerol), [Emim][OAc] is demonstrated to be a promising plasticiser for starch
35 to develop “green” flexible antimicrobial materials for novel applications.

36

37 *Keywords:*

38 Starch; Ionic liquid; 1-ethyl-3-methylimidazolium acetate; Plasticization; Crystalline structure;

39 Biodegradability

40

41 Chemical compounds studied in this article

42 Starch (PubChem CID: 24836924); Water (PubChem CID: 962); Glycerol (PubChem CID: 753); 1-

43 Ethyl-3-methylimidazolium acetate (PubChem CID: 11658353)

44

45 **1. Introduction**

46 In recent years, polymers from renewable resources have attracted great attention due to their
47 availability, renewability, biocompatibility, and biodegradability (Yu, Dean, & Li, 2006). Starch,
48 among this group of polymers, can be processed with conventional processing techniques such as
49 compression moulding, extrusion, and injection moulding and is therefore considered as an important
50 alternative to traditional non-renewable, non-biodegradable petroleum-based polymers (Liu, Xie, Yu,
51 Chen, & Li, 2009; Xie, Halley, & Av érous, 2012; Xie, Liu, & Yu, 2014).

52 Starch, nonetheless, has unique characteristics naturally: in plants, it exists in the form of granules
53 (<1 µm~100 µm); the granule is composed of alternating amorphous and semicrystalline shells (growth
54 rings) (100~400 nm); and the semicrystalline shell is stacked by crystalline and amorphous lamellae
55 (periodicity, 9~10 nm); while starch consists of two major biomacromolecules called amylose (mainly
56 linear) and amylopectin (hyper-branched) (~nm) (Fu, Wang, Li, Wei, & Adhikari, 2011; Jane, 2009;
57 P érez, Baldwin, & Gallant, 2009; P érez, & Bertoft, 2010). The 3D structure of native starch may be
58 disrupted with a plasticiser and elevated temperature, a process known as “gelatinisation” or “melting”.
59 If suitable conditions are reached, this results in a homogeneous amorphous material known as
60 “thermoplastic starch” or “plasticised starch”, which is the essential principle in starch processing
61 (Av érous, 2004; Liu et al., 2009). While water is the most commonly used and effective plasticiser for
62 starch, many other substances have been used to plasticise starch, such as polyols (glycerol, glycol,
63 sorbitol, etc.), compounds containing nitrogen (urea, ammonium derived chemicals, amines), and citric

64 acid (Liu et al., 2009; Xie et al., 2012). Favourable attributes of a plasticiser for starch include being
65 stable (non-volatile) both during thermal processing and in the post-processing stages, having little
66 effect on starch macromolecular degradation, being safe to humans and environmentally friendly, and
67 being advantageous for reducing the inherent hydrophilicity of starch and for long-term stability.
68 Unfortunately, plasticisers such as some of those mentioned above rarely meet all the attributes and
69 therefore finding a better plasticiser for starch is of interest.

70 Ionic liquids (ILs), now commonly defined as salts which melt below 100 °C, have recently attracted
71 much interest for aiding the processing of biopolymers like starch. Many ILs, especially ones based on
72 the imidazolium cation, have been shown to be capable of dissolving polysaccharides such as starch and
73 thus can be used as an excellent media for polysaccharide plasticisation and modification (Biswas,
74 Shogren, Stevenson, Willett, & Bhowmik, 2006; El Seoud, Koschella, Fidale, Dorn, & Heinze, 2007;
75 Wilpiszewska, & Szychaj, 2011; Zakrzewska, Bogel-Łukasik, & Bogel-Łukasik, 2010). Moreover, the
76 use of ILs could also allow the development of starch-based ionically conducting polymers or solid
77 polymer electrolytes (Liew, Ramesh, Ramesh, & Arof, 2012; Ramesh, Liew, & Arof, 2011; Ramesh,
78 Shanti, Morris, & Durairaj, 2011; Ramesh, Shanti, & Morris, 2012; Wang, Zhang, Liu, & He, 2009;
79 Wang, Zhang, Wang, & Liu, 2009; Wang, Zhang, Liu, & Han, 2010b). Nevertheless, most of the work
80 done before in this area involved the processing in solution, although melt processing is seen to be more
81 suitable for industrial production as much less solvent is required and higher efficiency is expected.
82 Sankri et al. (2010) and Leroy, Jacquet, Coativy, Reguerre, and Lourdin (2012) have done pioneering
83 work using an IL (1-butyl-3-methylimidazolium chloride) as a new plasticiser in melt processing of
84 starch-based materials where improvements in plasticisation, electrical conductivity, and hydrophobicity
85 were demonstrated.

86 This paper reports the preparation by a simple one-step compression moulding process of IL–
87 plasticised starch-based films, which are compared with glycerol-plasticised starch-based films. It is
88 noted that many of the ILs used previously with starch contained the corrosive $[Cl^-]$ anion (e.g., 1-butyl-
89 3-methylimidazolium chloride) (Wilpiszewska, & Szychaj, 2011) and this type of IL could contribute to
90 macromolecular degradation of starch (Kärkkäinen, Lappalainen, Joensuu, & Lajunen, 2011; Stevenson,
91 Biswas, Jane, & Inglett, 2007), resulting from the acidic hydrolysis of glycosidic bonds in starch-based
92 materials, due to the formation of HCl. Therefore, 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]),
93 an IL with a non-halogen-containing, weaker acid, anion, was chosen in the current work. [Emim][OAc]
94 has very low vapour pressure, high thermal stability, and relatively low viscosity at room temperature
95 (Liu, & Budtova, 2012), which enables it to be used with starch in a wide range of processing conditions.
96 Our recent finding (Mateyawa et al., 2013) has shown that the best plasticisation of starch may be
97 achieved once a certain ratio of water/[Emim][OAc] is met. In the current study, this effect of
98 water/[Emim][OAc] ratio was also used, and the plasticiser effects on the morphology, crystalline
99 structure, mechanical properties, glass transition temperature, thermal stability, and biodegradability of
100 the starch-based films are thus reported, which provides valuable information for understanding the
101 starch–IL interactions and for designing starch-based materials with desired properties. While in
102 extrusion or kneading different formulation can change the viscosity and cause different degrees of shear
103 degradation of starch macromolecules, which alters the final properties, a simple compression moulding
104 method is used here to minimise the effect of shear-induced macromolecular degradation during
105 processing.

106

107 **2. Materials and Methods**

108 *2.1. Materials*

109 A commercially available maize starch, Gelose 80, was used in this work, which was supplied by
110 Ingredion ANZ Pty Ltd (Lane Cove, NSW, Australia). It was chemically unmodified and the amylose
111 content was 82.9% as measured before (Tan, Flanagan, Halley, Whittaker, & Gidley, 2007). The
112 original moisture content of the starch was 14.4 wt.%. Deionised water was used in all instances.
113 Glycerol (AR) was supplied by Chem-Supply Pty Ltd (Gillman, SA, Australia) and was used as received.
114 [Emim][OAc] of purity $\geq 95\%$, produced by IoLiTec Ionic Liquids Technologies GmbH (Salzstraße 184,
115 D-74076 Heilbronn, Germany), was supplied by Chem-Supply Pty Ltd as well. [Emim][OAc] was used
116 as received. As [Emim][OAc] was in liquid form at room temperature, different weight ratios of water–
117 [Emim][OAc] mixture could be easily prepared in vials for subsequent usage. Water and [Emim][OAc]
118 were completely miscible as shown in our previous study (Mateyawa et al., 2013).

119

120 *2.2. Sample preparation*

121 Formulations for sample preparation are shown in Table 1. In Table 1 and the following text, the
122 plasticised starch samples are coded in the format of “G18” or “I18”, where “G” denotes glycerol and “I”
123 the ionic liquid, and the number stands for the content of the plasticiser (either glycerol or ionic liquid).
124 Water was used with the plasticiser and the liquid mixture (either water–glycerol or water–
125 [Emim][OAc]) content was fixed at 30 wt.% based on our preliminary trials. The liquid mixture was
126 added dropwise into starch, accompanied by careful blending by using a mortar and a pestle to ensure
127 the uniform distribution of the liquid in starch. Then, the blended samples were hermetically stored in
128 ziplock bags in a refrigerator (4 °C) at least overnight before compression moulding. This allowed a
129 further equilibration process for the samples. The powder was carefully and evenly spread onto the

130 moulding area with polytetrafluoroethylene glass cloths (Dotmar EPP Pty Ltd, Acacia Ridge, Qld,
131 Australia) located between the starch and the mould, and then compression moulding was carried out at
132 160 °C and 6 MPa for 10 min, followed by rapidly cooling to room temperature with circulation of
133 water before opening the mould and collecting the sample. All the samples were conditioned at 52%
134 relative humidity by placing them in a desiccator with saturated magnesium nitrate solution at room
135 temperature for one month before any characterisation work.

136

137

138 [Insert Table 1 here]

139

140

141 2.3. Characterisation

142 2.3.1. Scanning electron microscopy (SEM)

143 The starch samples were cryo-ground in liquid nitrogen and then put on circular metal stubs
144 previously covered with double-sided adhesive before platinum coating at 5 nm thickness using an Eiko
145 Sputter Coater, under vacuum. The morphology of the starch samples was examined using a scanning
146 electron microscope (SEM, JEOL XL30, Tokyo, Japan). An accelerating voltage of 3 kV and spot size
147 of 6 nm were used.

148

149 2.3.2. X-ray diffraction (XRD)

150 The starch samples were placed in the sample holder of a powder X-ray diffractometer (D8 Advance,
151 Bruker, Madison, USA) equipped with a graphite monochromator, a copper target, and a scintillation
152 counter detector. XRD patterns were recorded for an angular range (2θ) of 3–40 °, with a step size of

153 0.02 ° and a step rate of 0.5 s per step, and thus the scan time lasted for approximately 15 min. The
154 radiation parameters were set as 40 kV and 30 mA, with a slit of 2 mm. Traces were processed using
155 the Diffracplus Evaluation Package (Version 11.0, Bruker, Madison, USA) to determine the X-ray
156 diffractograms of the samples. The degree of crystallinity was calculated using the method of Lopez-
157 Rubio, Flanagan, Gilbert, and Gidley (2008) with the PeakFit software program (Version 4.12, Systat
158 Software, Inc. San Jose, USA), using Equation 1:

$$159 \quad X_c = \frac{\sum_{i=1}^n A_{ci}}{A_t} \quad (1)$$

160 where A_{ci} is the area under each crystalline peak with index i , and A_t is the total area (both amorphous
161 background and crystalline peaks) under the diffractogram.

162 The V-type crystallinity (the crystalline amylose-lipid complex) was calculated based on the total
163 crystalline peak areas at 7.5, 13, 20, and 23 ° (van Soest, Hulleman, de Wit, & Vliegenthart, 1996).

164

165 2.3.3. Nuclear magnetic resonance (NMR)

166 The rigid components of the starch-based films were examined by solid-state ^{13}C cross-polarization
167 magic angle spinning nuclear magnetic resonance (^{13}C CP/MAS NMR) experiments at a ^{13}C frequency
168 of 75.46 MHz on a Bruker MSL-300 spectrometer. Using scissors, the sheets were cut into small evenly
169 sized pieces and were packed in a 4-mm diameter, cylindrical, PSZ (partially-stabilized zirconium oxide)
170 rotor with a Kelf end cap. The rotor was spun at 5 kHz at the magic angle (54.7 °). The 90 ° pulse width
171 was 5 μs and a contact time of 1 ms was used for all samples with a recycle delay of 3 s. The spectral
172 width was 38 kHz, acquisition time 50 ms, time domain points 2 k, transform size 4 k, and line
173 broadening 50 Hz. At least 2400 scans were accumulated for each spectrum. Spectra were referenced
174 to external adamantane. Spectra were analysed by resolving the spectra into ordered and amorphous

175 subspectra and calculating the relative areas as described previously (Tan et al., 2007). To examine the
176 mobile components of the samples, single pulse excitation, direct polarization (^{13}C SPE/MAS NMR)
177 was used as well. The recycle time was 60 s and 20 k spectra were accumulated.

178

179 2.3.4. *Tensile testing*

180 Tensile tests were performed with an Instron[®] 5543 universal testing machine (Instron Pty Ltd,
181 Bayswater, Vic., Australia) on dumbbell-shaped specimens cut from the sheets with a constant
182 deformation rate of 10 mm/min at room temperature. The specimens corresponded to the Type 4 of the
183 Australian Standard AS 1683:11 (ISO 37:1994), and briefly the testing section of the specimen was
184 12 mm in length and 2 mm in width. Young's modulus (E), tensile strength (σ_t), and elongation at break
185 (ε_b) were determined by the Instron[®] computer software program, from at least 7 specimens for each of
186 the plasticised starch sample.

187

188 2.3.5. *Dynamic mechanical thermal analysis (DMTA)*

189 Dynamic mechanical thermal analysis (DMTA) was performed on the rectangular tensile bars of the
190 plasticised starch samples by using a Rheometric Scientific[™] DMTA IV machine (Rheometric
191 Scientific, Inc., Piscataway, NJ, USA) with the dual cantilever bending mode from -100 to 110 °C, with
192 a heating rate of 3 °C/min, a frequency of 1 Hz, and a strain value of 0.05% . The dynamic storage
193 modulus (E'), loss modulus (E''), and loss tangent ($\tan \delta = E''/E'$) were obtained from the tests. To
194 prevent water evaporation during the tests, the specimens were coated with Vaseline grease. No swelling
195 of the specimens was observed, suggesting no adverse effect of Vaseline.

196

197 2.3.6. *Thermogravimetric analysis (TGA)*

198 A Mettler Toledo TGA/DSC1 thermogravimetric analyser was used with 40 µL aluminium crucibles.
199 A sample mass of about 5 mg was used for each run. The samples were heated from 25 °C to 550 °C at
200 3 °C/min under nitrogen environment.

201

202 2.3.7. *Biodegradation*

203 The biodegradability of the starch samples was determined according to the Australian Standard AS
204 ISO 14855. The test material was reduced in size to achieve maximum surface area of each individual
205 piece of the test material, approximately 2 cm × 2 cm. Each composting vessel contained 100 g of the
206 test material and 600 g of the compost inoculum, both on dry weight basis. Each material was tested in
207 triplicate including the blank (the compost only) and positive (a mixture of cellulose and the compost)
208 references. All composting vessels were then placed inside an in-house built respirometer unit (Way,
209 Wu, Dean, & Palombo, 2010) and the temperature was maintained at 58±2 °C for all the testing period.
210 During this degradation period, the compost moisture content was maintained at 48–50% and the pH at
211 7.8–8.5 to ensure favourable conditions for the compost microorganisms involved in the biodegradation
212 process. Aerobic conditions were maintained by continuous supply of sufficient airflow to the
213 bioreactors and the contents of each of the bioreactors were mixed once a week to ensure uniform
214 distribution of air throughout the compost. The evolved CO₂ and flow rate data were continually data-
215 logged by computer for each respective bioreactor. The theoretical amounts of CO₂ produced by the test
216 and reference materials were assessed and the degree of biodegradation, D_t , was calculated (for the test
217 and reference materials) using the following equation, as described in the Australian Standard AS ISO
218 14855:

219
$$D_t = \frac{(CO_2)_T - (CO_2)_B}{THCO_2} \times 100 \quad (2)$$

220 where $(\text{CO}_2)_T$ is the cumulative amount of carbon dioxide evolved in each bioreactor containing the test
221 material (in grams per bioreactor), and $(\text{CO}_2)_B$ is the mean cumulative amount of carbon dioxide evolved
222 in the blank vessel (in grams per bioreactor).

223

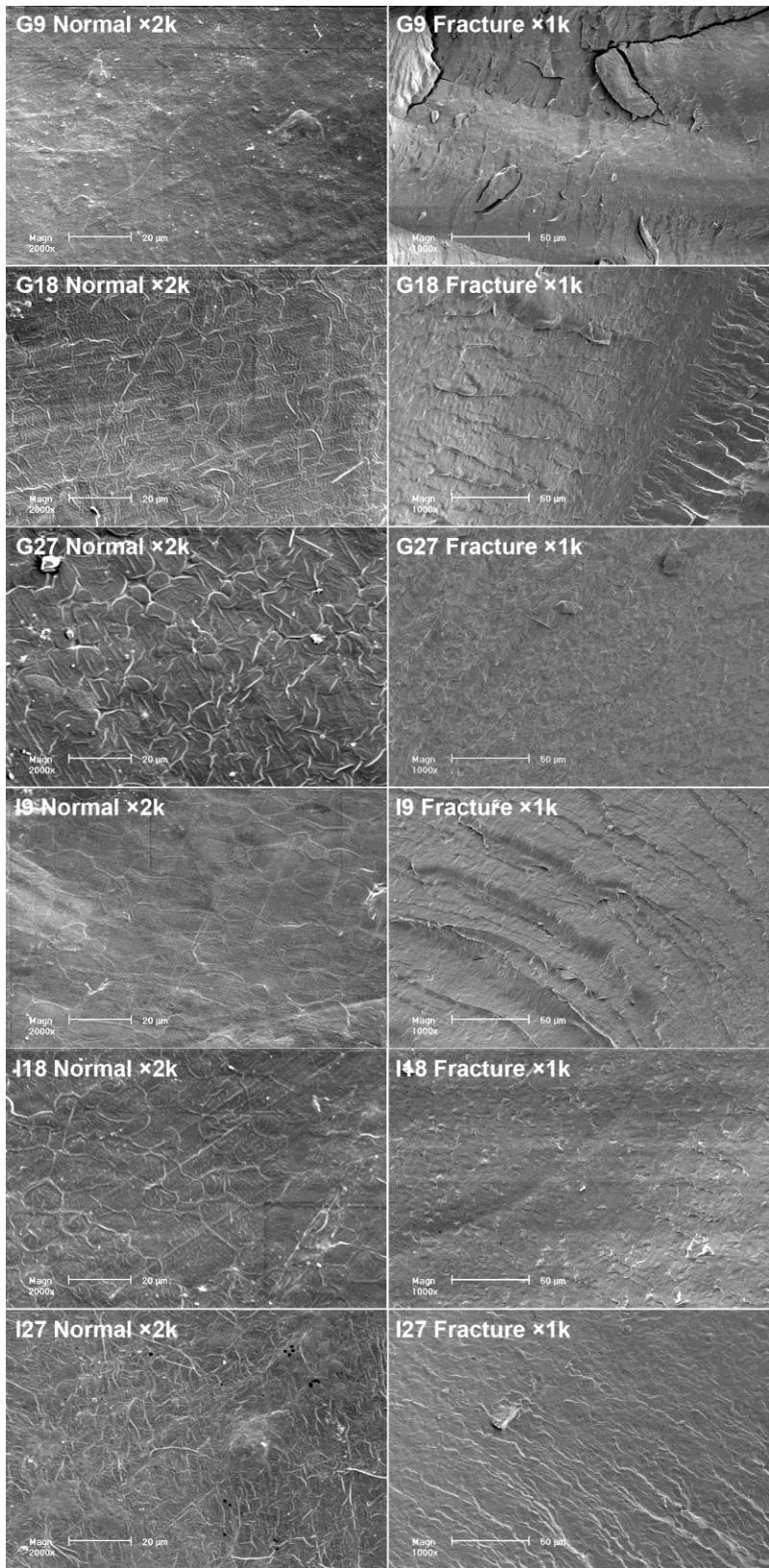
224 **3. Results and Discussion**

225 *3.1. Morphology*

226 In order to understand the morphology of the samples after processing, SEM work was carried out
227 and the images are shown in Figure 1. Different kinds of morphology are shown by the different
228 samples. From the normal surface images, a higher amount of glycerol could result in a more apparent
229 granular morphology. In particular, most remaining starch granules could be observed as contained in
230 G27, while G9 showed a smooth surface. This was reasonable as the use of glycerol instead of water
231 should increase the gelatinisation temperature, in other words making gelatinisation more difficult (Liu
232 et al., 2011). Among the [Emim][OAc]-plasticised samples, I18 seemed to have the most granular
233 surface, which however was still less granular than G27. These results demonstrate a stronger effect of
234 [Emim][OAc] on the disruption of granules during processing. This is especially true noting that in this
235 study only a simple compression moulding process was used which involved little shear and Gelose 80
236 (a high-amylose content starch) was used which, though is desirable for producing films with better
237 properties, is known to have poor processibility due to the difficulty in granule disruption (Li et al., 2011;
238 Wang et al., 2010a).

239

240



241

242 Figure 1 SEM images of both normal and fracture surfaces of the different starch samples.

243

244

245 The fracture surface images in Figure 1 show that the samples with lower content of the plasticiser
246 (either glycerol or [Emim][OAc]) appeared to be more brittle, which was as expected as plasticisers
247 generally make polymers more ductile. G9 seemed to be more brittle than I9, again showing a better
248 plasticisation effect of [Emim][OAc] than that of glycerol. This property will be further shown later in
249 the mechanical results.

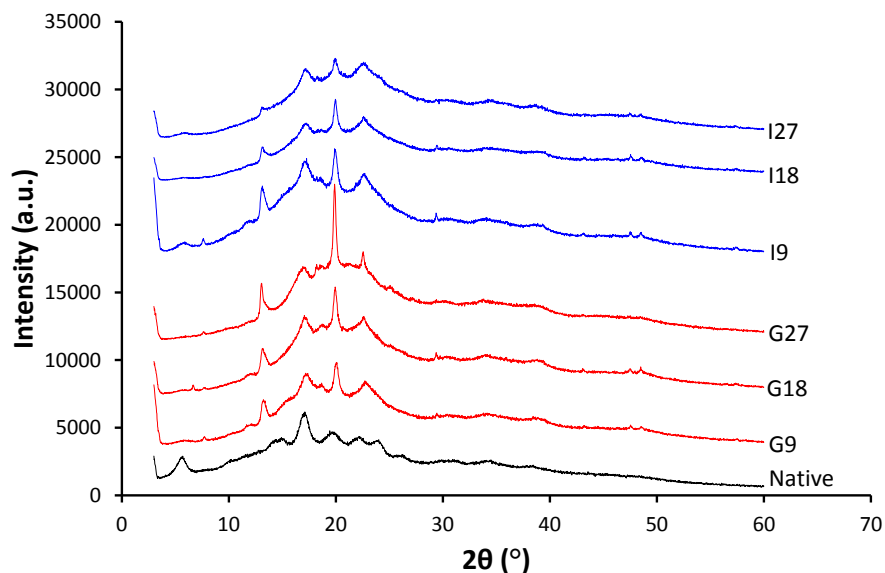
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251 3.2. XRD and NMR analyses

252 Figure 2 shows the XRD patterns of native Gelose 80 starch and the different starch samples. The
253 native starch showed the strongest diffraction peaks at 2θ of around 17° , with a few smaller peaks at 2θ
254 of around 5° , 10° , 14° , 15° , 19° , 22° , 23° , 26° , 31° , and 34° , which were indicative of the B-type
255 crystalline structure (Cheetham, & Tao, 1998; Lopez-Rubio et al., 2008). After processing, in addition
256 to the original B-type characteristic peaks (main peak at $2\theta = 17.1^\circ$), all the starch samples displayed
257 peaks at 2θ of around 7° , 13° , 20° , and 22° , which were characteristic of the V_H -type crystalline
258 structure, a single-helical amylose structure. This is similar to the one formed by amylose–lipid helical
259 complexes which is well known for thermally processed (by e.g. compression moulding and extrusion)
260 starch-based materials (van Soest et al., 1996). In other words, the plasticised samples contained some
261 crystalline structures that were not destroyed by the compression moulding process and some newly
262 formed V-type crystalline structures mainly induced by the processing.

263

264



265

266 Figure 2 XRD patterns of native starch (Gelose 80) and the different starch samples.

267

268

269 Table 1 also shows the crystallinity of the samples calculated from the XRD results. For the
 270 glycerol-plasticised samples, an increase in the glycerol content resulted in an increase in the intensity of
 271 2θ peak at 20° (the V-type crystallinity increased from 5.5% to 6.8% with the increased content of
 272 glycerol from 9% to 27%), suggesting that glycerol could promote the formation of the single-helical
 273 amylose structure. However, the B-type crystallinity largely decreased (from 26.6% to 13.9%) with a
 274 higher glycerol content, resulting in a decrease in the total crystallinity (from 32.1% to 20.7%). Along
 275 with the previous morphological results, it is interesting to summarise that a higher glycerol content (less
 276 water content) could result in more granule remains but less B-type crystallinity with the processing
 277 method used in this study.

278 On the other hand, for the [Emim][OAc]-plasticised samples, an increase in the [Emim][OAc]
 279 content contributed to a decrease in both the B-type crystallinity and the V-type crystallinity and thus a
 280 decrease in the total crystallinity. As observed for the glycerol-plasticised samples, a higher water

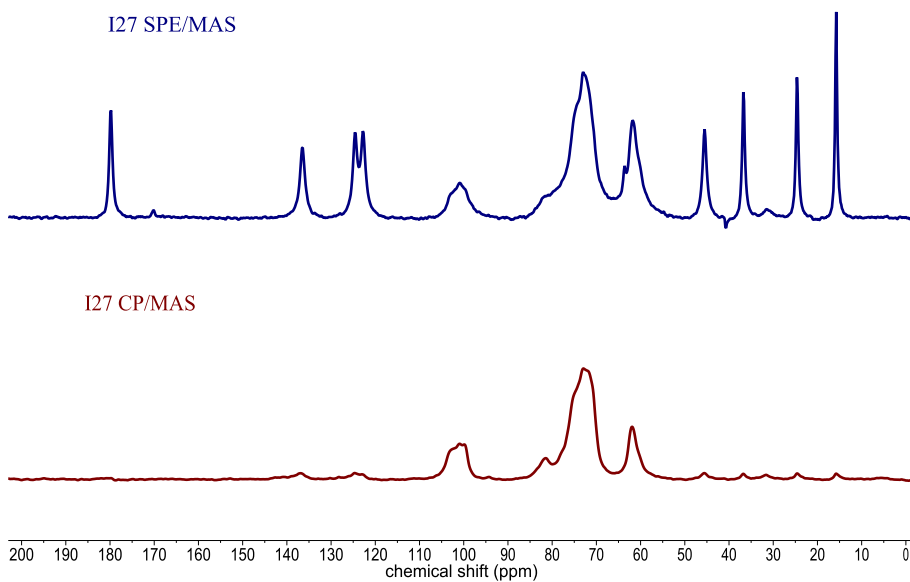
281 content (lower [Emim][OAc] content) resulted in less complete melting of granular crystallites. Unlike
282 glycerol, the use of [Emim][OAc] seemed to be able to hinder the formation of the single-helical
283 structure. Single helices of starch are formed via hydrogen bonding between the O3' and O2 oxygen
284 atoms of sequential residues. Additionally, a helical amylose has hydrogen-bonding O2 and O6 atoms
285 on the outside surface of the helix, forming a double-helical structure via hydrogen bonding of two
286 strand-adjacent glucose molecules and holding the two strands of the double helix together. It is
287 proposed that the effect of hindering either helix formation is due to the strong interaction between
288 acetate anion in [Emim][OAc] and starch hydroxyl groups, disrupting hydrogen bonding in the starch
289 polymer and making it difficult for the amylose molecules to form single (and double) helices. The fact
290 that [Emim][OAc]-plasticised starch has low crystallinity can be beneficial to the production of
291 electrically conductive materials which need to be essentially amorphous.

292 When the XRD data of starch granule are fitted using the crystal-defect method (Lopez-Rubio et al.,
293 2008), there is usually a close agreement between the crystallinity values from XRD and from ^{13}C
294 CP/MAS NMR. However, in the presence of a plasticiser, analysis of the ^{13}C CP/MAS spectra reveals
295 highly ordered sub-spectra and the percentages of amorphous starch are much lower than the values
296 found from XRD analysis. A similar effect is observed when starch is hydrated, the amorphous starch
297 becomes more mobile and is no longer observed in the ^{13}C CP/MAS spectrum as the cross-polarization
298 efficiency is reduced (Bogracheva, Wang, & Hedley, 2001; Colquhoun, Parker, Ring, Sun, & Tang,
299 1995; Tang, & Hills, 2003; Veregin, Fyfe, Marchessault, & Taylor, 1986).

300 To examine the mobile elements of the starch sheets, ^{13}C SPE/MAS spectra were recorded, which
301 revealed the presence of amorphous starch as shown in Figure 3. To calculate the amount of mobile
302 amorphous starch, it was assumed that all the crystalline starch was described by the XRD crystal-defect
303 fitting. Then, the difference in the percentage between amorphous starch calculated from XRD and that

304 from ^{13}C CP/MAS was considered to be due to the mobile amorphous starch. Table 1 shows that as the
305 amount of [Emim][OAc] increased, the degree of crystallinity decreased in much the same way as for
306 glycerol but the mobility of the amorphous starch was greatly increased indicating that the starch was
307 more plasticised.

308
309



310

311 Figure 3 ^{13}C CP/MAS and SPE/MAS NMR spectra of the sample I27 revealing the presence of
312 [Emim][OAc] and amorphous starch in the SPE/MAS spectrum and the highly ordered
313 material in the CP/MAS spectrum.

314

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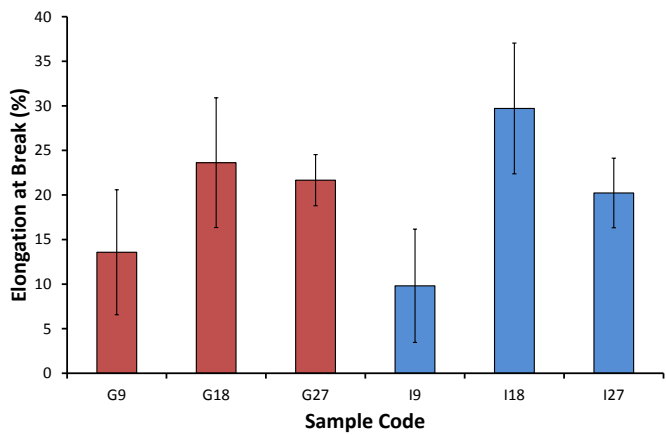
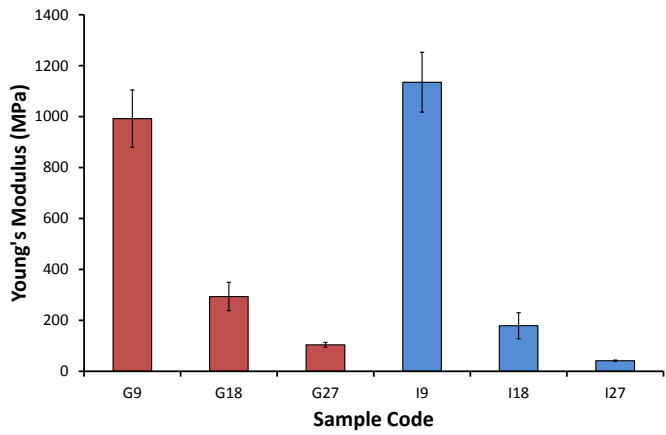
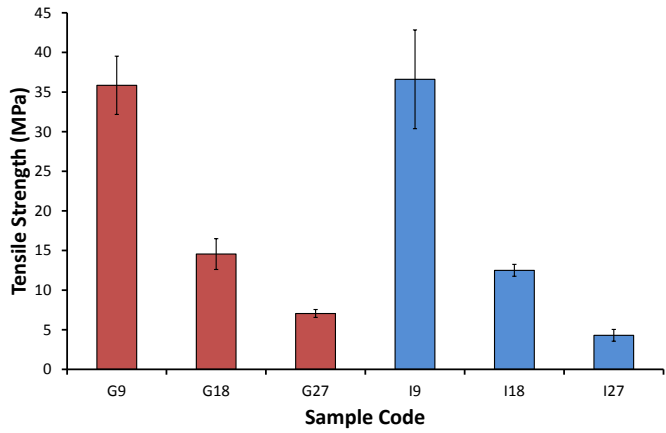
316 3.3. Mechanical properties

317 Figure 4 shows the tensile properties of the different starch samples. Both glycerol and
318 [Emim][OAc] impacted on the tensile properties in the same way. It can be seen that the sample with a

319 lower plasticiser (either glycerol or [Emim][OAc]) content had a higher tensile strength (σ_t). Comparing
320 the samples with the different plasticisers, I9 had the same tensile strength as G9, while I18 and I27 have
321 similar or slightly lower tensile strength than G18 and G27, respectively. The modulus (E) values
322 showed the same trends as for σ_t . G9 or I9 showed the lowest elongation at break (ϵ_b) while G18 or I18
323 had the highest (30%). These results confirmed the brittle nature of fracture surfaces of G9 and I9.
324 With a further increase in the plasticiser content from 18% to 27%, the plasticisation effect decreased
325 the ϵ_b . This could be because when the material became too soft by the plasticiser there was no work
326 hardening to stabilise drawing; this could also be ascribed to possible phase separation when the
327 plasticiser content was too high. Taking into account of the high variance of ϵ_b , the overall trend of the
328 mechanical property data was that [Emim][OAc] provided similar or perhaps slightly better
329 plasticisation effects than glycerol.

330

331



332

333 Figure 4 Tensile strength (σ) (top), Young's modulus (middle), and elongation at break (ϵ_b) (bottom) of
 334 the different starch samples. The errors bars represent standard deviations.

335

336

337 Mechanical properties can be affected by various factors such as the plasticiser type and content,
338 granule remains in the matrix, the crystalline structure and crystallinity, and the extent of plasticisation
339 of the amorphous parts. The highest σ_t and E , and the lowest ε_b of G9 or I9 could be mainly attributed to
340 the less melting of original granule crystallites. Noting the highest ε_b values of G18 and I18, it is
341 suggested that the large amount of mobile amorphous phase contributed by the plasticiser (cf. XRD and
342 NMR results) could be the reason. However, when the [Emim][OAc] content was even higher, the
343 material (G27 and I27) became too soft (as discussed above) so the ε_b value was reduced.

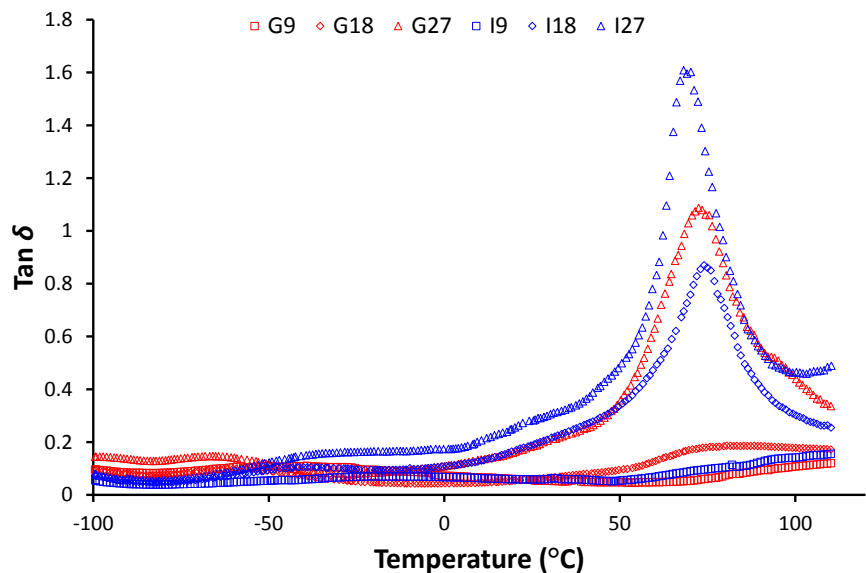
344

345 3.4. DMTA analysis

346 Figure 5 shows the DMTA results of the different starch samples. For some of the samples, a
347 prominent peak was shown between 30 °C and 100 °C. Based on previous studies (Madrigal, Sandoval,
348 & Müller, 2011; Perdomo et al., 2009), this peak can be undoubtedly attributed to the glass transition of
349 starch (T_g), which will be the main focus discussed below; at a lower temperature, another peak might be
350 shown which can be ascribed to the glass transition of the plasticiser-rich domains.

351

352



353

354 Figure 5 Tan δ results of the different starch samples.

355

356

357 It can be seen that, while the T_g could hardly be seen for G9 or I9, a higher plasticiser content
 358 contributed to a more prominent peak representing the starch glass transition. Compared to glycerol, the
 359 use of [Emim][OAc] as the plasticiser gave a stronger glass transition peak and the peak temperature
 360 was lower. For example, the T_g of I27 was 68.2 °C while that of G27 was 71.5 °C. This phenomenon
 361 shows that both a higher content of the plasticiser and the use of [Emim][OAc] instead of glycerol
 362 resulted in less crystallinity as well as more amorphous structure which was more mobile, which is in
 363 good agreement with the XRD and NMR results.

364

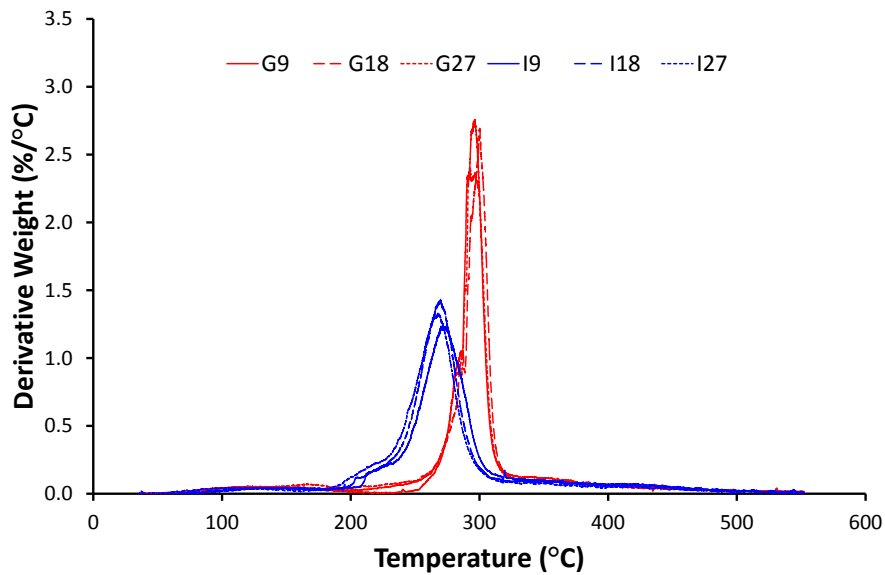
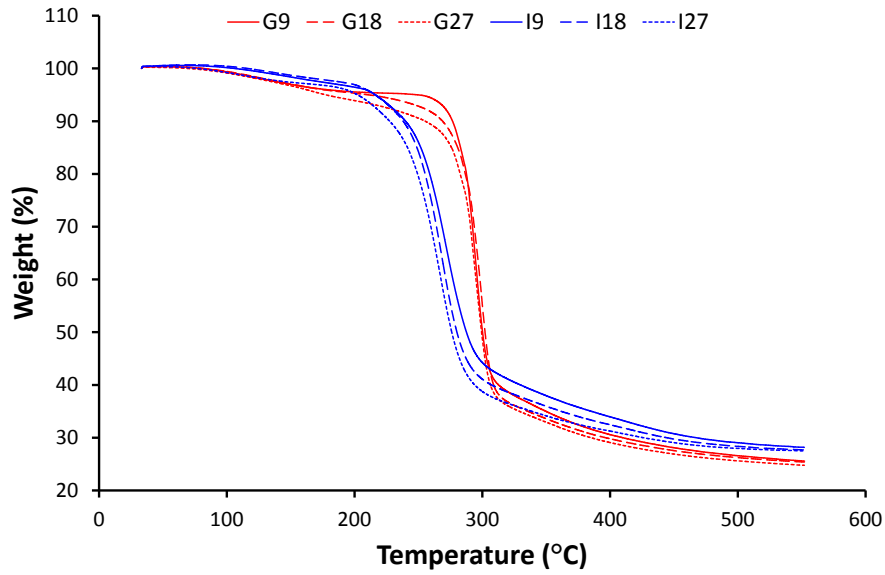
365 3.5. TGA

366 Figure 6 shows the TGA results of the different starch samples, in terms of both percentage of
 367 weight loss and its derivative value. The results clearly suggest that with use of [Emim][OAc], thermal
 368 degradation happened at a lower temperature. Judging from the peak temperature of the derivative

369 weight percentage, I27 had a thermal degradation temperature of 266 °C, which is 30 °C lower than that
370 of G27. While no reports have been released regarding the TGA of ionic liquid-plasticised starch-based
371 materials, previously studies have shown that ionic liquid has an effect of reducing the molecular weight
372 of starch during solution processing with heat (Kärkkäinen et al., 2011; Stevenson et al., 2007). It is
373 thus proposed that the existence of [Emim][OAc] could promote the thermal degradation of starch
374 molecules.

375

376



377

378 Figure 6 TGA results in terms of weight percentage (a) and derivative weight percentage (b) of the
 379 different starch samples.

380

381

382 *3.6. Biodegradation*

383 Table 1 also shows the biodegradation results after 1, 2, and 3 month(s) of aerobic composting. It
 384 can be clearly seen that there is a big difference between the glycerol-plasticised samples and the ionic

385 liquid-plasticised samples: the former experienced much greater biodegradation than the latter. For all
386 glycerol-plasticised samples, the biodegradation percentages were over 50%, and increased with time; in
387 contrast, the 1-month results for I9, I18, and I27 were just 34%, 14%, and 17%, respectively, which
388 increased only slightly over another two months. Along with the morphological and crystalline structure
389 results, it is apparent that the plasticiser played a dominant role in biodegradation, whilst the effect of
390 crystalline structure was minor. [Emim][OAc] seemed to be able to inhibit the attack of bacteria to
391 starch. While this has not been reported yet, the literature has already shown the antibacterial activity of
392 some other ionic liquids (Thuy Pham, Cho, & Yun, 2010). The mechanism regarding the antibacterial
393 effect of [Emim][OAc] needs further investigation. Nevertheless, the results here provide us a
394 promising way for developing antimicrobial starch-based materials with ILs.

395

396 **4. Conclusion**

397 In this study, the plasticisation effect of [Emim][OAc], as compared with glycerol, which is the most
398 commonly used plasticiser for starch, on the characteristics of starch-based films was investigated.
399 Despite choosing a high-amylose starch, Gelose 80, which is known to have difficulty in granule
400 disruption and poor processibility, [Emim][OAc] was shown to be effective in plasticisation of this
401 starch although only a compression moulding process involving little shear treatment was employed.
402 Compared with glycerol, [Emim][OAc] contributed to less granule remains as observed by SEM. The
403 XRD and NMR results reveal that [Emim][OAc] at a low concentration disrupted the original B-type
404 crystalline structure, generated less V-type crystalline structure, and increased the mobility of the
405 amorphous starch. As a result, a highly amorphous structure contributed to higher flexibility as shown
406 by mechanical tests and a lower glass transition temperature but stronger glass transition peak as
407 revealed by DMTA. Although the TGA results show the accelerated thermal degradation of starch by

408 [Emim][OAc] as compared with glycerol, the biodegradation study reveals that [Emim][OAc] inhibited
409 bacterial attack to the starch-based materials. However, [Emim][OAc] could be a promising plasticiser
410 for starch to develop “green” materials with controlled biodegradation rates to meet application needs.
411 In this regard, research is still under way in our lab.

412

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527

528

529 **Figure captions**

530 Figure 1 SEM images of both normal and fracture surfaces of the different starch samples.

531 Figure 2 XRD patterns of native starch (Gelose 80) and the different starch samples.

532 Figure 3 ^{13}C CP/MAS and SPE/MAS NMR spectra of the sample I27 revealing the presence of
533 [Emim][OAc] and amorphous starch in the SPE/MAS spectrum and the highly ordered
534 material in the CP/MAS spectrum.

535 Figure 4 Tensile strength (σ) (top), Young's modulus (middle), and elongation at break (ϵb) (bottom) of
536 the different starch samples. The errors bars represent standard deviations.

537 Figure 5 Tan δ results of the different starch samples.

538 Figure 6 TGA results in terms of weight percentage (a) and derivative weight percentage (b) of the
539 different starch samples.

540

Tables

Table 1 Sample formulations, and the XRD, ¹³C CP/MAS NMR and biodegradation results of the starch-based films

Code	Formulation ^a				XRD results (%)			NMR results (%) ^b					% Biodegradability (%)		
	Starch	Water	Glycerol	IL	B-type	V-type	Total	Double	Single	Total	Rigid	Mobile	1	2	3
					cryst.	cryst.	cryst.	helix	helix	ordered	amorph.	amorph.			
Native starch	–	–	–	–	33.7	ND ^c	33.7	–	–	–	–	–	–	–	–
Cellulose	–	–	–	–	–	–	–	–	–	–	–	–	77.4 (5) ^d	78.3 (<5)	79.9 (<5)
G9	100	21	9	0	26.6	5.5	32.1	28	4	32	40	28	68.6 (5)	76.2 (<5)	71.2 (7)
G18	100	12	18	0	21.8	5.6	27.4	20	7	27	43	30	74.0 (8)	82.5 (9)	86.8 (10)
G27	100	3	27	0	13.9	6.8	20.7	18	3	21	50	29	65.3 (10)	70.9 (8)	75.1 (9)
I9	100	21	0	9	18.0	6.1	24.1	13	11	24	52	24	34.1 (<5)	35.0 (<5)	39.0 (<5)
I18	100	12	0	18	16.6	4.6	21.2	14	7	21	44	35	13.7 (<5)	13.7 (<5)	14.3 (<5)
I27	100	3	0	27	14.7	2.5	17.2	14	3	17	24	59	16.7 (<5)	16.7 (<5)	16.7 (<5)

^a Portions in weight; ^b Based on the assumption that the crystal defect fitting method for XRD (Lopez-Rubio et al., 2008) describes all the crystallinity present and the difference between the results from the XRD and NMR measurements is a result of the amorphous starch in the mobile phase; ^c Unable to be determined as the V-type crystallinity pattern was difficult to be differentiated from the B-type crystallinity pattern; ^d Standard deviation in the brackets.