

1 **THE EFFECT OF CELLULOSE AND STARCH ON THE**
2 **VISCOELASTIC AND THERMAL PROPERTIES OF ACID-**
3 **SWOLLEN COLLAGEN PASTE**
4

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ABSTRACT

Collagen pastes are processed materials obtained through the swelling of minced bovine hides using acids into a fibrous swollen structure. Depending on the application, there is a need to improve the performance of these pastes in terms of rheological properties and mechanical strength of the final product. In this work, the addition of cellulose fibres and starch granules as fillers in acid swollen collagen paste was investigated. The influence of cellulose fibre length and starch granules with different amylose and amylopectin content on the viscoelastic and thermal properties of acid swollen paste were studied as a function of mixing ratio and collagen paste concentration. Addition of cellulose and starch granules resulted in an increase in the elastic modulus of the collagen paste with the starch granules having the highest impact. Addition of cellulose and starch also affected the $\tan \delta$ peak of collagen paste at different collagen concentrations as a function of temperature. The micro differential scanning calorimetry (microDSC) results indicated that the denaturation temperature value of collagen was not influenced by the presence of cellulose and starch. However, upon reheating the denaturation temperature of collagen pastes with starch granules shifted to lower temperatures.

Keywords: Corn starch, Denaturation, Rheology, Micro differential scanning calorimetry (DSC).

52 1 INTRODUCTION

53 Collagen is the major structural protein of connective tissues such as tendons, bones and
54 skins. It consists approximately 30% of the total protein in human and animal bodies (Hashim
55 et al., 2015). At least 27 forms of collagen have been identified in mammalian tissue of which
56 collagen type 1 has been identified as the major structural component of the connective tissue
57 (Pati et al., 2010;Zhang et al., 2010;Wang et al., 2017). Collagen's primary structure consists
58 of repeating units of glycine (Gly)-proline (X)-hydroxyproline(Y). The collagen molecule is
59 made of three polypeptide chains which are coiled in a left-handed helix, these chains are
60 further twisted into a right-handed superhelix that are stabilised by hydrogen bonds (Komsa-
61 Penkova et al., 1996;Schroepfer and Meyer, 2017). Due to its unique properties, such as weak
62 antigenicity, biodegradability, biocompatibility, bioactivity and tensile strength, collagen has
63 been extracted into different forms such as gels, pastes, films, sponges and fibres. It is widely
64 used in the food, pharmaceutical, chemical and cosmetics industries and for scaffolds in tissue
65 engineering (Friess, 1998;Ding et al., 2014). However, there are a number of challenges such
66 as low thermal stability and poor water vapour barrier properties, which limits wider
67 applications (Bigi et al., 2004;Mu et al., 2007). A possible means to circumvent the suboptimal
68 properties of collagen and increase its application, is to incorporate other natural polymers
69 such as polysaccharide fibres or fillers (Wang et al., 2018). These will act as a reinforcement
70 by enhancing the strength, barrier properties and thermal stability of the resulting composite
71 structures (Wolf et al., 2009) . In the food industry, collagen type 1 has been extracted into a
72 paste that is used for sausage casings. Collagen casings have been applied successfully as
73 an alternative to the relatively high cost of casings made from animal intestines (Barbut, 2010).
74 There is an interest in modifying the mechanical properties of collagen casings through the
75 addition of natural biopolymers such as cellulose fibres and starch granules. These natural
76 biopolymers are of a particular interest as particulate fillers due to their natural abundance,
77 biodegradability, and low cost (Ding et al., 2014;Basiak et al., 2017). They have also been
78 shown to provide great reinforcement to polymer matrices due to their high mechanical

79 strength (Dufresne and Vignon, 1998;Bledzki and Gassan, 1999). Cellulose is the most
80 abundant natural polymer on earth and is a major component of cell walls of higher plants and
81 some bacteria, such as *Acetobacter Xylinum* strains. Cellulose possess a complex and highly
82 ordered chemical structure akin to that of collagen giving high mechanical strength, albeit of
83 polysaccharide rather than protein origin. It is a linear homopolymer composed of β -D-
84 glucopyranose units linked with β -1, 4 glycosidic linkages. It is used in the food industry as a
85 texturiser, non-caloric bulking agent, thickener, stabiliser and raising agent (Yoon and Lee,
86 1990;Ang and Miller, 1991;Harris and Smith, 2006).

87 Starch is a well-known storage carbohydrate and is found in the form of insoluble granules
88 within plant cells (Sullo and Foster, 2010). Semi-crystalline starch granules are made up of
89 two polysaccharides. The first is amylose which contains the amorphous region of the granule
90 and has long linear (1-4) linked α -D-glucopyranose residues. The second is amylopectin,
91 which is composed mainly in the crystalline region of the granule and is a highly branched
92 molecule consisting of shorter chains of (1-4) α -D-glucopyranose residues with (1-6) - α -D-
93 glycosidic branched linkages (Buléon et al., 1998;Jane et al., 1999). Starch is obtained from
94 various sources such as cereal, tuber and root crops and used in wide range of applications,
95 such as food, pharmaceutical, paper and plastic. Starch has unique physicochemical and
96 functional properties hence it is used as a thickener, colloidal stabiliser, gelling agent, adhesive
97 and water retention agent in various industries (Singh et al., 2003;Copeland et al., 2009).

98 In recent years, there has been an increased interest in the blending of collagen with other
99 natural biopolymers in order to develop products with new and modified functional properties
100 as well as providing cost and processing advantages. Several studies demonstrated that the
101 addition of polysaccharides into collagen solutions can modify the thermal and rheological
102 properties of collagen solutions. (Ding et al., 2014) reports enhancement upon the
103 Hydroxypropyl Methylcellulose (HPMC) improved the on the rheological and thermal stability
104 of collagen solutions and was attributed to a hydrogen bond interactions as well as a
105 compatibility between the collagen and HPMC molecules. Nicoleti and Telis (2009) reported
106 that concentrations of about 0.1% xanthan gum increased the gel strength of collagen solution,

107 when heated. Increasing the concentration of xanthan to about 0.3% resulted in a weaker gel
108 while the addition of Maltodextrin led to a more fluid-like structure and this was suggested to
109 be due to the thermodynamic incompatibilities between the biopolymers. Similarly, Oechsle et
110 al. (2015) studied the influence of incorporating co-gelling biopolymers of different molecular
111 weights; low molecular weight (whey protein isolate and blood plasma protein), or high
112 molecular weight (soy protein isolate and gluten) into a collagen matrix and they reported that
113 they displayed effects when added to the collagen gels, affecting the collagen network and
114 embedding within it.

115 Therefore, the aim of this study was to understand how the addition of cellulose with different
116 fibre lengths and starch granule fillers affects the viscoelastic and thermal properties of acid
117 swollen collagen pastes. The viscoelastic properties were investigated using small oscillation
118 rheological measurements and the thermal changes were studied using a microDSC. Both
119 rheological and thermal measurements were studied as a function of collagen paste
120 concentration. The effect of biopolymer morphology and aspect ratios was also considered.
121 The hypothesis underpinning this research is that the viscoelastic and thermal properties can
122 be modified by the addition of non-charged polysaccharides. Also, it was hypothesised that
123 similar effect will be observed when the phase volumes of the polysaccharide are matched.

124 **2 Materials and Methods**

125 **2.1 Materials**

126 Two types of cellulose with different aspect ratios were used in this study i.e. Solka-Floc 300
127 (SF3) and Solka-Floc 900 (SF9) with fibre lengths of 22 and 100 μm respectively. Cellulose
128 powders were supplied by the International Fibre Corporation (New York, USA). Two types of
129 starches with different amylose and amylopectin contents were used, waxy maize starch (WS:
130 1-2% amylose) and high amylose starch (HAS: 70% amylose) were both supplied by Ingredion
131 (Manchester, UK). A pH 2.0 acid (HCl) swollen paste was made from fibrous collagen donated
132 by Devro plc (Scotland, UK).

133 **2.2 Determination of Phase Volume (Φ)**

134 The phase volume of cellulose and starch dispersions were measured by centrifugation
135 (Thermo Electron Corporation, Ohio, USA) using a centrifugation force of 2000g for 20 mins
136 at 20 °C. Cellulose and starch dispersions at a total solid concentration ranging from 1% to
137 7% were transferred into 50 ml conical bottom centrifuge tubes. After centrifugation, the total
138 height H_T of the sample and the height of the sediment H_S were measured and the phase
139 volume of the cellulose and starch dispersions were calculated by the total height divided by
140 the height of the sediment. The concentrations of cellulose and starch dispersions needed for
141 a 15% phase volume were extrapolated from a graph of phase volume against concentration
142 (not shown).

143 **2.3 Preparation of Collagen-Cellulose and Collagen-Starch blends.**

144 The concentrations of cellulose and starch (refer to **Error! Reference source not found.**)
145 corresponding to a phase volume of 15% were prepared by dispersing the powders in
146 deionised water. The dispersions were mixed with collagen paste at different
147 collagen/cellulose (solka floc 300 and solka floc 900) and collagen/starch (waxy starch and
148 high amylose starch) mixing ratios i.e., 80:20, 70:30 and 50:50. The final concentrations of the
149 collagen pastes at the various mixing ratios were 4% (w/w), 3.5% (w/w) and 2.5% (w/w). The
150 collagen paste with cellulose dispersions and starch dispersions were mixed by gently stirring
151 at room temperature for 15 mins using an overhead stirrer (yellow line IKA OST 20 high torque
152 Overhead Stirrer). The pH of the final blends was between 2 and 2.5. Samples were degassed
153 using an Audionvac VMS 53 multivac vacuum packager (Audion Elektron, Netherlands) in
154 order to remove the air bubbles incorporated during mixing, before further analyses were
155 performed. The blends formulation and sample code is shown in Table 1.

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157 Table 1. Formulation of collagen-cellulose and collagen-starch blends at various mixing ratios
 158 and different concentrations of collagen. SF3 = Solka floc 300, SF9 = Solka floc 900, WS =
 159 Waxy starch, HAS = High amylose starch, Φ = phase volume

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Collagen Paste: Suspension	Conc. of Collagen in the mixture (%wt)	Conc. (%wt) of cellulose and starch for $\Phi = 15\%$ in the mixture (%wt)
80:20 ColISF3	4	2.7
80:20 ColISF9		1.8
80:20 ColIWS		7
80:20 ColIHAS		6.6
70:30 ColISF3	3.5	2.7
70:30 ColISF9		1.8
70:30 ColIWS		7
70:30 ColIHAS		6.6
50:50 ColISF3	2.5	2.7
50:50 ColISF9		1.8
50:50 ColIWS		7
50:50 ColIHAS		6.6

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166 **2.4 Measurement of paste viscoelasticity**

167 Small amplitude oscillatory rheology of the collagen pastes with and without cellulose/starch
 168 was performed using a controlled stress rheometer (Physica MCR 301, Anton Paar, Austria),

169 using a parallel plate (diameter 50 mm, gap 1.5 mm) geometry. To prevent the pastes from
170 drying during the experiment, the edges of the measuring geometry was covered using a low
171 viscosity mineral oil, which is known to have no effect on the rheological measurements. The
172 samples were equilibrated for 5 mins before measurements were conducted, this was
173 performed in order to prevent temperature variations within the samples. The dynamic
174 viscoelastic measurements were performed by subjecting the samples to various profiles: (i)
175 amplitude sweeps were conducted at a constant angular frequency (10 rad/s) to determine
176 the maximum deformation attained by the sample in the linear viscoelastic range (ii) frequency
177 sweeps were performed from 0.1-100 rad/s at 20 °C and a constant strain of 0.1% (selected
178 from LVE region) (iii) dynamic temperature sweeps were conducted at a constant strain of
179 0.1% and frequency of 10 rad/s. Collagen pastes with/without cellulose and starch were
180 heated from 5 to 80 °C at a rate of 5 °C/min. The storage modulus (G'), loss modulus (G''),
181 and loss factor ($\tan \delta = G''/G'$) were recorded as a function of frequency or temperature. The
182 data reported represent the mean values from three replicates.

183 **2.5 Differential Scanning Calorimeter (microDSC)**

184 The denaturation temperature (T_d) of the pastes was determined using a micro DSC
185 (MicroDSC III, SETARAM Instrumentation, Calurie, France). Approximately 0.8 g of the
186 samples were weighed into Hastelloy cells and sealed with O rings and Hastelloy screw tops.
187 Samples were loaded in the DSC instrument at 20°C and then cooled to 3°C at 1°C/min.
188 Collagen pastes with/without cellulose and starch were heated up to 95 °C. Samples were
189 then held at 95°C for 15 mins and cooled down to 3°C and reheated to 95 °C at 1°C/min. A
190 Hastelloy cell filled with RO water was used as a reference. The onset (T_o), offset, peak
191 temperatures (T_p) and enthalpy of transition (ΔH) were processed using Calisto Processing
192 software v1.43 (AKTS, Switzerland). The peak temperature of the thermogram was taken as
193 the melting or denaturation temperature of collagen. Runs were performed in duplicate and
194 the average and standard deviation reported.

195 **2.6 Statistical analysis**

196 A one-way ANOVA was used to determine the difference between samples using Statistical
197 Package for Social Science Software (SPSS Inc.). When there were any differences between
198 samples, Tukey's test was used to determine the significance of the average ($P < 0.05$).

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200 **3 Results and Discussion**

201 **3.1 Dynamic Frequency Sweep**

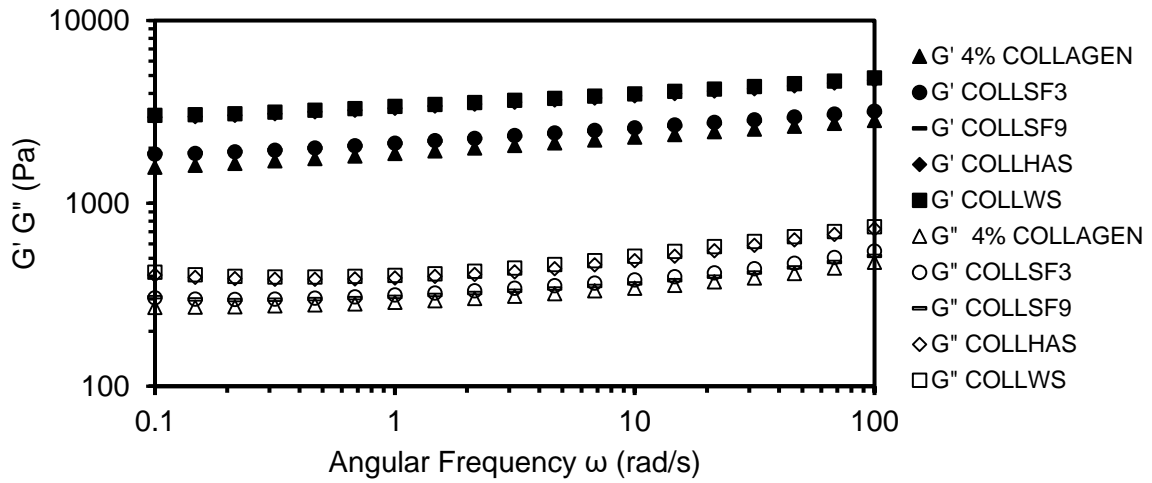
202 Figure 1 and Figure 2 shows the frequency dependency of the storage modulus (G'), the loss
203 modulus (G'') and the loss tangent ($\tan \delta$) for COLLSF9, COLLSF3, COLLWS and COLLHAS
204 pastes at the different mixing ratios and collagen concentrations. The mechanical spectra of
205 collagen pastes with the addition of starch granules and cellulose fibres at all mixing ratios
206 showed that all the pastes had a solid like behaviour as the storage modulus was higher than
207 the loss modulus throughout the frequency range investigated. This is the typical behaviour
208 of biopolymer gel where G' (storage modulus) is usually greater than the G'' (loss modulus) at
209 the range of angular frequencies investigated (Ross-Murphy, 1995). Similar behaviour has
210 also been reported by (Oechsle et al., 2015) who studied the influence of gluten, soy isolate
211 and blood plasma proteins on the viscoelastic properties of collagen gels. The magnitude of
212 the dynamic mechanical spectra of the collagen paste was modified after the addition of
213 cellulose and starch, although the enhancement in G' was dependent on collagen
214 concentration. COLLWS and COLLHAS pastes had higher values of storage modulus than
215 that of collagen/cellulose pastes which suggests that the addition of starch promoted the
216 formation of a stronger network structure (Figure 1A-C). However, at mixing ratio of 80:20 the
217 effect of starch was more pronounced than cellulose and this was attributed to the poor
218 dispersibility of the cellulose fibres in the collagen matrix. Ahmed and Jones (1990) reported
219 that the viscoelastic properties of composite materials are dependent on factors such as size,
220 shape, concentration and distribution of the reinforcing polymers. Thus, the higher storage
221 modulus observed for COLLWS and COLLHAS pastes might be due to the higher
222 concentration of starch granules needed to match the phase volume of cellulose, as well as
223 differences in particle shape, sizes, and packing. Also, according to (Tatsumi et al., 2002), it
224 was reported that fibre suspensions would give higher elasticity than spherical suspensions
225 when matched at equal volume concentration. In contrast, results of this study show that when

226 starch granules and cellulose fibres were compared at the same phase volume, starch
227 granules were more effective at reinforcing the elastic modulus of acid swollen collagen
228 pastes. In addition, at all the mixing ratios studied, cellulose fibres with longer (COLLSF9) and
229 shorter (COLLSF3) fibre lengths both had similar effects on the G' and G'' of the pastes. This
230 could be attributed to the fact that the volume fraction of the shorter fibre length (SF3) and
231 longer fibre length (SF9) were matched in the final mixtures at all the mixing ratios studied.
232 Hemar et al. (2011) indicated that the rheological behaviour of dispersions is related to the
233 volume fraction that the particles occupy. Moreover, if the volume fraction of the cellulose
234 fibers with different fibre lengths were not matched, differences in their packing densities and
235 aspect ratios might have an effect on the viscoelastic properties of collagen. Philipse (1996)
236 reported that the packing densities of high aspect ratio rod-like colloids are lower compared to
237 rods with small aspect ratios. . The $\tan \delta$ values of the blends at the various formulations were
238 lower than one which indicates that the elastic component dominated over the viscous
239 component (Figure 2A-C). The $\tan \delta$ value was dependent on the collagen concentrations,
240 higher $\tan \delta$ values were observed for COLLSF3, COLLSF9, COLLWS and COLLHAS pastes
241 at a ratio of 50:50 (2.5% collagen concentration). This indicates that the pastes were weaker
242 in structure as compared to pastes at ratios 80:20 (4% collagen concentration) and 70:30
243 (3.5% collagen concentration). The most striking observation to emerge from the $\tan \delta$ data is
244 the difference in the $\tan \delta$ shape at the various formulations. The $\tan \delta$ values of collagen with
245 cellulose and starch mixed at a ratio of 80:20 and 70:30 decreased when the oscillation
246 frequency increased from 0.1 to 1 angular frequency with a further increase in $\tan \delta$ values at
247 higher frequencies. A similar frequency dependency of $\tan \delta$ was found for chicken-wheat
248 flour doughs (Mohammed et al., 2011). However, at a ratio of 50:50, $\tan \delta$ values decreased
249 when the frequency increased from 0.1 to 10 angular frequency and was almost constant at
250 higher frequencies. This indicates that the pastes were solid-like when a slow change in stress
251 is imposed but when subjected to fast motions the pastes behaves more like a liquid .

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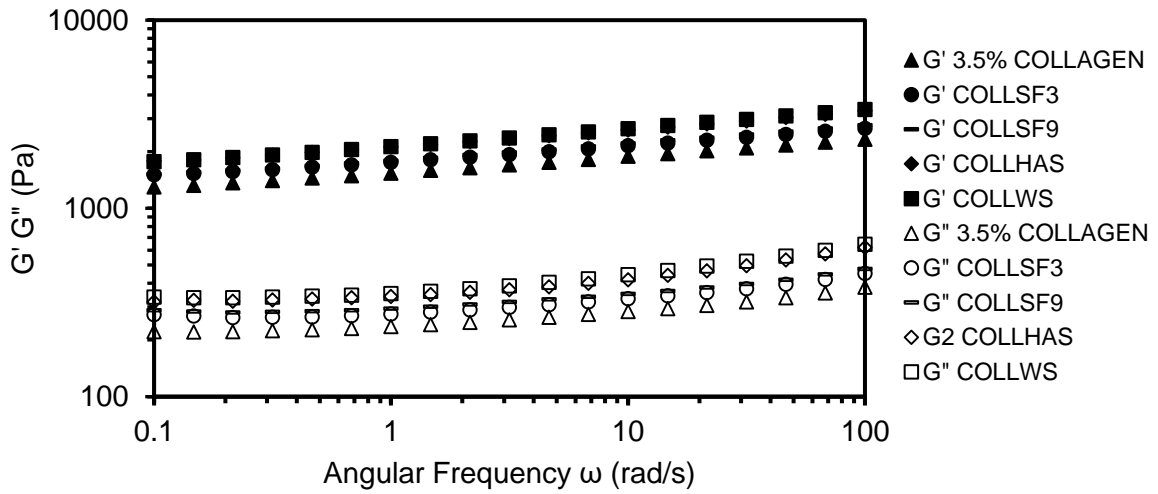
(A)



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(B)

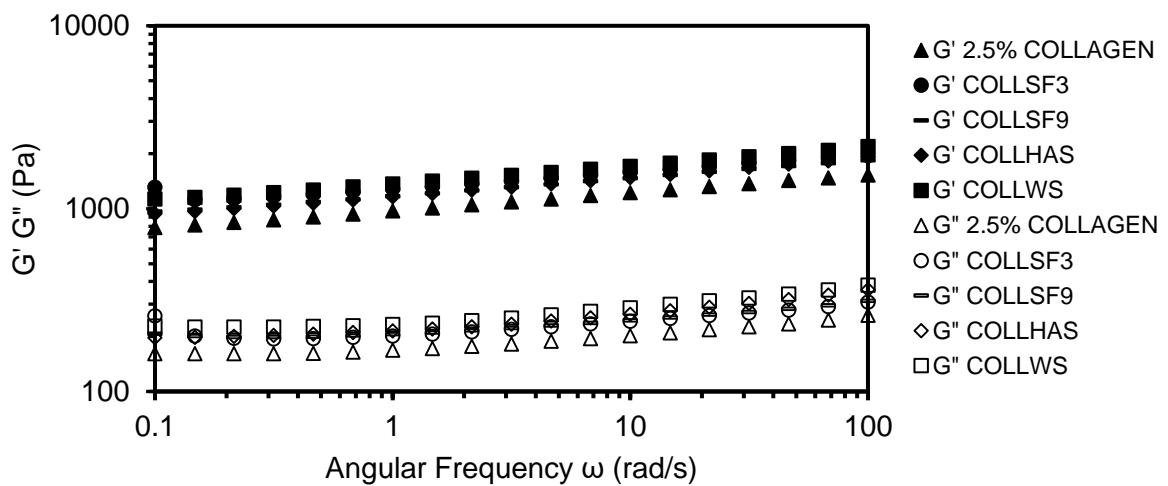


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(C)



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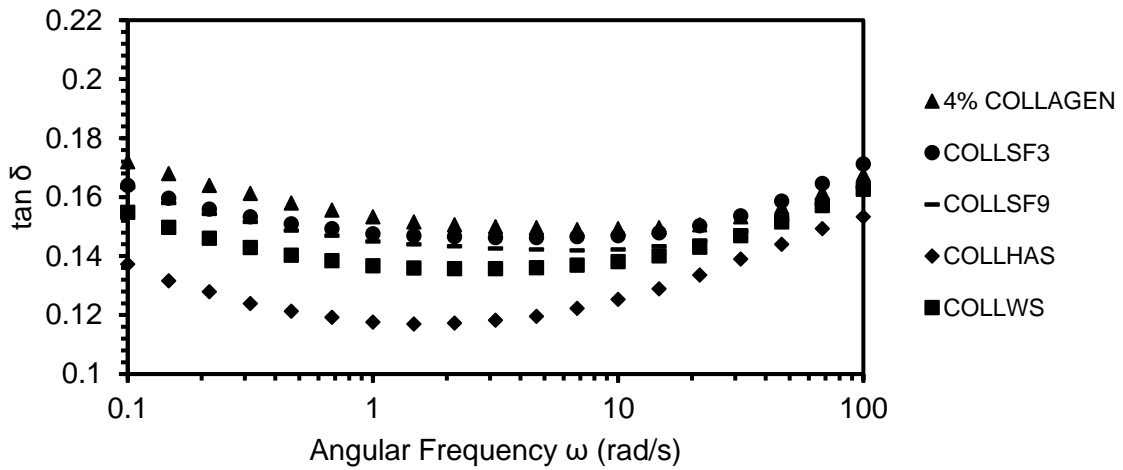
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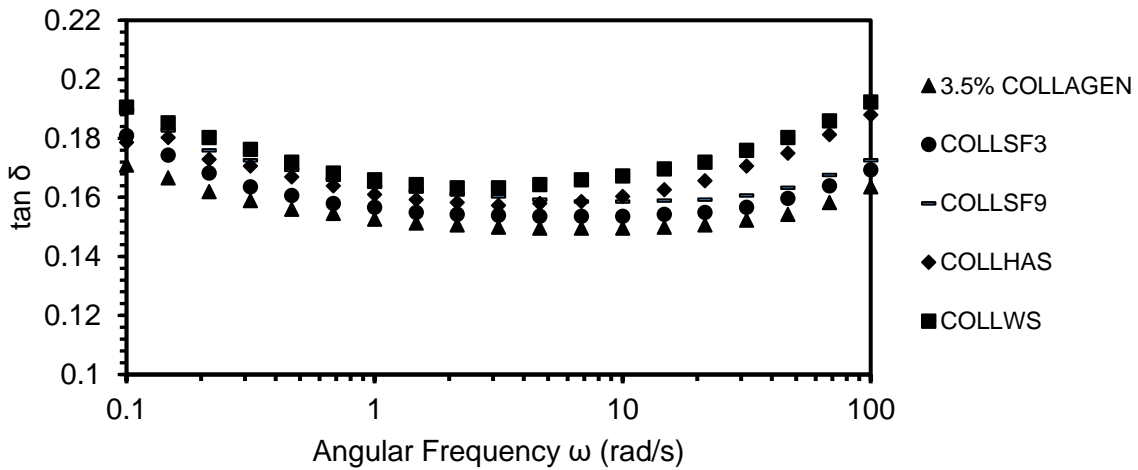
Figure 1. Storage modulus G' (closed symbols) and loss modulus G'' (open symbols) as a function of angular frequency for COLLSF3 (●), COLLSF9 (—), COLLWS (■), and COLLHAS (◆) and COLLAGEN

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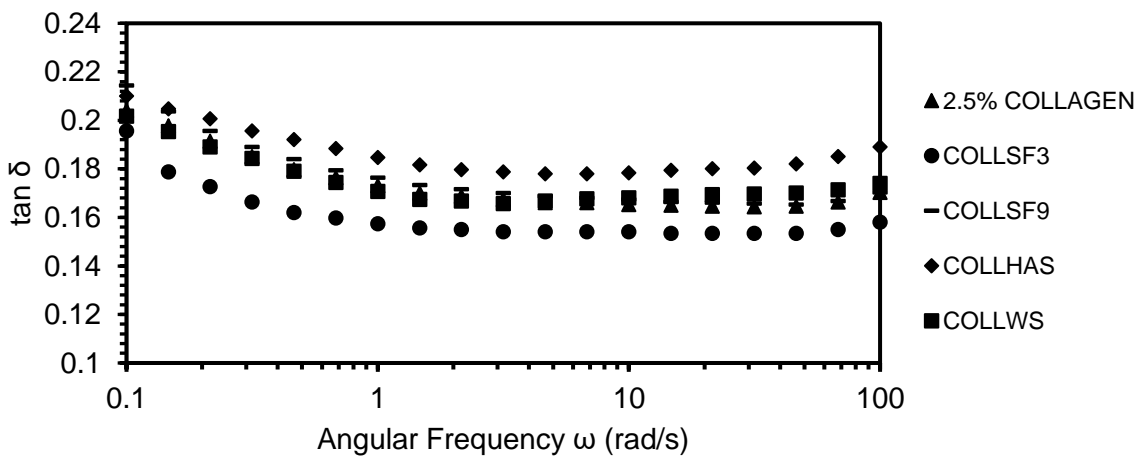
263 (A)



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266 (B)
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269 (C)
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272 Figure 2. Loss factor $\tan \delta$ as a function of angular frequency for COLLSF3 (●), COLLSF9 (—),
273 COLLWS (■), and COLLHAS (◆) and COLLAGEN (▲) pastes at different collagen concentrations (A)
274 4% collagen (B) 3.5% collagen (C) 2.5% collagen.

275 3.2 Dynamic Temperature Sweep

276 **Error! Reference source not found.**Figure 3 and Figure 4 shows the changes in storage
277 modulus (G'), loss modulus (G'') and loss factor ($\tan \delta$) of COLLSF3, COLLSF9, COLLWS
278 and COLLHAS pastes at various collagen concentrations as a function of temperature. All the
279 samples at the various mixing ratios showed no changes in G' with stable $\tan \delta$ values at
280 temperatures from 10 °C to 33 °C, however, the G' rapidly decreased at approximately 34 °C
281 (Figure 3) with a corresponding increase in $\tan \delta$ (Figure 4). Similar behaviour was observed
282 when a collagen solution was studied as a function of concentration and temperature (Lai et
283 al., 2008). This behaviour was attributed to the loss of mechanical strength as a result of the
284 structural transition of the collagen triple helix to a random coil conformation. The temperature
285 where G' decreased steeply closely matched the onset of denaturation (T_o , about 34 °C) as
286 measured by the micro differential scanning calorimeter, indicating helix to random coil
287 transition (detailed later in section 3.3). Furthermore, as depicted in Figure 3A-C, the addition
288 of waxy starch to different concentrations of collagen pastes resulted in an increase in G'
289 between 68 °C and 76 °C and a maximum at 76 °C with a corresponding decrease in $\tan \delta$
290 values at a temperature of 72 °C. This increase in G' and decrease in $\tan \delta$ can be ascribed
291 to the swelling of the starch granules as a result of the melting of the amylopectin region
292 (Donovan, 1979; Tester and Morrison, 1990; Hsu et al., 2000). However, with further increases
293 in temperature above 76 °C, a decrease in G' was observed for COLLWS pastes which was
294 attributed to the loss of granule integrity and subsequent break down of the starch granules at
295 higher temperature. Similar findings were reported by Sullo and Foster (2010) for waxy maize
296 starch/hydrocolloid mixtures. It was shown in this work that the G' of the mixture increased at
297 about 60 °C with a further decrease at higher temperatures (Sullo and Foster, 2010). On the
298 contrary, the addition of high amylose starch to different concentrations of collagen paste did
299 not result in an increase in G' at higher temperatures, which means that the granules of
300 amylose starch did not swell under the range of temperatures the rheological measurements

301 were performed. This is expected, as it has been reported that amylose molecules swell in the
302 temperature range of 104 °C and 125 °C (Kibar et al., 2010).

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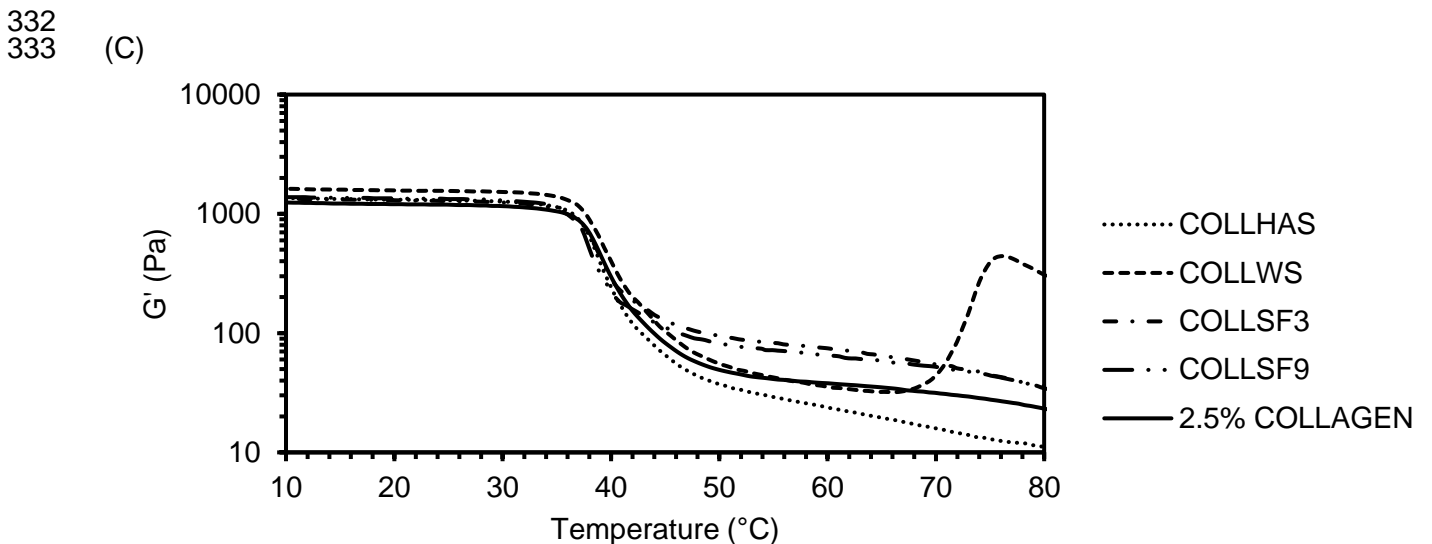
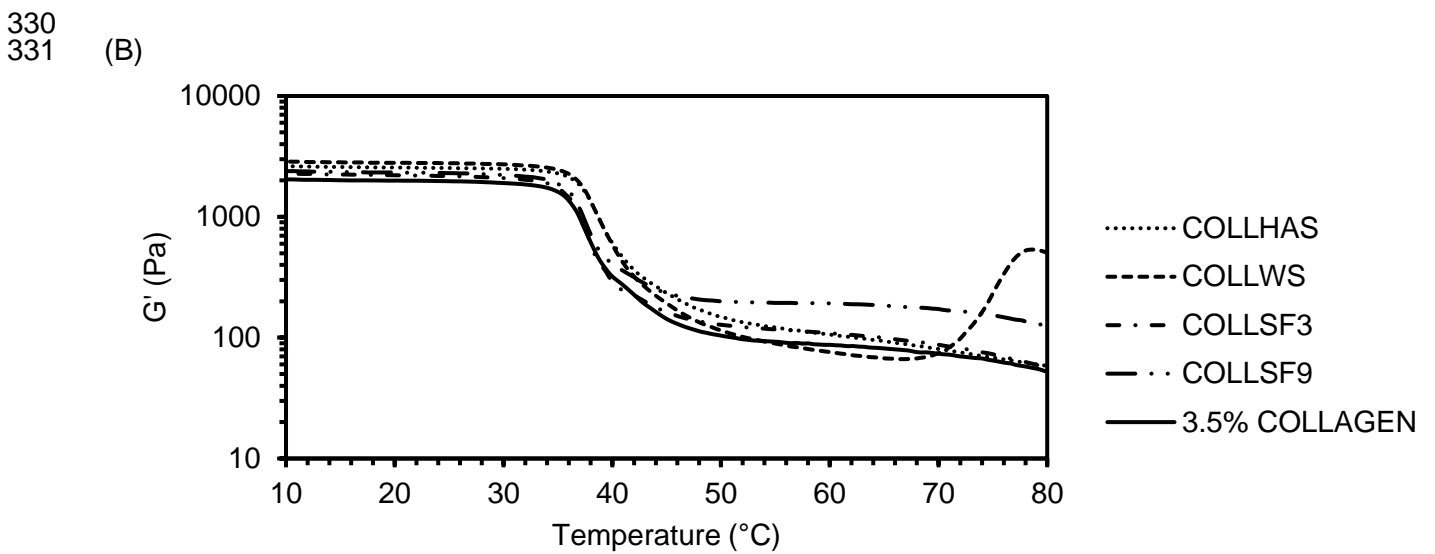
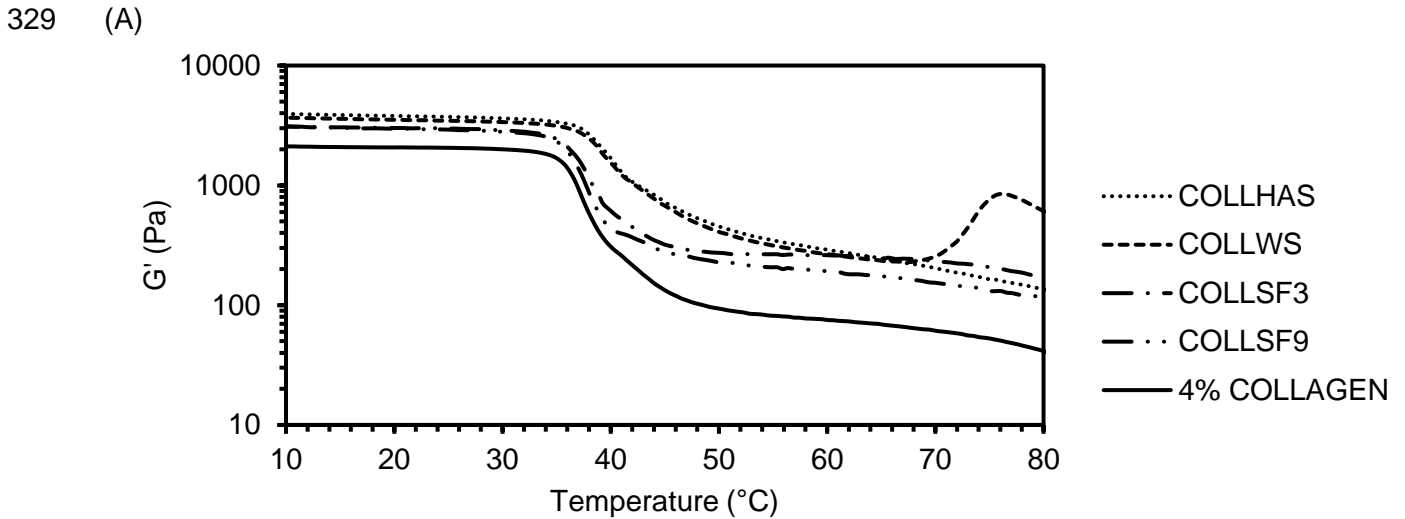
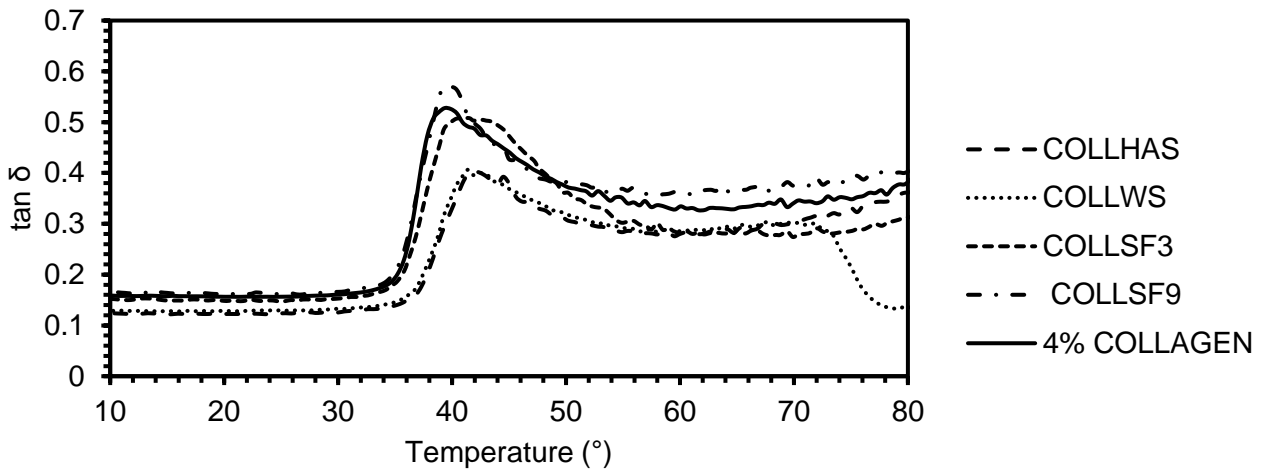
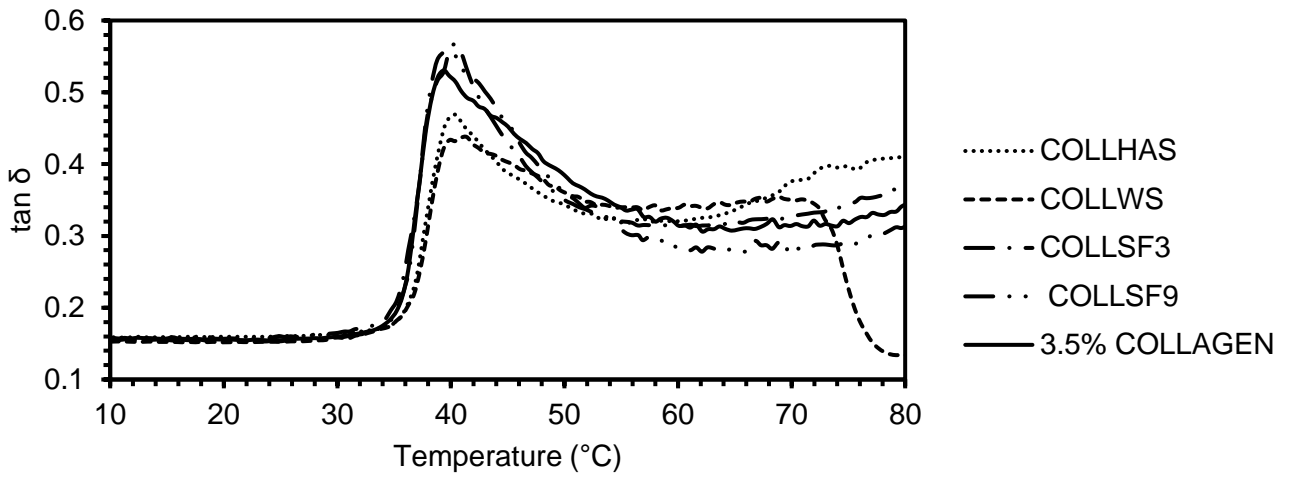


Figure 3. Storage modulus G' as a function of temperature for COLLHAS (.....), COLLWS (----), COLLSF3 (- · -), COLLSF9 (— ·) and COLLAGEN (—) pastes at different collagen concentrations (A) 4% collagen (B) 3.5% collagen and (C) 2.5% collagen.

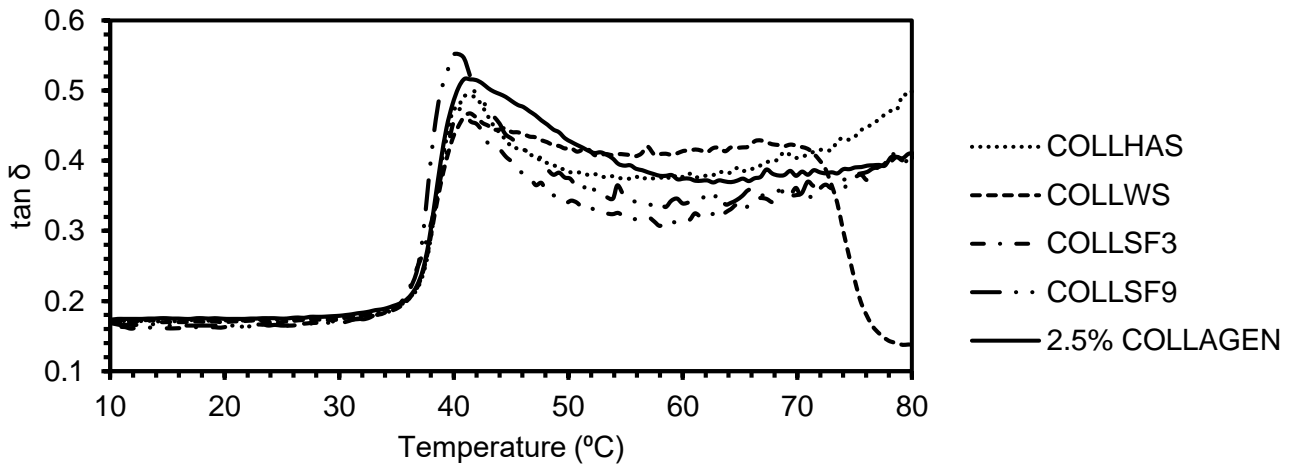
339 (A)



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341 (B)



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343 (C)



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345 Figure 4. Storage modulus G' as a function of temperature for COLLHAS (.....), COLLWS (---),
346 COLLSF3 (- · -), COLLSF9 (- · ·) and COLLAGEN (—) pastes at different collagen concentrations
347 (A) 4% collagen (B) 3.5% collagen and (C) 2.5% collagen.
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349 The loss factor ($\tan \delta$) values of collagen with and without cellulose/starch pastes at the various
350 collagen concentrations were lower than 1 indicating that their overall behaviour was solid-
351 like. Some studies have reported that during oscillatory rheological measurements, the
352 temperature at which $\tan \delta$ reached a peak value could be taken as the dynamic denaturation
353 temperature of collagen (T_{dd}) (Lai et al., 2008; Zhang et al., 2010; Ding et al., 2014). Hence the
354 dynamic denaturation temperature of COLLSF3, COLLSF9, COLLWS and COLLHAS at the
355 different collagen paste concentrations was recorded as 42 °C, which was slightly higher than
356 that measured by micro DSC (37 °C). In addition, the different measurements report on the
357 melting at different length scales. DSC report on the helix-coil transition directly, whereas the
358 rheological measurement is dependent on the melting and softening of the helical aggregates
359 as the helices melt. This result implies that the thermal denaturation of the collagen triple helix
360 could be influenced by differences in the heating rate used by each instrument. Furthermore,
361 a drastic drop in $\tan \delta$ was observed for all the samples at about 42 °C. This has been
362 attributed to the glass/rubbery transition of the denatured collagen (Pietrucha, 2005). It is also
363 interesting to note that the $\tan \delta$ peak value of COLLWS and COLLHAS pastes were lower
364 than that of COLLSF3 and COLLSF9 pastes, indicating that the collagen pastes with starch
365 granules were more rigid in comparison to collagen pastes with cellulose fibres. The lower \tan
366 δ values observed for COLLWS and COLLHAS pastes might be due to the presence of closer
367 packing of the starch granules in the collagen matrix, which reduced the molecular mobility of
368 the collagen triple helix molecules during heating. This is in comparison to cellulose fibres that
369 were not closely attached to the collagen matrix, thus allowing the easy mobility of the collagen
370 chains. Another explanation is that the higher concentrations of starch granules resulted in a
371 stronger network structure which led to an increase in the restriction of the collagen chains
372 and reduction in the damping values. Mohanty suggested that the magnitude of damping factor
373 ($\tan \delta$) peak of composite materials are affected by the incorporation of fillers, the extent of
374 packing and the concentration of the fillers (Mohanty et al., 2006).

375 **3.3 Thermal properties**

376 The thermal properties of the collagen pastes with and without the addition of cellulose and
377 starch at various mixing ratios are summarised in

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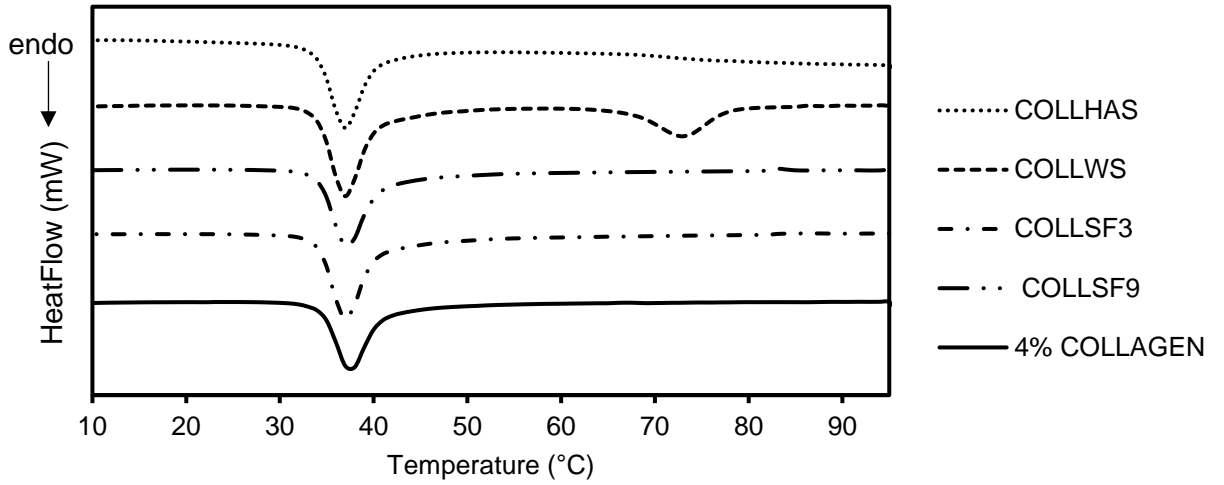
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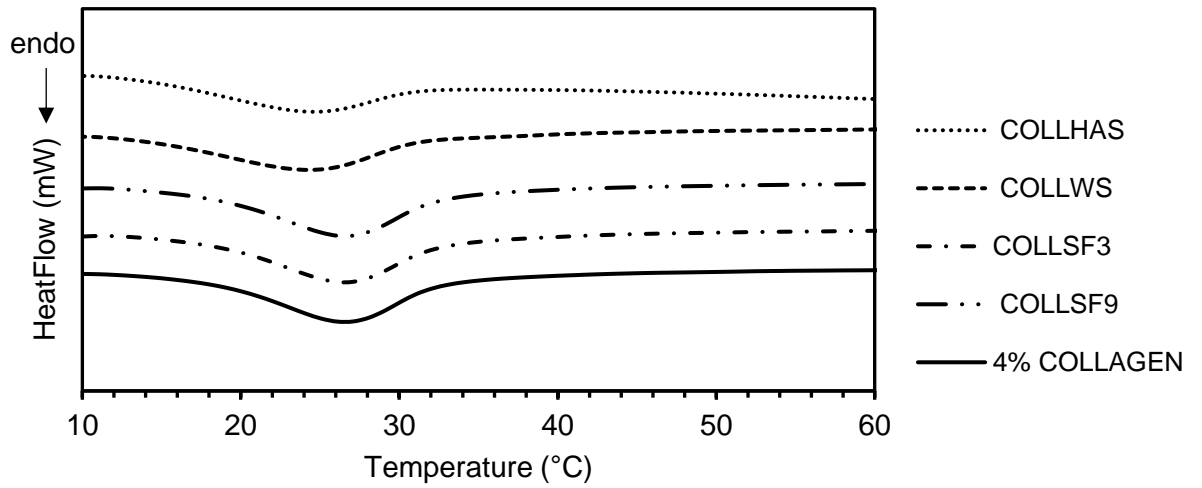
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397 (A)



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399 (B)



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401 Figure 5. DSC thermograms of (A) first heating and (B) second heating of COLLHAS (), COLLWS
402 (), COLLSF3 (), COLLSF9 () and 4% COLLAGEN () pastes.

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409 Table 2. As depicted in (Figure 5A), pure collagen, COLLSF3 and COLLSF9 pastes (various
410 formulations) exhibited a single endotherm at approximately 37°C. The transition temperature
411 was associated with the transition of collagen molecules from the triple helix to a randomly
412 coiled conformation which is induced by the thermal disruption of hydrogen bonds in the
413 collagen molecule (Bigi et al., 2004). In contrast, COLLWS and COLLHAS exhibited two
414 endothermic peaks, the first peak was related to the denaturation of collagen and the second
415 peak corresponds gelatinisation of the starch granules (Figure 5A). Starch gelatinisation
416 involves the irreversible swelling of starch granules when starch is heated in excess water
417 (Morris, 1990;Tester and Morrison, 1990) It was observed that the COLLWS showed a
418 pronounced gelatinisation peak at 73°C similar to previous reports (Liu et al., 2006). This
419 endotherm can be attributed to the widely accepted gelatinisation of the amylopectin starch
420 granules which is due to the disruption of the amylopectin crystallites (Donovan, 1979;Tester
421 and Morrison, 1990;Koganti et al., 2011). However, the endotherms for COLLHAS pastes
422 were not as sharp as those of the waxy maize starch which contains mainly amylopectin,
423 instead the beginning of a broad endotherm can be observed from 68 °C. Such a broad peak
424 was also observed previously for corn starch with high amylose content, within the
425 temperature range of 65 to 115 °C (Liu et al., 2006), suggesting it resulted from a composite
426 consisting of both gelatinisation and a phase transition of the amylose lipid complex. In
427 measurements of pasting properties of high amylose starch up to 140 °C, we have observed
428 gelatinisation peaks beginning at 100 °C and peaking at 120 °C (data not shown). Therefore,
429 we would support the fact that the broad peak seen in the DSC endotherms would be
430 consistent with starch-lipid complexes. These results were expected because waxy maize
431 contains about 99% amylopectin which constitute the crystalline regions of the starch. On the
432 other hand, amylose restricts the swelling of the starch due to the leaching out of amylose
433 during the gelatinisation and formation of complexes with the lipid's presence in starch
434 granules, which limits the rate at which water penetrates into the starch granules. In addition,
435 it was observed that decreasing the collagen concentrations from 4% to 2.5% resulted in a
436 slight increase of the peak value temperatures of the collagen/cellulose and collagen/starch

437 pastes. Overall, the transition temperatures of COLLSF3, COLLSF9, COLLWS and COLLHAS
438 pastes were not significantly different ($P>0.05$) from pure collagen pastes at the collagen
439 concentrations studied. The endothermic peaks of the COLLSF3, COLLS9, COLLWS and
440 COLLHAS pastes at a mixing ratio of 80:20 observed on reheating are shown Figure 5B and
441 the thermal transition parameters of all formulations are detailed in Table 3. The endothermic
442 peak appeared at lower temperatures between 24°C - 26°C (Figure 5B) for all the samples.
443 During the thermal denaturation of collagen, the triple helix is disrupted to give a random coil
444 confirmation (gelatin). However, on cooling, the random coils undergo a conformational
445 disorder-order transition and partly renatures to a triple-helix structure which is similar to that
446 of collagen but with a lower molecular weight (Machado et al., 2002; Bigi et al., 2004). Gilsean
447 and Ross-Murphy (2000) suggested that lower molecular weight gels melt at lower
448 temperatures than gels of high molecular weight. Also, the lower transition temperature could
449 indicate the formation of lower and less ordered helical bundles as well as reduced helix length
450 (Michon et al., 1997; Aymard et al., 2001). Thus, this might explain the lower denaturation
451 temperatures observed on the second DSC scans. Furthermore, as shown in Table 3, the
452 thermal transitions temperatures of COLLWS and COLLHAS pastes were significantly lower
453 ($P<0.05$) than those of the COLLSF3, COLLSF9 and pure collagen pastes. The decrease in
454 the denaturation temperature and enthalpy can be related to a decrease in the ordering and
455 semi-crystalline nature of the reformed gelatin-like matrix, after collagen melting, upon cooling.
456 Such a decrease might not be expected if the two materials do not affect one another. Lorén
457 et al. (2001) have shown that phase separation and subsequent phase concentration does
458 not impact on the amount of gelatin helices formed. Therefore we do not expect that
459 confinement effects would reduce the ability of the collagen to reform triple helices. Therefore,
460 an unexpected molecular interaction between collagen chains and starch may reduce the
461 creation of gelatin-like network. Indeed, when mixtures highlighted in table 2 are dried, then
462 mixtures containing starch also show a decrease in enthalpy. This is a phenomenon that is
463 currently under further exploration.

464 Finally, the transition enthalpy for COLLWS pastes was slightly higher than COLLHAS pastes,
465 however, this increase in enthalpy for COLLWS was not significantly different ($P>0.05$) from
466 enthalpy values of COLLHAS (Table 2). An explanation for this might be due to the high
467 molecular weight and branched chains of amylopectin, indicating a larger amount of energy
468 was needed to melt the regenerated triple helix of the COLLWS paste.

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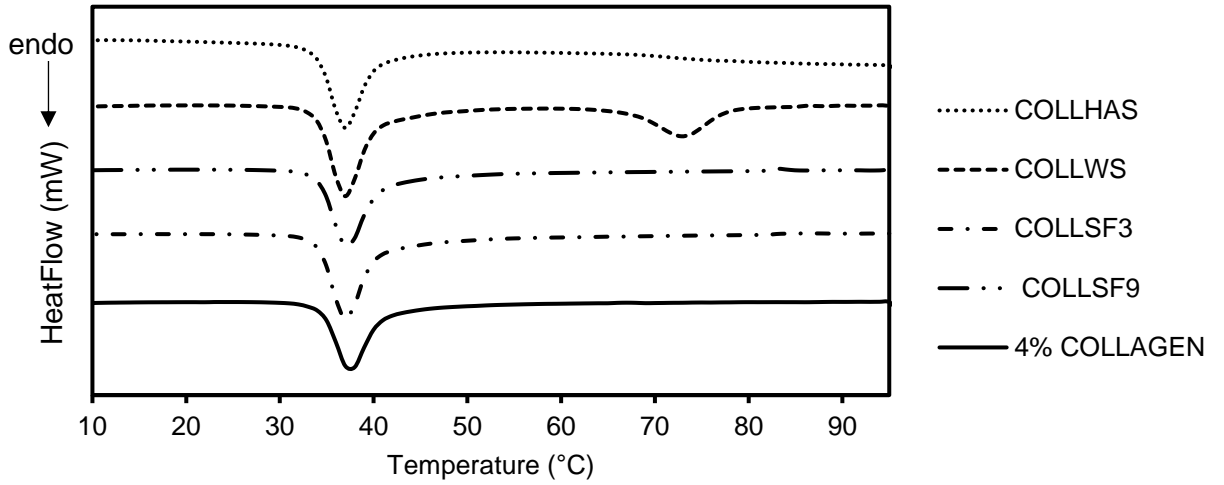
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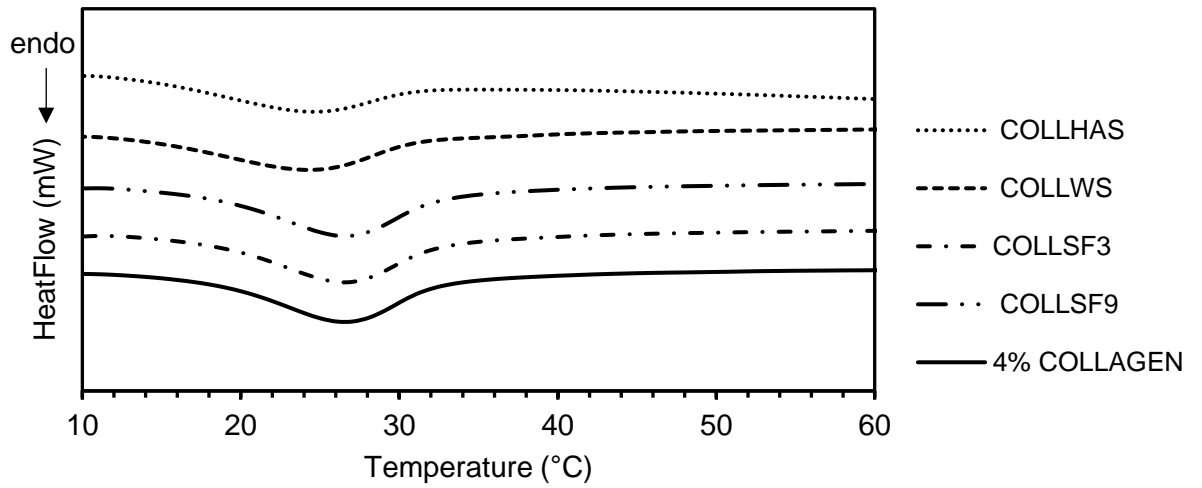
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488 (A)



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490 (B)



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492 Figure 5. DSC thermograms of (A) first heating and (B) second heating of COLLHAS (·····), COLLWS
493 (-----), COLLSF3 (- · - ·), COLLSF9 (- · · ·) and 4% COLLAGEN (—) pastes.

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500 Table 2. DSC parameters after first heating of collagen/cellulose and collagen/starch pastes
 501 at different collagen paste concentrations and mixing ratios. T_P = peak temperature; T_O= Onset
 502 temperature; T_e= end set temperature and ΔH= enthalpy

Samples	T _{onset} (°C)	T _{Peak} (°C)	ΔH (J/g of collagen)	T _{end} (°C)
4% COLLAGEN	33.90 ± 0.01 ^a	36.80 ± 0.02 ^a	40.36 ± 0.02 ^a	0.47 ± 0.02 ^a
COLLSF3 80:20	34.00 ± 0.05 ^a	37.01 ± 0.07 ^a	40.91 ± 0.05 ^a	0.43 ± 0.01 ^{ab}
COLLSF9 80:20	33.99 ± 0.01 ^a	37.01 ± 0.00 ^a	40.60 ± 0.23 ^a	0.45 ± 0.45 ^{ab}
COLLWS 80:20	34.05 ± 0.01 ^a	36.97 ± 0.02 ^a	40.38 ± 0.00 ^a	0.46 ± 0.01 ^{ab}
COLLHAS 80:20	34.03 ± 0.03 ^a	36.97 ± 0.02 ^a	40.37 ± 0.06 ^a	0.41 ± 0.01 ^b
3.5% COLLAGEN	34.16 ± 0.07 ^a	37.23 ± 0.05 ^{bc}	40.91 ± 0.27 ^b	0.42 ± 0.06 ^a
COLLSF3 70:30	34.10 ± 0.02 ^a	37.21 ± 0.07 ^{abc}	40.96 ± 0.16 ^{ab}	0.46 ± 0.00 ^a
COLLSF9 70:30	34.17 ± 0.02 ^a	37.29 ± 0.07 ^c	40.99 ± 0.99 ^b	0.49 ± 0.01 ^a
COLLWS 70:30	34.12 ± 0.02 ^a	37.06 ± 0.02 ^{ab}	40.48 ± 0.12 ^{ab}	0.45 ± 0.01 ^a
COLLHAS 70:30	34.01 ± 0.11 ^b	37.06 ± 0.02 ^{ab}	40.30 ± 0.12 ^a	0.48 ± 0.02 ^a
2.5% COLLAGEN	34.63 ± 0.05 ^a	37.77 ± 0.03 ^a	41.63 ± 0.08 ^a	0.48 ± 0.00 ^b
COLLSF3 50:50	34.46 ± 0.07 ^a	37.66 ± 0.09 ^a	41.09 ± 0.03 ^a	0.45 ± 0.01 ^{ab}
COLLSF9 50:50	34.62 ± 0.04 ^a	37.77 ± 0.07 ^a	41.49 ± 0.03 ^a	0.44 ± 0.00 ^{ab}
COLLWS 50:50	34.35 ± 0.36 ^a	37.79 ± 0.15 ^a	41.02 ± 0.06 ^a	0.40 ± 0.01 ^a
COLLHAS 50:50	34.63 ± 0.01 ^a	37.67 ± 0.02 ^a	41.01 ± 0.13 ^a	0.42 ± 0.00 ^{ab}

503 ^{a-c} Mean ± standard deviation. Means in the same column with different superscript letters
 504 are significantly different (P<0.05).

505 Table 3. DSC parameters (second heating) of collagen/cellulose and collagen/starch at
 506 various collagen concentrations (4%, 3.5% and 2.5%). T_P = peak temperature; T_O= Onset
 507 temperature; T_e= end set temperature and ΔH= enthalpy

Samples	T_{onset} (°C)	T_{Peak} (°C)	ΔH (J/g of denatured collagen)	T_{end} (°C)
4% COLLAGEN	17.67 ± 0.11 ^b	26.66 ± 0.11 ^b	0.27 ± 0.01 ^c	33.29 ± 0.14 ^b
COLLSF3 80:20	17.68 ± 0.13 ^b	26.49 ± 0.05 ^b	0.27 ± 0.00 ^c	33.38 ± 0.05 ^b
COLLSF9 80:20	17.46 ± 0.10 ^b	26.55 ± 0.02 ^b	0.27 ± 0.01 ^c	33.41 ± 0.05 ^b
COLLWS 80:20	13.76 ± 0.20 ^a	24.37 ± 0.09 ^a	0.18 ± 0.00 ^b	31.67 ± 0.00 ^a
COLLHAS 80:20	14.33 ± 0.42 ^a	24.22 ± 0.00 ^a	0.14 ± 0.01 ^a	31.33 ± 0.03 ^a
3.5% COLLAGEN	18.13 ± 0.05 ^b	26.81 ± 0.04 ^b	0.26 ± 0.00 ^b	33.60 ± 0.04 ^b
COLLSF3 70:30	18.09 ± 0.13 ^b	26.79 ± 0.04 ^b	0.27 ± 0.00 ^b	33.61 ± 0.02 ^b
COLLSF9 70:30	19.07 ± 0.17 ^b	26.72 ± 0.02 ^b	0.27 ± 0.00 ^b	33.57 ± 0.00 ^b
COLLWS 70:30	14.06 ± 0.37 ^a	24.45 ± 0.02 ^a	0.18 ± 0.00 ^a	31.47 ± 0.02 ^a
COLLHAS 70:30	14.14 ± 0.37 ^a	24.22 ± 0.09 ^a	0.16 ± 0.01 ^a	31.41 ± 0.01 ^a
2.5% COLLAGEN	18.96 ± 0.04 ^b	27.35 ± 0.02 ^b	0.24 ± 0.02 ^b	33.98 ± 0.06 ^b
COLLSF3 50:50	19.21 ± 0.36 ^b	27.48 ± 0.09 ^b	0.23 ± 0.02 ^b	33.89 ± 0.16 ^b
COLLSF9 50:50	19.13 ± 0.02 ^b	27.38 ± 0.02 ^b	0.24 ± 0.00 ^b	34.02 ± 0.08 ^b
COLLWS 50:50	15.48 ± 0.07 ^a	25.08 ± 0.11 ^a	0.14 ± 0.01 ^a	31.80 ± 0.02 ^a
COLLHAS 50:50	15.98 ± 0.20 ^a	24.99 ± 0.06 ^a	0.12 ± 0.01 ^a	31.56 ± 0.13 ^a

508 ^{a-c} Mean ± standard deviation. Means in the same column with different superscript letters are
 509 significantly different (P<0.05).

510 **4 Conclusions**

511 The elastic properties of collagen pastes were modified by the addition of fillers i.e. cellulose
 512 fibres and starch granules at a range of different collagen concentrations. The results indicate
 513 that different materials, when matched for phase volumes, have very different effects on the
 514 elastic properties of collagen pastes. In addition, these materials did not affect the thermal
 515 stability of collagen. However, on reheating, the addition of starch granules had an impact on

516 the melting of denatured collagen. The findings in this study may serve as a platform to
517 improve the rheological properties and processing performance of collagen pastes in order to
518 improve their product or processing performance. Further research is still necessary to
519 understand the mechanisms or interactions underlying the effect of starch granules on the
520 melting of denatured collagen.

521 **5 Acknowledgements**

522 The authors would like to acknowledge the financial support obtained from Engineering and
523 Physical Sciences Research Council (EP/M50810X/1), in collaboration with Devro Plc.

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