

Formulation and additive manufacturing of polysaccharide-surfactant hybrid gels as gelatin analogues in food applications

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1 **Formulation and additive manufacturing of polysaccharide-surfactant hybrid gels as**
2 **gelatin analogues in food applications.**
3

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8 **Abstract**

9 A vegetarian alternative to gelatin, for use in food applications was proposed as a synergistic
10 combination of 0–2 wt% low acyl gellan gum (LAG) and 0–2 wt% tamarind seed xyloglucan (TSX). The
11 mechanical, thermal and temperature-mediated release properties of the gels were examined using
12 rheology and conductivity. The influence of the addition of a food grade emulsifier, Tween® 20, was
13 also investigated. It was found that both the total concentration of biopolymers and the ratio of
14 polymer blends influenced thermal (gelling and melting temperatures) and mechanical (storage
15 modulus and phase angle) properties, however the total polymer concentration was the major factor.
16 The addition of Tween® 20 led to small increases gelling and melting temperatures, elastic modulus
17 and a small reduction phase angle in most of the LAG/TSX samples. Using rheological data the LAG/TSX
18 samples were predicted to be printable using extrusion-based additive manufacturing, which was then
19 performed on a custom-made printer. The rheological and release data suggested that 0.5 wt%
20 LAG/1.5 wt% TSX/1 wt% Tween® 20 was the most similar to a tested sample of 5 wt% porcine gelatin
21 in terms of viscoelastic moduli, gelling & melting temperatures and release profile, and could
22 therefore be developed as a printable gelatin replacement. No difference was found between the
23 release properties of moulded versus printed gels.

24 **1. Introduction**

25 Across many industries, notably the food and pharmaceutical industries, there is a need for the
26 development of vegetarian gels that provide ‘melt-in-the-mouth’ behaviour (Ikeda & Talashek, 2007),
27 as an alternative to porcine gelatin-based formulations. This due to concerns over gelatin’s animal-

28 based origins. Additionally, the junction zones in gelatin continually re-arrange to become gradually
29 more thermally stable, hence the gel strength and melting temperature of gelatin is a direct function
30 of its age (Ledward, 2000). Gelatin alternatives must be firm at room temperature yet must soften
31 significantly at body temperature (37 °C) to provide the correct mouthfeel and release properties. In
32 such edible formulations it is often advantageous to include an emulsifier to allow for the stable
33 inclusion of oil-based flavourings and additives (Kralova & Sjöblom, 2009). Initial characterisation of a
34 gel-surfactant system would pave the way for future gel-oil-surfactant formulations for the food
35 industries and beyond.

36 Gellan gum is an extracellular polysaccharide secreted by *Sphingomonas elodea* (syn. *Pseudomonas*
37 *elodea*) micro-organisms (Moorhouse, Colegrove, Sandford, Baird, & Kang, 1981). Its primary structure
38 consists of a linear repetition of four saccharide derivatives: $\rightarrow 3\text{-}\beta\text{-D-Glcp-(1}\rightarrow 4\text{)-}\beta\text{-D-GlcpA-(1}\rightarrow 4\text{)-}\beta\text{-}$
39 $\text{D-Glcp-(1}\rightarrow 4\text{)-}\alpha\text{-L-Rhap-(1}\rightarrow$ (Jansson, Lindberg, & Sandford, 1983). In its native high acyl form, the
40 polymer has two acyl substituents present on the 3-linked glucose (Kuo, Mort, & Dell, 1986), however
41 these can be removed through heating under alkaline conditions to yield the deacylated (low acyl)
42 form (Chilvers & Morris, 1987). Both gellans undergo gelation in the presence of sufficient gelling
43 cations through the formation and subsequent aggregation of double helices, achieved by hydration
44 at an elevated temperature followed by cooling (Grasdalen & Smidsrød, 1987). The degree of acyl
45 substitution determines the resultant gel properties: high acyl gellan (HAG) gum typically forms
46 thermoreversible, soft, elastic gels whereas low acyl gellan (LAG) gum generally forms non-
47 thermoreversible, hard, brittle gels (Lee, Fisher, Kallos, & Hunter, 2011). Divalent cations are much
48 more effective at promoting gelation in gellan compared to monovalent cations (Grasdalen &
49 Smidsrød, 1987).

50 Tamarind seed xyloglucan (TSX) is a non-ionic, branched polysaccharide which is extracted from the
51 seed of the tamarind tree *Tamarindus indica* (Kaur, Yadav, Ahuja, & Dilbaghi, 2012; Nayak, Pal, &
52 Santra, 2014). TSX has a backbone of $(1\rightarrow 4)\beta\text{-D-glucans}$ substituted with side chains of $\alpha\text{-D-}$

53 xylopyranose which are linked (1→6) to glucose molecules (Kaur et al., 2012). In water, TSX swells and
54 causes a rise in viscosity which sees it used mainly as a thickener and stabiliser in the food industry
55 (Nayak et al., 2014), however, it does not form a gel on its own.

56 A synergistic combination of two biopolymers describes a case whereby a mixture of polymers shows
57 different properties than what is possible from any of the individual substituent polymers. The origin
58 of this synergistic effect is due to the formation of either a phase-separated or a coupled network,
59 depending on whether direct binding takes place or not between the two polymers. In either case a
60 synergistic effect is often indicated by the viscosity of a mixture being greater than the sums of the
61 viscosities of its substituents (Nishinari, Kim, Fang, Nitta, & Takemasa, 2006). Morris (1995) noted that
62 many synergistic interactions arise from mixtures of a polysaccharide that undergoes a coil-helix
63 transition, such as carrageenan, xanthan gum and gellan gum, mixed with another polysaccharide with
64 a $\beta(1\rightarrow4)$ linked cellulosic backbone, such as locust bean gum, konjac glucomannan or tamarind seed
65 xyloglucan (Nishinari et al., 2006). Work by Grisel *et al.* (2015), who investigated the synergistic
66 interactions between xanthan and galactomannans, suggested that the mechanism of synergy
67 between the two polymers is dependent on the degree of compatibility between the two molecular
68 structures: in the attractive scenario, unsubstituted areas on the galactomannan can interact with
69 linear, non-helical regions on the xanthan which allows the formation of hybrid junction zones,
70 whereas if the structures are geometrically incompatible, then such junction zones cannot form, and
71 instead the synergy results from a repulsive, phase-separated microstructure.

72 In an unmodified state, both HAG and LAG have a melting temperature that far exceeds the target for
73 a gelatin replacement ($T_m > 70$ °C). The synergistic combination of LAG with TSX has been briefly
74 reported in the literature previously (Nayak et al., 2014; Nishinari et al., 2006; Nitta et al., 2003),
75 however its use as a potential gelatin replacement has not been exploited to the authors' knowledge.
76 Ikeda *et al.* (2004) attempted to elucidate the nature of the interaction between TSX and LAG using
77 rheology and atomic force microscopy (AFM). It was found that by mixing two non-gelled mixtures of

78 LAG and TSX, a gel network was formed, however it could not be definitively stated if this synergism
79 was the result of phase separation or coupling between the polymers. Nitta *et al.*, (2003) also reported
80 synergy between LAG and TSX as detected by rheology and differential scanning calorimetry, but once
81 again, mechanistically the nature of the interaction was not defined.

82 Surfactants are used widely in edible formulations, primarily as an emulsifier to allow the stable
83 inclusion of hydrophobic additives, such as fats, flavourings and colourings, to water-based
84 formulations (Kralova & Sjöblom, 2009). Polysorbate 20, or Tween® 20, is a non-ionic surfactant widely
85 used as an additive in foodstuffs to stabilise oil-in-water emulsions (Genot, Kabri, & Meynier, 2013).
86 Generally, non-ionic surfactants are preferred in edible formulations due to their low toxicity
87 compared to their charged counterparts (Fasolin, Picone, Santana, & Cunha, 2013). It has generally
88 been reported that charged biopolymers, such as gellan, and polysorbates do not associate (Fasolin
89 *et al.*, 2013) even up to concentrations of 10% w/v, except for chitosan-polysorbate 80 which formed
90 structures on a nanometre and micrometre scale at extremely low (below 0.01% w/v) and extremely
91 high (above 50% w/v) surfactant concentrations (Picone & Cunha, 2013). However, new studies have
92 indicated that non-ionic surfactant micelles can contribute to electrostatic shielding of gelling
93 biopolymers through weak hydrophobic interactions (Yang & Pal, 2020).

94 Additive manufacturing (3D printing) has recently found its way in food applications (Lipton, Cutler,
95 Nigl, Cohen, & Lipson, 2015; Sun, Peng, Yan, H Fuh, & Soon Hong, 2015; Fan; Yang, Zhang, & Bhandari,
96 2017). Among the current 3D printing methods for food applications (Chia & Wu, 2015; Guo & Leu,
97 2013), extrusion is a prevailing technique because it is easy to develop and it has the broadest set of
98 'inks' (Guvendiren *et al.*, 2016; Tan, Toh, Wong, & Lin, 2018) which can be tailored to match certain
99 rheological requirements (Daffner *et al.*, 2021; Godoi, Prakash, & Bhandari, 2016). Among all the
100 available extrusion techniques, cold extrusion 3D food printing has emerged as the technology which
101 enables the manufacture of food in different compositions, textures, tastes or shapes, and offers huge
102 potential for personalised food products beyond what is achievable with conventional moulding.

103 However, the high cost of production compared to existing manufacturing techniques is a barrier to
104 commercialisation of 3D printing (Godoi et al., 2016). Warner, Norton and Mills (2019) reported that
105 due to the slow gelation time of gelatin, 3D printing of gelatin-based formulations, without a gelation
106 accelerant, results in distortion of printed shapes after printing. Hence, the development of a printable
107 gelatin analogue would be advantageous.

108 This study aimed to explore LAG and TSX, firstly as a gelatin replacement, and secondly as an 'ink' to
109 manufacture 3D printed gelled structures. The influence of a common food-grade emulsifier, Tween®
110 20, was also investigated. Rheology gave information on the thermal and mechanical properties of the
111 gels through temperature and frequency sweeps. The release performance of the gels was measured
112 with respect to temperature in water using conductivity – made possible due to the efflux of cations
113 upon gel erosion. The release profiles from both quiescent and 3D printed gels were compared.

114 **2. Experimental**

115 **2.1 Materials**

116 Kelcogel® F (batch 8F0778A), a commercially available low acyl gellan gum was kindly gifted by CP
117 Kelco (USA). Tamarind seed xyloglucan (batch UG660FJ) was purchased from Tokyo Chemical Industry
118 (UK). Gelatin from porcine skin (batch BCBH5042V, 240 - 270 g bloom, type A) was purchased from
119 Sigma Aldrich (Germany). Tween® 20 was purchased from Merck (Germany). All materials were used
120 as received without further purification or modifications. The ion content of Kelcogel® F and tamarind
121 seed xyloglucan was determined by Inductively Coupled Plasma-Optical Emission Spectroscopy
122 (PerkinElmer Optima 8000) as shown in Table 1.

123

124

125

126 *Table 1 – Ion content for polysaccharide samples. Values given are mean averages from at least three successive*
 127 *measurements and the associated standard deviation. (* = negative values for ion contents were generated due to the fact*
 128 *that the concentration was below the detectable limits, hence these values can be considered to be 0).*

Ion content ($10^5 \times \% \text{ w/w}$)				
	Na^+	K^+	Mg^{2+}	Ca^{2+}
Kelcogel® F	31.31 ± 0.08	229.9 ± 1.8	2.906 ± 0.473	6.908 ± 0.847
TSX	2.427 ± 0.064	3.283 ± 0.072	$-0.472 \pm 0.020^*$	$-0.847 \pm 0.028^*$

129

130 2.2 Methods

131 2.2.1 Preparation of gel solutions

132 Polymer powders were weighed (wet basis) and were slowly added to a vessel of deionized water
 133 (Milli-Q, Millipore®) at 80 °C under agitation from a magnetic stirrer bar at a moderate speed to avoid
 134 clumping. The flask was covered to prevent evaporation and was kept isothermal under agitation for
 135 several hours until no powder clumps remained, and the solution was homogeneous. If required,
 136 Tween® 20 was then added dropwise and stirred for 10 minutes under gentle agitation to minimise
 137 foaming. Solutions were then stored overnight at room temperature (20 °C) until testing, at which
 138 time they were re-heated to 80 °C under gentle agitation. The total concentrations of biopolymers
 139 tested were 1 – 3 wt%, and surfactant concentrations were 0 – 1 wt%.

140 2.2.2 Rheological measurements

141 An MCR 302 rheometer (Anton Paar, Austria) equipped with a PP50-TG parallel plate geometry (D =
 142 50.0 mm) and a P-PTD200/62/TG lower plate geometry (D = 62 mm) was used to characterise the
 143 rheology of the samples. In all measurements, samples were loaded in liquid form at 80 °C and
 144 trimmed to a gap of 1 mm, with the geometry pre-heated to 60 °C to avoid pre-gelation ($\delta > 45^\circ$). A
 145 thin layer of silicone oil was immediately added to the outer edge of the samples to prevent
 146 evaporation and a Peltier hood (H-PTD-200) was lowered. For the amplitude and frequency sweep

147 measurements the temperature of the geometry was reduced to 20 °C and held isothermally for 5
148 minutes before proceeding.

149 *2.2.2.1 Amplitude sweep to determine the linear viscoelastic region (LVR)*

150

151 An amplitude sweep was performed from 0.01 to 100% strain, at a frequency of 6.28 rad s⁻¹ (1 Hz) and
152 a temperature of 20 °C. The linear viscoelastic region was determined as the range of strain values
153 which showed no significant degradation ($\pm 1\%$) in the value of the storage modulus (G').

154 *2.2.2.2 Temperature sweep to determine the gelling and melting temperatures*

155

156 A temperature sweeps was performed from 60 °C to 10 °C and back to 60 °C for each sample, in
157 triplicate. The applied strain was 1% as this was found to be within the LVR of the samples and all
158 sweeps were performed at a frequency of 6.28 rad s⁻¹ (1 Hz). The rate of temperature change was 1
159 °C per minute. Gelling and melting temperatures were determined by the crossover of the elastic (G')
160 and viscous (G'') modulus.

161 *2.2.2.3 Frequency sweep to determine the mechanical spectra and printability*

162

163 A frequency sweep was performed in triplicate, with the frequency varying between 62.8 rad s⁻¹ to
164 0.628 rad s⁻¹ (10 Hz to 0.1 Hz), at a strain of 1%. The measurements were performed at 20 °C.

165 *2.2.1 3D Printing*

166

167 A custom-built food 3D printing system was used in this study to conduct extrusion-based printing. 3D
168 digital design of the object was generated with Cura 15.04.6 (Ultimaker B.V., Netherlands). A 10 mL
169 syringe and a 22G needle (inner diameter 0.413 mm) were used for all samples. The syringe was filled
170 with liquid samples (80 °C). All samples were printed at a flow level of 60% (Derossi et al., 2018). The
171 cubes (15 mm × 15 mm × 15 mm) were printed for printability assessment. All objects were printed at
172 a printing bed temperature of 50 °C.

173

174 2.2.2 Release Measurements

175

176 A SevenEasy conductivity meter (Mettler Toledo, USA) was used to monitor conductivity. Gel cubes
177 (dimensions 15 mm × 15 mm × 15 mm) were formed in a custom 3D-printed mould by pouring liquid
178 samples (50 °C) in to the mould recess, covering with plastic film and were then stored at room
179 temperature overnight in an air-tight container before measurement. The bottom portion and sides
180 of the mould were wrapped in Parafilm® M tape to prevent sample leakage through the slightly porous
181 plastic. A 1 litre beaker was filled with 900 mL of distilled water and a custom 3D printed lid was placed
182 on top of the beaker. The lid contained holes, through which a conductivity probe and a temperature
183 probe were inserted, and a net which extended down in to the beaker for placement of the gel cubes.
184 The beaker was placed on a stirrer hotplate and the water was kept isothermal at either 20, 30 or 40
185 °C. The water was agitated gently at a constant rate of 100 rpm with a 70 mm pivoted PTFE stirrer bar.
186 Conductivity was logged via Mettler Toledo LabX Direct-pH software, which recorded the measured
187 conductivity once per second until it was manually stopped. The endpoint of the release measurement
188 was determined once the conductivity value was steady ($\pm 0.01 \text{ mS cm}^{-1}$) for 5 minutes.

189 2.2.3 Statistical Analysis

190

191 Data was analysed by calculating the Pearson correlation coefficient (ρ): the further the value of ρ
192 from 0 indicates a strong positive or negative correlation, depending on if the value of ρ is positive or
193 negative respectively. Data was fitted using the linear regression analysis and data populations were
194 compared using two-sample T-tests: both in the Analysis Toolpak for Microsoft Excel. A linear fit was
195 assumed statistically accurate if $R^2 \geq 0.90$ and populations were considered statistically different if
196 $P(\text{one-tail}) \leq 0.05$. Non-linear regression was performed using the curve fitting tool in SigmaPlot 14.5,
197 and a fit was assumed accurate if $R^2 \geq 0.90$.

198

199

200 3. Results

201

202 3.1 Rheology

203

204 3.1.1 *Temperature sweeps to determine the gelling and melting temperatures*

205

206 The values of the gelling (T_g) and melting (T_m) temperatures of LAG/TSX gels were obtained as a
207 function of total polymer concentration (at a constant polymer ratio) and a function of polymer ratio
208 (at a constant total polymer concentration). The addition of 1 wt% Tween[®] 20 on the value of the
209 gelling and melting temperatures was also investigated. All of these data are shown in Figure 1.

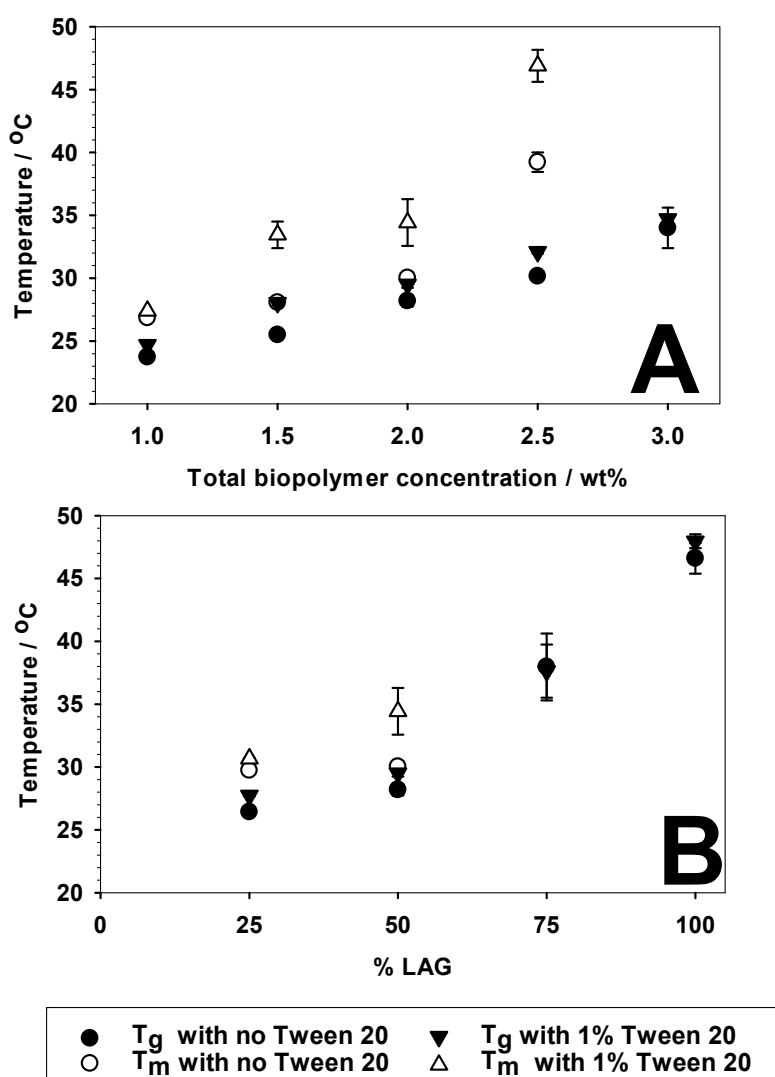
210 In general it was found that the values of the gelling and melting temperatures increased with total
211 biopolymer concentration ($\rho = 0.99$ and 0.90 respectively): at 1 wt% these values were ca. $24\text{ }^\circ\text{C}$ and
212 ca. $27\text{ }^\circ\text{C}$, increasing up to ca. $34\text{ }^\circ\text{C}$ and ca. $39\text{ }^\circ\text{C}$ at 2.5 wt% respectively.

213 At a total biopolymer concentration of 3 wt%, there was no crossover of the viscoelastic moduli
214 detected in the heating step of the temperature sweep i.e. the gel samples did not melt in the
215 experimental temperature range. The increase in gelling temperature values with the total biopolymer
216 concentration has a good linear fit ($R^2 = 0.99$), however this is untrue for melting temperatures ($R^2 =$
217 0.81), likely due to the discontinuous rise in T_m at 2.5 wt% total polymer concentration. The
218 introduction of Tween[®] 20 led to statistically significant increases in the gelling temperatures (ca. 2
219 $^\circ\text{C}$), and the melting temperatures (ca. $5\text{ }^\circ\text{C}$) as indicated by $P(\text{one-tail}) = 0.005$ and 0.03 respectively.

220 The increase in gelling temperature upon addition of surfactant was independent of total biopolymer
221 concentration ($\rho = -0.24$) yet the melting temperature was found to be dependent ($\rho = 0.92$), hence
222 indicating that the surfactant had a greater influence on the melting temperature of more
223 concentration formulations.

224 In the second set of data shown in Figure 1, the total biopolymer concentration was kept constant at
225 2 wt% and the polymer blend was varied: this concentration was chosen as it was a sensible value
226 based on the previous data set. At 0% LAG content, i.e. 100% TSX content, no gelling or melting was

227 detected, and at and beyond 75% LAG content, no melting was detected, hence there are no data at
 228 these points. Increasing the LAG content from 25% to 50% only lead to a very small increase in the
 229 values of the gelling temperature (ca. 1 °C), and the melting temperature remained constant,
 230 indicating very similar gel microstructures. However, as the LAG content was then increased to 75%
 231 and 100%, the gelling temperatures increased dramatically (ca. 10 °C per additional 25% LAG) and the
 232 melting temperatures increased to beyond the experimental maximum temperature (60 °C),
 233 indicating a significant microstructural change



234

235 *Figure 1 - Gelling and melting temperature data for LAG/TSX samples. In figure A, the ratio of LAG:TSX was kept at 1:1 and*
 236 *the total concentration of biopolymer was changed. In figure B, the total biopolymer concentration was kept constant at 2*
 237 *wt% and the ratio of LAG:TSX was varied. Values given are mean averages from three measurements and error bars*
 238 *represent standard deviation.*

239 Over the range of polymer blends tested, there was a strong positive correlation between the gelling
240 temperatures and the percentage of LAG ($\rho = 0.97$) however there were too few data points to
241 perform statistical analysis for the melting temperatures. The addition of 1 wt% Tween[®] 20 to these
242 samples, produced no statistical change in the phase transition temperatures of the LAG/TSX gels at a
243 constant biopolymer concentration ($P(\text{one-tail}) = 0.49$).

244 In summary, it was found that the only the total concentration of biopolymers was sensitive to the
245 addition of Tween[®] 20 with greater shifts in gelling and melting temperatures seen at higher total
246 polymer concentrations. In contrast, small increases in gelling and melting temperatures were seen
247 across all the blends tested, regardless of the polymer ratio.

248 For comparison, samples of 5 wt% gelatin with and without 1 wt% Tween[®] 20 were made, and the
249 measured gelling and melting temperatures were ca. 21.5 °C and 30.3 °C in each case, regardless of
250 the presence of surfactant. This data can be used to determine which, if any, of the LAG/TSX samples
251 would be potentially suitable as a gelatin replacement. Osorio *et al.* (2007) obtained gelling and
252 melting temperatures for gelatin using the crossover of the viscous and elastic moduli, and these
253 values are approximately 18 – 28 °C and 32 – 37 °C respectively, with thermal hysteresis values in the
254 order of ca. 10 °C: this agrees well with the measured values in our study.

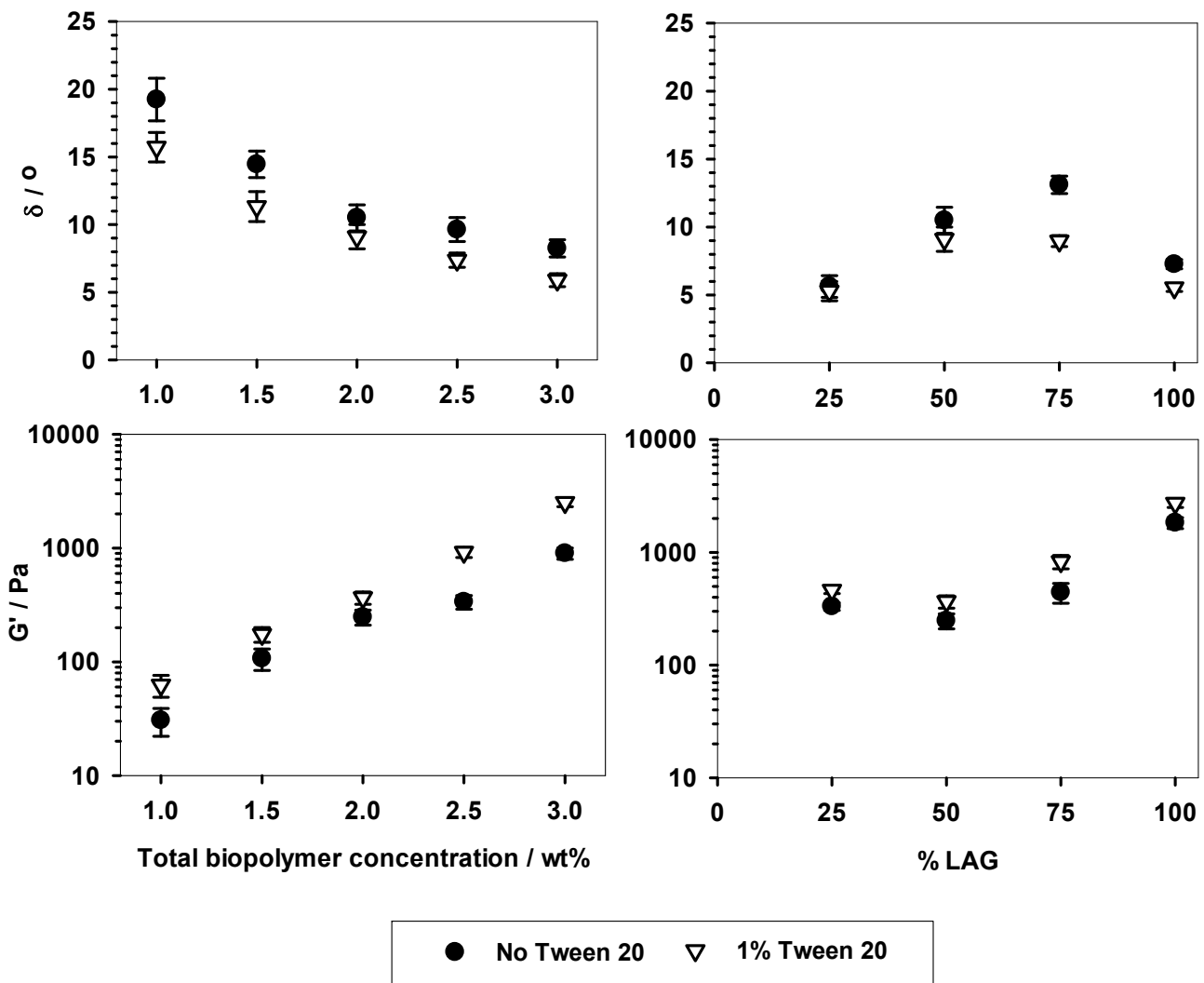
255 3.1.2 Frequency sweeps to determine the mechanical spectra

256 Frequency sweeps were conducted to determine two important rheological parameters: the phase
257 angle (δ) and storage modulus (G'), and their dependence on the measurement frequency. This was
258 performed for the LAG/TSX gels with or without the addition of 1 wt% Tween[®] 20, and the results are
259 shown in Figure 2. A larger error bar indicates that the sample was more frequency-dependent, and
260 hence more liquid-like.

261 As the total concentration of biopolymer was increased – again, at a fixed blend of 50:50 LAG:TSX – a
262 steady decrease in the phase angle was seen ($\rho = -0.95$), dropping from ca. 20° at a concentration of
263 1 wt% to ca. 7° at a concentration of 3 wt%. The standard deviation in the phase angle significantly

264 decreased as the total biopolymer concentration was increased ($\rho = -0.91$), so the samples became
265 less frequency-dependent. Conversely, the value of the storage modulus (G') increased exponentially
266 ($\rho = 0.93$, $R^2 = 0.98$) as the total biopolymer concentration was increased, from ca. 30 Pa to ca. 1000
267 Pa. All of these data suggested that increasing the total concentration of biopolymer increased gel
268 strength and made the gel more solid-like.

269 At a fixed biopolymer concentration of 2 wt%, the phase angle showed a steady increase from ca. 5°
270 to ca. 13° from 25 to 75% LAG content, followed by a decrease as the blend reached 100% LAG back
271 to ca. 7° , hence indicating that the gel was **least solid-like** at 75% LAG content. This was accompanied
272 by an initial decrease in the value of the storage modulus of 300 Pa to 250 Pa from 25% to 50% LAG
273 content, followed by another exponential increase to ca. 2000 Pa up to 100% LAG, hence indicating a
274 minimum in gel strength was measured at 50% LAG. A negative second-order polynomial trendline
275 showed good agreement for both the phase angle ($R^2 = 0.94$) and the storage modulus ($R^2 = 0.98$) with
276 respect to percentage LAG content.



277

278 *Figure 2 - Frequency sweep data for LAG/TSX samples, plotting phase angle (δ) or elastic modulus (G') against formulation*
 279 *parameters. Values given are mean averages from all data points in the three frequency sweeps, and error bars represent*
 280 *standard deviation.*

281 The frequency dependence was low ($SD \leq 1^\circ$) in all blends tested, yet it was still found to correlate in
 282 a strong negative relationship with the percentage of LAG ($\rho = -0.97$), indicating that the higher the
 283 percentage of LAG in a blend, the less frequency dependent, and more solid-like, the gel was.

284 In the constant blend experiments, the addition of 1 wt% Tween[®] 20 led to statistically significant
 285 decreases ($P(\text{one-tail}) = 0.0014$) in measured phase angles, in the order of 1 - 5°, compared to samples
 286 without surfactant. There was some indication that the storage modulus increased upon addition of
 287 Tween[®] 20 however these were found to be not statistically significant ($P(\text{one-tail}) = 0.098$). The size

288 of the reduction in phase angle upon addition of Tween® 20 was found to be mostly independent of
289 the total biopolymer concentration ($\rho = -0.64$) in this case.

290 In the constant total polymer concentration experiments, the addition of Tween® 20 showed the
291 largest decreases to the phase angle of LAG/TSX gels with the highest phase angle ($\rho = 0.87$): i.e., the
292 largest change was seen for the 75% LAG, 25% TSX sample which had the largest starting phase angle.
293 Furthermore, the magnitude of the increase in the storage modulus upon addition of Tween® 20 was
294 found to be strongly dependent on both the percentage of LAG in the blend ($\rho = 0.91$) and the initial
295 storage modulus ($\rho = 0.97$): the two are related. Hence, once again it is seen that the addition of
296 surfactant had the greatest influence on the weaker, low thermal hysteresis gels compared to the
297 stronger, high thermal hysteresis gels.

298 For comparison, frequency sweeps with formulations consisting of 5% gelatin with and without 1 wt%
299 Tween® 20 were performed. It was found that the storage moduli were 360 ± 40 Pa and 445 ± 48 Pa,
300 and the phase angles were $1.20 \pm 0.43^\circ$ and $1.17 \pm 0.44^\circ$ for the sample without and with Tween® 20
301 respectively. This provided a target storage modulus and phase angle for a gelatin replacement.

302 3.2 3D Printing

303

304 3.2.1 Rheology to predict printability

305

306 The printability of a range of LAG/TSX and gelatin gels was predicted using rheological data obtained
307 from frequency sweeps. Phase angles (δ) and the relaxation exponents (m) can be used to predict the
308 suitability of a material to being manufactured using a 3D printing process (Gholamipour-Shirazi,
309 Norton, & Mills, 2019). One can obtain the relaxation component by noting the dependence of the
310 elastic modulus (G') on angular frequency (ω) in a power-law relationship as shown in Equation 1
311 (Kavanagh & Ross-Murphy, 1998), using frequency sweep data. Previous work by Gholamipour-Shirazi,
312 Norton and Mills (2019) showed that if $3^\circ < \delta < 15^\circ$ and $0.03 < m < 0.13$, the formulation was printable.
313 The summary of printability results for LAG/TSX and LAG/TSX/Tween® 20 gels are shown in Tables 2A

314 – 2D. Formulations were stated to be ‘feasibly printable’ if the required rheological parameters were
 315 partially in the required range, and ‘printable’ if the parameters were fully in the required range,
 316 including any standard deviation. As can be seen, $R^2 \geq 0.95$ in all LAG/TSX samples, with and without
 317 surfactant, indicating a good fit to the power-law equation.

318

319 $G'(\omega) \approx k_1 \omega^m$ (1)

320

321 *Table 2A – Table of rheological parameters used to determine printability based on the formula given in Equation 1. All*
 322 *samples consisted of a 50:50 blend of LAG:TSX whilst the total polymer concentration was varied. Values given are mean*
 323 *averages from three frequency sweeps with standard deviation. († = mean values are in the required range however*
 324 *printability is uncertain due to calculated error).*

Total gelling biopolymer concentration (wt %)	δ (°)	k_1 (Pa s rad ⁻¹)	m	R ²	Printable?
1	19.24 ± 1.57	20.75 ± 0.87	0.190 ± 0.002	0.9990	No
1.5	14.45 ± 0.98	79.93 ± 2.96	0.14 ± 0.04	0.9992	No
2	10.50 ± 0.96	203.6 ± 10.3	0.10 ± 0.05	0.9970	Yes [†]
2.5	9.42 ± 0.81	359.4 ± 16.2	0.09 ± 0.05	0.9964	Yes [†]
3	8.24 ± 0.64	811.1 ± 31.6	0.07 ± 0.04	0.9965	Yes

325

326 *Table 2B – Table of rheological parameters used to determine printability based on the formula given in Equation 1. All*
 327 *samples consisted of 1 wt% Tween 20 with a 50:50 blend of LAG:TSX whilst the total polymer concentration was varied.*
 328 *Values given are mean averages from three frequency sweeps with standard deviation. († = mean values are in the required*
 329 *range however printability is uncertain due to calculated error).*

Total gelling biopolymer concentration (wt %)	δ (°)	k_1 (Pa s rad ⁻¹)	m	R ²	Printable?
1	15.72 ± 1.09	51.91 ± 5.25	0.14 ± 0.10	0.9947	No
1.5	11.33 ± 1.10	152.9 ± 8.8	0.10 ± 0.06	0.9974	Yes [†]
2	9.10 ± 0.90	326.2 ± 13.4	0.083 ± 0.041	0.9968	Yes
2.5	7.38 ± 0.53	838.2 ± 29.1	0.068 ± 0.035	0.9967	Yes
3	5.90 ± 0.48	2334 ± 61	0.055 ± 0.03	0.9962	Yes

330

331 *Table 2C – Table of rheological parameters used to determine printability based on the formula given in Equation 1. All*
 332 *were formulated at 2 wt% total gelling biopolymer concentration whilst ratio of LAG:TSX was varied. Values given are mean*
 333 *averages from three frequency sweeps with standard deviation. ([†] = mean values are in the required range however*
 334 *printability is uncertain due to calculated error).*

% LAG	δ (°)	k_1 (Pa s rad⁻¹)	m	R²	Printable?
100	7.26 ± 0.34	1578 ± 4	0.078 ± 0.002	0.9992	Yes
75	13.11 ± 0.64	336.8 ± 7.2	0.14 ± 0.02	0.9994	No
50	10.50 ± 0.96	203.6 ± 10.3	0.10 ± 0.05	0.9970	Yes [†]
25	5.63 ± 0.80	302.7 ± 12.6	0.049 ± 0.042	0.9913	Yes

335

336 *Table 2D – Table of rheological parameters used to determine printability based on the formula given in Equation 1. All*
 337 *were formulated with 1 wt% Tween 20 at 2 wt% total gelling biopolymer concentration whilst the ratio of LAG:TSX was*
 338 *varied. Values given are mean averages from three frequency sweeps with standard deviation.*

% LAG	δ (°)	k_1 (Pa s rad⁻¹)	m	R²	Printable?
100	5.53 ± 0.27	2455 ± 6	0.057 ± 0.002	0.9972	Yes
75	8.96 ± 0.40	701.1 ± 9.1	0.092 ± 0.013	0.9992	Yes
50	9.10 ± 0.90	326.2 ± 13.4	0.083 ± 0.041	0.9968	Yes
25	5.28 ± 0.71	443.1 ± 16.6	0.043 ± 0.037	0.9900	Yes

339

340 *Table 2E - Table of rheological parameters used to determine printability based on the formula given in Equation 1. All were*
 341 *formulated with 5 wt% gelatin whilst the concentration of Tween 20 was varied. Values given are mean averages from*
 342 *three frequency sweeps with standard deviation.*

Concentration Tween 20 (wt %)	δ (°)	k_1 (Pa s rad⁻¹)	m	R²	Printable?
0	1.20 ± 0.43	518.6 ± 106.6	-0.040 ± 0.21	0.8730	No
1	1.17 ± 0.44	633.0 ± 124.6	-0.039 ± 0.203	0.8847	No

343

344 Upon addition of 1 wt% Tween 20 to the samples ascribed in Table 2A (Table 2B), the phase angle and
 345 the relaxation exponent decreased ($P(\text{one-tail}) = 0.01$), hence the samples became more printable.
 346 Without surfactant, formulations at 2 - 2.5 wt% total biopolymer concentration were feasibly printable
 347 and 3 wt% was predicted to be printable. Whereas upon addition of 1 wt% surfactant, 1.5 wt% samples
 348 were now feasibly printable, and printable at 2 wt% and beyond.

349 The ratio of biopolymers in the blend was also investigated to see if it influenced printability (Table
 350 2C). It was discussed previously that the phase angle had a parabolic relationship with the percentage
 351 of LAG (at a constant total concentration of gelling biopolymers). In this case the parabolic behaviour
 352 can be seen to carry over, with 75% LAG being the only blend not deemed printable due to its high

353 mean phase angle. As previously, the addition of 1 wt% Tween 20 was investigated (Table 2D). Apart
354 from the 25% LAG blend, both the phase angle relaxation exponent showed significant changes upon
355 addition of Tween 20 ($P(\text{one-tail}) = 0.04$), and all samples became printable.

356

357 For comparison, a sample of 5 wt% gelatin with and without 1 wt% Tween 20 was investigated in an
358 identical manner (Table 2E). It was found that the formulations were not printable since the phase
359 angle and relaxation component were too small for the printing requirements.

360 3.2.2 3D printing of samples

361 Following the printability predictions, three LAG/TSX samples of varying formulation were tested for
362 real-world printability. The samples tested were 0.5 wt% LAG/1.5 wt% TSX, 1.25 wt% LAG/1.25 wt%
363 TSX, and – 1 wt% LAG/1 wt% TSX. Concentrations were chosen to represent a range of printability
364 values within the permitted values for printability (Gholamipour-Shirazi et al., 2019). When comparing
365 the predicted printability of the samples to the printed samples, shown in Figure 3, the images suggest
366 that the rheological rules outlined by Gholamipour-Shirazi, Norton and Mills (2019) were generally a
367 good indicator of printability. All samples were predicted to be printable, based on the value of the
368 phase angle and the release exponent, however samples B and C could only be predicted to be feasibly
369 printed because of the error in the measurements extended outside the printable parameters. In each

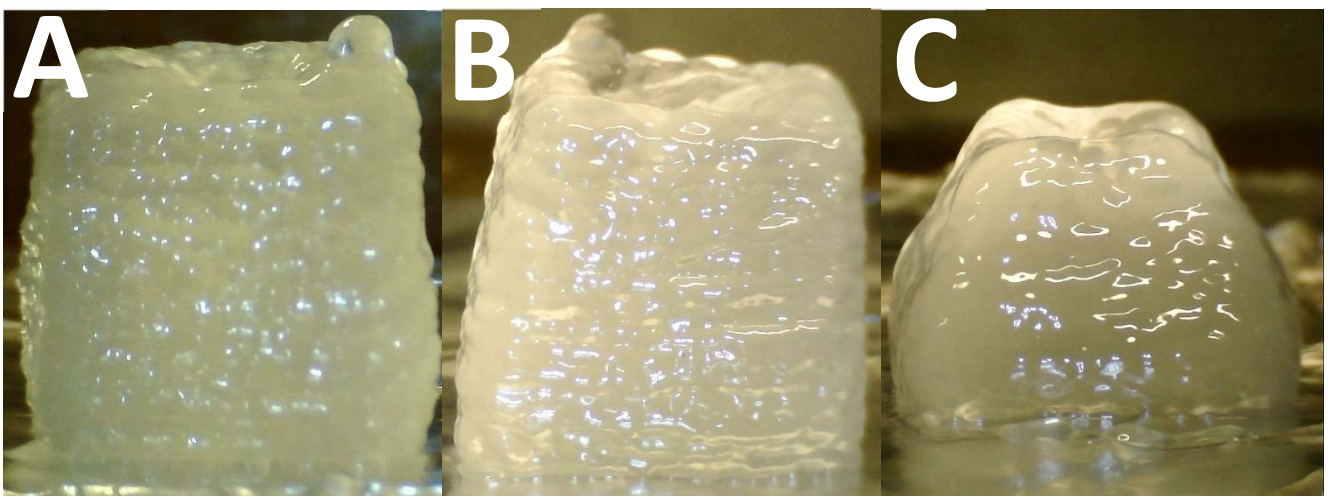


Figure 3 - Printed LAG/TSX samples. (A) – 0.5 wt% LAG/1.5 wt% TSX, (B) – 1.25 wt% LAG/1.25 wt% TSX, (C) – 1 wt% LAG/1 wt% TSX.

370 sample successful printing was achieved, with varying degrees of accuracy. Samples A and B were
371 printed with good accuracy and the finished gel resembled a cube geometry. Sample C, did form a
372 self-supporting gel, however it is clear that the gel sagged post-printing made evident by the
373 narrowing of the cube nearer the top.

374 3.3 Measuring temperature-mediated release from moulded and printed gels

375 Following from the rheological data in section 3.1 and the printing data in 3.2, two LAG/TSX/Tween®
376 20 blends were chosen to represent potential gelatin replacements in release studies: these samples
377 were 0.5 wt% LAG/1.5 wt% TSX/1 wt% Tween® 20 and 1 wt% LAG/1 wt% TSX/1 wt% Tween® 20. These
378 concentrations were chosen because the measured melting temperatures were ca. 31 °C and 36 °C:
379 at each extremity for the theoretical melting requirements of a gelatin replacement (greater than
380 ambient temperature but below body temperature), and the storage moduli were very similar (within
381 100 Pa) of the measured storage modulus of 5 wt% gelatin with 1 wt% Tween® 20, hence the gel
382 strength was similar. Additionally, based on the printing of samples in section 3.2.2, both samples
383 were predicted to be printable and so the influence of moulding versus printing the samples on the
384 release behaviour could be investigated. To this end, a sample of 0.5wt% LAG/1.5 wt% TSX/1 wt%
385 Tween 20 was printed and compared to a moulded sample: all other samples in the release studies
386 were moulded. The release profiles of the gels were measured in water at 20, 30 and 40 °C using
387 conductivity, and the results are shown in Figure 4.

388 The 5 °C difference in melting temperatures between the two LAG/TSX blends led to significant
389 differences in the release profiles. At 20 °C, the release time for both the gels was similarly slow
390 (several hours), however, at 30 °C the 0.5 wt% LAG/1.5 wt% TSX/1 wt% Tween® 20 gel showed rapid
391 melting behaviour, reaching a maximum conductivity value in under ten minutes, with 40 °C taking
392 less than three minutes. Conversely, the 1 wt% LAG/1 wt% TSX/1 wt% Tween® 20 took over an hour
393 to reach maximum conductivity at 30 °C, and under ten minutes at 40 °C.

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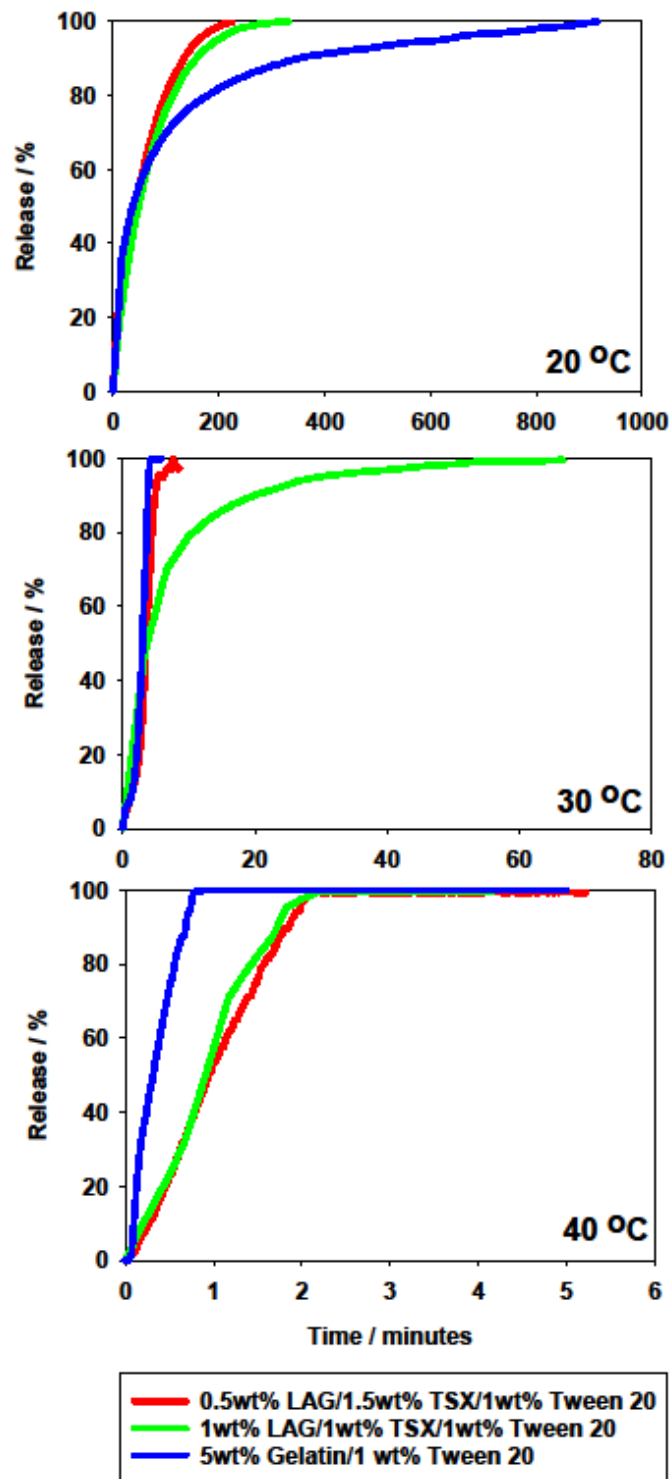
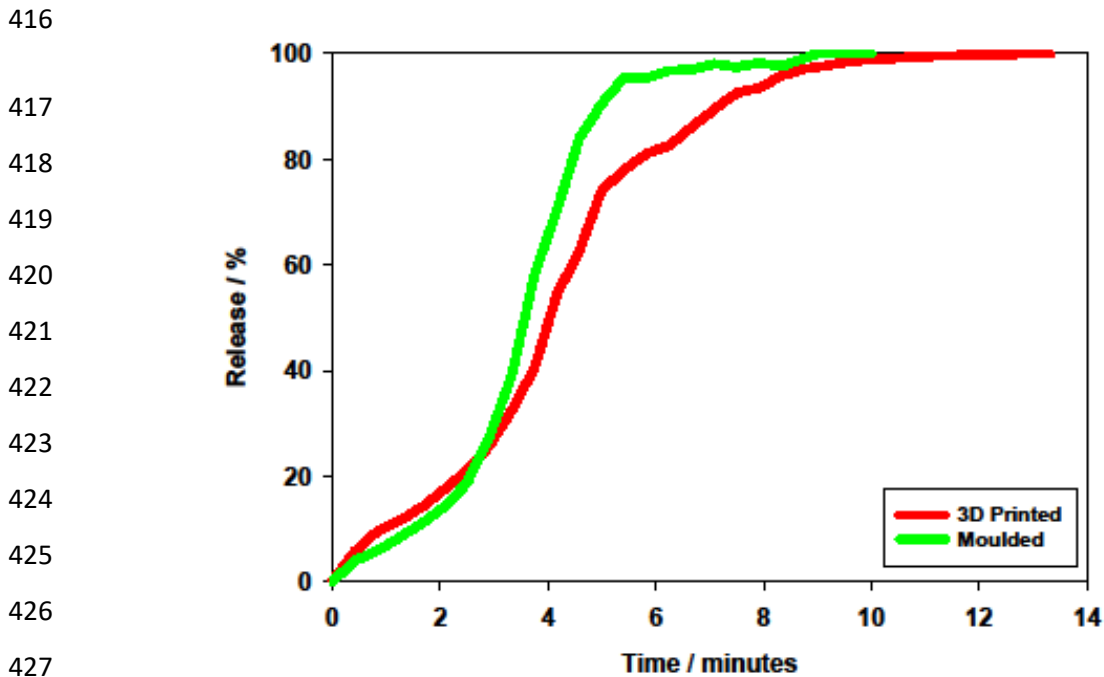


Figure 4 – Release data obtained from conductivity measurements of LAG/TSX/Tween and Gelatin/Tween Gels.

413 As a reference the release of 5 wt% gelatin with 1 wt% Tween® 20 was analysed in the same manner:
414 release at 20 °C took nearly sixteen hours, yet at 30 °C and 40 °C maximum conductivity was achieved
415 in approximately five minutes and one minute respectively.



429 *Figure 5 - A comparison of release data from a 3D printed and a moulded sample of 0.5wt% LAG/1.5wt% TSX/1wt% Tween®*
430 *20.*

431 Furthermore, a comparison between gel samples manufactured using 3D printing and moulding was
432 performed to see if the release profiles were similar, as shown in Figure 5. To this end, a sample of
433 0.5wt% LAG/1.5wt% TSX/1wt% Tween® 20 was prepared, printed and tested for release at 30 °C. The
434 initial findings suggest that there was no statistical difference between the release profiles for the
435 moulded gel and the printed gel. Further investigation between moulded and 3D printed samples was
436 intended, however complications due to COVID-19 made further production of 3D printed samples
437 unattainable for the foreseeable future.

438 **4. Discussion**

439
440 The rheological data in section 3.1 showed a statistically significant relationship that as the total
441 biopolymer concentration was increased this led to a greater gelling temperature, melting

442 temperature, storage modulus and a lower phase angle. This is a common occurrence for many gelling
443 biopolymers that gel with a cation-driven helical-aggregation mechanism, such as kappa-carrageenan
444 or gellan. At a higher polymer concentration – and therefore gelling cation concentration – greater
445 aggregation of helices can take place, owing to better screening of repulsive charges on adjacent
446 helical aggregates. This leads to more thermally stable aggregates that can form at higher
447 temperatures and consequently need a higher temperature to overcome, explaining the greater
448 gelling and melting temperatures. The increased helical aggregation also explains the greater storage
449 modulus and lower phase angle as gel strength and solid-like character of the gels increases in such
450 circumstances. Similar rheological trends in gellan with polymer and cation concentration have been
451 reported by previous authors (Lee et al., 2011; Picone & Cunha, 2011).

452 The blend ratio, however, showed a more variable influence on the rheological properties of the gels.
453 The gelling temperature showed a strong positive correlation with LAG content: this is expected due
454 to the high gelling temperatures of LAG, and as the blend of LAG increased, the more LAG aggregates
455 can form. Hence it is predicted that the gelling temperatures are controlled by the LAG content. In
456 contrast, the storage modulus and phase angle had parabolic relationship with the LAG content, with
457 a maximum in phase angle achieved at 75% LAG and a minimum in storage modulus at 50% LAG. If
458 the morphology of the LAG/TSX was phase separated, one would expect a gradual decrease in phase
459 angle and an increase in storage modulus as the LAG content was increased, due to the fact that TSX
460 does not form a gel network on its own – such as the trends observed in the gelling temperature.
461 Therefore, the strong mechanical properties at 25% LAG content suggest the existence of some
462 coupled polymer network. Nevertheless, the purpose of this paper is not to unravel the mechanism
463 of LAG and TSX interactions, however these observations invoke some interesting questions.

464 In the constant blend experiments, the introduction of Tween® 20 to the LAG/TSX gel mixture led to
465 an increase in gelling temperature, melting temperature and storage modulus, and a reduction in the
466 phase angle and frequency dependence across the tested samples. Whereas in the constant total

467 biopolymer concentration experiments, only the frequency dependence and the magnitude of the
468 increase in the storage modulus showed change upon the addition of Tween® 20. Pragmatically, this
469 means that the total biopolymer concentration is the primary factor in determining the rheological
470 properties of the gels, and the blend of LAG and TSX is secondary to this.

471 The alteration in rheological properties upon the addition of Tween® 20 is unlikely to simply be due
472 to an increase in the effective concentration of the gelling biopolymers: an increase in polymer
473 concentration by 1% would not explain the magnitude observed change in rheological behaviour.
474 Volume exclusion effects could theoretically increase the effective polymer concentrations, however
475 this is unlikely to be responsible for the shift in properties due because Tween® 20 micelles occupy a
476 very similar volume to the equivalent mass of water that they replace, i.e. in micellar form, the density
477 of Tween® 20 is very similar to that of water. Previous work concerning formulations of an anionic
478 polymer with a non-ionic surfactant showed a similar increase in the gelling and melting temperature
479 compared to the data reported here, and this was attributed to electrostatic shielding of the charged
480 polymer helices by the surfactant micelles which enhanced helical aggregation (Fenton, Kanyuck,
481 Mills, & Pelan, 2021; Yang & Pal, 2020). Fortunately, the magnitude of the increase in gelling, and more
482 importantly, the melting temperatures was such that the LAG/TSX system could still be viable as a
483 gelatin replacement.

484 Work set out in section 3.2, used the previously obtained rheological data to evaluate the printability
485 of the samples. Generally, the samples found to be most printable were those whose total gelling
486 biopolymer concentration was at least 2 wt%, or 1.5 wt% if 1 wt% Tween 20 was added. Printability
487 was predicted to improve as biopolymer concentration was increased up to 3 wt%. Furthermore,
488 printability was influenced by the LAG:TSX blend, with the 75% LAG blend being the least printable of
489 all samples tested, and the 25% LAG blend the most printable. The addition of 1 wt% Tween® 20
490 improved the printability parameters of almost all samples tested, by decreasing the phase angle and
491 the relaxation exponent and in no case did the printability parameters become less favourable. This is

492 likely because there was a small microstructural change upon the addition of Tween® 20, as discussed
493 previously, and this simply led to samples which were more rheologically suited for a printing process.
494 When examining the data for 5 wt% gelatin, it is clear that the samples were unprintable, hence the
495 novelty of this formulation can be extended beyond providing similar mechanical and melting
496 behaviours from a renewable, vegan formulation to being processible using additive manufacturing.

497 The significant difference in real-world printability between samples B & C is contrasted by their
498 apparent similarities in both their measured phase angles and relaxation exponents, with differences
499 of ca. 1° and 0.01 respectively, with both samples showing feasible printability. This suggests that the
500 rules set out by Gholamipour-Shirazi, Norton and Mills (2019) successfully predicted if a sample was
501 *at all* printable, but for samples that have printability parameters that are close to printability limits,
502 the variability in rheological measurements make it difficult to successfully predict printability.
503 Additionally, there may be another factor affecting the accuracy of the printed shape such as gelling
504 temperature or gelation time. According to the measured values in section 3.1, sample B had a gelling
505 temperature of ca. 2 °C higher than sample C: whether such a small difference in gelling temperature
506 would lead to a marked difference in printability is debatable. If so, reducing the extrusion
507 temperature or the bed temperature may improve the printability of samples with a lower gelling
508 temperature.

509 The release profiles obtained in 3.3 are useful for indicating if the gel formulations could be suitable
510 gelatin replacements, by seeing if they mimic the same melt-in-the-mouth behaviour that gelatin
511 shows. From the release profiles obtained for the 5 wt% gelatin gel, it is clear that the requirement
512 must be quick melting at 30 °C (under 10 minutes) and even quicker melting at 40 °C (1-2 minutes).
513 Similar melting behaviour has been reported previously for gelatin (Mills, Spyropoulos, Norton, &
514 Bakalis, 2011). At 20 °C, the release profiles for both of the moulded LAG/TSX gels was similarly slow
515 – ca. 2-3 hours – and the release at this temperature for the LAG/TSX gels was driven mainly by
516 diffusion of counter-ions out of the gel and subsequent erosion of the weakened gel layer. Slight

517 differences between different LAG/TSX blends arise at this temperature due to slightly different gel
518 porosities and counter-ion concentrations within the gel, even though both gels have a melting
519 temperature well above 20 °C. Gelatin, on the other hand, took significantly longer to show 100%
520 release at 20 °C and that because the chemical nature of the gel network is markedly different: in the
521 case of LAG/TSX, it relies on the existence of counter-ions to 'glue' the helices together in an
522 aggregated network, and hence if these counter ions diffuse out of the gel in to water then the gel
523 network collapses and is simply eroded from the outside, inwards, as diffusion of ions from the surface
524 of the gel is much faster compared to diffusion from the bulk gel. As the gel is eroded this speeds up
525 further diffusion of counter-ions from the gel by increasing the porosity of the gel and reducing the
526 diffusion distance between counter-ions and the water. However, in the case of gelatin the movement
527 of counter ions from the gel is pure-diffusion since there is no erosion, resulting in much slower
528 diffusion (Mills et al., 2011). This mechanism of erosion in LAG/TSX versus pure diffusion in gelatin is
529 further evidenced by the fact that at the end of the release experiments at 20 °C, there was no solid
530 material left in the LAG/TSX experiments, whereas for gelatin there was no observable change in the
531 physical dimensions of the gel compared to the start of the experiment. This diffusion-erosion
532 mechanism is shown graphically in Figure 6. The results suggest that the 0.5 wt% LAG/1.5 wt% TSX
533 polymer blend has a much more similar melting behaviour to gelatin compared to 1 wt% LAG/1 wt%
534 TSX, because at 30 °C release was comparatively much slower in the latter blend, than the former,
535 which was much closer to those results obtained for gelatin.

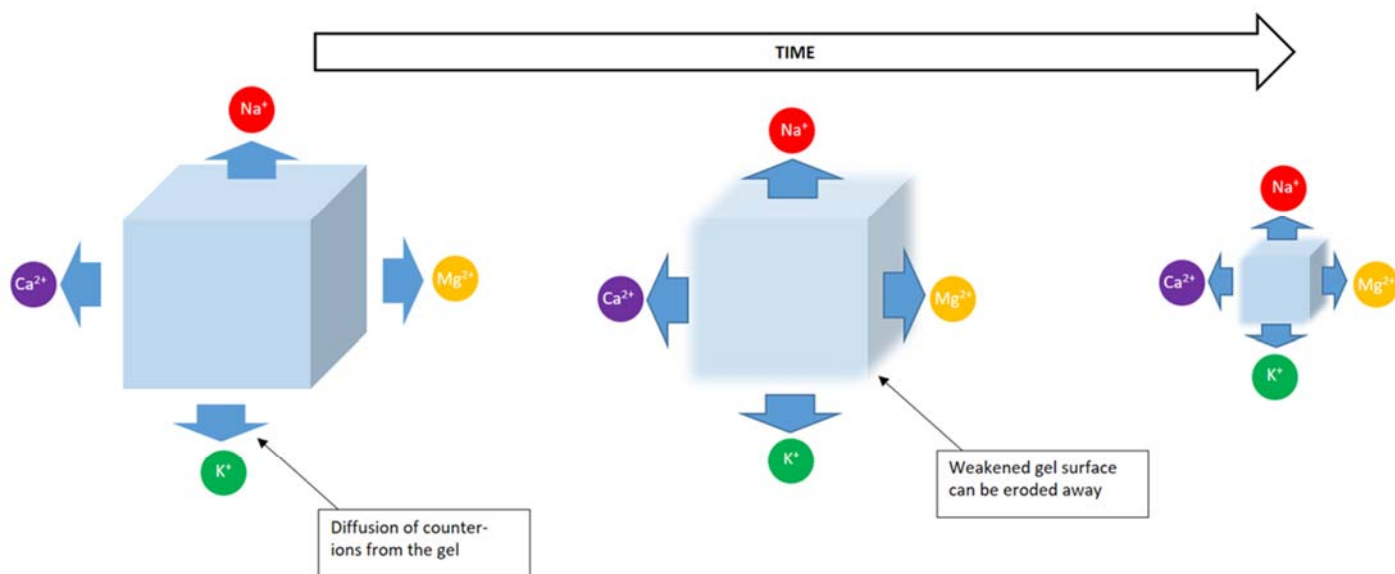


Figure 6 - Mechanism of erosion in LAG/TSX gels in water at temperatures below their melting temperature.

537 When comparing the release curves for a moulded sample compared to the 3D printed sample, the
 538 profiles are indistinguishable from one another, indicating that the 3D printing process did not
 539 significantly alter the structure of the final gel, and either technique could be used to manufacture gel
 540 samples. However, the authors recognise that further data in this area would be advantageous.

541 5. Conclusions

542

543 Overall these data have shown that the formulation engineering approach of a vegetarian gelatin
 544 replacement using low acyl gellan and tamarind seed xyloglucan is possible. The most similar blend
 545 tested was 0.5 wt% LAG/1.5 wt% TSX, which showed very similar gel strength and melting behaviour
 546 to 5 wt% gelatin. Furthermore it was shown that LAG/TSX gels can be manufactured using 3D printing,
 547 whereas this is not possible for gelatin gels. The introduction of Tween® 20 to the LAG/TSX
 548 formulations had no detrimental effect on the printability of the LAG/TSX samples, and in many cases
 549 improved printability. Additionally, there was a small increase in the measured gelling and melting
 550 point temperatures which could influence efficacy in some applications if melting is required. The
 551 origin of these phenomena is currently unknown due to poor understanding of the mechanism of

552 LAG/TSX interactions and how the formation of the hybrid gel network forms and exactly what
553 influence the surfactant has on the junction zones of the LAG/TSX system. Further work could involve
554 more clearly elucidating the nature of this interaction with advanced imaging techniques such as
555 scanning electron microscopy (SEM). The LAG/TSX system represents an exciting avenue for further
556 research in to gelatin replacements.

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558

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563 7. References

564

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