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Improvements in gelatin cold water solubility after electrospinning and associated physicochemical, functional and rheological properties

Behrouz Ghorani[1], Bahareh Emadzadeh[1], Hassan Rezaeinia[1] & Stephen.J.Russell[2]

[1]Department of Food Nanotechnology, Research Institute of Food Science and Technology (RIFST), km 12 Mashhad-Quchan Highway, Mashhad, Iran, P.O.Box : 91895-157-356

[2]School of Design, University of Leeds, Leeds, LS2 9JT, UK

Correspondingauthor* :Dr.BehrouzGhorani , Department of Food Nanotechnology

Research Institute of Food Science & Technology (RIFST), km 12 Mashhad-QuchanHighway,KhorasanRazavi,Mashhad,Iran,P.O.Box : 91895-157-356

E-mail: b.ghorani@rifst.ac.ir

Phone : +98-513-5425386

Fax : +98-513-5425406

Abstract

A major limitation of gelatin feedstocks for industrial food and pharmaceutical applications is the lack of solubility at room temperature, necessitating use of drum/dry blending processes, combined with additives. Herein, electrospinning is investigated as an alternative route for producing cold water soluble 100% gelatin feedstock in place of powders. The physicochemical, rheological and functional properties of electrospun gelatin and an industrially available gelatin powder feedstocks were compared. Optimal conditions for producing gelatin nanofiber sheets were found to be 25% (w/v) polymer concentration in a binary solvent system of acetic acid: water (3:1), a spinning voltage of 25 kV, a flow rate of 0.5 ml/h and a tip-to-collector distance of 150 mm. The production of nanofibers from gelatin powder did not change the nature of the material. The glass transition temperature of gelatin nanofibers was lower than gelatin powder. Conversion of gelatin powder into nanofiber sheets also increased the dissolution rate in water at ambient temperature and promoted emulsion and foam forming ability, as well as increasing foam stability. Loss tangent measurements revealed that the gel formed by the gelatin nanofibers could be characterized as a weak gel. No difference was observed in the Young's modulus of samples made from gelatin nanofibers and powder, and the 0.2% (w/v) gelatin nanofiber sample yielded a higher viscosity than the 0.1% (w/v) concentration. Gelatin nanofibers have promising potential to be used as feedstock in food technology when cold water solubility and improved control of physical, functional and textural properties are required.

Keywords: Gelatin; Nanofibers; Electrospinning; Physicochemical properties; Functional properties; Gel

1. Introduction

Gelatin is one of the most widely used hydrocolloids in the food industry for production of for example, desserts, jellies, sauces, baked goods, meat and low-fat products. Its unique properties and ability to improve stability and viscosity, as well as carrying capacity for bioactive and pharmaceutical compounds, has created demand across various industry sectors (Karim & Bhat, 2008). Given its biocompatibility, biodegradability, and wide availability, it is one of the most commonly used FDA approved biopolymers (Karim, et al., 2008). However, there are limitations in terms of its basic properties for food and pharmaceuticals processing. In cold water, gelatin hydrates and swells, and above 40ºC, a colloidal solution is produced (sol). The lack of cold water solubility is problematic in a variety of food and pharmaceutical manufacturing processes (M. Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011). Methods of increasing the cold water solubility of gelatin, particularly those that confer instant solubility are therefore of high industrial importance. There is a particular need in the manufacture of products such as whipped cream powders, ready-to-use cake powders, and heat-sensitive foodstuffs (Alting & van de Velde, 2012; Muller, 1987; Phillips & Williams, 2011; Williams & Phillips, 2009).

Currently, cold-soluble gelatin is produced by drum drying or dry-blending using carbohydrates, acids or urea that results in amorphous gelatin powder. However, there are some limitations associated with instantly water soluble gelatin products made by these systems. For example, dry blending relies heavily on additives to overcome sensitivity to moisture and the low wettability and modulus of resulting cold gels is also problematic (Steyaert, Rahier, Van Vlierberghe, Olijve, & De Clerck, 2016).

Nanotechnology in the form of electrospinning is an alternative approach that has potential to address current limitations and enable cold water soluble gelatin products to be made that are easy to handle and mix with other foodstuff components. Gelatin

3

95   nanofiber feedstocks may also provide scope for higher rates of dissolution than can be

96   currently achieved using powders, and without the use of additives.

97   Electrospinning or electrostatic spinning is an established method of producing

98   nanofibers for various industries, including the food and pharmaceutical sectors. The

99   process can take various forms, but in its simplest configuration, a polymeric solution

100   is pumped into the tip of a hollow needle, where the effects of a high voltage electric

101   field on the polymer stream, and the resulting instabilities produces thin polymer

102   streams that solidify into fibers, forming a self-supporting fibrous web on a collector

103   (Ghorani & Tucker, 2015). The nanofibrous web is porous and consists of a planar,

104   sheet-like network of amorphous fibers, with a high surface to volume ratio (Aytac,

105   Ipek, Erol, Durgun, & Uyar, 2019; Li, Yang, Yu, Du, & Yang, 2018). Electrospinning

106   has been proposed as a promising method of producing instant cold-water soluble

107   gelatin, with gelatin nanofibers dissolving in cold water up to concentrations of 5%

108   (w/w), and having the capability to form gels at room temperature (Steyaert, et al.,

109   2016). However, the physical, functional and rheological properties of electrospun

110   gelatin structures, in relation to gelatin powder are still not fully understood, and the

111   purpose of this paper is to address this gap to enable the potential for nanofiber

112   feedstocks in food technology to be comprehensively addressed.

113

114   **2. Materials ad Methods**

115   **2.1 Materials**

116   Gelatin powder (CAS number: 9000-70-8/Type A, Bloom value about 110), acetic acid glacial

117   (purity >99.8%, Molar Mass=60.05 g/mol, boiling point =118ºC and flash point =39ºC) and

118   ethanol (absolute, HPLC grade, ≥99.8%) were purchased from Merck company (Germany). All

119   the chemicals used without any further purification. The water was deionized (DI water) using

120   a Milli-DI water purification system and used as received.

4

121

2.2 Methods

2.2.1 Preparation of Gelatin Solution

A binary solvent system of acetic acid: water (3:1) was used to electrospin gelatin at various concentrations from 10% to 25% (w/v). To this end, 7.5 ml of acetic acid was carefully mixed with 2.5 ml water (making a total volume of 10 ml). This particular solvent system and ratio were selected based on preliminary experimental work and previously published papers (Erencia, et al., 2015). Optimal conditions for electrospinning of gelatin nanofibers were selected by considering morphology (freedom from beads and defects) and needle blockages affecting the continuity of the process. Spinning solutions were prepared by dissolving gelatin in 10 ml of a binary mixture of acetic acid: water (3:1) and slowly stirring for 60 min at room temperature. All the solutions were then stored at room temperature to remove air bubbles before electrospinning.

2.2.2 Electrospinning

A fully automatic triple-head lab-scale electrospinning machine (ES-Lab RN/X, ANSTCO, Iran) was used for gelatin electrospinning experiments. The polymer solution was loaded into a 10 ml plastic syringe connected to a to a blunt-ended Luer Lock metal needle (Gauge18-Sigma-Aldrich) with a nominal inner diameter of 0.84mm. Electrospinning parameters were fixed at 0.5 ml/hr flow rate, 25 kV applied voltage and a needle tip-to-collector distance of 150 mm. A stationery collector (5×5 cm^2) wrapped in aluminum foil from both-sides were used for the collection of nanofibers. Electrospinning was carried out at ambient temperature (25 ± 5ºC) and a relative humidity of 50% for 30 min.

2.2.3 Field Emission Scanning Electron Microscopy (FESEM)

147 A field emission scanning electron microscope (FESEM) (MIRA3, TESCAN - Czech

148 Republic) was used to study the morphology of gelatin powder and the electrospun

149 nanofibers. Samples were carefully adhered to a SEM stub (diameter=12 mm) and sputter

150 coated with gold (Q150R Rotary-Pumped Sputter Coater, Quorum Technologies Ltd.,

151 UK) under vacuum (2mbar) for 5 min at an accelerating potential of 20 kV. The average

152 fiber diameter of the electrospun gelatin nanofibers was determined by measuring 50

153 randomly selected fibers in the FESEM images using Image-Pro Plus 7.0 Software (US)

154 (Ghorani, Kadkhodaee, Rajabzadeh, & Tucker, 2019).

155

156 **2.2.4 Atomic force microscopy (AFM)**

157 The surface morphology of the electrospun gelatin nanofibers was studied by atomic force

158 microscopy (AFM) (Ara-AFM Research, Model 0103/A, Iran). The images were scanned

159 in contact mode with silicon tip at a resonance frequency of 180 kHz (Miri, et al., 2016).

160 The captured images were then processed using Imager software (AFM Ara-Imager, V.2).

161

162 **2.2.5 Fourier-transform infrared (FTIR) spectroscopy**

163 FTIR spectroscopy enabled identification of functional groups in all samples as well as

164 insights into any structural changes in the gelatin powders associated with the solvent

165 system and electrospinning process. Samples were weighed, mixed with KBR and then

166 pressed into pellets. Scanning was regulated from 4000 to $400cm^{-1}$ with a resolution of

167 $4cm^{-1}$ and a scanning interval of $2cm^{-1}$ taking an average of 64 scans per sample using a

168 FTIR spectrometer (Thermo Nicolet Avatar 370, US) (Erencia, et al., 2015).

169

170 **2.2.6 X-Ray diffraction analysis (XRD)**

171 X-ray diffraction (XRD) was used to study crystallinity changes in the gelatin materials.

172 This analysis was carried out in an X-ray diffractometer equipment (Unisants, XMD-300,

173 Singapore) using Cu Kα radiation source (λ=1.5418) operating at irradiation conditions

174 40 kV and 40 mA. X-ray diffractogram was obtained in the range of 2θ=5-40º,scanning

175 rate of 1º/min at ambient temperature (25ºC) (Ki, et al., 2005).

176

177 **2.2.7 Differential scanning calorimetry (DSC)**

178 Thermal properties of the gelatin materials were evaluated by Differential Scanning

179 Calorimetry method (DSC, Spicotech, Model 100, China). The samples were weighed,

180 placed in an aluminum pan and heated from 0 to 300°C at a 10°C/min heating rate under

181 a nitrogen atmosphere with a flow rate of 150 mL/min. Change in glass transition

182 temperature were calculated from the thermogram data(Laha, Sharma, & Majumdar,

183 2016).

184

185 **2.2.8 Zeta Potential**

186 Zeta potential experiments were conducted to study the surface-charge changes of pure

187 gelatin powder and electrospun nanofiber samples produced at different voltages (15 kV,

188 20 kV and 25 kV). The magnitude of the net-charge of the samples was measured using

189 a dynamic light scattering system (Malvern Zeta-sizer Nano ZS, Worcestershire, UK) at

190 25°C with three replicates. Since gelatin is insoluble in ethanol, this solvent was used as

191 the dispersing medium for all samples (Okutan, Terzi, & Altay, 2014). Note that each

192 sample at a concentration of 1mg/ml was completely dispersed by a probe-type ultrasonic

193 homogenizer (VCX750-Sonics-USA) with a frequency of 20 KHz and at 750 W. The

194 electrophoretic mobility of the gelatin dispersion was measured at pH =8.1 by the

195 instrument and then converted into zeta-potential values via the *Smoluchowsky* equation

196 (Fathollahipour, Abouei Mehrizi, Ghaee, & Koosha, 2015).

197

198 **2.2.9.1 Dissolution rate**

199 Dissolution rate was determined using a digital high-speed camera based on the method

200 developed by Rezaeinia *et al.* (with some modifications). Equal amounts of gelatin

201 nanofiber and gelatin powder were dissolved in 10 ml distilled water and mixed for 20s

202 at 600 rpm continuously. Disintegration time was considered to be the point at which no

203 residue could be visually detected in the medium (Rezaeinia, Emadzadeh, & Ghorani,

204 2020).

205

206 **2.2.9.2 Emulsifying ability and stability**

207 The emulsifying capability and stability were evaluated based on the method proposed

208 by Alpizar-Reyes *et al.* (2017) with minor modification. Herein, 6.5 g sunflower oil was

209 added to 50 ml distilled water containing 0.06 and 0.12 g gelatin nanofiber. The samples

210 were homogenized using an Ultra-Turrax homogenizer (IKA, USA) at 6400 rpm for 3

211 min. The samples were then, centrifuged for 10 min at 520×g. In case of the samples

212 prepared using gelatin powder, the hydrocolloid solutions were prepared through heating

213 for 10 min at 40°C. The emulsifying ability (EA) was calculated according to the below

214 equation(Alpizar-Reyes, et al., 2017):

215

216 $$\%\text{EA} = \frac{Emulsion\ Volume}{Total\ volume} \times 100 \qquad \textbf{Eq. (1)}$$

217

218 Emulsion stability (ES) was determined as indicated in Eq. 2. Being homogenized, the

219 samples were heated for 30 min in a 80°C water bath and then cooled down to ambient

220 temperature. ES was calculated as follow:

221

222 $$\%\text{ES} = \frac{Final\ Emulsion\ Volume}{Initial\ Emulsion\ Volume} \times 100 \qquad \textbf{Eq. (2)}$$

223

8

2.2.9.3 Foam capacity and stability

Solutions of 0.2 and 0.4 % (w/v) gelatin were prepared by dissolving the nanofibers and powder separately in distilled water. Each samples were then mixed in an Ultra-Turrax homogenizer (IKA, USA) at 6400 rpm for 5 min. Foaming capacity (FC) and stability (FS) were measured immediately, and after15 min aeration, respectively (Alpizar-Reyes, et al., 2017):

$$\%FC = \frac{Initial\ Foam\ Volume}{Total\ Suspension\ Volume} \times 100 \qquad \textbf{Eq. (3)}$$

$$\%FS = \frac{Final\ Foam\ Volume}{Total\ Suspention\ Volume} \times 100 \qquad \textbf{Eq. (4)}$$

Dissolution of the gelatin powder was achieved by heating the suspension at 40°C for 10 min.

2.2.10 Flow behavior

The viscosity of the 0.1and 0.2%(w/v) gelatin nanofiber and powder solutions were determined using a Brookfield viscometer (UK) equipped with a SC4-18 spindle at 1-350 s^{-1} shear rates after being overnighted at 4°C. To remove the time dependency effect from the samples, they were sheared at $100s^{-1}$ until no changes in viscosity was observed over time.

2.2.11 Gel properties

2.2.11.1 Viscoelastic behavior of gels

A controlled stress/ strain Physica MCR301 rheometer (Anton Paar GmbH, Germany) equipped with a cone-plate geometry (40 mm of diameter, 4° cone angel, and 0.206 mm gap) was used to determine the small oscillatory shear behavior. The temperature was

249 fixed by means of a Peltier system at 25°C and each sample was equilibrated at least for

250 5 min before the rheological test. To avoid the evaporation during the experiment, around

251 the periphery was coated by light silicon oil.

252 The strain sweep test was performed at 1Hz frequency in the range of 0.1- 10% strain at

253 room temperature. The 0.5% strain was selected as the appropriate strain for the frequency

254 sweep experiment since it was located in the linear region for all samples.

255 The frequency range of 0.1-10 Hz and 0.5% strain was considered for running the

256 frequency sweep experiment and all the experiments were done at ambient temperature.

257 To study the viscoelastic behavior, the elastic modulus (G'), and viscous modulus (G'')

258 were determined. The loss tangent was also measured according to the following

259 equation:

260 $$\tan \delta = \frac{G''}{G'}$$ **Eq. (5)**

261

262

263 **2.2.11.2 Textural properties of gels**

264 To evaluate the textural characteristics, 1% (w/v) nanofiber solutions were prepared and

265 poured into a cylindrical mold with a diameter and height of 1 cm. It was overnighted at

266 4°C for complete hydration. The gelatin powder was prepared in a similar way, but before

267 molding, it was heated at 40°C for 10 min to ensure complete dissolution. A TA-XT Plus

268 texture analyzer (UK) equipped with a ball probe (P/0.25s) was used for the penetration

269 texture evaluation. The trigger point, test speed, and strain were set at 2 g, 1 mm/s, and

270 20% respectively.

271

272 **3. Results and Discussion**

273 **3.1 Characterization of samples**

3.1.1 Morphological characterization of gelatin nanofibers and powder

Continuous electrospinning of gelatin is impractical if water is used as the only solvent. This can be attributed to: (a) the propensity to form gel structure in water, which is related to the degree of hydroxylation of proline residues (M. C. Gómez-Guillén, et al., 2002; Ledward, 1986) leading to gelation within the syringe at room temperature. Electrospinning therefore requires temperatures above 36ºC (Ki, et al., 2005; Sajkiewicz & Kołbuk, 2014);(b) Low volatility and the high boiling point of water (Ki, et al., 2005);(c) High surface tension at the needle tip and inability to form a stable Taylor cone (Sajkiewicz, et al., 2014) and; (d) the low probability of forming random-coil structures in the solution because of the tendency to gel (Kriegel, Arrechi, Kit, McClements, & Weiss, 2008; Nieuwland, et al., 2013; Sajkiewicz, et al., 2014).

Consequently, to improve the feasibility of gelatin electrospinning, acetic acid was added to the solvent to increase the rate of evaporation during spinning. The value of acetic acid in promoting fiber formation is well reported (Aytac, et al., 2019; Erencia, et al., 2015; Song, Kim, & Kim, 2008; Steyaert, et al., 2016).

Insert figure.1 about here

A binary solvent system of acetic acid: water (3:1v/v) effectively promoted continuous electrospinning of gelatin and the formation of coherent fibrous sheets. The resultant fibers at a gelatin concentration of 25% (w/v) contained ultrathin and bead-free nanofibers, with a mean fiber diameter of 122±39.79 nm (**Fig.1 A-B**). Uniformly surfaced fibers with a mixture of round and flat morphologies were also observed following electrospinning (**Fig.1C**). The spinnability and continuity of electrospinning conditions, deteriorated at gelatin concentrations higher than 25 %(w/v) due to the high viscosity of the polymer solution. Similarly, at polymer concentrations lower than 25% (w/v),

11

300 specifically 10%, 15% and 20% (w/v), the fibers tended to have large, spindle-like or

301 bead-on-string structures on their surface (Data not shown). This may be related to

302 insufficient molecular chain entanglement and difficulties in forming a stable jet during

303 electrospinning (Ghorani, et al., 2015).Therefore, satisfactory conditions for producing

304 gelatin nanofibers were identified as a gelatin concentration of 25%(w/v), a binary solvent

305 of acetic acid: water (3:1), a flow rate of 0.5 ml/hr, a voltage of 25 kV and a tip-to-

306 collector distance of 150 mm.

307 The morphology of pure gelatin powder is shown in **Figure 1.** Unlike the electrospun

308 fibers, which were generally less than 1μm in diameter, the gelatin powders comprised

309 particle sizes in the range80 μm–763.5 μm, and were of irregular geometric shape (**Fig**

310 **1.D**). Differences in cold water solubility might therefore be partly explained by the

311 difference in surface-to-volume ratio between gelatin nanofibers and powder (Steyaert,

312 et al., 2016).

313

314 **3.1.2 FTIR analysis**

315 The FTIR spectra of the gelatin powder, solution and electrospun nanofibers are

316 compared in **Figure 2**. All samples showed major absorption bands at 1700-1600 cm^{-1}

317 ,1575-1480cm^{-1} and 1330-1230cm^{-1}(Garidel & Schott, 2006; Muyonga, Cole, & Duodu,

318 2004). These regions are attributable to Amide I (mainly C=O stretch), Amide II (N–H

319 bend in plane and C–N stretch) and Amide III bands (stretching of N-H groups),

320 respectively (Garidel, et al., 2006) . The broad absorption bands at 3400-3250 cm^{-1} are

321 characteristic of Amide A, which is highly sensitive and depends on O–H stretching

322 (Krimm & Bandekar, 1986). These four functional groups of Amide A, I, II and III are

323 characteristic features of gelatin (fingerprint regions) and confirm that gelatin is still

324 present after the electrospinning process.

325

Insert Figure.2 about here

328

329   The band at 1730-1700 cm^{-1} for gelatin dissolved in a solvent system of acetic acid: water

330   (3:1) corresponds to the carbonyl groups (C=O) of acetic acid (Erencia, et al., 2015),

331   which were entirely absent in the electrospun nanofibers due to evaporation (**Fig.2**).

332   Similarly, significant changes can be observed in the functional group of Amide A at

333   3400-3250 cm^{-1} so that the peak width for the electrospun nanofibers was considerably

334   reduced (**Fig.2**). This behavior can be attributed to the hydroxyl group (O-H) in this

335   region and mostly to the evaporation of water subject to the high voltage field during

336   electrospinning.

337   Importantly, considering the changes in the Amide I group absorption spectrum for the

338   gelatin powder at 1646.52 cm^{-1}, and the polymer solution at 1654.148 cm^{-1}(**Fig.2**), the

339   Amide I band shifted slightly to a higher wavenumber, suggesting conformational

340   changes in gelatin when in solution (Aceituno-Medina, Lopez-Rubio, Mendoza, &

341   Lagaron, 2013; Kong & Yu, 2007). The amide I band located at 1654 cm^{-1} reflected the

342   structure of gelatin and represented the distinctive coiled conformations which is

343   responsible for stabilizing the triple helical structure (Cebi, Durak, Toker, Sagdic, &

344   Arici, 2016; Utomo & Suryanti, 2018).

345   Solubility in an appropriate solvent system and the formation of a random coil structure,

346   are basic features of protein-based polymer electrospinning (Nieuwland, et al., 2013).

347   Intermolecular interactions or aggregation of proteins in the solution, or on the other hand,

348   even insufficient interaction and entanglements between proteins, particularly for

349   globular structures, hamper the electrospinning process (Nieuwland, et al., 2013).The

350   findings described herein in terms of gelatin solvation and random coil formation in the

351 electrospinning solution is in good agreement with other publications (Kong, et al., 2007;

352 Nieuwland, et al., 2013; Sajkiewicz, et al., 2014; Utomo, et al., 2018).

353

354 **3.1.3 XRD analysis**

355 **Figure 3** shows the X-ray diffraction pattern of gelatin powder and electrospun gelatin

356 nanofibers. Unlike the gelatin nanofibers, gelatin powder produces a broad distinct peak

357 at $2\theta=19.84°$, which is a diffraction peak attributable to gelatin (Nagahama, et al., 2009).

358 This peak is characteristic of the α-helix and triple-helical structure of pure gelatin powder

359 (Peña, de la Caba, Eceiza, Ruseckaite, & Mondragon, 2010; Yakimets, et al., 2005).

360

361   **Insert Figure.3 about here**

362

363 The electrospun gelatin nanofibers presented no discernible peak, and only hollow

364 scattering was observed in the XRD patterns (**Fig.3**), which is indicative of a change in

365 the crystalline structure to an amorphous state (Kwak, Woo, Kim, & Lee, 2017; Peña, et

366 al., 2010). Some researchers have explained these changes as being due to the removal of

367 hydrogen bonds (O-H) between amine and hydroxyl groups (R-O-H) in the gelatin

368 structure (Ba Linh, Min, & Lee, 2013; Heidari, Bahrami, & Ranjbar-Mohammadi, 2017),

369 but they may also be linked to the electrospinning process itself (Andrady, 2007).

370 When electrospinning is initiated, the spinneret meniscus deforms from the apex into the

371 familiar geometry of the Taylor cone (Reneker, Yarin, Fong, & Koombhongse, 2000). In

372 other words, the surface tension drawing the droplet into its characteristic shape is

373 overcome by internal electrostatic repulsion, and a charged jet of liquid polymer is

374 emitted from the tip of the Taylor cone (Doshi & Reneker, 1995). The jet has a linear

375 flight path, but eventually the flight path changes into a helix of increasing diameter and

376 pitches, which is often loosely described as "whipping instability" (Ghorani, et al., 2015).

377 This observation is the combined result of surface tension, electrostatic, and viscoelastic

378 forces, viscous drag, and gravity effects. The amount of stretching and elongation forces

379 applied to the fluid jet is very high and, therefore, this can lead to a decrease in the

380 crystallinity of nanofibers (Andrady, 2007).

381 Another important point is that the formation of crystalline regions or well-ordered

382 molecular chains require sufficient time (Reiter, 2014). In the electrospinning process the

383 polymer jet continues to be drawn, with a consequent reduction in diameter even after the

384 solvent is evaporated. This drawing process continues until the jet hits the earthed

385 collector (Reneker & Yarin, 2008). However, the jet formation and solvent evaporation

386 occurs within a very short timeframe (roughly 0.01 to 1s) (Wu, Salkovskiy, & Dzenis,

387 2011). Subsequently, formation and expansion of crystalline regions slow down or are

388 largely interrupted (Andrady, 2007). Moreover, the nanoscopic size of the electrospun

389 nanofibers could potentially accentuate the amorphous-unordered regions in the gelatin

390 nanofibers, as compared to pure gelatin powder (Erencia, et al., 2015; Ki, et al., 2005).

391 This behavior as well as the lack of crystalline regions in electrospun nanofibers has been

392 widely reported (Andrady, 2007; Bognitzki, et al., 2001; Qian, Zhang, Zheng, Song, &

393 Zhao, 2014; Zong, et al., 2003).

394

395 **3.1.4 Thermal analysis**

396 The DSC results for all samples are shown in **Figure 4**. The gelatin powder produced a

397 broad region in the DSC trace, from almost room temperature to above 150˚C, with the

398 onset temperature at 94.2˚C and a calorimetric enthalpy of H = 29.85 J/g. This confirms

399 the findings of other authors, and is associated with water loss and the glass transition

400 temperature (T_g) due to triple helix to random coil transition (Mukherjee & Rosolen,

401 2013; Nguyen & Lee, 2010).

402

403   **Insert Figure.4 about here**

404

405   A weak transition was also observed around 200-240 °C (with an onset temperature of

406   220.6°C), which may be due to polymer decomposition due to breakage of peptide bonds

407   (**Fig.4**) (Fraga & Williams, 1985; Ki, et al., 2005; Peña, et al., 2010) .

408   Unlike the gelatin powder, the water loss and glass transition region of the electrospun

409   gelatin nanofibers occurred at lower temperature, and the endothermic region for the

410   electrospun nanofibers was limited from 60ºC to 120ºC, with an onset temperature (T_g)

411   at 86.5°C and a calorimetric enthalpy of H = 50.64 J/g (**Fig.4**). These values and thermal

412   behavior are consistent with other reports (Koosha, Habibi, & Talebian, 2017), where

413   similar glass transition temperature ranges were reported for electrospun gelatin

414   nanofibers dissolved in pure acetic acid (Koosha, Habibi, & Talebian, 2017).

415   The degradation temperature for gelatin nanofibers was not clearly discernible at 220ºC

416   in the thermograms and no distinct thermal decomposition peak could be detected for

417   electrospun gelatin nanofiber samples. Such observations were reported in relation to

418   gelatin films and nanofibers manufactured using a binary solvent system of formic acid:

419   water (Ki, et al., 2005). The thermal behavior can be attributed to the bound water content

420   in gelatin samples, and the amorphous structure of nanofibers because of the low degree

421   of helical conformation (Ki, et al., 2005).These results are consistent with the results of

422   the FTIR and XRD studies in sections3.1.2 and 3.1.3.

423

424   **3.1.5 Zeta Potential**

425   The isoelectric point (pI) of gelatin type B varies in the range of 4.8-5.2 (Kuan, Nafchi,

426   Huda, Ariffin, & Karim, 2017) so that the net-charge of the gelatin creates Zwitterions

427   (Kebede, Dube, & Nindi, 2018). At solution pHs above the pI, the surface-charge of the

428   protein predominantly turns negative, and the carboxylic group of the amide functional

429 bonds will be more negatively charged. However, protonation of the amino acid groups

430 occurs in acidic conditions (i.e pH< 4.8) promoting positive charges on the gelatin

431 backbone (Kebede, et al., 2018).

432 **Table.1** compares the zeta potential of gelatin powder and electrospun nanofiber samples.

433 The gelatin powder is characterized by a negative zeta potential equal to 2.56 mV, but

434 interestingly positive values are obtained for electrospun nanofibersat pH 8.1 (**Table.1**).

435 The high positive electric field at the needle-tip may be responsible for shifting the

436 surface-charge of gelatin nanofibers from negative to positive values. Previous studies

437 have suggested an increase in surface charge on electrospun nanofibers, due to the applied

438 voltage during electrospinning (Okutan, et al., 2014).

439

440   **Insert Table.1 about here**

441

442 As the applied voltage decreased from 25 kV to 15 kV, the zeta potential values

443 significantly increased in the electrospun nanofibers ($p < 0.05$). This may be attributed to

444 the fact that the thickness of the surface-layer of nanofibers increases with decreasing

445 voltage during spinning and therefore, higher zeta potential results because of the

446 increased interaction in the dispersing medium (Okutan, et al., 2014). A few previous

447 studies have reported measurements of zeta potential for electrospun nanofibers, and

448 highlighted surface-charge changes from positive to negative or vice versa. Further

449 investigations would be worthwhile to shed more light on this behavior (Cho, Lee, &

450 Frey, 2012; Okutan, et al., 2014).

451 Such surface-charge changes might also be relevant to the design and manufacture of bio-

452 carriers. Mucin has negative charges, with a zeta potential approximately between -5mV

453 to -7.9 mV., and the positive surface charges associated with gelatin nanofibers could

454 potentially improve mucin's biocompatibility and mucoadhesive properties as a result of

455   strong electrostatic interactions (Mendes, et al., 2018; Van Vlierberghe, Graulus, Keshari

456   Samal, Van Nieuwenhove, & Dubruel, 2014).

457

458   **3.1.6 Dissolution rate**

459   As is observed in **Fig.5**, gelatin nanofibers were completely dissolved in aqueous medium

460   at room temperature, whereas the gelatin powder led to sedimentation at the bottom of

461   the container.

462

463   **Insert Figure.5 about here**

464   Solubility is an important parameter governing the performance of hydrocolloids in

465   dispersed systems because of its influence on other functional properties (Alpizar-Reyes,

466   et al., 2017). While gelatin nanofibers could be dissolved in 2-4 s at ambient temperature,

467   the gelatin powder required stirring at 40ºC for at least 10 min. This ability to be rapidly

468   solubilised at room temperature is one of most valuable properties of nanofibers in the

469   food industry, as compared to gelatin powder. The difference in solubility is largely due

470   to the amorphous nature of the nanofibers but is also linked to the difference in the bulk

471   format of powder and nanofibers. The high surface area and porosity of nanofiber webs

472   enable the dissolving medium to diffuse quickly into the material because of the very

473   large solid free surface. Zhang *et al.* (2015) also demonstrated improved solubility of

474   pectin by the formation of nanofibers (Zhang, et al., 2015).

475   The ability to dissolve gelatin at room temperature, without the need for heating, has the

476   potential to greatly reduce industrial production costs and simplify processing. Moreover,

477   in the production of heat-sensitive food products, gelatin nanofiber feedstocks in place of

478   powders could also create potential to protect nutritional components.

479

3.2 Functional properties

3.2.1 Emulsifying ability and stability

One of the most important functions of hydrocolloids is their ability to form and stabilize emulsion systems. Although most have the ability to stabilize, a few can be considered to be emulsifiers (Dickinson, 2009). The emulsifying capability and stability of gelatin powders and nanofibers were found to decrease at higher concentrations (**Table. 2**). It is worth noting that although gelatin has some emulsifying capability, it is mainly valued for its stabilizing and gelling effects (Xu, et al., 2017). Emulsifiers are surface active compounds which reduce the emulsion particle size during the homogenization process and also prevent coalescence. The increased emulsifying capability at low hydrocolloid concentrations might be due to unfolding of polypeptides during the homogenization process (Binsi, Shamasundar, Dileep, Badii, & Howell, 2009). It could also be linked to an increase in protein-protein interactions at higher hydrocolloid concentrations which in turn, lead to a lower protein content at the oil-water interface (Lawal, 2004). The relation between adsorption kinetics and protein concentration also needs to be considered. At a low protein content, protein adsorption is governed by the diffusion phenomenon, while at higher concentrations, the activation energy barrier does not allow protein migration and protein accumulation in aqueous conditions occurs. The opposite relation between protein concentration and emulsification capability has also been observed (Giménez, Alemán, Montero, & Gómez-Guillén, 2009; Huidobro, Montero, & Borderías, 1998; Montero & Borderías, 1991; Thiansilakul, Benjakul, & Shahidi, 2007). Surh *et al.* (2006) reported that increasing fish extracted gelatin concentration from 0.5 to 6% decreases the emulsion particle size, and its instability (Surh, Decker, & McClements, 2006).

The emulsifying capability of gelatin nanofibers was found to be greater than in the gelatin powder samples. It may be that the faster dissolution rate in the nanofiber samples

19

505 resulted in a greater coverage rate of oil droplets in the emulsion system. This observation

506 is in agreement with the findings of Zhang *et al.* (2015) who compared the emulsifying

507 capability of chitin in its native and nanofiber formats (Zhang, et al., 2015)

508 The improved emulsion stability of gelatin powder compared to the nanofiber samples is

509 evident in **Table 2.** The heating process in the emulsion stability evaluation procedure

510 (section 2.2.9.2) possibly led to improved dissolution of gelatin powder, which

511 subsequently, during the cooling step, produced a harder gel structure. The results of the

512 rheometry experiments also demonstrated greater elasticity of the gel produced by the

513 gelatin powder, compared to the nanofibers. The loss tangent values confirmed that a

514 harder gel structure was obtained for the gelatin powder as compared to the nanofibers

515 (section 3.3.2).

516

517 **3.2.2 Foam Capacity and stability**

518 Foam formation by proteins requires rapid adsorption to the interface layer and reduction

519 in surface tension. Therefore, the adsorption rate and the ability to unfold and restructure

520 at the surface are important factors in foam forming capacity (Giménez, et al.,

521 2009).Gelatin powder and nanofibers were both able to adsorb to the air-water interface

522 and produce a foam system. After 15 min relaxation, the foam stability was evaluated for

523 the gelatin nanofibers and powder containing systems. Increasing the hydrocolloid

524 concentration improved the foam stability in both the gelatin nanofibers and powder

525 samples. It appeared that the higher gelatin concentrations produced stiffer foam

526 structures. Sila *et al.* (2017) suggested that higher concentrations of protein can be

527 expected to result in more stable foam systems (Sila, Martinez-Alvarez, Krichen, Gómez-

528 Guillén, & Bougatef, 2017). The gelatin nanofibers produced more stable foam systems

529 than those produced from powder (**Table 2**). Foam stability is primarily dependent on the

530   protein-protein interactions in the film matrix surrounding the air bubbles (Giménez, et

531   al., 2009), and it is possible that more hydrogen bonds are formed in the gelatin nanofiber

532   system, resulting in the denser network necessary for the foam stabilization. Nagarajan *et*

533   *al.* (2012) reported that foam capacity and stability were positively influenced by the

534   concentration of splendid squid (*Loligo formosana*) and skin gelatin present in the system

535   (Nagarajan, Benjakul, Prodpran, Songtipya, & Kishimura, 2012).

536

537   **Insert Table 2 about here**

538

539   **3.3 Rheological properties**

540   **3.3.1 Flow behavior**

541   The flow behavior of 0.1 and 0.2% (w/v) associated with gelatin nanofiber and powder

542   feedstocks are reported in **Fig. 6.** In the studied range of shear rates, almost all of the

543   samples exhibited Newtonian behavior.

544

545   **Insert Figure.6 about here**

546

547   There was no marked difference in the flow behavior between the 0.1% (w/v) nanofiber

548   solution and the samples containing 0.1 and 0.2% (w/v) gelatin powder. However, the

549   shear stress in the 0.2% nanofiber solution was clearly higher than in other samples. For

550   gelatin, which is mostly known for its stabilizing effect rather than being an emulsifier,

551   production of higher viscosities at equal amounts is a notable advantage of nanofiber

552   gelatin feedstock compared to powder. Marcotte *et al.* (2001) reported Newtonian

553   rheological behavior for 2-4% concentrations of gelatin (Marcotte, Taherian Hoshahili,

21

554 & Ramaswamy, 2001), whereas Binsi *et al.* (2009) reported shear-thinning non-

555 Newtonian behavior for gelatin extracted from fish (Binsi, et al., 2009).

556

557 **3.3.2 Viscoelastic behavior**

558 The elastic and viscous moduli of samples made from gelatin nanofibers and powder

559 versus frequency are shown in **Fig. 7**. Results consistent with the behavior of gels were

560 observed for both sample groups, while G' values were much higher than G''. It indicates

561 that mechanical energy was stored in the structure with a minimal amount dissipated

562 (Tkaczewska, Morawska, Kulawik, & Zając, 2018). For the sample made from gelatin

563 nanofibers, the elastic modulus remained higher than the viscous modulus, without cross

564 over. This is characteristic of viscoelastic solid behavior. The elastic modulus was almost

565 constant over the studied range of frequencies, which reflects the high strength of the

566 sample. Similarly, for the sample prepared using gelatin powder, over the studied range

567 of frequencies, the elastic modulus dominated the viscous modulus with an increasing

568 trend. Both the values of G'' and G' for the gelatin powder were lower than for the

569 nanofiber samples.

570

571 **Insert Figure.7 about here**

572

573 For further comparison between the gels produced with the gelatin nanofibers and

574 powder, the loss tangent changes against frequency are shown in **Fig. 8.** A trend of

575 increasing loss tangent values in nanofiber gel samples was observed, while there was no

576 obvious variation in the gelatin powder gel data. The loss tangent values for the nanofiber

577 samples were higher than the gelatin powder over the studied range of frequencies.

578 Therefore, the gel strength of the gelatin powder was higher than for the nanofiber

22

579   samples. The loss tangent values in the nanofiber samples were between 0.1 and 1,

580   indicating that they were not true gels, with a structure between that of a concentrated

581   biopolymer and a true gel.

582

583   **Insert Figure.8 about here**

584

585   **3.3.3 Gel texture**

586   No marked difference was observed in the Young modulus values of gelatin nanofiber

587   and powder gel samples (4.32±0.02 for nanofiber and 4.44±0.64 for powder). The very

588   slight difference in the Young Modulus values could be attributed to the amorphous

589   structure of nanofibers. In an amorphous structure, the molecular 3D network is weakly

590   connected, so that water is able to easily penetrate the structure. A gel-like network or

591   semi-gel, is produced, which is not as strong as the system produced using gelatin powder

592   (Schrieber & Gareis, 2007).

593

594   **4 Conclusions**

595   Gelatin has limitations in terms of its ambient or cold water solubility, which restricts its

596   ability to be processed simply and cost-effectively in food and pharmaceutical

597   manufacture. Cold-water soluble gelatin feedstocks can be made by electrospinning

598   gelatin powder to make a sheet-like feedstock suitable for industrial use. After

599   electrospinning of gelatin, the amorphous structure, shift in Tg and the large fiber surface

600   area to volume ratio, enable rapid dissolution in cold water. Gelatin nanofibers possessed

601   improved emulsifying capability, foam capability, and also foam stability properties

602   compared to gelatin powder. However, the emulsion stability of nanofibers was lower

603   than for gelatin powders. Loss tangent measurements revealed greater gel strength values

604 for the gelatin nanofiber samples, as compared to the powder; whereas both groups of

605 samples had similar Young modulus values. It may be suggested that electrospinning has

606 strong potential to be used for the manufacture of cold-water soluble gelatin feedstocks

607 suitable for future food and pharmaceutical manufacturing processes.

608

609 **5 References**

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