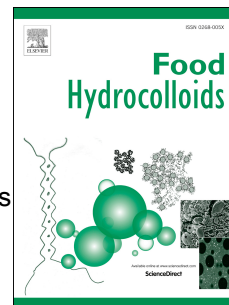# Journal Pre-proof

Effect of concentrations of alginate, soy protein isolate and sunflower oil on water loss, shrinkage, elastic and structural properties of alginate-based emulsion gel beads during gelation

Duanquan Lin, Alan L. Kelly, Valentyn Maidannyk, Song Miao
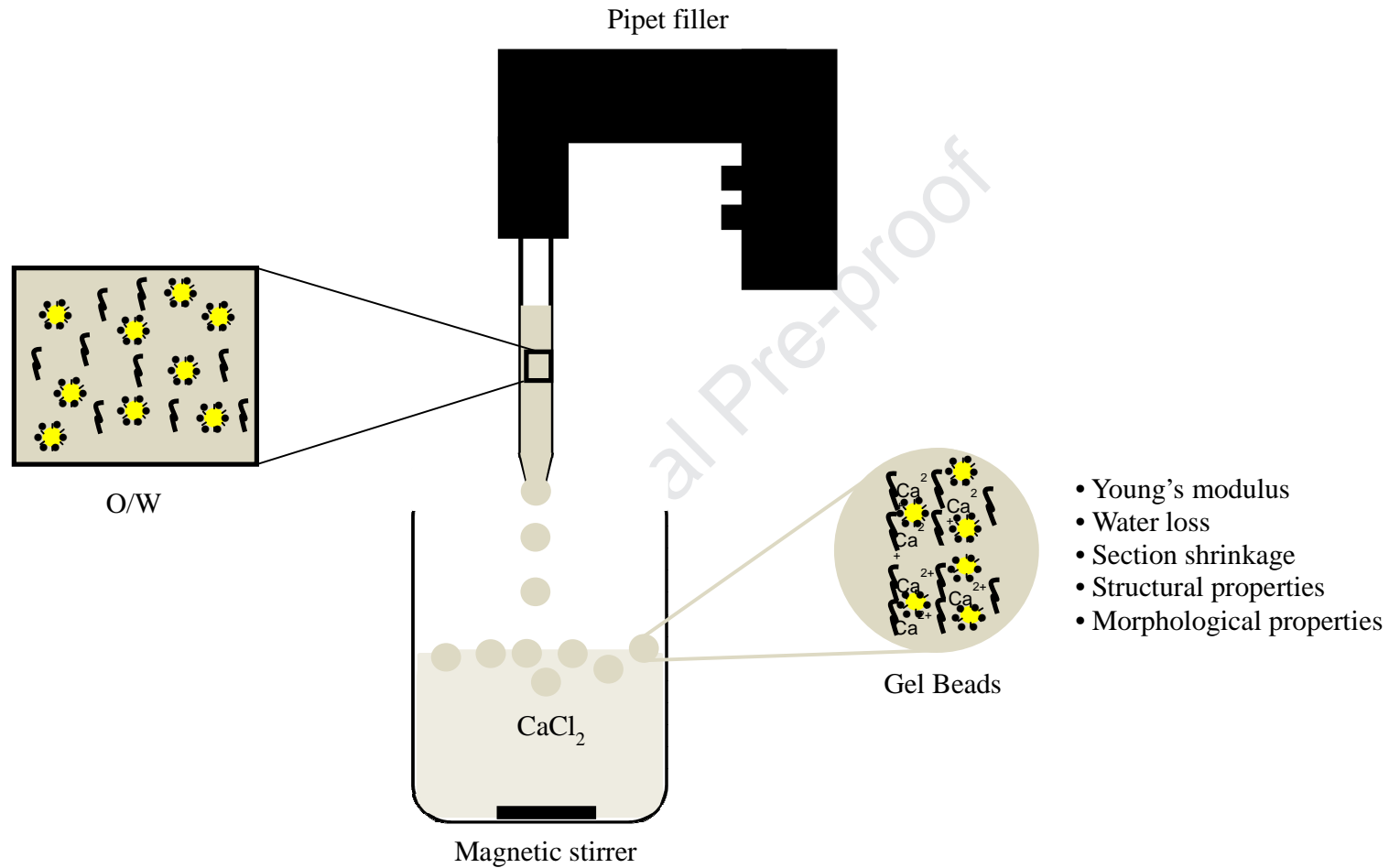
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
Graphical Abstracts

Pipet filler

O/W

CaCl$_2$

Magnetic stirrer

- Young's modulus
- Water loss
- Section shrinkage
- Structural properties
- Morphological properties

Gel Beads

SPI

Sodium alginate

O/W droplet structure

Alginate gel structure

1   **Effect of concentrations of alginate, soy protein isolate and sunflower oil on water loss,**

2   **shrinkage, elastic and structural properties of alginate-based emulsion gel beads during**

3   **gelation**

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15 **Abstract**

16 The aim of this study was to investigate the influence of concentrations of sodium alginate

17 (0.5%–1.5% in the water phase of an emulsion), soy protein isolate (SPI, 0.5%–2.0% in the

18 water phase) and oil phase (10%–40% in the emulsion) on the properties (including water

19 loss, shrinkage, morphological, elastic, and structural properties) of emulsion gel beads

20 during gelation (0–30 min). Gel beads were prepared with external gelation by dropping

21 emulsions into $CaCl_2$ solutions using pipettes. The Young's modulus of emulsion gel beads

22 kept increasing during gelation before reaching a plateau accompanied by syneresis (i.e.,

23 water loss), shrinkage, and structural tightening. SPI absorbed at the surface of oil droplets

24 could prevent re-coalescence of droplets during gelation. Additionally, increasing

25 concentrations of sodium alginate and oil increased the Young's modulus of gel beads. Water

26 loss decreased with increasing contents of alginate, SPI and oil, and shrinkage could be

27 diminished by increasing alginate and oil contents.

28 **Keywords:** Alginate; Elastic property; Emulsion gel bead; Microstructure; Shrinkage; Soy

29 protein isolates.

30   **1. Introduction**

31   Emulsion gels, also called emulgels, are a complex colloidal material which have some

32   properties of both emulsions and gels (Dickinson, 2012). During the last decade, emulsion

33   gels have received growing interest, due to their advantages compared to emulsions, such as

34   higher storage stability by reducing oil and water phase movement and lipid oxidation (Ma,

35   Wan, & Yang, 2017) and slower intestinal drug release, due to improved protective effects

36   against gastric and intestinal phases (Corstens, Berton-Carabin, Elichiry-Ortiz, Hol, Troost,

37   Masclee, et al., 2017; Guo, Bellissimo, & Rousseau, 2017). In order to produce emulsion

38   gels, emulsions are first prepared by mixing gelling agent, emulsifier and oil and then turned

39   into gels by different gelation mechanisms.

40   The choice of matrix material and emulsifier is the key factor in structuring emulsion gels.

41   Proteins (e.g., soy protein isolate (SPI) and whey protein isolate (WPI)) and polysaccharides

42   (e.g., agar and gellan gum) have been widely investigated as gelling agents in the formation

43   of emulsion gels (Brito-Oliveira, Bispo, Moraes, Campanella, & Pinho, 2017; Geremias-

44   Andrade, Souki, Moraes, & Pinho, 2017; Guo, et al., 2017). Different gelling agents can form

45   different gelation structures, and the gelation mechanism (e.g., heat, high pressure,

46   acidification, enzymatic treatment, and addition of ions) for different gelling agents differs

47   (Dickinson, 2012), which can affect the properties of emulsion gels and encapsulated food

48   nutrients. Both synthetic (e.g., Tween 80 and Span 80) and natural (e.g., proteins, egg

49   lecithin, and soy lecithin) emulsifiers can be used to prepare emulsion gels. Lipid droplets in

50   emulsion gels can be divided into active and inactive fillers according to the interactions

51   between gelling agents and emulsifier-coated lipid droplets (Van Vliet, 1988; Yang et al.,

52   2020), which can also influence the properties of emulsion gels (Geremias-Andrade, et al.,

53   2017).

54 Alginate, a linear unbranched natural polysaccharide, is derived from brown seeweed extracts

55 (*Phaeophyceae*) (King, 1983) and composed of two monomeric isomers: β-(1→4)-linked D-

56 mannuronic acid (M) residues and α-(1→4)-linked L-guluronic acid (G) residues (Ching,

57 Bansal, & Bhandari, 2017). Alginate-based emulsion gels received high attention in recent

58 years (Lević, Pajić Lijaković, Đorđević, Rac, Rakić, Šolević Knudsen, et al., 2015; Qu, Zhao,

59 Fang, Nishinari, Phillips, Wu, et al., 2016; Zeeb, Saberi, Weiss, & McClements, 2015).

60 Alginate monomers can form gels by ionic crosslinking with divalent cations (mostly calcium

61 cations in the food industry) (King, 1983). External gelation and internal gelation are two

62 methods used to prepare alginate-based emulsion gels. Pintado, Ruiz-Capillas, Jimenez-

63 Colmenero, Carmona, & Herrero (2015) added $CaSO_4$ into an alginate-based emulsion to

64 directly produce an alginate-based emulsion gel. Sato, Moraes, & Cunha (2014) used internal

65 method to produce emulsion gels, in which CaEDTA was added to an alginate-based

66 emulsion first, after which acid was introduced to liberate calcium ions. Compared these two

67 methods, Puguan, Yu, and Kim (2014) found that gels formed by external gelation had a

68 smoother surface and denser inner structure. In addition, alginate-based gels are not sensitive

69 to gastric fluids, and can protect the encapsulated nutrients from harsh gastric environment,

70 and the remaining gel structures can be further disrupted during intestinal digestion

71 accompanied by the release of encapsulated compounds (Zhang, et al., 2016).

72 Previous studies mainly focused on the formulation, structural properties, mechanical

73 properties, stability, and digestion of alginate-based emulsion gels. However, there are few

74 reports on the gelation process of alginate-based emulsion gels. It has been indicated that,

75 during the gelation process of alginate hydrogels prepared by external gelation, calcium

76 cations can diffuse into alginate drops after being dropped into a calcium chloride solution

77 (Rehm, 2009). Syneresis also occurs during this gelation process, with a consequent decrease

78 in dimensions of gel beads (Quong, Neufeld, Skjåk-Bræk, & Poncelet, 1998; Rehm, 2009).

79 However, the gelation process of alginate-based emulsion gels may differ from that of

80 alginate gels, because the presence of lipids and emulsifers in emulsions may affect the

81 gelation process. Understanding the gelation process of alginate-based emulsion gels may

82 help to produce emulsion gels with specific properties (e.g., size, water content, mechanical

83 properties) by controlling gelation time, formulation, preparation methods and processing

84 technologies. Therefore, further studies are needed to understand how alginate, emulsifiers

85 and oil affect the gelation process of emulsion gel beads.

86 The purpose of this study was thus to investigate the gelation process of alginate-based

87 emulsion gel beads. In order to improve the encapsulation efficiency and hygroscopicity of

88 alginate-based emulsion gels, proteins (e.g., lupin protein and WPI) can be used as

89 emulsifiers (Corstens, et al., 2017; Piornos, Burgos-Díaz, Morales, Rubilar, & Acevedo,

90 2017), and polysaccharides (e.g., *Prosopis alba* exudate gum and chitosan) can be used as

91 structural strengthening agents (Natrajan, Srinivasan, Sundar, & Ravindran, 2015; Vasile,

92 Judis, & Mazzobre, 2018). In this study, denatured SPI was thus introduced as surfactant,

93 because SPI has a huge potential value in producing emulsion gels, due to its good

94 emulsifying property, and denatured SPI has increased emulsifying capacity compared to

95 natural SPI (Lin, Lu, Kelly, Zhang, Zheng, & Miao, 2017; Nishinari, Fang, Guo, & Phillips,

96 2014). In addition, the external gelation was used, in order to obtain gel beads with denser

97 structures, compared to internal gelation. Effect of concentrations of alginate, SPI, and

98 sunflower oil on the shrinkage, water loss, elastic and structural properties of alginate-based

99 emulsion gel beads during gelation were investigated in this study.

100 **2. Materials and methods**

101 *2.1. Materials*

5

102 Defatted soy flour (Bob's Red Mill, Milwaukie, Oregon, USA) and sunflower oil (Aldi Stores

103 Ltd., Kildare, Ireland) were purchased from iHerb and Aldi, respectively. Sodium alginate

104 was obtained from Special Ingredients (Chesterfield, UK). Calcium chloride, sodium

105 hydroxide, and hydrochloric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

106 *2.2. Preparation of soy protein isolate*

107 SPI was prepared according to the method described by Urbonaite, Jongh, Linden and

108 Pouvreau (2015). The defatted soy flour was suspended in distilled water at a ratio of 1:10

109 (w/w) at 45 ℃ and stirred for 30 min. The pH value was then adjusted to 8.0 with 5 M

110 NaOH, and the solution was stirred for 30 min in the water bath. The supernatant was

111 collected by centrifugation (30 min, 6000×g, 13 ℃) (Sorvall LYNX 6000 Superspeed

112 Centrifuge, Thermo Fisher Scientific, Waltham, USA). Protein isolates were obtained by

113 isoelectric precipitation by adjusting the pH value to 4.5 with 6 M HCl. After mild stirring for

114 12 h at 5 ℃, the suspension was centrifuged (30 min, 6000×g, 7 ℃). The sediment was re-

115 suspended three times in deionized water at a ratio of 1:3 (w/w) and filtered by multilayer

116 gauze to remove any remaining insoluble material, and the filtrate was centrifuged (30 min,

117 6000×g, 7 ℃) again. The sediment was finally suspended in deionized water at a ratio of 1:4

118 (w/w), and the pH value was justified to 7.0 with 5 M NaOH. Then, the solution was freeze-

119 dried (Free Zone 12 Freeze Dry System, Labconco Corpotation, Kansas, MO, USA). The

120 dried SPI was kept in polyethylene bags and stored at room temperature. The protein content

121 of SPI powder was $96.29 \pm 0.03\%$.

122 *2.3. Preparation of alginate-based and SPI-stabilized emulsions and gel beads*

123 A dispersion of soy protein isolate (5% wt in distilled water) was stirred at room temperature

124 for 30 min using a magnetic stirrer, heated at 90 ℃ for 30 min, and then cooled to room

125 temperature. For the production of continuous phase, sodium alginate (0.5, 1.0, and 1.5% wt)

126   was added into the pre-heated soy protein isolate solution with adding water to reach final

127   concentrations of SPI (0.5, 1.0, and 2.0% wt) by shearing at 400 rpm for 30 min with a

128   magnetic stirrer and then allowed to rest for 24 h to permit hydration. For the production of

129   o/w emulsion, sunflower oil (10, 20, and 40% wt) was added to above continuous phase and

130   mixed at 18,000 rpm for 2 min with an Ultra-Turrax (IKA-25, Staufen, Germany). Solutions

131   containing 1.0% alginate (1A) and dispersions containing 1.0% alginate and 1.0% SPI

132   (1A1S) were prepared as control groups without mixing at 18,000 rpm for 2 min. Table 1

133   shows the formulations used for preparing emulsions.

134   For producing gel beads, the resulting dispersions/solutions were dropped into 2 % (w/w)

135    $CaCl_2 \cdot 2H_2O$ solutions using 5-ml measuring pipettes and a S1 pipette filler (Thermo Fisher

136   Scientific Inc., Waltham, USA). The distance between the tip of pipette and the surface of

137    $CaCl_2$ solutions was fixed at 10 cm. The samples were allowed to gel in $CaCl_2$ solutions for

138   30 min with mild magnetic stirring, and the resulting beads were rinsed with distilled water.

139   Samples were analyzed immediately for measurement of Young's modulus, shrinkage, water

140   loss, and morphology, and samples were kept in distilled water for observing their structures

141   within 3 hours after being prepared.

142   *2.4. Properties of dispersions/solutions*

143   2.4.1. Structures

144   Confocal scanning laser microscopy (CLSM) was used to observe microstructures of

145   dispersions/emulsions. Dispersion/emulsion samples (500 µl) were transferred to a glass slide

146   and stained with 50 µl of a mixture of Nile red (0.1%, w/v, in polyethylene glycol-200) and

147   fast green (0.1%, w/v, in distilled water) at a ratio of 3:1. Confocal observation was

148   performed using a Leica TCS SP5 microscope (Leica Microsystems GmbH, Wetzlar,

149   Germany) at excitation and emission wavelengths of 488 nm and 633 nm, provided by an

150   argon laser and a HeNe laser, respectively.

151   2.4.2. Viscosity

152   The viscosity of dispersions/solutions was tested at 25 ºC using an AR 2000ex rheometer (TA

153   Instruments, Crawley, UK) with an aluminium parallel plate (60 mm in diameter, and 0.5 mm

154   in gap). Each sample was added in the middle of Peltier plate and allowed to stand for 2 min

155   before testing. The flow measurement was performed over a shear rate range of 0.1 to 100 s^{-1},

156   and viscosity (η) was obtained from the data analysis software.

157   *2.5. Microstructures of gel beads*

158   CLSM was used to observe microstructures of gel beads. Each gel bead was cut into a thin

159   layer (~ 1 mm), transferred to a glass slide, and stained with a mixture of Nile red (0.1%, w/v,

160   in polyethylene glycol-200) and fast green (0.1%, w/v, in distilled water) at a ratio of 3:1.

161   Confocal observation was performed by the method described in section 2.4.1.

162   *2.6. Young's modulus of gel beads*

163   The Young's modulus of gel beads during gelation at 1, 2, 3, 4, 5, 6, 8, 10, 20, and 30 min

164   were analysed by a TA.XT Plus texture analyser (Stable Micro System, Godalming, UK)

165   according the method described by Ching, Bansal, & Bhandari (2016) with a minor change.

166   The surface of samples was dried with dry paper before testing. Compression tests were

167   performed using a cylinder probe of 10-mm diameter and a 5-kg load cell. The samples were

168   compressed to 30% strain at a crosshead speed of 0.1 mm/s, and five beads with same

169   composition were examined one after another. Due to their ellipsoidal shapes, the cross-

170   sectional area of samples was calculated after measuring the major axis and minor axis of

171   samples after being placed on the platform of texture analyser. The Young's modulus of each

8

172 sample was calculated as the gradient of the stress vs. strain curve in the 5–15% strain region,

173 where stress and strain showed good linearity. The experiment was performed in triplicate.

174 *2.7. Water loss of gel beads*

175 The water loss of samples during gelation was determined at 1, 2, 3, 4, 5, 6, 8, 10, 20, and 30

176 min after dispersions/solutions were dropped into calcium chloride solutions. In this study,

177 the water loss means the decreased water in gel beads during gelation compared to the

178 original dispersions/solutions. Five gel particles were obtained from calcium chloride

179 solutions and washed with distilled water. After drying the surface, the initial weight of 5

180 beads was weighted (W'_i) and then they were dried in an oven at 80 ℃ until constant weight

181 (W'_d). The initial weight (W_i) and the weight after drying (W_d) of dispersions/solutions (5

182 drops) were determined by the same method. Thus, the water loss was calculated from Eq.

183 (1), if we assumed that the main content (i.e., alginate, SPI, and oil) of gel beads have no

184 significant change during gelation, and the experiment was replicated three times.

185 $$\text{Water loss (\%)} = (\frac{W_i - W_d}{W_i} - \frac{W_d}{W_i} \times \frac{W'_i - W'_d}{W'_d}) \times 100\% \qquad (1)$$

186 *2.8. Shrinkage of gel beads*

187 The section shrinkage rate of gel beads was determined at 2, 4, 6, 8, 10, 20, and 30 min after

188 dispersions/solutions were dropped into calcium chloride solutions. Five gel beads were

189 obtained from the calcium chloride solutions and washed with distilled water. After drying

190 the surface, photographs of gel beads were taken using a camera (iPhone 7 plus, Apple Inc.,

191 California, USA). The major semi-axis (r'_{max}) and minor semi-axis (r'_{min}) of gel beads were

192 measured by using a digital vernier calliper, and the section area (A'_s) was calculated from

193 Eq. (2). The major semi-axis (r_{max}) and minor semi-axis (r_{min}) of gel beads after gelation for 1

194 min were measured, and the section area (A_s) was also calculated from Eq. (2). The section

9

195 shrinkage rate of gel beads was calculated from Eq. (2), and the experiment was replicated

196 three times. It should be noted that the section shrinkage rate of samples during gelation

197 process was compared to the section area of samples after gelation for 1 min in this study.

198 $Section\ shrinkage\ rate\ (\%) = \frac{A_s - A'_s}{A_s} = \frac{3.14 \times r_{max} \times r_{min} - 3.14 \times r'_{max} \times r'_{min}}{3.14 \times r_{max} \times r_{min}} \times 100\%$ (2)

199 **3. Results and discussion**

200 *3.1 Structural properties of gel beads*

201 Structural properties are important for emulsion gels because they can influence mechanical

202 properties of emulsion gels and release behavior of encapsulated nutrients. Many factors

203 (e.g., structures of the gel matrix, structures of emulsion droplets, and interactions between

204 the gel matrix and droplets) can influence the structures of overall emulsion gels. Therefore,

205 the effect of concentrations of alginate, SPI and oil on the structures of emulsions and

206 emulsion gels was investigated in this study.

207 Fig. 1 shows the structures of emulsions/dispersions before gelation and gel beads after

208 gelation for 30 min. In sample 1A1S, SPI formed aggregates and dispersed in alginate

209 solutions (Figs. 1A and 1V). This was because SPI was heated at 90℃ for 30 min in this

210 study, and thus the solubility of SPI decreased, due to denaturation; additionally, denatured

211 SPI exposed hydrophobic residues and thus formed aggregations in alginate solutions

212 (Wagner & Añón, 1990). After mixing the 1A1S dispersion with oil, SPI modules can move

213 from the continuous phase to the O/W interfaces and are absorbed at the surface of oil

214 droplets (Figs. 1A and 1B), due to their amphipathic nature and emulsifying capacity.

215 Hydrophobic groups of SPI absorbed onto the surface of oil droplets, and hydrophilic groups

216 connected with the water phase, acting as a steric barrier against coalescence of oil droplets

217 (Nishinari, et al., 2014). However, increasing the alginate concentration to 1.5% led to more

10

218   SPI aggregations in the water phase (Figs. 1B and 1C), because the higher viscosity of the

219   continuous phase of emulsions hindered SPI from moving to the oil–water interface

220   (Tavernier, Patel, Van der Meeren, & Dewettinck, 2017). Higher SPI concentrations resulted

221   in more SPI being absorbed at the surface of oil droplets but led to more obvious flocculation

222   of oil droplets (Figs. 1B and 1D), probably due to the depletion flocculation of droplets

223   coated by excessive amount of SPI (Moschakis, Murray, & Biliaderis, 2010). In addition,

224   increasing oil content of emulsions resulted in more compacted gel structures (Figs. 1B and

225   1E), due to the decreased ratio of the water phase to the oil phase.

226   Fig. 1 also indicates that there were more dark sections in emulsions/dispersions than gel

227   beads in all samples, which indicates that syneresis and shrinkage of the water phase during

228   gelation led to more compact filler structures. In addition, the concentrations of SPI, alginate

229   and oil could affect the stability of droplets during gelation. As shown in Figs. 1B and 1W

230   SPI-coated droplets in sample 1A1S20O could maintain their structures during gelation. This

231   was because SPI could stabilize the o/w emulsions, and gelation, syneresis and shrinkage

232   mainly occurred in the water phase during gelation, which had no significant effects on the

233   structures of emulsion droplets. Similarly, it was found that WPI-aggregate-stabilized

234   emulsions were stable during the gelation period (Rosa, Sala, Van Vliet, & Van De Velde,

235   2006). Additionally, higher SPI concentration resulted in more stable droplet structures

236   during gelation, probably because of more SPI being absorbed at the surface of oil droplets

237   (Figs. 1D and 1Y). However, increasing the alginate concentration to 1.5% led to re-

238   coalescence of droplets during gelation (Figs. 1C and 1X), because increased viscosity of the

239   continuous phase of emulsions hindered SPI from moving to the oil–water interface and thus

240   resulted in decreased stability of emulsion droplets during gelation. In addition, increasing the

241   oil content to 40% also led to re-coalescence of droplets during gelation (Figs. 1E and 1Z),

242   probably because 1.0% SPI in the water phase was not enough to stabilize 40% oil.

243 *3.2. Young's modulus of gel beads*

244 3.2.1. The profiles of Young's modulus during gelation

245 Compression tests were carried out to study the elastic properties of gel beads during

246 gelation. Firstly, the effect of introducing SPI and oil into alginate gels on the profiles of

247 Young's modulus during gelation was investigated. As shown in Fig. 2A, the changes of

248 Young's modulus of gel beads containing 1% alginate (1A in short) included three steps:

249 increasing up to 5 min, decreasing between 5 and 10 min, and then reaching a plateau. The

250 Young's modulus of gel beads containing 1% alginate and 1% SPI (sample 1A1S) had a

251 similar trend to that of sample 1A, but the Young's modulus of emulsion gel beads containing

252 1% alginate, 1% SPI, and 20% oil (sample 1A1S20O) increased first and then reached a

253 plateau at 8 min directly (Fig. 2A). It can be seen that the gelation process of alginate-based

254 gel beads includes the maturation step (increased Young's modulus), the structural collapse

255 step (decreased Young's modulus), and the equilibrium step (unchanged Young's modulus).

256 Therefore, it was assumed that the gelation mechanism of alginate beads prepared by the

257 external gelation has a direct effect on the changes in Young's modulus during gelation.

258 After being dropped into calcium chloride solutions, the surface of alginate drops can gel

259 instantaneously, and then Ca^{2+} can diffuse from the $CaCl_2$ solutions into the interior of

260 alginate drops, which leads to the gelation of gel beads from outside to inside and increased

261 Young's modulus (Ching, et al., 2017). This process is called the maturation step (Puguan, et

262 al., 2014). The concentration of alginate solutions and the size of gel beads are the main

263 factors affecting the Ca^{2+} diffusion into alginate gel beads during gelation. It has been

264 reported that higher alginate concentrations incorporated more calcium ions in alginate gel

265 beads (Quong, et al., 1998). In this study, all gel beads were prepared with 2% (w/w)

266 $CaCl_2 \cdot 2H_2O$ solutions, and the size of samples 1A ($r_{max} = 2.1$ mm and $r_{min} = 2.0$ mm), 1A1S

267   (r_{max} = 2.2 and r_{min} = 2.1), and 1A1S20O (r_{max} = 2.1 and r_{min} = 2.0) did not significantly differ

268   at the end the maturation step. Therefore, it was assumed that the changes in Young's

269   modulus of samples 1A, 1A1S, and 1A1S20O during the maturation step showed a similar

270   trend probably because oil and SPI had no significant effect on the Ca^{2+} diffusion from the

271    $CaCl_2$ solutions in alginate gel beads during the maturation step.

272   After the maturation step, the Young's modulus of samples 1A and 1A1S decreased before

273   reaching a constant value (Fig. 2A). The concentrations of Ca^{2+} and alginate in alginate gel

274   breads decreases from the gel surface to gel core (Quong, et al., 1998), which indicates that

275   the gel structure of the outer regions of beads is stronger than that of inside gel beads.

276   Therefore, the fragile core structure of gel beads can not support the whole structure, which

277   leads to the collapse of the inner structure at the end of the maturation step and thus a

278   decreased Young's modulus (Puguan, et al., 2014). However, the Young's modulus of

279   sample 1A1S20O reached the balance directly after the maturation step during gelation (Fig.

280   2A). This was probably because the structures of sample 1A1S20O are totally different from

281   that of samples 1A and 1A1S. After introducing oil into 1A1S dispersions, oil droplets

282   disperse in the alginate solutions during homogenization, and SPI molecules move to the

283   surface of oil droplets from the water phase, due to their emulsifying capacity. The resulting

284   emulsions can turn into emulsion gels after alginate monomers are crosslinked by calcium

285   cations, and shrinkage also occurs during this process. However, oil droplets may act as

286   fillers and help to support the structure of gel beads from collapse after the maturation step

287   during gelation. It has also been indicated that the oil core could support the shell of silica

288   gels from fracture during the sol-gel process (Liang, Liu, Zhang, Qu, Li, & Yang, 2011).

289   Therefore, it was also assumed that oil played an important role on preventing the structural

290   collapse of alginate gel beads during gelation.

13

291   The effect of concentrations of alginate (Fig. 2B), SPI (Fig. 2C) and sunflower oil (Fig. 2D)

292   on the profiles of Young's modulus of emulsion gel beads during gelation was further

293   investigated. Fig. 2B shows that the Young's modulus of sample 0.5A1S20O increased

294   initially, decreased between 4 and 8 min, and then increased again, before reaching a plateau.

295   In this sample, the structure of gel matrix formed by 0.5% alginate is fragile during the

296   maturation step, which results in severe structural collapse before compact emulsion droplets

297   can support emulsion gel structures. However, samples 1A1S20O and 1.5A1S20O showed a

298   similar trend, in which the Young's modulus increased up to 8 min and then reached a

299   plateau (Fig. 2B). This indicates that increasing alginate concentrations from 0.5% to 1.5%

300   not only slowed Ca^{2+} diffusion and thus caused a slowing of the maturation step but also

301   formed stronger alginate-based matrix structures and thus protected emulsion gel structures

302   from collapse during gelation. Figs. 2C and 2D show that increasing SPI concentrations from

303   0.5% to 2.0% and oil contents from 10% to 40% had no significant effect on the profiles of

304   Young's modulus during gelation (i.e., reaching the plateau directly after the maturation step

305   at around 8 min during gelation). This was probably because increasing concentrations of SPI

306   and oil had no significant impact on calcium diffusion in emulsion gel beads, and 10% oil

307   was high enough to prevent structural collapse of emulsion gel beads after the maturation step

308   during gelation.

309   3.2.2. Effect of alginate, SPI and oil on the Young's modulus of gel beads after gelation

310   Mechanical properties are important for emulsion gels because they are closely associated

311   with other properties (e.g., storage stability, oral perception, and controlled release of

312   encapsulated nutrients). Many factors can affect mechanical properties of emulsion gels, such

313   as gel strength of gel matrix structures (i.e., protein and polysaccharide), modulus of filler

314   droplets, and interactions between oil droplets and the gel matrix. Therefore, the effect of

315   concentrations of alginate, oil and SPI on the Young's modulus of emulsion gel beads was

316   investigated in this study, and all samples were compared after they were allowed to gel for

317   30 min in CaCl$_2$ solutions (Figs. 2B–D).

318   Fig. 2B shows that increasing alginate concentrations from 0.5 to 1.5% significantly

319   increased the Young's modulus of emulsion gel beads. This was expected because increasing

320   alginate concentration could increase gel strength of alginate-based gel matrix and thus

321   increase the Young's modulus of overall emulsion gels. Similarly, it has previously been

322   reported that increasing agar content (from 1.0 to 1.8%) in o/w emulsions containing 0.1

323   volume fraction of corn oil decreased the overall volume of void spaces and increased strand

324   compactness of emulsion gels (Kim, Gohtani, Matsuno, & Yamano, 1999).

325   Fig. 2D indicates that increasing oil contents from 10% to 40% had no significant effect on

326   the Young's modulus of emulsion gel beads. According to the interactions between

327   emulsifier-coated emulsion droplets and the gel matrix, oil droplets can be divided into active

328   and inactive fillers (also known as bound and unbound fillers) in emulsion gels (Dickinson,

329   2012; Yang et al., 2020). Active fillers are mechanically connected to the gel network by

330   noncovalent and/or covalent bonds through emulsifiers. For examples, it has been reported

331   that WPI-coated oil droplets could be bound to a WPI-based gel matrix by covalent

332   interactions (e.g., hydrophobic interactions and sulphur bridges) (Sala, de Wijk, van de

333   Velde, and van Aken, 2008); it has been also indicated that lactoferrin-stabilised emulsion

334   droplets could bind to a κ-carrageenan gel, probably because of electrostatic interactions

335   between positively charged lactoferrin (pI = 8.2) and negatively charged κ-carrageenan at pH

336   7–8 (Sala, van Vliet, Cohen Stuart, Aken, and van de Velde, 2009). In addition, the Kerner

337   model can explain the effect of active fillers on the mechanical properties of emulsion gels

338   (Kerner, 1956). According to this model, increasing the volume fraction (ϕ_f) of active fillers

339   can increase the mechanical properties of emulsion gels, which has been supported by many

340   studies (Oliver, Berndsen, van Aken, & S□holten, 2015; Sala, et al., 2009). However, in this

341 study, SPI (pI = 4.5) and alginate were both negatively charged at pH 6.5–7.0, so there are no

342 electrostatic interactions between SPI-coated droplets and alginate-based gel matrix. It is also

343 unlikely that SPI-coated droplets can connect to the alginate-based gel network by covalent

344 interactions. Additionally, the results obtained in this study were in a disaccord with the

345 Kerner model. Therefore, it was assumed that SPI-coated droplets were inactive fillers in

346 alginate-based emulsion gel beads.

347 Fig. 2C shows that increasing SPI concentrations decreased the Young's modulus of

348 emulsion gel beads. According to the state of emulsion droplets in gels, structures of

349 emulsion gels can be divided into two categories: emulsion droplet-filled gels and emulsion

350 droplet-aggregated gels (Dickinson, 2012). In emulsion droplet-filled gels, the continuous

351 phase (e.g., protein- and polysaccharide-based gels) forms a continuous gel matrix, and

352 emulsion droplets are embedded in this gel matrix. In emulsion droplet-aggregated gels,

353 emulsion droplets aggregate together and form a network structure, such that the gel matrix is

354 disrupted by the aggregated emulsion droplets. As shown in Figs. 1B and 1D, more

355 aggregations of emulsion droplets occurred in sample 1A2S20O compared to sample

356 1A1S20O, probably because increasing SPI concentration led to more depletion flocculation

357 of SPI-coated droplets in emulsions (Lam & Nickerson, 2013). In active droplet-aggregated

358 gels, the crowding effect of fillers (particle interactions) increases the shear modulus of the

359 overall gels (Oliver, et al., 2015). However, SPI-coated droplets in alginate-based gel matrix

360 may act as inactive fillers as disccussed before in this study. Therefore, it was assumed that

361 increased aggregation of SPI-coated droplets (inactive fillers) had a negative effect on the

362 Young's modulus of alginate-based emulsion gel beads, probably because aggregated

363 droplets (i.e., the increased phase separation between alginate-based gel matrix and SPI-

364 coated droplets) may disturb the formation of alginate-based network structures (Dickinson,

365 2012; Lin, Lu, Kelly, Zhang, Zheng, & Miao, 2017).

366   *3.3 Water loss of gel beads*

367   During the maturation step, inter-chain interactions between stretches of alginate monomers

368   and Ca^{2+} occurred with the diffusion of Ca^{2+} from the surface to interior of gel beads, and the

369   formation of junctions between these stretches forced water out, which led to shrinkage and

370   increased water loss of gel beads during gelation (Puguan, et al., 2014; Rehm, 2009). Fig. 3

371   shows the effects of concentrations of alginate, SPI, and oil on the water loss from emulsion

372   gel beads during gelation. It indicates that increasing alginate contents (from 0.5 to 1.5%) or

373   SPI concentration (from 0.5 to 2.0%) had no significant effect on the rares of water loss, but

374   increasing oil content (from 10 to 40%) could slow the water loss in terms of the profiles of

375   water loss during gelation, probably because lower water content of the original emulsions

376   results in slower water loss of emulsion gels during gelation.

377   Fig. 3 also indicates that the water loss of emulsion gel beads after gelation for 30 min

378   decreased with increasing alginate contents (from 0.5 to 1.5%), SPI concentration (from 0.5

379   to 2.0%) and oil content (from 10 to 40%). Many factors can affect the water loss of emulsion

380   gel beads during gelation, such as the concentration of $CaCl_2$ solutions, the water content of

381   original emulsions, the strength of gel matrix, and the hydrophilicity and rigidity of fillers. It

382   has been reported that increasing the concentration of $CaCl_2$ solution (from 0.08 M to 0.3 M)

383   reduced the final weight of alginate gel beads due to the increased water loss (Puguan, et al.,

384   2014), but in this study all samples were dropped into the $CaCl_2$ solutions with the same

385   concentration. Therefore, increasing alginate concentration from 0.5% to 1.5% decreased the

386   water loss of beads from $42.3 \pm 1.2\%$ to $36.9 \pm 0.3\%$ after gelation (Fig. 3A), which was

387   probably because elastic modulus of gel beads increased with increasing alginate

388   concentration (Fig. 2B), and gels with stronger matric structures had better water-bolding

389   capacity.

390   In addition, increasing SPI concentration from 0.5% to 2.0% decreased the water loss of

391   emulsion gel beads from $40.2 \pm 0.6\%$ to $37.3 \pm 0.3\%$ after gelation as well (Figs. 3B),

392   probably due to increased water-absorption capacity of SPI-coated droplets. Denatured SPI

393   has emulsifying capacity because it has both hydrophobic and hydrophilic groups (Nishinari,

394   et al., 2014). As shown in Figs. 1A and 1B, SPI aggregated in sample 1A1S but formed a film

395   at the oil-water interface in sample 1A1S20O, in which hydrophobic groups of SPI connected

396   to oil droplets and hydrophilic groups connected to water. Therefore, more SPI was absorbed

397   at the surface of emulsion droplets by increasing SPI concentration (Fig. 1D), which resulted

398   in increased hydrophilicity of SPI-coated droplets and increased water-retention capacity of

399   emulsion gel beads (Wang, Marcone, Barbut, & Lim, 2012). This explanation could be

400   supported by previous conclusions by Wagner, et al. (1990) that SPI with highly denatured

401   proteins and high surface hydrophobicity exhibited the highest water-absorption capacity.

402   Additionally, increasing oil content from 10% to 40% led to the decreased water loss of

403   emulsion gel beads (from $46.1 \pm 0.2\%$ to $25.1 \pm 0.4\%$ after gelation) (Figs. 3C), probably

404   because the water content in original emulsions significantly decreased with increasing oil

405   contents from 10% to 40%, and emulsion droplets could protect gel structures from collapse

406   as well. A similar finding has been reported where increasing the oil volume fraction (13%–

407   31.1%) in β-lactoglobulin-based oil-in-water emulsions improved the water-retention

408   capacity of emulsion gels (Line, Remondetto, & Subirade, 2005).

409   *3.4 Morphological properties and shrinkage of gel beads*

410   As shown in Fig. 4, alginate gel beads (1A) were transparent, but the presence of SPI

411   decreased the transparency of alginate gel beads (1A1S) because of its yellow colour, and

412   introducing oil led to ivory gel beads, due to the formation of emulsions. In addition, gel

413   beads in all groups were not completely spherical, and samples 1.5A1s20O, 1A2S20O, and

414   1A1S40O had small tails. This was because increasing the concentrations of alginate, SPI and

18

415   oil could raise the viscosity of emulsions (Fig. 5), which could affect the morphological

416   properties of emulsion gel beads. In this study, we used the simple dripping method to

417   produce emulsion gel beads. The emulsions were pushed out from pipette and one droplet

418   was formed at the tip before the droplet grew in size gradually and dropped into $CaCl_2$

419   solutions. During this process, spherical emulsion droplets were formed because of the

420   surface tension of liquid (Ching, et al., 2017). However, Lević, et al. (2015) found that D-

421   limonene could increase the viscosity and reduce the conductivity of the alginate liquid

422   systems by changing structural ordering of alginate, which indicates that the high viscosity of

423   emulsion was against the formation of spherical bead at the tip of pipet because of poor flow

424   properties. For example, the introduction of hydroxypropylmethylcellulose (0.2%–1%)

425   changed the rheological properties of 2% alginate solutions and produced beads with small

426   tails (Bellich, Borgogna, Cok, and Cesàro, 2011).

427   Fig. 4 also shows that the size of all samples decreased during gelation and, in order to

428   compare their shrinkage during gelation, the section shrinkage rates were calculated (Fig. 6).

429   The profiles of shrinkage rates show that shrinkage rates of all samples increased during

430   gelation, probably due to syneresis (i.e., water loss) and structural collapse (Rehm, 2009).

431   However, in terms of the profiles of shrinkage rate, increasing contents of alginate and oil

432   could slow the shrinkage, but increasing SPI content had no significant effect on the rate of

433   shrinkage during gelation. Fig.6 also shows that the shrinkage rates decreased from $26.7 \pm$

434   2.1% to $18.2 \pm 2.2\%$ and from $27.1 \pm 1.6\%$ to $13.6 \pm 2.5\%$ after gelation with increasing

435   concentrations of alginate (from 0.5% to 1.5%) and oil (from 10% to 40%), respectively, but

436   increasing SPI concentration from 0.5% to 2.0% had no significant effect on the shrinkage

437   rates of emulsion gel beads after gelation for 30 min. Many factors can affect the shrinkage of

438   emulsion gels during gelation, such as water loss, gel stiffness, the content and properties of

439   fillers, and interactions between fillers and the continuous phase (Smith, Scherer, &

19

440   Anderson, 1995). In terms of alginate, increasing its concentration could increase the elastic

441   modulus of emulsion gel beads (Fig. 2B), which may provide resistance to shrinkage

442   (Brinker, et al., 1994). Increasing oil concentration led to more compact filler structures,

443   which resisted further shrinkage during gelation as seen on comparing emulsion gel structures

444   of samples 1A1S20O and 1A1S40O in Fig. 1. Eichler, Ramon, Ladyzhinski, Cohen, &

445   Mizrahi (1997) also indicated similar conclusions, in that fructose or polydextrose being

446   introduced into polyacrylamide (PAAm) gels could act as a mechanical barrier against further

447   volume shrinkage of PAAm gels during dehydration. However, increasing SPI concentration

448   reduced the Young's modulus (Fig. 2C) but increased water retention (i.e., decreased water

449   loss) (Fig. 3B) of emulsion gel beads, which may explain why increasing SPI concentration

450   had no significant effects on shrinkage rates of emulsion gel beads.

4. Conclusions

452   The Young's modulus of alginate-based emulsion gel beads kept increasing before reaching a

453   plateau during gelation process. This gelation process was accompanied by syneresis (i.e.,

454   water loss) and shrinkage, which resulted in an increased compactness of emulsion gel beads.

455   SPI-coated droplets could maintain their structures during gelation. With increasing alginate

456   concentration (0.5%–1.5%), the water loss decreased, the Young's modulus increased, and

457   shrinkage rate decreased. Increasing SPI concentration (0.5%–2.0%) led to decreased

458   Young's modulus and water loss, and undifferentiated shrinkage. Higher oil content (10%–

459   40%) decreased water loss and section shrinkage rates, and had no significant effect on the

460   Young's modulus. These findings underlined the effect of concentrations of components on

461   the properties of emulsion gel beads during gelation, which are very important because the

462   properties of emulsion gel beads may affect encapsulation, stability, and release of

463   hydrophobic functional ingredients encapsulated in emulsion gel beads.

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464 **Acknowledgement**

469 **References**

470 Bellich, B., Borgogna, M., Cok, M., & Cesàro, A. (2011). Release properties of hydrogels: Water

471   evaporation from alginate gel beads. *Food Biophysics, 6*, 259-266.

472 Brinker, C. J., Sehgal, R., Hietala, S., Deshpande, R., Smith, D., Loy, D., & Ashley, C. (1994). Sol-

473   gel strategies for controlled porosity inorganic materials. *Journal of Membrane Science, 94*,

474   85-102.

475 Brito-Oliveira, T. C., Bispo, M., Moraes, I. C. F., Campanella, O. H., & Pinho, S. C. (2017). Stability

476   of curcumin encapsulated in solid lipid microparticles incorporated in cold-set emulsion filled

477   gels of soy protein isolate and xanthan gum. *Food Research International, 102*, 759-767.

478 Ching, S. H., Bansal, N., & Bhandari, B. (2016). Rheology of emulsion-filled alginate microgel

479   suspensions. *Food Research International, 80*, 50-60.

480 Ching, S. H., Bansal, N., & Bhandari, B. (2017). Alginate gel particles–A review of production

481   techniques and physical properties. *Critical Reviews in Food Science & Nutrition, 57*, 1133-

482   1152.

483 Corstens, M. N., Berton-Carabin, C. C., Elichiry-Ortiz, P. T., Hol, K., Troost, F. J., Masclee, A. A.

484   M., & Schroën, K. (2017). Emulsion-alginate beads designed to control in vitro intestinal

485   lipolysis: Towards appetite control. *Journal of Functional Foods, 34*, 319-328.

486 Dickinson, E. (2012). Emulsion gels: The structuring of soft solids with protein-stabilized oil droplets.

487   *Food Hydrocolloids, 28*, 224-241.

488 Eichler, S., Ramon, O., Ladyzhinski, I., Cohen, Y., & Mizrahi, S. (1997). Collapse processes in

489   shrinkage of hydrophilic gels during dehydration. *Food Research International, 30*, 719-726.

21

490   Geremias-Andrade, I. M., Souki, N. P. D. B. G., Moraes, I. C. F., & Pinho, S. C. (2017). Rheological

491   and mechanical characterization of curcumin-loaded emulsion-filled gels produced with whey

492   protein isolate and xanthan gum. *LWT - Food Science and Technology, 86*, 166-173.

493   Guo, Q., Bellissimo, N., & Rousseau, D. (2017). Role of gel structure in controlling in vitro intestinal

494   lipid digestion in whey protein emulsion gels. *Food Hydrocolloids, 69*, 264-272.

495   Kerner, E. (1956). The elastic and thermo-elastic properties of composite media. *Proceedings of the*

496   *Physical Society. Section B, 69*, 808.

497   Kim, K., Gohtani, S., Matsuno, R., & Yamano, Y. (1999). Effects of oil droplet and agar

498   concentration on gel strength and microstructure of o-w emulsion gel. *Journal of Texture*

499   *Studies, 30*, 319-335.

500   King, A. (1983). Brown seaweed extracts (alginates). *Food Hydrocolloids, 2*, 115-188.

501   Lam, R. S., & Nickerson, M. T. (2013). Food proteins: A review on their emulsifying properties using

502   a structure-function approach. *Food Chemistry, 141*, 975-984.

503   Lević, S., Pajić Lijaković, I., Đorđević, V., Rac, V., Rakić, V., Šolević Knudsen, T., Pavlović, V.,

504   Bugarski, B., & Nedović, V. (2015). Characterization of sodium alginate/$_D$-limonene

505   emulsions and respective calcium alginate/$_D$-limonene beads produced by electrostatic

506   extrusion. *Food Hydrocolloids, 45*, 111-123.

507   Liang, F., Liu, J., Zhang, C., Qu, X., Li, J., & Yang, Z. (2011). Janus hollow spheres by emulsion

508   interfacial self-assembled sol-gel process. *Chemical Communications, 47*, 1231-1233.

509   Lin, D., Lu, W., Kelly, A. L., Zhang, L., Zheng, B., & Miao, S. (2017). Interactions of vegetable

510   proteins with other polymers: Structure-function relationships and applications in the food

511   industry. *Trends in Food Science & Technology, 68*, 130-144.

512   Line, V. L.S., Remondetto, G. E., & Subirade, M. (2005). Cold gelation of *β*-lactoglobulin oil-in-

513   water emulsions. *Food Hydrocolloids, 19*, 269-278.

514   Ma, D., Tu, Z.-C., Wang, H., Zhang, Z., & McClements, D. J. (2017). Fabrication and

515   characterization of nanoemulsion-coated microgels: Electrostatic deposition of lipid droplets

516   on alginate beads. *Food Hydrocolloids, 71*, 149-157.

517   Ma, L., Wan, Z., & Yang, X. (2017). Multiple water-in-oil-in-water emulsion gels based on self-

518   assembled saponin fibrillar network for photosensitive cargo protection. *Journal of*

519   *Agricultural and Food Chemistry, 65*, 9735-9743.

520   Moschakis, T., Murray, B. S., & Biliaderis, C. G. (2010). Modifications in stability and structure of

521   whey protein-coated o/w emulsions by interacting chitosan and gum arabic mixed

522   dispersions. *Food hydrocolloids, 24*, 8-17.

523   Natrajan, D., Srinivasan, S., Sundar, K., & Ravindran, A. (2015). Formulation of essential oil-loaded

524   chitosan-alginate nanocapsules. *Journal of Food and Drug Analysis, 23*, 560-568.

525   Nishinari, K., Fang, Y., Guo, S., & Phillips, G. O. (2014). Soy proteins: A review on composition,

526   aggregation and emulsification. *Food Hydrocolloids, 39*, 301-318.

527   Oliver, L., Berndsen, L., van Aken, G. A., & Scholten, E. (2015). Influence of droplet clustering on

528   the rheological properties of emulsion-filled gels. *Food Hydrocolloids, 50*, 74–83.

529   Pintado, T., Ruiz-Capillas, C., Jimenez-Colmenero, F., Carmona, P., & Herrero, A. M. (2015). Oil-in-

530   water emulsion gels stabilized with chia (*Salvia hispanica L.*) and cold gelling agents:

531   Technological and infrared spectroscopic characterization. *Food Chemistry*, 185, 470–478.

532   Piornos, J. A., Burgos-Díaz, C., Morales, E., Rubilar, M., & Acevedo, F. (2017). Highly efficient

533   encapsulation of linseed oil into alginate/lupin protein beads: Optimization of the emulsion

534   formulation. *Food Hydrocolloids, 63*, 139-148.

535   Puguan, J. M., Yu, X., & Kim, H. (2014). Characterization of structure, physico-chemical properties

536   and diffusion behavior of Ca-Alginate gel beads prepared by different gelation methods.

537   *Journal of Colloid and Interface Science, 432*, 109-116.

538   Quong, D., Neufeld, R., Skjåk-Bræk, G., & Poncelet, D. (1998). External versus internal source of

539   calcium during the gelation of alginate beads for DNA encapsulation. *Biotechnology and*

540   *Bioengineering, 57*, 438-446.

541   Rehm, B. H. A. (Ed.). (2009). *Alginates: biology and applications*. Heidelberg: Springer.

542   Rosa, P., Sala, G., Van Vliet, T., & Van De Velde, F. (2006). Cold gelation of whey protein

543   emulsions. *Journal of Texture Studies, 37*, 516-537.

544 Sala, G., de Wijk, R. A., van de Velde, F., & van Aken, G. A. (2008). Matrix properties affect the

545   sensory perception of emulsion-filled gels. *Food Hydrocolloids, 22*, 353-363.

546 Sala, G., van Vliet, T., Cohen Stuart, M. A., Aken, G. A. v., & van de Velde, F. (2009). Deformation

547   and fracture of emulsion-filled gels: Effect of oil content and deformation speed. *Food*

548   *Hydrocolloids*, 23, 1381–1393.

549 Sato, A. C. K., Moraes, K. E. F. P., & Cunha, R. L. (2014). Development of gelled emulsions with

550   improved oxidative and pH stability. *Food Hydrocolloids*, 34, 184–192.

551 Smith, D., Scherer, G., & Anderson, J. (1995). Shrinkage during drying of silica gel. *Journal of Non-*

552   *Crystalline Solids, 188*, 191-206.

553 Tavernier, I., Patel, A. R., Van der Meeren, P., & Dewettinck, K. (2017). Emulsion-templated liquid

554   oil structuring with soy protein and soy protein: κ-carrageenan complexes. *Food*

555   *Hydrocolloids, 65*, 107-120.

556 Urbonaite, V., de Jongh, H. H. J., van der Linden, E., & Pouvreau, L. (2015). Water holding of soy

557   protein gels is set by coarseness, modulated by calcium binding, rather than gel stiffness.

558   *Food Hydrocolloids, 46*, 103-111.

559 Van Vliet, T. (1988). Rheological properties of filled gels: Influence of filler matrix interaction.

560   *Colloid and Polymer Science, 266*, 518-524.

561 Vasile, F. E., Judis, M. A., & Mazzobre, M. F. (2018). Impact of Prosopis alba exudate gum on

562   sorption properties and physical stability of fish oil alginate beads prepared by ionic gelation.

563   *Food Chemistry, 250*, 75-82.

564 Wagner, J., & Añón, M. C. (1990). Influence of denaturation, hydrophobicity and sulfhydryl content

565   on solubility and water absorbing capacity of soy protein isolates. *Journal of Food Science,*

566   *55*, 765-770.

567 Wang, S., Marcone, M., Barbut, S., & Lim, L. T. (2012). The impact of anthocyanin-rich red

568   raspberry extract (ARRE) on the properties of edible soy protein isolate (SPI) films. *Journal*

569   *of Food Science, 77*, C497-505.

570   Wang, Z., Neves, M. A., Kobayashi, I., Uemura, K., & Nakajima, M. (2013). Preparation,

571   characterization, and in vitro gastrointestinal digestibility of oil-in-water emulsion-agar gels.

572   *Bioscience Biotechnology and Biochemistry, 77*, 467-474.

573   Yang, N., Feng, Y. N., Su, C. X., Wang, Q., Zhang, Y. M., Wei, Y. H., Zhao, M., Nishinari, K., Fang,

574   Y. P. (2020). Structure and tribology of κ-carrageenan gels filled with natural oil bodies. *Food

575   hydrocolloids,* 105945.

576   Yin, L., Zhao, Z., Hu, Y., Ding, J., Cui, F., Tang, C., & Yin, C. (2008). Polymer–protein interaction,

577   water retention, and biocompatibility of a stimuli-sensitive superporous hydrogel containing

578   interpenetrating polymer networks. *Journal of Applied Polymer Science, 108*, 1238-1248.

579   Zhang, Z. P., Zhang, R. J., Zou, L. Q., Chen, L., Ahmed, Y., Bishri, W. A., Balamash, K., &

580   McClements, D. J. (2016). Encapsulation of curcumin in polysaccharide-based hydrogel

581   beads. *Food Hydrocolloids, 58*, 160–170.

582

583 **Table 1**

584 Formulations of experimental emulsions.

Group	Water phase[a]		Oil Con. (% wt)
	Alginate Con. (% wt)	Soy protein Con. (% wt)	
1A (Control 1)	1.0	0	0
1A1S (Control 2)	1.0	1.0	0
1A1S20O	1.0	1.0	20
0.5A1S20O	0.5	1.0	20
1.5A1S20O	1.5	1.0	20
1A0.5S20O	1.0	0.5	20
1A2S20O	1.0	2.0	20
1A1S10O	1.0	1.0	10
1A1S40O	1.0	1.0	40

585 [a] The content of water phase was adjusted according to the oil content in the formulation.

Figure Legends

Fig. 1. CLSM images of dispersions/emulsions (A–E) and gel beads (V–Z) after gelation for 30 min. SPI and sunflower oil were stained by red and green, respectively.

Fig. 2. Kinetics of Young's modulus of alginate-based gel beads during gelation: (A) control groups; (B) effect of alginate concentrations (0.5–1.5% in the water phase); (C) effect of SPI concentrations (0.5–2.0% in the water phase); and (D) effect of oil contents (10–40% in the emulsion).
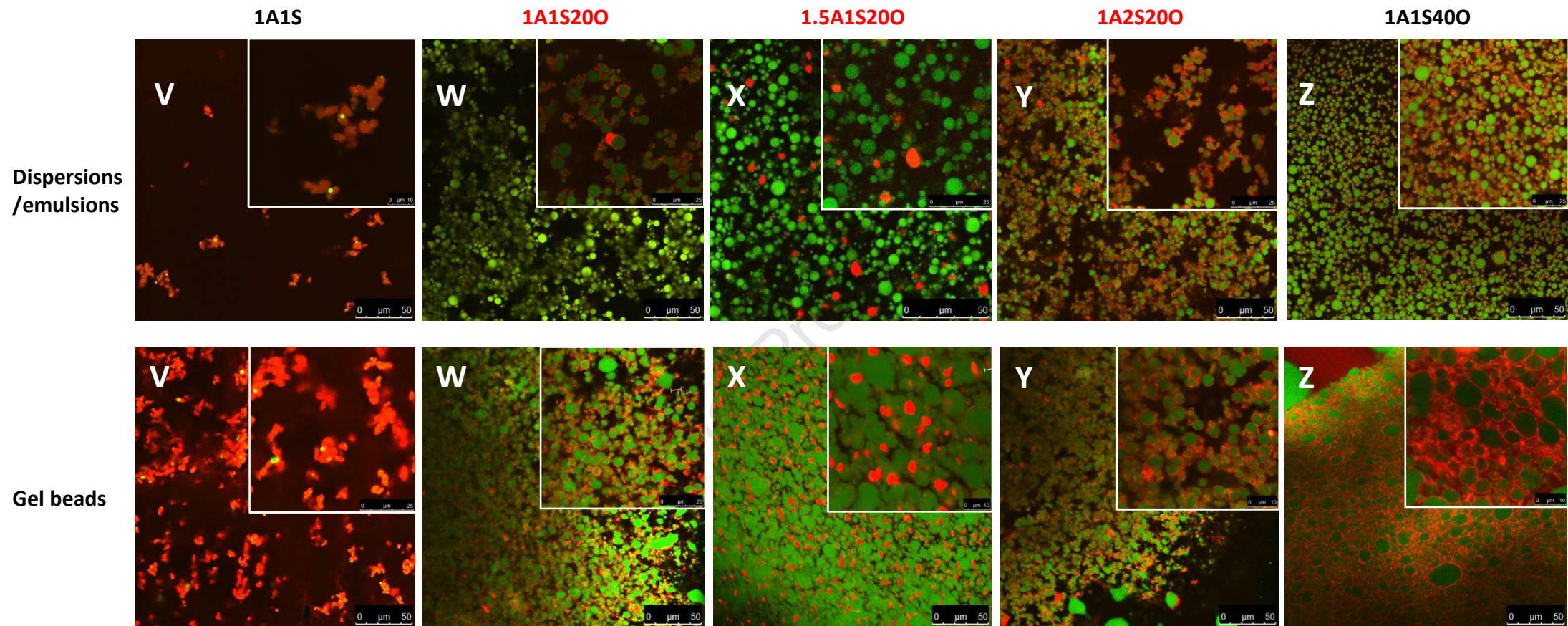
Fig. 3. Kinetics of water loss from alginate-based gel beads during gelation: (A) effect of alginate concentrations (0.5–1.5% in the water phase); (B) effect of SPI concentrations (0.5–2.0% in the water phase); and (C) effect of oil contents (10–40% in the emulsion).
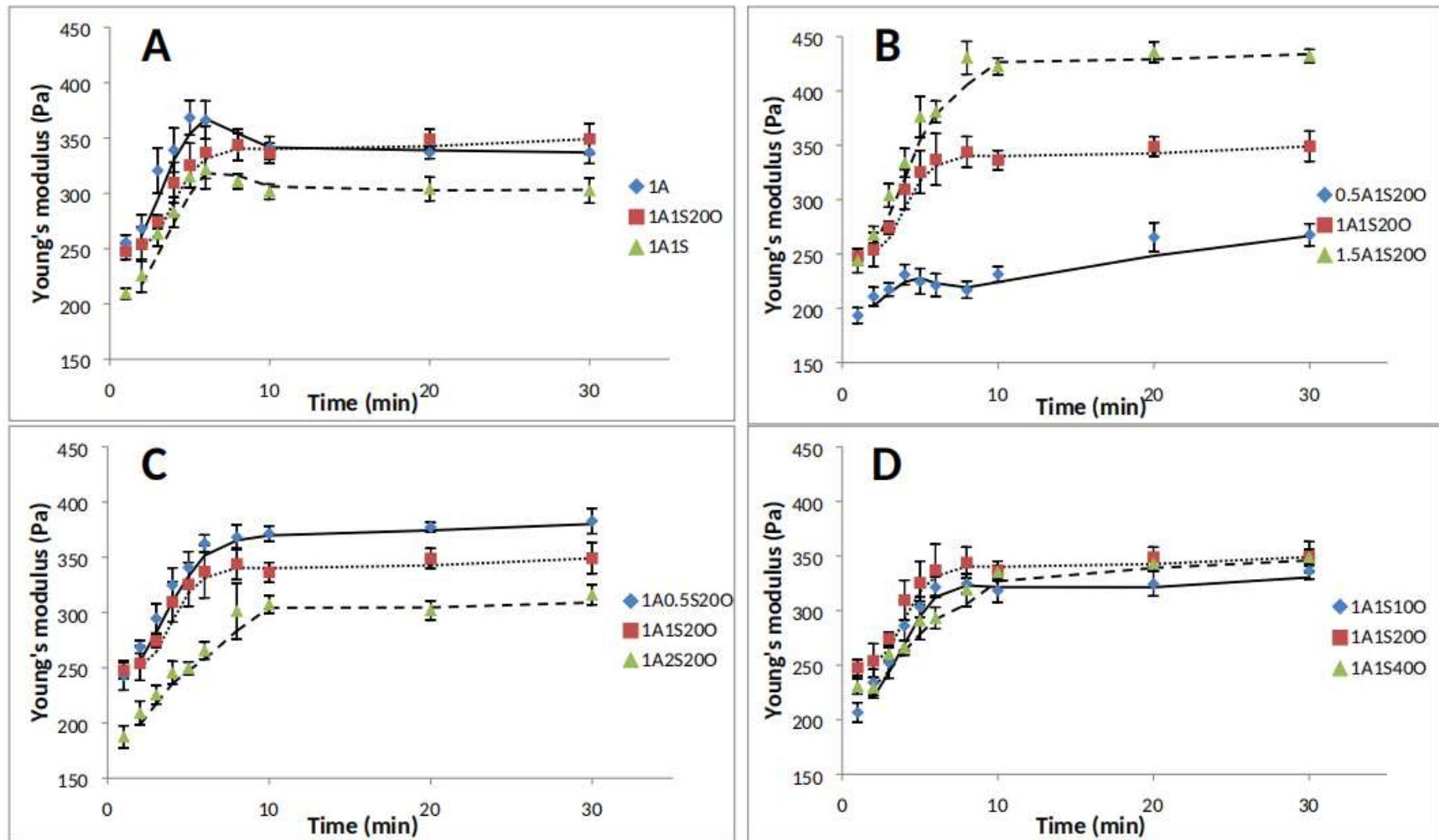
Fig. 4. Visual aspects of alginate-based gel beads during gelation (minimum scale mark = 1 mm).

Fig. 5. Viscosity of dispersions/emulsions with different component concentrations.

Fig. 6. Kinetics of section shrinkage of alginate-based gel beads during gelation: (A) effect of alginate concentrations (0.5–1.5% in the water phase); (B) effect of SPI concentrations (0.5–2.0% in the water phase); and (C) effect of oil contents (10–40% in the emulsion).

1A1S **1A1S20O** **1.5A1S20O** **1A2S20O** **1A1S40O**

Dispersions /emulsions

Gel beads

Fig. 1

Fig. 2

Fig. 3

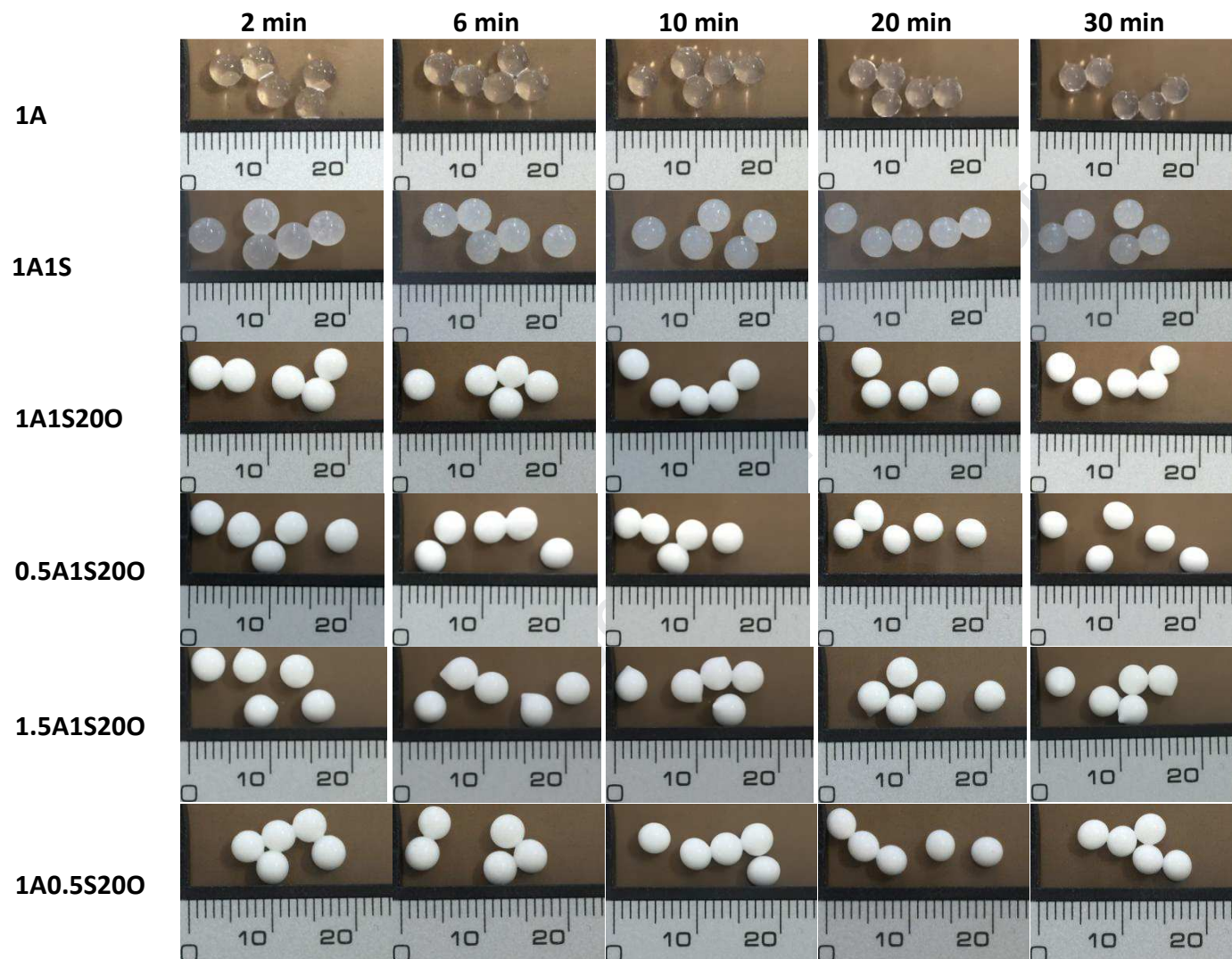**1A2S20O**
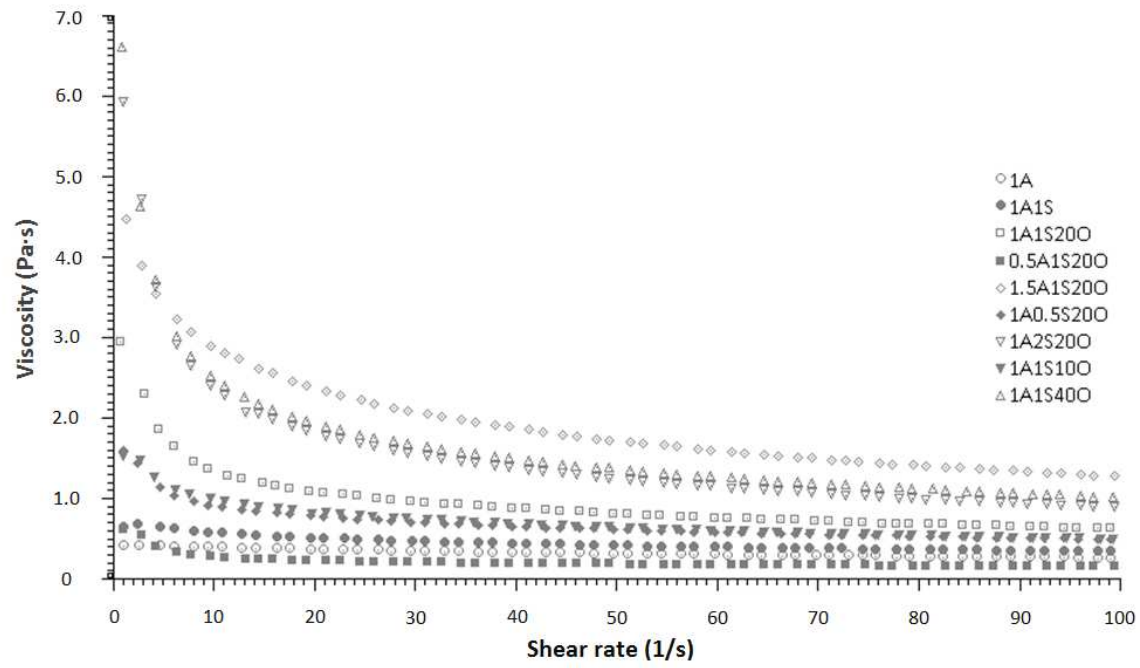
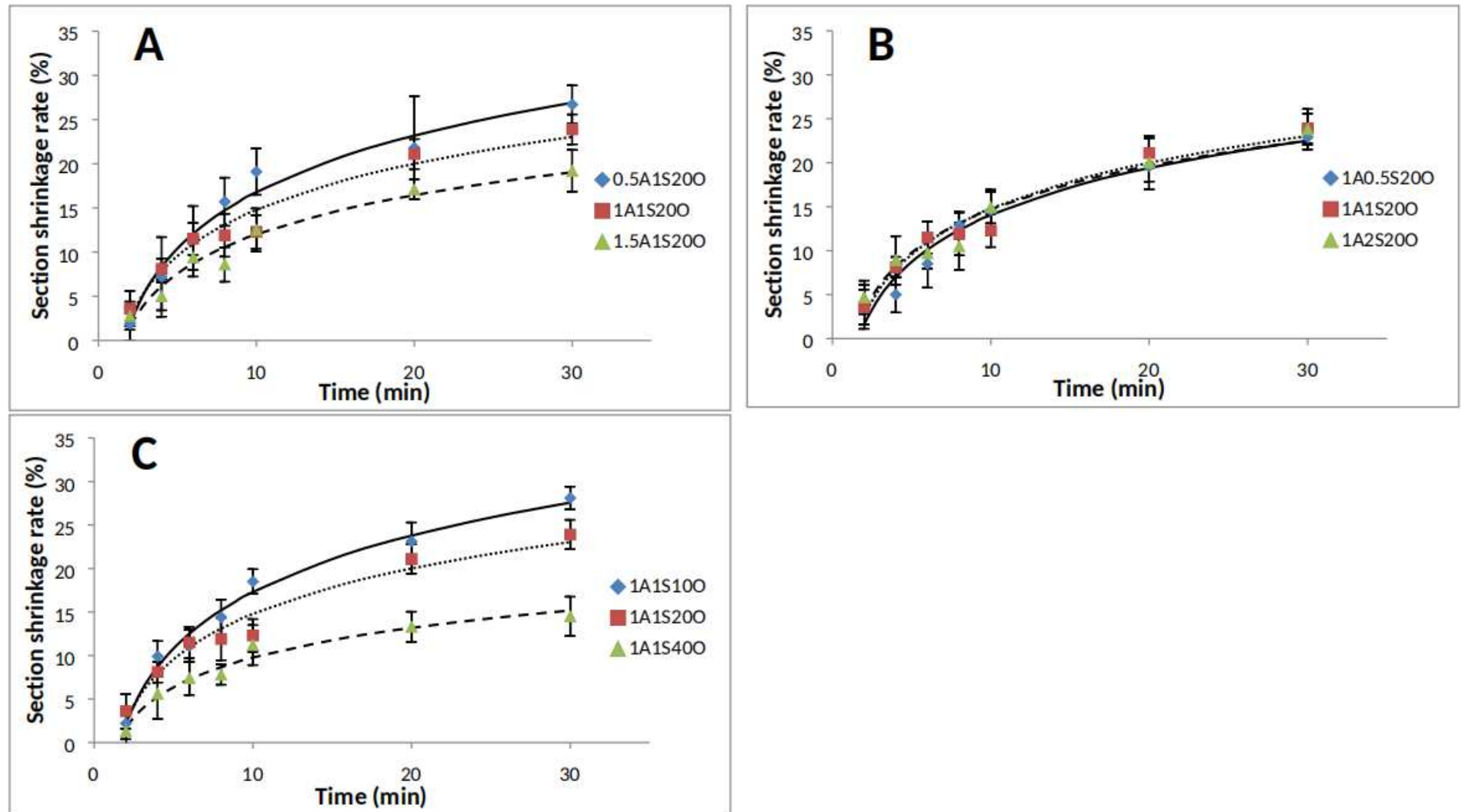**1A1S10O**

1A1S40O
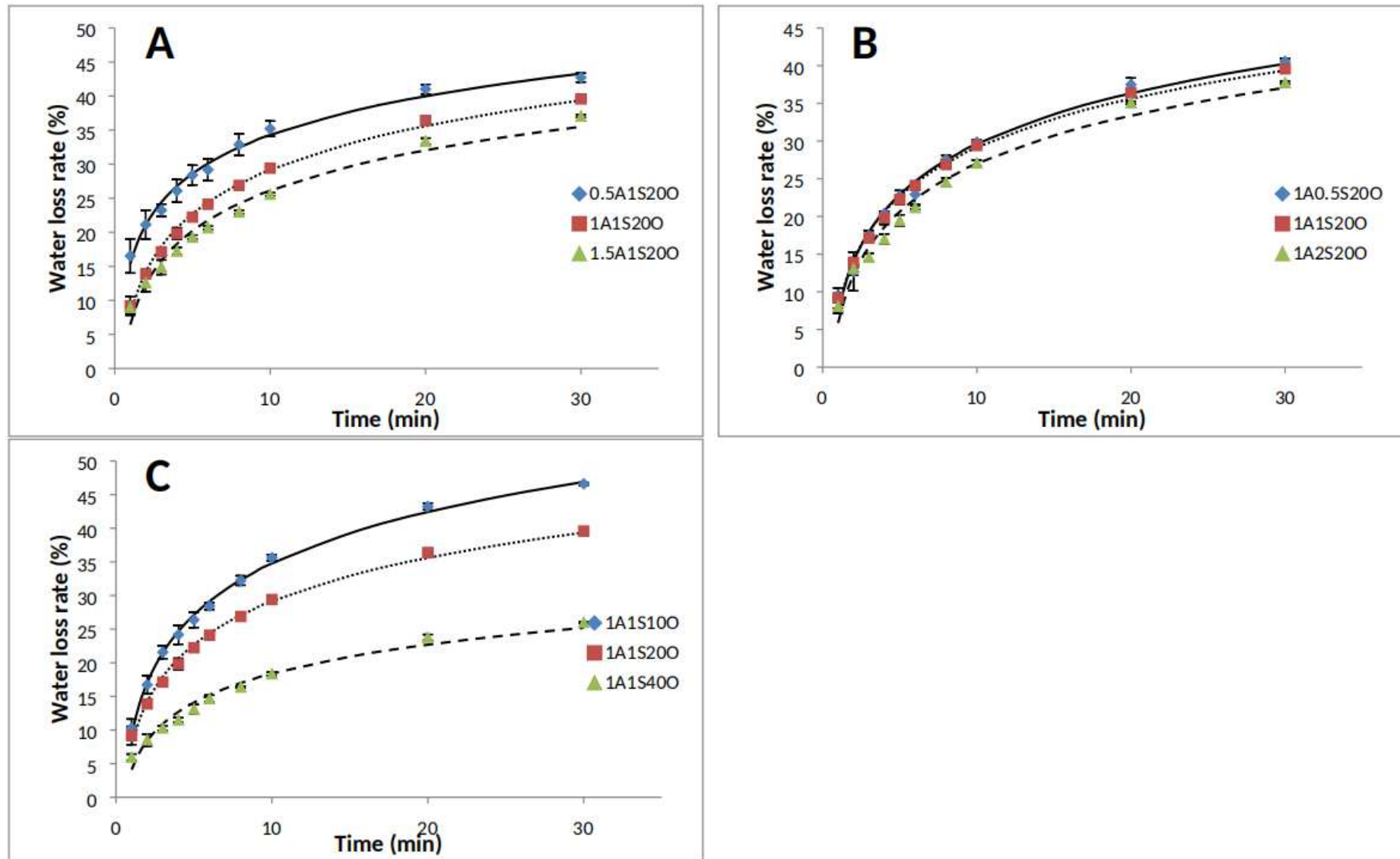
Fig. 4

Fig. 5

Fig. 6

Highlights

• The Young's modulus of emulsion gel beads increased before reaching a plateau during gelation.

• The gelation of emulsion gel beads was accompanied by syneresis and shrinkage.

• High SPI and oil content led to re-coalescence of emulsion droplets during gelation.

• Increasing SPI content decreased the Young's modulus of emulsion gel beads.

• Increasing oil content decreased the shrinkage of emulsion gel beads.

Duanquan Lin: Conceptualization, Methodology, Writing - original draft, Data curation, investigation.

Alan Kelly: Supervision, writing - review & editing.

Valentyn Maidannyk: Investigation.

Song Miao: Supervision, Conceptualization, Writing - review & editing, Funding acquisition, Project administration, Investigation.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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