1   STUDY OF INTRAGASTRIC STRUCTURING ABILITY OF SODIUM ALGINATE BASED O/W

2   EMULSIONS UNDER *IN VITRO* PHYSIOLOGICAL PRE-ABSORPTIVE DIGESTION CONDITIONS

3   Christos Soukoulis[1],*, Ian D. Fisk[2], Torsten Bohn[1] and Lucien Hoffmann[1]

4   [1]Environmental Research and Innovation (ERIN) Department, Luxembourg Institute of Science and

5   Technology (LIST), 41, rue du Brill, L-4422, Belvaux, LUXEMBOURG

6   [2]Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington, LE12

7   5RD, Leicestershire, UNITED KINGDOM

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9   *Author to whom correspondence should be sent:

10   Dr. Christos Soukoulis

11   Phone: +352470261436

12   Fax: +352470264

13   E-mail address: christos.soukoulis@list.lu

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24   Running title: Structured alginate o/w emulsions for satiety

25 ABSTRACT

26 In the present work, the intragastric structuring ability of o/w emulsions either stabilised (1-4% w/w of

27 sodium alginate (SA)) or structured with sheared ionic gel (1-3% w/w of SA crosslinked with Ca^{2+}) in

28 the absence (saliva and gastric phases constituted of deionised water) or presence of *in vitro* pre-

29 absorptive conditions (physiological simulated saliva and gastric fluids) was investigated. Visualisation

30 of the morphological aspects of the gastric chymes, in the absence of multivalent counterions,

31 demonstrated that SA stabilised systems underwent a remarkable swelling in the pH range of 2-3, whilst

32 at the same pH range, ionic SA gel structured systems maintained their major structure configuration.

33 When the aforementioned systems were exposed to physiological intragastric fluids, a reduction of

34 length and the hydrodynamic volume of the alginate fibres was detected regardless the structuring

35 approach. On their exposure to physiological intragastric conditions (pH = 2), SA stabilised emulsions

36 underwent sol-gel transition achieving ca. 3- to 4-order increase of storage modulus (at 1 Hz). In the

37 case of ionic sheared gel structured emulsions, exposure to physiological intragastric fluids resulted in

38 a 10-fold reduction ability of their acid structuring ability, most likely due to the dialysis of egg-box dimer

39 conformations by monovalent cations and protons and the sterical hindering of hydrogen bonding of

40 MM and GG sequences under acidic conditions. Using of non-physiological simulated intragastric fluids

41 was associated with overestimated structuring performance of SA regardless its physical state.

42 Keywords: acid gelation; ionotropic gelation, rheology; gastric fluids; pre-absorptive digestion conditions

43 1. INTRODUCTION

44 Over the last two decades an alarming increase of obesity rates and obesity-associated chronic health

45 complications has been observed: these include type II diabetes, cardiovascular disease, stroke,

46 hypertension, obstructive sleep apnoea and several forms of cancer e.g. postmenopausal breast,

47 colorectal adenoma, endometrial and kidney cancer (Lavie, Milani, & Ventura, 2009; Vigneri, Frasca,

48 Sciacca, Frittitta, & Vigneri, 2006). Obesogenic lifestyle conditions are mainly diet and physical activity

49 driven, and solutions related to eating behaviour, control of food intake via satiety enhancement and

50 suppression of appetite have been under increasing research interest over recent years. In general,

51 satiety is recognised as a neurobiological-physiological construct involving food choice and intake

52 based on orosensory (cross-modally perceived food quality), pre-absorptive (gastric stretching and

53 emptying, suppression of digestive enzymes, conformational changes of the food matrix) and post-

54 absorptive (macronutrients absorption, modification of microbiota and gut biomarkers) factors

55 (Benelam, 2009). Furthermore, it is well established that macronutrients such as proteins and

56 polysaccharides play a prominent role at regulating satiety post-absorption (Brownlee, 2011;

57 Chambers, McCrickerd, & Yeomans, 2015; Fiszman & Varela, 2013).

58 From a mechanistic point of view, polysaccharides can modulate satiety signalling via several non-

59 absorptive routes such as prolongation of orosensory exposure and modification of sensory perception

60 patterns associated with satisfaction (Morell, Fiszman, Varela, & Hernando, 2014; Tárrega, Martínez,

61 Vélez- Ruiz, & Fiszman, 2014), intragastric structuring (Fiszman & Varela, 2013), suppression of the

62 gastric and intestinal enzymatic activity (Brownlee, 2011; Houghton et al., 2015), and reduction of

63   glucose absorption rates (Fiszman & Varela, 2013). In terms of intragastric structuring, anionic

64   polysaccharides such as sodium alginate, low methoxyl pectins and low acyl gellan gum have been

65   demonstrated to have the potential as acid structuring ingredients when adopting either *in vitro*

66   (Bradbeer, Hancocks, Spyropoulos, & Norton, 2014; Norton, Cox, & Spyropoulos, 2011; Norton, Frith,

67   & Ablett, 2006; Spyropoulos, Norton, & Norton, 2011) or *in vivo* testing (Hoad et al., 2009; Hoad et al.,

68   2004). Following on *in vitro* evaluation of the gastric structuring of anionic biopolymers, it has been

69   demonstrated that parameters such as particle shape, gel strength, physical form and micro- and

70   macro-structure substantially impact gastric emptying and distension and therefore, satiety signalling

71   (Norton et al., 2006) possibly via mechanical receptors (Carmagnola, Cantù, & Penagini, 2005).

72   Although the aforementioned studies have provided a valuable *in vitro* understanding of the intragastric

73   structuring potential of anionic biopolymers, in most cases the adopted conditions are quite different

74   from the physiological expected ones. The latter may refer either to the adoption of slow acidification

75   conditions (e.g. use of slowly hydrolysed δ-glucono-lactone), absence of the saliva phase dilution step,

76   adoption of gastric conditions that are not relevant physiologically, e.g. very narrow pH range (typically

77   pH=1-2), absence of multivalent counterions and digestive enzymes (Bradbeer et al., 2014; Hoad et al.,

78   2004; Norton et al., 2011; Spyropoulos et al., 2011).

79   Based on this knowledge gap, in the present work we aimed to assess the impact of two pre-absorptive

80   digestion protocols: a) *in vitro* simulated physiological (as adopted by Minekus et al., 2014) pre-

81   absorptive digestion conditions (oral and gastric phase) and b) direct acidification of initial systems

82   using de-ionised water as diluting medium, on the acid structuring ability of a widely used material,

83   targeting nutraceutical applications including satiety modulation, specifically sodium alginate. The

84   aforementioned digestion protocols were assessed in sodium alginate stabilised and Ca^{2+} sheared gel

85   structured o/w emulsions.

86   2. MATERIALS AND METHODS

87   *2.1 Materials*

88   Low viscosity sodium alginate (250cP, 2% in water at 25°C, M/G ratio = 1.6, mannuronic to guluronic

89   acid content 61-31, Mw = 1.43 x 10^{-5} g mol^{-1}), Tween 80, calcium carbonate, δ-glucono-lactone, porcine

90   pepsin (≥ 250 U/mg) were purchased from Sigma Aldrich (Leuven, Belgium). All other chemicals, unless

91   otherwise stated, were from the same supplier and of analytical grade quality. Sodium alginate was

92   used for the preparation of o/w emulsions without further purification. Canola oil (Mazola, Bekkevoort,

93   Belgium) was obtained from the local market.

94   *2.2 Preparation of the sodium alginate based solutions and Ca^{2+} mediated hydrogels*

95   Sodium alginate was dispersed into 400 mL deionised 18 MΩ (Millipore, USA) water (2-8% w/w), heated

96   at 75°C and left to fully dissolve and hydrate overnight. A small amount of sodium azide (0.002% w/w)

97   was added to prevent microbial spoilage. Two hundred mL aliquots of sodium alginate solutions (2-6%

98   w/w) were mixed with $CaCO_3$ in order to achieve a final concentration of 15, 30 and 45 mM as previously

99   reported by Fernández Farrés & Norton (2014). The biopolymer solutions were successively

100   ultrasonicated (5 min, 90% amplitude, Hielscher UP200S, GmbH, Teltow, Germany) to ensure uniform

101   distribution of $CaCO_3$. Finally, the solutions were mixed with of δ-glucono-lactone (at a 2:1 GDL to

102   $CaCO_3$ ratio) to trigger the slow *in situ* release of Ca^{2+} ions and kept under agitation at 1000 rpm using

103   a paddle stirrer for 6h. The obtained sodium alginate solutions and sheared gels were stored overnight

104   at ambient temperature ($20\pm2\,^{\circ}$C) prior to successive use.

105   *2.3 Preparation of the o/w emulsion-sodium alginate systems*

106   Sub-micron o/w emulsions (6% w/w in oil) were prepared via the spontaneous emulsification method at

107   ambient temperature ($20\pm2^{\circ}$C), as described by Komaiko and McClements (2015). The lipid phase

108   comprising Tween 80 and canola oil at the ratio of 3:7 w/w (kept at ambient temperature under constant

109   agitation for at least 45 min prior to use) was dropwise (ca. 0.5 g/min) added into Millipore water under

110   constant magnetic stirring (1000 rpm) and the obtained o/w emulsion was kept stirring for 10 min.

111   Finally, the obtained o/w emulsions were blended (1:1) with either the sodium alginate solutions or

112   sheared gels resulting in sodium alginate stabilised (1-4% w/w, dissolved state) or structured (1-3%

113   w/w, sheared gel state) o/w emulsions, respectively.

114   *2.4 In vitro pre-absorptive digestion of the o/w emulsions*

115   The gastric structuring ability under simulated physiological conditions was studied adopting a static

116   standardised *in vitro* model as recently descripted by Minekus et al. (2014). In brief, 10 mL of the model

117   food matrix (o/w emulsions) were transferred with a pipette into 50 mL plastic centrifuge tubes and

118   blended with simulated salivary fluid (SSF) (pH = 7, K^+ = 18.8, Na^+ = 13.6, Mg^{2+} = 0.15, Ca^{2+} = 1.5 mM).

119   Then, the oral phase was blended (1:1) with the simulated gastric fluid (SGF) (pH = 2, K^+ = 7.8, Na^+ =

120   72.2, Mg^{2+} = 0.1, Ca^{2+} = 0.15 mM) and incubated at $37\,^{\circ}$C for 1h into a shaking water bath (GFL GmbH,

121   Germany) operated at 100 rpm simulating a physiologically achievable antral shear rate (Vardakou et

122   al., 2011). Simulated gastric chyme systems were cooled down to 25 $^{\circ}$C and were successively

123   characterised for rheological properties. *In vitro* digestion experiments were carried out in triplicate. In

124   addition, a series of model gastric chymes adjusted to a pH ranging from 1 to 4, corresponding to

125   stomach conditions varying from the fasted (starvation) to fed (full stomach) state respectively, were

126   also prepared adopting either physiological (emulsions diluted with SSF & SGF) or non-physiological

127   (emulsions diluted with Millipore water) pre-absorptive digestion conditions.

128   *2.5 Rheological measurements*

129   Steady state shear flow and dynamic oscillatory rheological measurements of the sodium alginate o/w

130   emulsions as well as of the obtained model oral and gastric phase systems were carried out in an

131   Anton-Paar rheometer (MCR 302, WESP, Graz, Austria) using a double gap concentric cylinder

132   geometry (DG 26.7). All measurements were performed at $25 \pm 0.03\ °C$.

133   Steady state shear flow measurements applying an upward-downward ramp shear stress ranged from

134   0.1 to 200 s^{-1} with a 60 s maintenance shear rate step (at 200 s^{-1}) to evaluate thixotropic behaviour of

135   the sodium alginate containing samples were carried out. Upward ramp shear stress (τ) – shear rate

136   data ($\dot{\gamma}$) were fitted to a Herschel-Bulkley model:

137     $$\tau = \tau_0 + K\dot{\gamma}^n \quad (1)$$

138   where: τ_0 equals the yield stress (Pa), K the consistency coefficient (mPa*s^{-n}) and n the rheological

139   behaviour index (dimensionless).

140   Strain-sweep measurements were performed on the sodium alginate containing o/w emulsions at 1 Hz

141   to determine the linear viscoelastic region (LVR). The viscoelastic properties of the sodium alginate

142   containing o/w emulsions as well as the obtained simulated gastric chyme systems were measured by

143   small frequency amplitude sweeps (0.1 to 10 Hz) at a constant strain of 0.1%.

144   *2.6 Light microscopy*

145   The structure conformation changes of alginate fibres during the pre-absorptive digestion conditions

146   (saliva and gastric phases) were qualitatively assessed by means of light microscopy. A small amount

147   (ca. 1 mL) of the biopolymer containing aliquots was mixed with 0.25 mL of toluidine blue solution

148   (0.05% w/w in distilled water) and vortexed for 30 s. Then, 20 μL of the stained biopolymer solution was

149   deposited on a glass slide and covered carefully by a glass cover slip to avoid the entrapment of air

150   bubbles. Samples were visualised at a magnification of 40x using a Zeiss microscope (Axio Vert A1,

151   Zeiss GmbH, Germany).

152   *2.7 Statistical analyses*

153   Normal distribution of data and equality of variance were verified by normal distribution plots and box-

154   plots, respectively. One-way ANOVA at the significance level of $\alpha=0.05$ followed by Tukey's means post

155   hoc comparison test was applied on the steady state flow rheological data. A two-way repeated

156   measurements ANOVA was performed on complex viscosity data of the simulated gastric chyme in

157   order to evaluate the significance of pH and ionic strength conditions. All analyses were performed

158   using SPSS v.19 statistical software (IBM Inc., Chicago, IL, USA).

159   3. RESULTS AND DISCUSSION

160   *3.1 Flow behaviour and thixotropy of the sodium alginate containing o/w emulsions*

161   As can be seen in Fig. 1 and Table 1 the viscosimetric response of the sodium alginate containing

162   emulsions was significantly influenced by the structure conformational state of the biopolymer

163   molecules present in the bulk aqueous phase. Based on the flow behaviour data, all systems exerted a

164   shear thinning behaviour with pseudoplasticity being more pronounced in the case of ionic sheared gel

165   structured systems. However, no clear impact of the biopolymer concentration on the rheological

166   behaviour index (n) of the prepared emulsions was found. In all cases, ionic sheared gel structured

167   emulsions exhibited a significantly ($p < 0.001$) higher consistency coefficient and apparent viscosity

168   values (the latter was calculated at a shear rate of 50 s^{-1} which is indicative of oral shear forces) for the

169   entire concentration range tested in the present work.

170   However, the gap between the stabilised and structured emulsions was diminished when the

171   biopolymer content was increased. Therefore, it may be postulated that the intermolecular

172   entanglement of the sodium alginate chains in the dissolved state is rather restricted leading to the

173   formation of very weak structures that are able to recover almost completely when shear stress is

174   suspended (Ma, Lin, Chen, Zhao, & Zhang, 2014). On the other hand, ionic sheared gel structured

175   emulsions exerted a stronger pseudoplastic (1.5-fold) and thixotropic character (14 to 30-fold)

176   compared to the biopolymer stabilised ones indicating the presence of a biopolymer network due to the

177   aggregation of Ca^{2+}-sodium alginate dimer (egg-box like) structures via their inter-clustering bonding

178   (Fernández Farrés & Norton, 2014).

179   *3.2 Gastric structuring of the o/w emulsions*

180   Acid self-structuring of anionic polysaccharides is regarded as one of the most efficient strategies to

181   promote control of gastric structuring and retard stomach emptying leading to an enhanced satiety

182   response (Bradbeer et al., 2014; Norton et al., 2011). Assessment of intragastric structuring ability of

183   sodium alginate under physiologically simulated conditions is essential as the multivalent counterion

184   composition complexity of saliva and gastric fluids can impact, under specific pH conditions, its

185   ionotropic complexation performance. In addition, the interaction of sodium alginate with other

186   biopolymers via e.g. electrostatic or hydrogen bond binding mechanisms as well as the physical state

187   transformation of the biopolymer matrix throughout pre-absorptive digestion passage due to the action

188   of digestive enzymes may also modify the acid self-assembly of sodium alginate when present in

189   complex food matrices (Brownlee, 2011). Hereby the acid self-assembly of high M/G ratio low viscosity

190   sodium alginate exposed at different pre-absorptive digestion conditions was assessed by means of

191   light microscopy and oscillatory dynamic rheology.

192   *3.2.1 Morphology*

193   In Figs. 2 and 3 the morphological changes of o/w emulsions containing sodium alginate on their

194   exposure to herein assessed pre-absorptive digestion protocol conditions are shown. It should be noted

195   that due to the compression of samples between the glass slide and the cover slip the captured images

196   are not necessarily representative of the actual phase volume (Wolf, Scirocco, Frith, & Norton, 2000).

197   As clearly depicted in Fig. 2, the microstructural aspects of the oral phases were affected primarily by

198   the compositional profile of the simulated saliva fluid (physiological vs. deionised water) and the physical

199   state of sodium alginate (dissolved vs. Ca^{2+} sheared gel state). Oral phases containing sodium alginate

200   in the dissolved state exerted an uneven morphology described by either jagged thread-like or compact

201   polymeric aggregates (Fig. 2a,b). Mixing of the o/w emulsions with physiologic saliva fluid did not

202   remarkably change their morphological aspects, apart from particle size e.g. thinner polymer cords or

203   finer aggregates were detected. In the case of the Ca^{2+} sheared gel structured emulsions (Fig. 2c,d)

204   mixed with deionised water, the presence of low width-to-length particles was confirmed which could

205   be attributed to the high viscosity of the continuous phase and the high shear forces imposed during

206   their fabrication (Wolf et al., 2000). The increase of sodium alginate concentration from 1 to 4% w/w did

207   not confer any remarkable modification of the morphology of the detected particles. When the

208   aforementioned structured emulsions were combined with physiological saliva fluids, the detected

209   biopolymer particles exhibited a more swollen – less compact structure which possibly is associated

210   with the rapid exchange of calcium ions by sodium ions, the latter being present in abundance in the

211   simulated saliva fluids. A similar effect on sodium alginate fibres crosslinked with Ca^{2+} immersed in

212   0.9% w/w saline solution, has been reported by Qin (2004). On the increase of the M/G ratio, the fibrous

213   alginate structures underwent a significant decrease in length which is well corroborating to our

214   observations (Qin, 2004).

215   Exposure of the biopolymer stabilised/structured o/w emulsions to acidic conditions resulted in

216   significant morphological changes as fairly illustrated in Fig. 3. Parameters such as the composition and

217   the pH of the simulated gastric juice, the biopolymer concentration as well as its physical state, e.g.

218   dissolved or Ca^{2+} crosslinked, influenced notably the intragastric structuring performance of the o/w

219   emulsions. Gastric chymes at pH=3 comprising sodium alginate in the dissolved state, exerted a rather

220   compact filamentous structure with protruding outstretched polymeric sheets and limited degree of

221   swelling. When the same systems were exposed to a highly acidic environment (pH=2), cloudy-like

222   polymer aggregates indicative of the formation of acid gel particulates were detected. Although the

223   implemented conditions for light microscope do not allow an accurate determination of the phase

224   volume it appears that the hydrodynamic volume of sodium alginate was increased by increasing the

225   biopolymer content and decreasing the pH, as expected. When the same gastric chyme systems were

226   brought to physiological pre-absorptive conditions, a noteworthy size reduction of the structure

227   polymeric elements was detected regardless the pH value (Fig. 3b,d1). Therefore, the observed pH

228   depending reduction of hydrodynamic volume of the gel particulates under the in-vitro gastric conditions

229   could attributed to a fibre contraction and solvent exclusion associated mechanism

230   (Andriamanantoanina & Rinaudo, 2010; Qin, 2004).

231   In the case of Ca^{2+} mediated structured emulsions (Fig. 3a,b2) the morphological changes found to be

232   strongly dependent on the composition of the gastric chyme. For gastric chymes comprising solely

233   deionised water, only minor changes in the gastric structuring performance of sheared ionic gels at the

234   herein tested sodium alginate content and pH conditions were observed. This is in agreement with the

235   findings of Fernández Farrés & Norton (2014) who demonstrated that ionic sheared gels acid structuring

236   ability shows a limited responsiveness to intragastric pH conditions. In all cases, the gel particulates

237   exerted an agglomerated structure configuration consisting of swollen biopolymer filaments. However,

238   when the Ca^{2+} gel structured emulsions were subjected to physiological in-vitro pre-absorptive digestion

239   conditions, the biopolymer gel particles underwent a noticeable structure conformational change

240   resembling that of the gastric chymes containing sodium alginate in the non-crosslinked state

241   (particularly at the low pH band). In addition, acid gel particulates sustained a hydrodynamic volume

242   reduction in a similar manner, though less extensive, to that of gastric chymes containing sodium

243   alginate in the non-crosslinked state. Hence, it can be deduced that the prevalence of Na^+ in both saliva

244   and gastric phases exerts a strong Ca^{2+} exchanging role (dialysis) which leads to the reduction of

245   amount of Ca^{2+} occupied in the GG blocks. This implies that the M/G ratio also plays a critical role on

246   the gastric structuring ability of sodium alginate, as the increase of the percentage of the MM blocks

247   could lead to a high responsiveness to the ionic composition and pH of the pre-absorptive digestion

248   fluids.

249   *3.2.2 Rheological characterisation*

250   Dynamic oscillatory frequency sweeps were carried out in order to assess the structural changes of the

251   sodium alginate containing o/w emulsions following pre-absorptive digestion conditions (Fig. 4). As

252   illustrated in the rheological spectra (Fig. 4a,b), the physical state of sodium alginate (dissolved or Ca^{2+}

253   mediated gel-like) in the continuous aqueous phase was the governing factor influencing the structure

254   of the o/w emulsions at pH = 7. Specifically, sodium alginate stabilised o/w emulsions (Fig. 4a) exerted

255   a fair viscous behaviour (G''>G'), with moduli being highly dependent on frequency, particularly at the

256   lower concentrations, that is, 1 and 2% w/w. On the other hand, o/w emulsions structured using sheared

257   Ca^{2+} mediated gels, exhibited a clear viscoelastic behaviour (tanδ ranging from 0.43 to 0.52) with the

258   storage modulus (G') being in all cases independent of frequency, which suggests the formation of a

259 loose gel primarily formed via the intermolecular junction of the egg-box dimer structures (Fernández

260 Farrés & Norton, 2014).

261 Mixing of the emulsions with simulated saliva and gastric fluids inducing a plausible 4-fold increase of

262 the gastric chyme bulk volume, accompanied by a direct acidification at pH=2, led to a diversified

263 intragastric structuring pattern (Fig. 4c,d). When emulsions containing sodium alginate in the dissolved

264 state underwent physiological pre-absorptive digestion conditions, a sol-gel transition was observed

265 (G'>G'') attaining a 3- to 4-order increase of G' values which were comparable to those obtained in the

266 case of initial sheared gel structured emulsions. On the contrary, pre-absorptive digestion of Ca^{2+}

267 sheared gel structured emulsions induced a 10-fold decrease of G' implying that both the dilution factor

268 (4-fold) but also the high monovalent cation (Na^+ and K^+) concentration affected adversely the

269 mechanical properties of the intragastric formed gels. However, it should be noted that the digested

270 Ca^{2+} sheared gel structured emulsions maintained their low frequency independent viscoelastic

271 character as a result of their loose gel-like structure. In both cases, the rheological analysis observations

272 corroborate the fairly depicted acid structuring of sodium alginate occurring on direct acidification.

273 To elucidate the magnitude of the impact of the simulated pre-absorptive conditions on the acid

274 structuring performance of the sodium alginate containing emulsions, the gastric chyme samples were

275 rheologically characterised over a broad pH range (reflecting both fasted (empty) and fed (full) stomach

276 conditions) and under diversified multivalent counterion composition of the oral and gastric phases

277 (deionised water vs. physiological saliva and gastric fluids; Figs. 5,6). Although the absence of

278 multivalent counterions did not alter the viscoelastic behaviour of the gastric chymes, the gastric

279   structuring potential remained higher for emulsions containing sodium alginate in the dissolved state.

280   Interestingly, we observed that the G' modulus of the formed acid gels was further increased achieving

281   an almost 10-fold increase compared to initial emulsions. Similarly, gastric chyme systems structured

282   using Ca^{2+} sheared gels (1 and 2% w/w in sodium alginate) exhibited a higher G' modulus (36 and 6-

283   fold, respectively) compared to those obtained adopting physiological conditions (Fig. 5b). On the

284   contrary, a 5-fold reduction of the G' modulus was detected in the case of chyme systems stabilised by

285   3% w/w fluid gel. Our findings underpin that the adopted pre-absorptive digestion conditions may lead

286   to notable discrepancies (under- or over-estimation) in the acid self-structuring of biopolymers and

287   therefore, the adoption of standardised *in-vitro* digestion protocols purposed for the assessment of

288   biopolymer based satiety enhancing processed food prototypes is recommended. In the case of sodium

289   alginate systems, their structural configuration (ratio of M- to G-residues, distribution of homopolymeric

290   and heteropolymeric blocks) and their functional properties (thickening, ion-exchange and gelation

291   properties) influence critically their intragastric structuring ability (Draget, 2009). Andriamanantoanina

292   & Rinaudo (2010) analysing the acid gel forming ability of sodium alginate dialysed with saline solution

293   (0.15% w/w NaCl), reported that in the case of high M/G ratio systems (ca. 1.3), acid gel formation

294   (approx. pH=2.5) is induced via the GG block junction zones interactions stabilised by a hydrogen bond

295   network. When the same biopolymer systems were pre-treated with Ca^{2+} and subsequently exposed to

296   highly acidic conditions (pH=1.35) the authors observed a significant loss of the mechanical gel strength

297   (decline of G'), and reduction of the gel hydrodynamic volume compared to the acid gels obtained in

298   the case of Na^{+} mediated dialysis, which was mainly attributed to the complete exchange of the Ca^{2+}

299   ions by H^+. This is in agreement with our observations for the o/w emulsions structured via Ca^{2+} sheared

300   gels and therefore, it can be hypothesised that direct acidification of the ionically structured o/w

301   emulsions triggers the replacement of Ca^{2+} ions by H^+ favouring the electrostatic repulsion of same

302   charged biopolymer segments and the formation of intermolecular junction zones due to hydrogen

303   bonding of both polyguluronate (GG) and polymannuronic (MM) sequences (Draget, Skjåk-Bræk, &

304   Stokke, 2006). The decrease of G' modulus observed in the gastric chymes containing 3% w/w Ca^{2+}

305   sheared gel is most likely associated with the insufficient dialysis of the highly entangled ionically set

306   alginate gels, which in turn obstructs sterically the formation of intermolecular junction zones (via

307   hydrogen bonding) between the homo-polymeric (GG and MM) blocks under highly acidic conditions

308   (Draget, Skjåk-Bræk, & Stokke, 2006). Thus, it is assumed that although the partial dialysis of the ionic

309   gel is able to induce a reduction of the hydrodynamic volume of alginate molecules lowering the swelling

310   and storage modulus of the ionic gel, at the same time the acid self-structuring of the protonated alginate

311   molecules remains restricted causing eventually the reduction of the G' modulus of the gastric chyme.

312   Screening the pH response of the gastric structuring ability of the sodium alginate containing emulsions

313   (Fig. 6), it was observed that gastric chymes prepared using physiological saliva and gastric fluids

314   exhibit a quite different pH response pattern of complex viscosity compared to that of deionised water

315   based ones. In the presence of a physiological multivalent counterion environment (Fig. 6a), acid

316   structuring was evidenced only at sufficiently low pH conditions associated mainly with gastric

317   conditions in the fasted state i.e. pH< 2.5 (Dressman et al., 1990). Increase of pH was accompanied by

318   a steep decrease of the complex viscosity of the chymes suggesting no prominent gastric structuring

319   ability. Inasmuch as model chyme systems in the absence of multivalent counterions (Fig. 6b), it was

320   observed that complex viscosity appears to be more sensitive to pH changes, with most of the tested

321   systems to exerting a fair acid self-structuring ability in the pH range of 1 to 3. The latter implies that

322   adopting non-physiological gastric conditions (e.g. direct acidification with no pre-adjustment of the ionic

323   composition and strength) may result to an overestimation of the acid self-structuring ability of sodium

324   alginate purposed for satiety promoting applications.

325   4. CONCLUSION AND PERSPECTIVES

326   Overall the present work demonstrated that the adoption of the *in vitro* physiological pre-absorptive

327   conditions affects the acid-self structuring ability of sodium alginate. It was observed that the multivalent

328   counterion composition (Ca^{2+}, Mg^{2+}, Na^+, K^+) of the simulated pre-absorptive digestion fluids, i.e. saliva

329   and gastric phases, critically influences the structuring ability. The structuring responsiveness of sodium

330   alginate was minimised in the presence of multivalent counterions and in the case of the sheared

331   calcium-mediated gels. Dilution of the initial structured/stabilised emulsions with the simulated saliva

332   phase affected pronouncedly the sheared gel containing systems, which however they retained their

333   viscoelastic character. Adoption of a physiological pre-absorptive digestion conditions protocol (dilution

334   factor, pH range and multivalent counterion concentration and composition) appears to be of paramount

335   importance when developing innovative food products with optimal intragastric ability purposed for

336   satiety modulation. Exploiting the acquired knowledge, in future studies are required to demonstrate

337   how, by adopting physiological *in vitro* pre-absorptive digestion conditions, the intragastric structuring

338   and colloidal destabilisation of complex model food systems is affected.

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TABLE 1: Steady flow rheological characteristics (as calculated according to the Herschel-Bulkley model) of the o/w emulsions containing sodium alginate either in the dilute (Sol) or Ca^{2+} mediated gel state (Gel).

Sample	Yield stress (τ_0) (Pa)	Consistency coefficient K (mPa*s^{-n})	Rheological behaviour index n	Apparent viscosity at 50 s^{-1} (mPa*s)	Thixotropy index (%)
1% Sol	ns	9.3[a]	0.98[a]	8.7[a]	0.01[a]
2% Sol	ns	28.3[b]	0.97[a]	25.6[b]	0.29[b]
3% Sol	ns	77.9[c]	0.96[a]	66.3[c]	0.18[b]
4% Sol	ns	193[d]	0.93[a]	149[d]	0.19[b]
1% Gel	1.13	276[e]	0.68[b]	99.6[e]	5.7[c]
2% Gel	1.49	530[f]	0.66[b]	174[f]	4.1[c]
3% Gel	2.06	1133[g]	0.61[b]	293[g]	2.9[d]

[a] Values in a column not sharing the same superscripts are significantly different ($p<0.05$) according to Tukey's post hoc means comparison test. ns = non-significant

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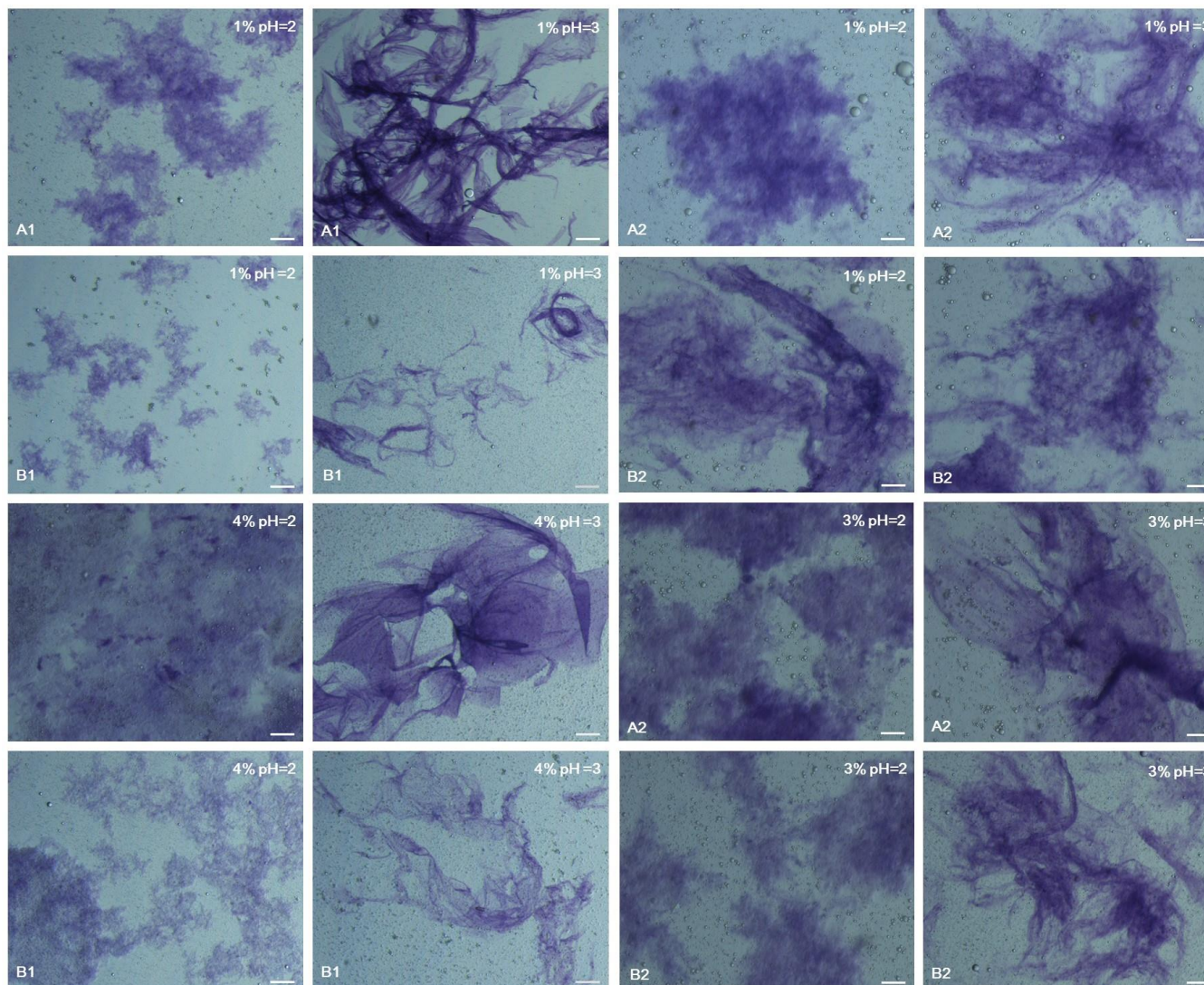
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FIGURE 1

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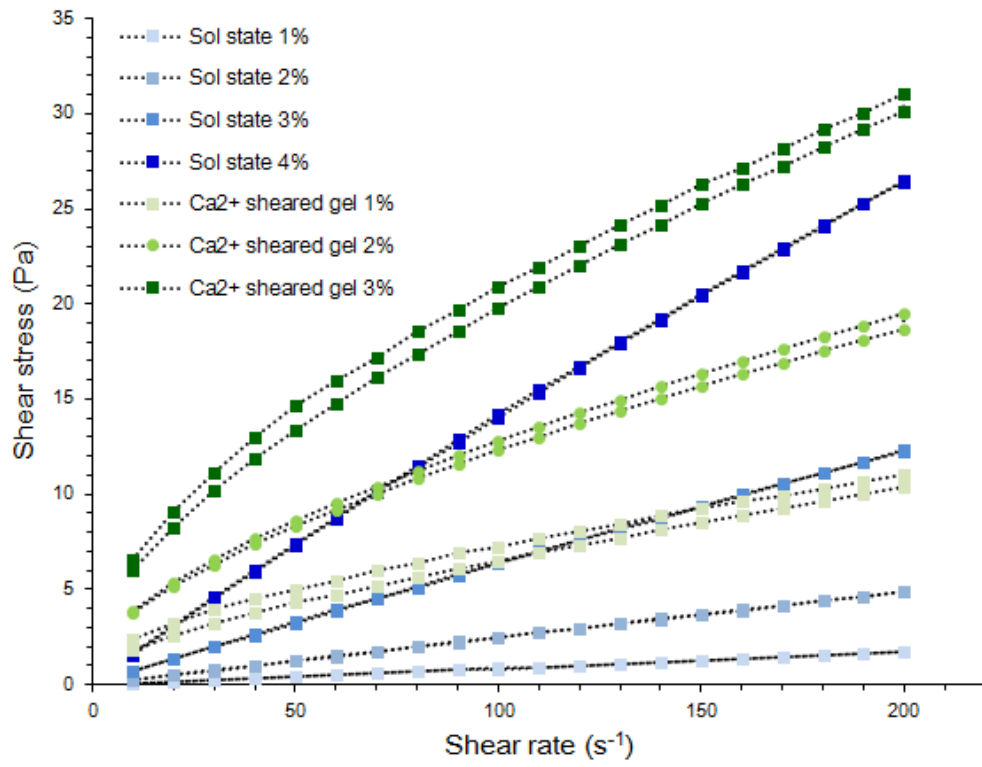
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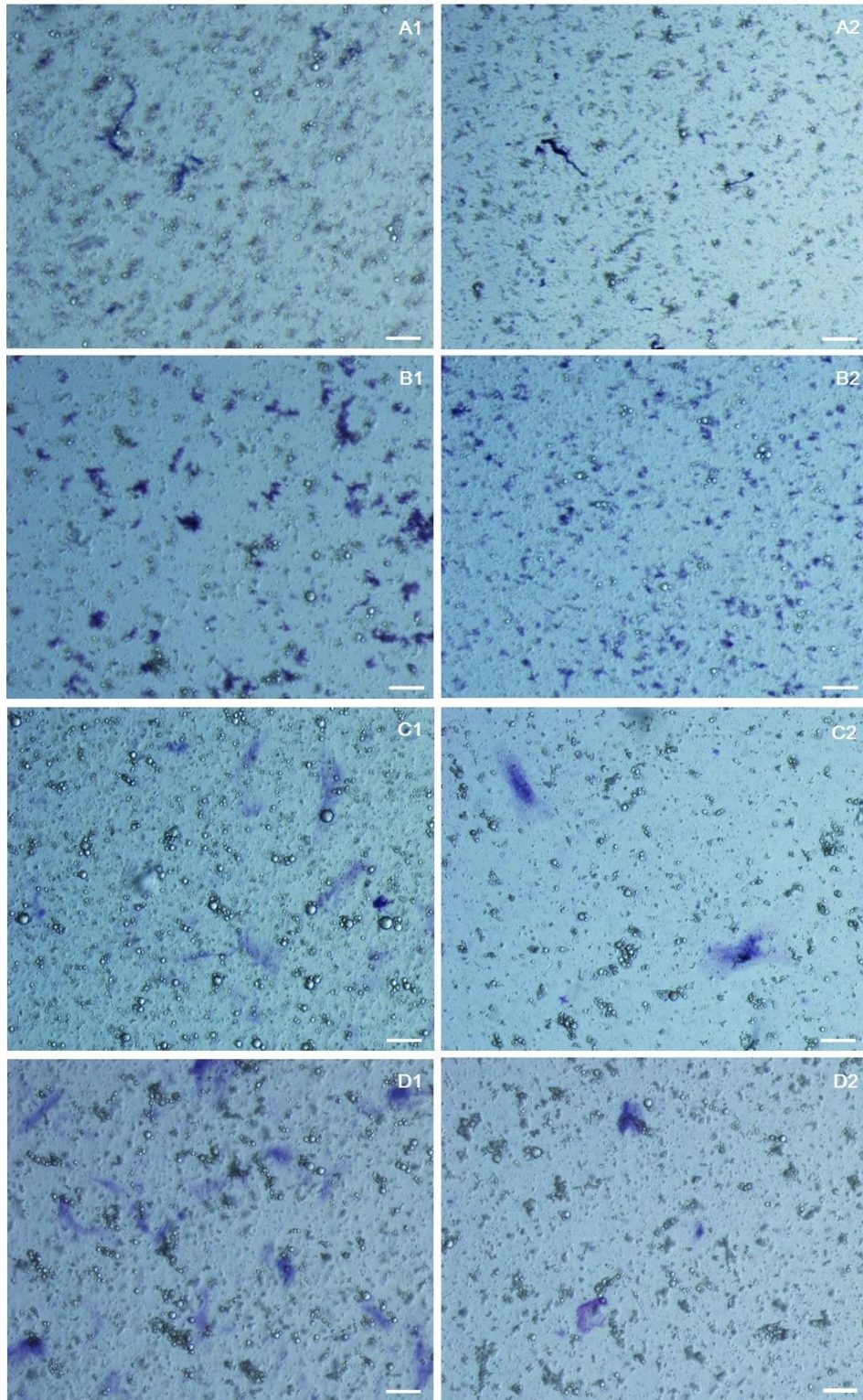
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430   FIGURE 2
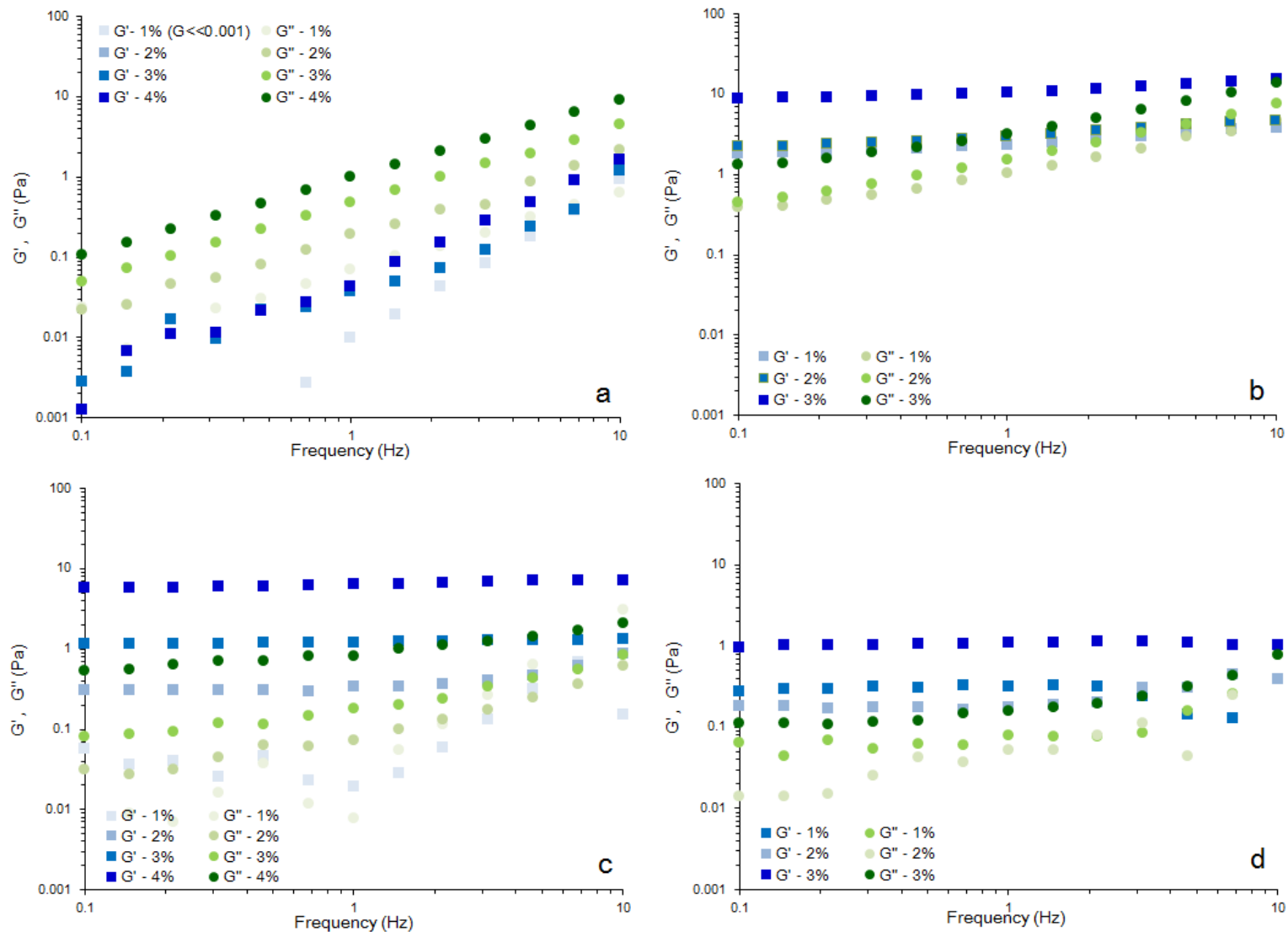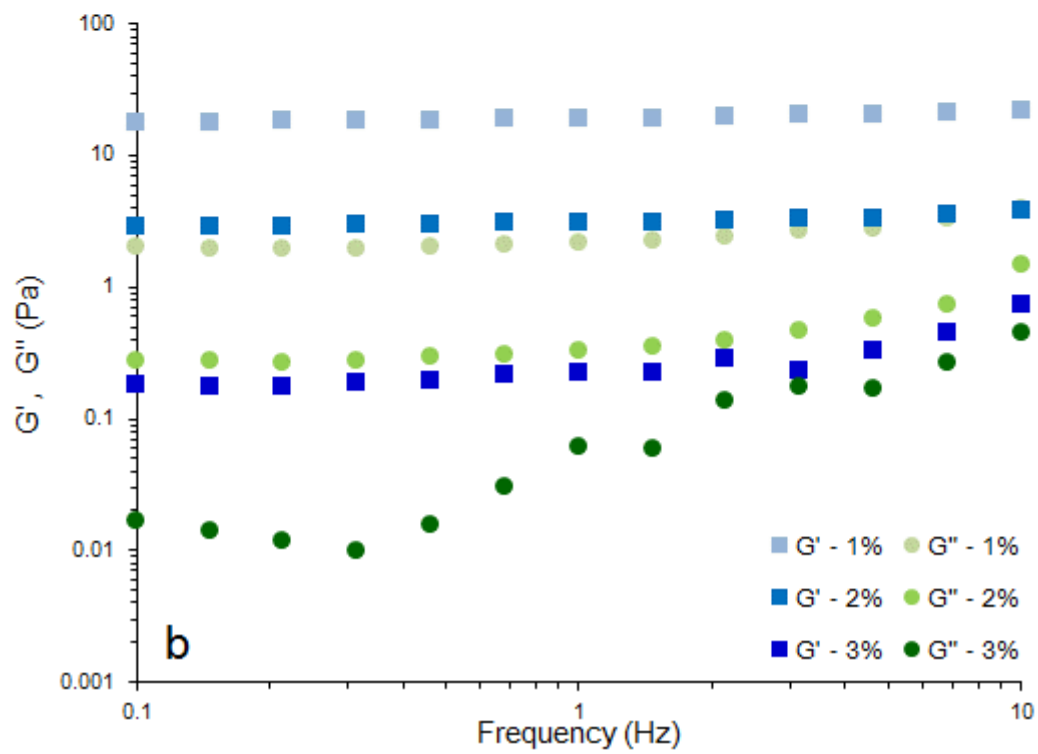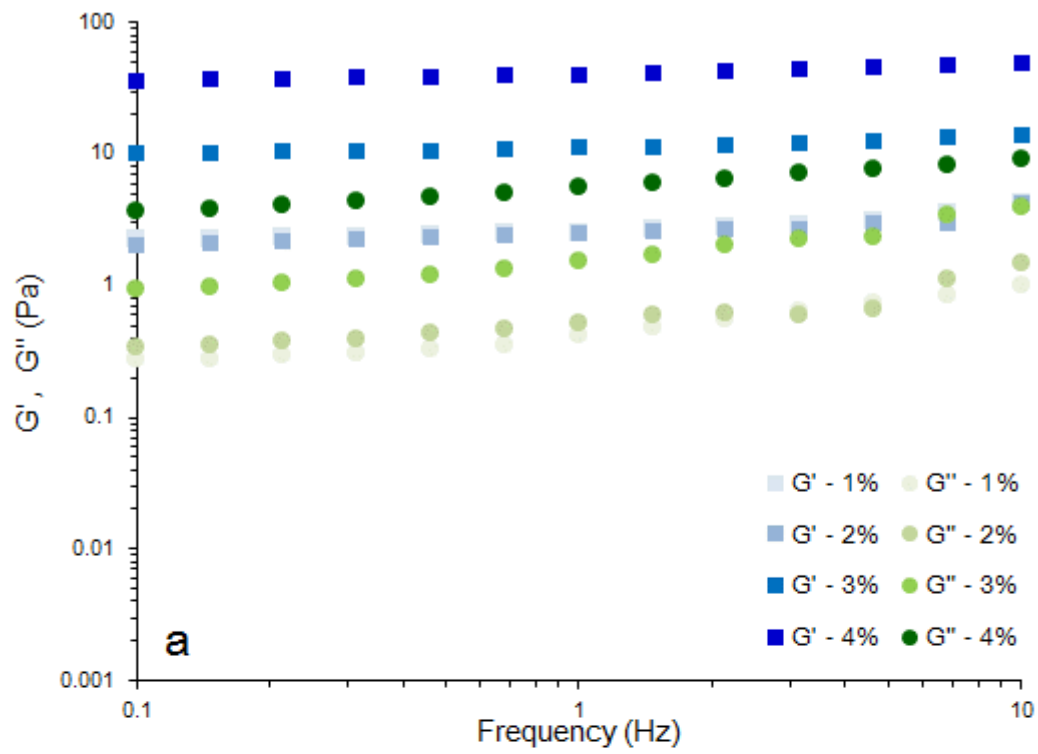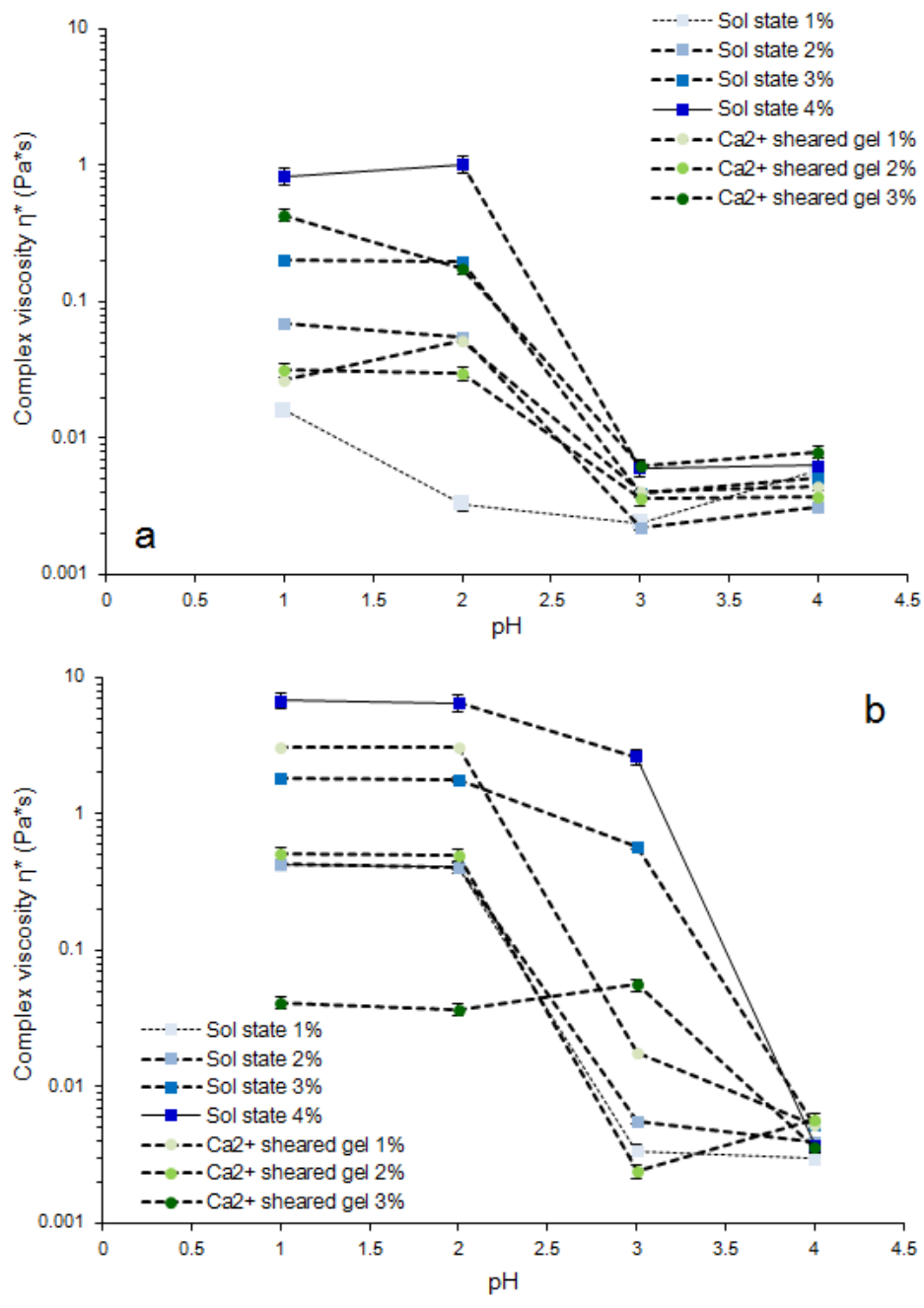
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FIGURE 3

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FIGURES CAPTION

FIGURE 1: Flow curves of o/w emulsions stabilised with sodium alginate hydrogels either in the dilute (squares) or in the Ca^{2+} mediated gel-like state (circles).

FIGURE 2: Optical micrographs illustrating the microstructural aspects of the model saliva phases obtained by mixing (1:1) the initial sodium alginate stabilised (A,B) or ionically structured (C,D) o/w emulsions with either deionised water (label 1) or simulated physiological saliva fluid (label 2). Structural configurations corresponding to sodium alginate were stained with toluidine blue solution (0.05% w/w). Magnification: 40x, Scale bar: 20 μm.

FIGURE 3: Optical micrographs illustrating the microstructural aspects of the model gastric chyme systems obtained by mixing (1:4) the initial sodium alginate stabilised (label 1) or ionically structured (label 2) o/w emulsions with either deionised water (A,C) or physiological saliva and gastric fluids (B,D). Gastric chymes were standardised at two different pH values and incubated at 37 °C for 1h and a shear rate of 100 rpm to simulate stomach antral forces. Structural configurations corresponding to sodium alginate were stained with toluidine blue solution (0.05% w/w). Magnification: 40x, Scale bar: 20 μm.

FIGURE 4: Dynamic oscillatory frequency sweep spectra of sodium alginate stabilised o/w emulsions (a: dilute state, b: fluid gel) and the resulting model gastric chyme systems adopting physiological saliva and gastric juice patterns (c: dilute state, d: fluid gel).

FIGURE 5: Dynamic oscillatory frequency sweep spectra of model gastric chyme systems (at pH =2) of pre-absorptively digested o/w emulsions containing sodium alginate either in the dissolved state (a) or in the Ca^{2+} mediated gel state (b) adopting non-physiological ionic strength conditions (deionised water).

FIGURE 6: pH and ionic composition responsiveness of complex viscosity of model gastric chyme systems of pre-absorptively digested o/w emulsions structured with sodium alginate (a: initial system diluted exclusively with deionised water b: physiological saliva and gastric juice ion composition and concentration).