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Study of intragastric structuring ability of sodium alginate based o/w emulsions under *in vitro* physiological pre-absorptive digestion conditions

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ABSTRACT

In the present work, the intragastric structuring ability of o/w emulsions either stabilised (1–4%, w/w of sodium alginate (SA)) or structured with sheared ionic gel (1–3%, w/w of SA crosslinked with Ca^{2+}) in the absence (saliva and gastric phases constituted of deionised water) or presence of *in vitro* pre-absorptive conditions (physiological simulated saliva and gastric fluids) was investigated. Visualisation of the morphological aspects of the gastric chymes, in the absence of multivalent counterions, demonstrated that SA stabilised systems underwent a remarkable swelling in the pH range of 2–3, whilst at the same pH range, ionic SA gel structured systems maintained their major structure configuration. When the aforementioned systems were exposed to physiological intragastric fluids, a reduction of the length and the hydrodynamic volume of the alginate fibres was detected regardless the structuring approach. On their exposure to physiological intragastric conditions (pH = 2), SA stabilised emulsions underwent sol–gel transition achieving a ca. 3- to 4-order increase of storage modulus (at 1 Hz). In the case of ionic sheared gel structured emulsions, exposure to physiological intragastric fluids resulted in a 10-fold reduction ability of their acid structuring ability, most likely due to the dialysis of egg-box dimer conformations by monovalent cations and protons and the sterical hindering of hydrogen bonding of MM and GG sequences under acidic conditions. Using of non-physiological simulated intragastric fluids was associated with overestimated structuring performance of SA regardless its physical state.

1. Introduction

Over the last two decades an alarming increase of obesity rates and obesity-associated chronic health complications has been observed: these include type II diabetes, cardiovascular disease, stroke, hypertension, obstructive sleep apnoea and several forms of cancer e.g. postmenopausal breast, colorectal adenoma, endometrial and kidney cancer (Lavie, Milani, & Ventura, 2009; Vigneri, Frasca, Sciacca, Frittitta, & Vigneri, 2006). Obesogenic lifestyle conditions are mainly diet and physical activity driven, and solutions related to eating behaviour, control of food intake via satiety enhancement and suppression of appetite have been under increasing research interest over recent years. In general, satiety is recognised as a neurobiological–physiological construct involving food choice and intake based on orosensory (cross-modally perceived food quality), pre-absorptive (gastric stretching and emptying, suppression of digestive enzymes, conformational changes of the food matrix) and post-absorptive (macronutrients absorption, modification of microbiota and gut biomarkers) factors (Benelam, 2009). Furthermore, it is well established that macronutrients such as proteins and polysaccharides play a prominent role at regulating satiety post-absorption (Brownlee, 2011; Chambers, McCrickerd, & Yeomans, 2015; Fiszman & Varela, 2013).

From a mechanistic point of view, polysaccharides can modulate satiety signalling via several non-absorptive routes such as prolongation of orosensory exposure and modification of sensory perception patterns associated with satisfaction (Morell, Fiszman, Varela, & Hernando, 2014; Tárrega, Martínez, Vélez-Ruiz, & Fiszman, 2014), intragastric structuring (Fiszman & Varela, 2013), suppression of the gastric and intestinal enzymatic activity (Brownlee, 2011; Houghton et al., 2015), and reduction of glucose absorption rates (Fiszman & Varela, 2013). In terms of intragastric

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structuring, anionic polysaccharides such as sodium alginate, low methoxyl pectins and low acyl gellan gum have been demonstrated to have the potential as acid structuring ingredients when adopting either *in vitro* (Bradbeer, Hancocks, Spyropoulos, & Norton, 2014; Norton, Cox, & Spyropoulos, 2011; Norton, Frith, & Ablett, 2006; Spyropoulos, Norton, & Norton, 2011) or *in vivo* testing (Hoad et al., 2004, 2009). Following on *in vitro* evaluation of the gastric structuring of anionic biopolymers, it has been demonstrated that parameters such as particle shape, gel strength, physical form and micro- and macro-structure substantially impact gastric emptying and distension and therefore, satiety signalling (Norton et al., 2006) possibly via mechanical receptors (Carmagnola, Cantù, & Penagini, 2005). Although the aforementioned studies have provided a valuable *in vitro* understanding of the intragastric structuring potential of anionic biopolymers, in most cases the adopted conditions are quite different from the physiological expected ones. The latter may refer to the adoption of slow acidification conditions (e.g. use of slowly hydrolysed δ-glucono-lactone), absence of the saliva phase dilution step, adoption of gastric conditions that are not relevant physiologically, e.g. very narrow pH range (typically pH = 1–2), absence of multivalent counterions and digestive enzymes (Bradbeer et al., 2014; Hoad et al., 2004; Norton et al., 2011; Spyropoulos et al., 2011).

Based on this knowledge gap, in the present work we aimed to assess the impact of two pre-absorptive digestion protocols: (a) *in vitro* simulated physiological (as adopted by Minekus et al., 2014) pre-absorptive digestion conditions (oral and gastric phase) and (b) direct acidification of initial systems using de-ionised water as diluting medium, on the acid structuring ability of a widely used material, targeting nutraceutical applications including satiety modulation, specifically sodium alginate. The aforementioned digestion protocols were assessed in sodium alginate stabilised and Ca^{2+} sheared gel structured o/w emulsions.

2. Materials and methods

2.1. Materials

Low viscosity sodium alginate (250 cP, 2% in water at 25 °C, M/G ratio = 1.6, mannuronic to guluronic acid content 61–31, Mw = 1.43×10^{-5} g mol^{-1}), Tween 80, calcium carbonate, δ-glucono-lactone, porcine pepsin (≥250 U/mg) were purchased from Sigma Aldrich (Leuven, Belgium). All other chemicals, unless otherwise stated, were from the same supplier and of analytical grade quality. Sodium alginate was used for the preparation of o/w emulsions without further purification. Canola oil (Mazola, Bekkevoort, Belgium) was obtained from the local market.

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

Sodium alginate was dispersed into 400 mL deionised 18 MΩ (Millipore, USA) water (2–8%, w/w), heated at 75 °C and left to fully dissolve and hydrate overnight. A small amount of sodium azide (0.002%, w/w) was added to prevent microbial spoilage. Two hundred mL aliquots of sodium alginate solutions (2–6%, w/w) were mixed with $CaCO_3$ in order to achieve a final concentration of 15, 30 and 45 mM as previously reported by Fernández Farrés and Norton (2014). The biopolymer solutions were successively ultrasonicated (5 min, 90% amplitude, UP200S, Hielscher GmbH, Teltow, Germany) to ensure uniform distribution of $CaCO_3$. Finally, the solutions were mixed with δ-glucono-lactone (at a 2:1 GDL to $CaCO_3$ ratio) to trigger the slow in situ release of Ca^{2+} ions and kept under agitation at 1000 rpm using a paddle stirrer for 6 h. The obtained sodium alginate solutions and sheared gels were stored overnight at ambient temperature (20 ± 2 °C) prior to successive use.

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

Sub-micron o/w emulsions (6%, w/w in oil) were prepared via the spontaneous emulsification method at ambient temperature (20 ± 2 °C), as described by Komaiko and McClements (2015). The lipid phase comprising Tween 80 and canola oil at the ratio of 3:7 w/w (kept at ambient temperature under constant agitation for at least 45 min prior to use) was dropwise (ca. 0.5 g/min) added into Millipore water under constant magnetic stirring (1000 rpm) and the obtained o/w emulsion was kept stirring for 10 min. Finally, the obtained o/w emulsions were blended (1:1) with either the sodium alginate solutions or sheared gels resulting in sodium alginate stabilised (1–4%, w/w, dissolved state) or structured (1–3%, w/w, sheared gel state) o/w emulsions, respectively.

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

The gastric structuring ability under simulated physiological conditions was studied adopting a static standardised *in vitro* model as recently described by Minekus et al. (2014). In brief, 10 mL of the model food matrix (o/w emulsions) were transferred with a pipette into 50 mL plastic centrifuge tubes and blended with simulated salivary fluid (SSF) (pH = 7, K^+ = 18.8, Na^+ = 13.6, Mg^{2+} = 0.15, Ca^{2+} = 1.5 mM). Then, the oral phase was blended (1:1) with the simulated gastric fluid (SGF) (pH = 2, K^+ = 7.8, Na^+ = 72.2, Mg^{2+} = 0.1, Ca^{2+} = 0.15 mM) and incubated at 37 °C for 1 h into a shaking water bath (GFL GmbH, Germany) operated at 100 rpm simulating a physiologically achievable antral shear rate (Vardakou et al., 2011). Simulated gastric chyme systems were cooled down to 25 °C and were successively characterised for rheological properties. *In vitro* digestion experiments were carried out in triplicate. In addition, a series of model gastric chymes adjusted to a pH ranging from 1 to 4, corresponding to stomach conditions varying from the fasted (starvation) to fed (full stomach) state respectively, were also prepared adopting either physiological (emulsions diluted with SSF & SGF) or non-physiological (emulsions diluted with Millipore water) pre-absorptive digestion conditions.

2.5. Rheological measurements

Steady state shear flow and dynamic oscillatory rheological measurements of the sodium alginate o/w emulsions as well as of the obtained model oral and gastric phase systems were carried out in an Anton-Paar rheometer (MCR 302, WESP, Graz, Austria) using a double gap concentric cylinder geometry (DG 26.7). All measurements were performed at 25 ± 0.03 °C.

Steady state shear flow measurements applying an upward–downward ramp shear stress ranged from 0.1 to 200 s^{-1} with a 60 s maintenance shear rate step (at 200 s^{-1}) to evaluate the thixotropic behaviour of the sodium alginate containing samples were carried out. Upward ramp shear stress (τ) – shear rate data ($\dot{\gamma}$) were fitted to a Herschel–Bulkley model:

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

where τ_0 equals the yield stress (Pa), K the consistency coefficient (mPa s^{-n}) and n the rheological behaviour index (dimensionless).

Strain–sweep measurements were performed on the sodium alginate containing o/w emulsions at 1 Hz to determine the linear viscoelastic region (LVR). The viscoelastic properties of the sodium alginate containing o/w emulsions as well as the obtained simulated gastric chyme systems were measured by small frequency amplitude sweeps (0.1–10 Hz) at a constant strain of 0.1%.

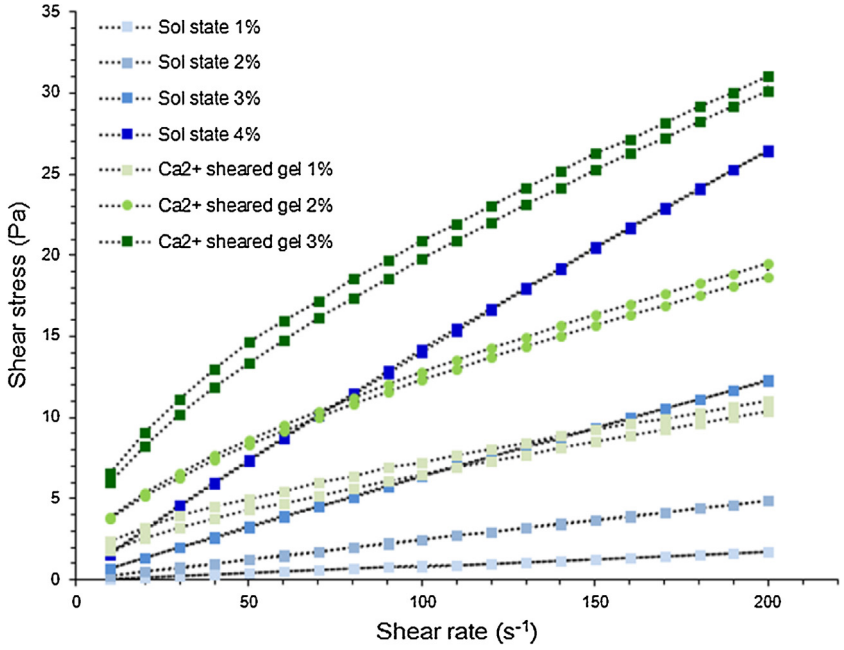

Fig. 1. Flow curves of o/w emulsions stabilised with sodium alginate hydrogels either in the dilute (squares) or in the Ca^{2+} mediated gel-like state (circles).

2.6. Light microscopy

The structure conformation changes of alginate fibres during the pre-absorptive digestion conditions (oral and gastric phases) were qualitatively assessed by means of light microscopy. A small amount (ca. 1 mL) of the biopolymer containing aliquots was mixed with 0.25 mL of toluidine blue solution (0.05%, w/w in distilled water) and vortexed for 30 s. Then, 20 μL of the stained biopolymer solution was deposited on a glass slide and covered carefully by a glass cover slip to avoid the entrapment of air bubbles. Samples were visualised at a magnification of 40× using a Zeiss microscope (Axio Vert A1, Zeiss GmbH, Germany).

2.7. Statistical analyses

Normal distribution of data and equality of variance were verified by normal distribution plots and box-plots, respectively. One-way ANOVA at the significance level of $\alpha = 0.05$ followed by Tukey's means post hoc comparison test was applied on the steady state flow rheological data. A two-way repeated measurements ANOVA was performed on complex viscosity data of the simulated gastric chyme samples in order to evaluate the significance of pH and ionic strength conditions. All analyses were performed using SPSS v.19 statistical software (IBM Inc., Chicago, IL, USA).

3. Results and discussion

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

As can be seen in Fig. 1 and Table 1 the viscosimetric response of the sodium alginate containing emulsions was significantly influenced by the structure conformational state of the biopolymer molecules present in the bulk aqueous phase. Based on the flow behaviour data, all systems exerted a shear thinning behaviour with pseudoplasticity being more pronounced in the case of ionic sheared gel structured systems. However, no clear impact of the biopolymer concentration on the rheological behaviour index (n) of the prepared emulsions was found. In all cases, ionic sheared gel structured emulsions exhibited significantly ($p < 0.001$) higher consistency coefficient and apparent viscosity values (the latter was calculated at a shear rate of 50 s^{-1} which is indicative of oral shear forces) for the entire concentration range tested in the present work.

However, the gap between the stabilised and structured emulsions was diminished when the biopolymer content was increased. Therefore, it may be postulated that the intermolecular entanglement of the sodium alginate chains in the dissolved state is rather restricted leading to the formation of very weak structures that are able to recover almost completely when shear

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}

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.

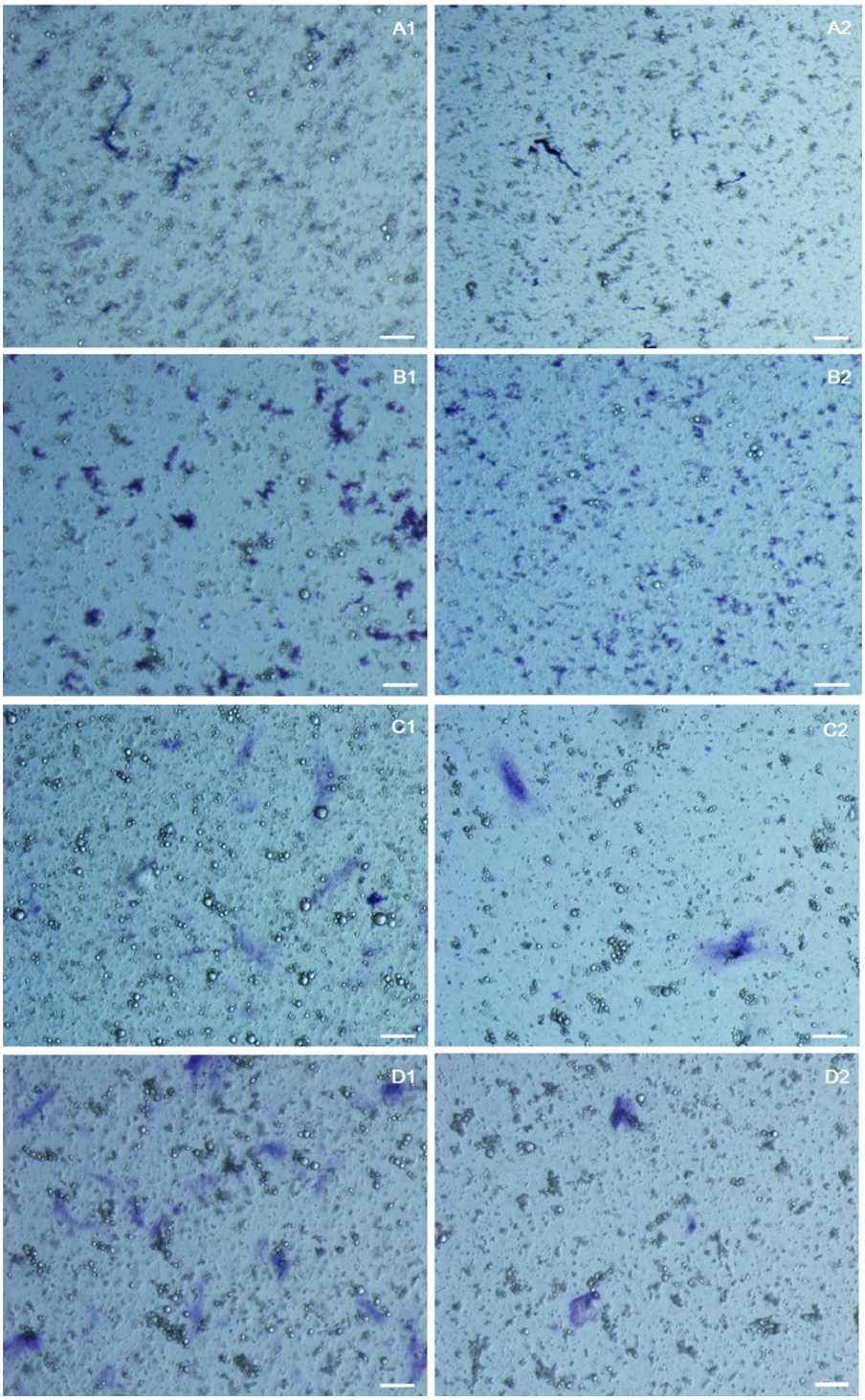

Fig. 2. Optical micrographs illustrating the microstructural aspects of the model saliva phases obtained by mixing (1:1) the initial sodium alginate stabilised (A and B) or ionically structured (C and D) o/w emulsions with either deionised water (label 1) or simulated physiological saliva fluid (label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

stress is suspended (Ma, Lin, Chen, Zhao, & Zhang, 2014). On the other hand, ionic sheared gel structured emulsions exerted a stronger pseudoplastic (1.5-fold) and thixotropic character (14–30-fold) compared to the biopolymer stabilised ones indicating the presence of a biopolymer network due to the aggregation of Ca^{2+}-sodium alginate dimer (egg-box like) structures via their inter-clustering bonding (Fernández Farrés & Norton, 2014).

3.2. Gastric structuring of the o/w emulsions

Acid self-structuring of anionic polysaccharides is regarded as one of the most efficient strategies to promote control of gastric structuring and retard stomach emptying leading to an enhanced satiety response (Bradbeer et al., 2014; Norton et al., 2011). Assessment of intragastric structuring ability of sodium alginate under physiologically simulated conditions is essential as the multivalent

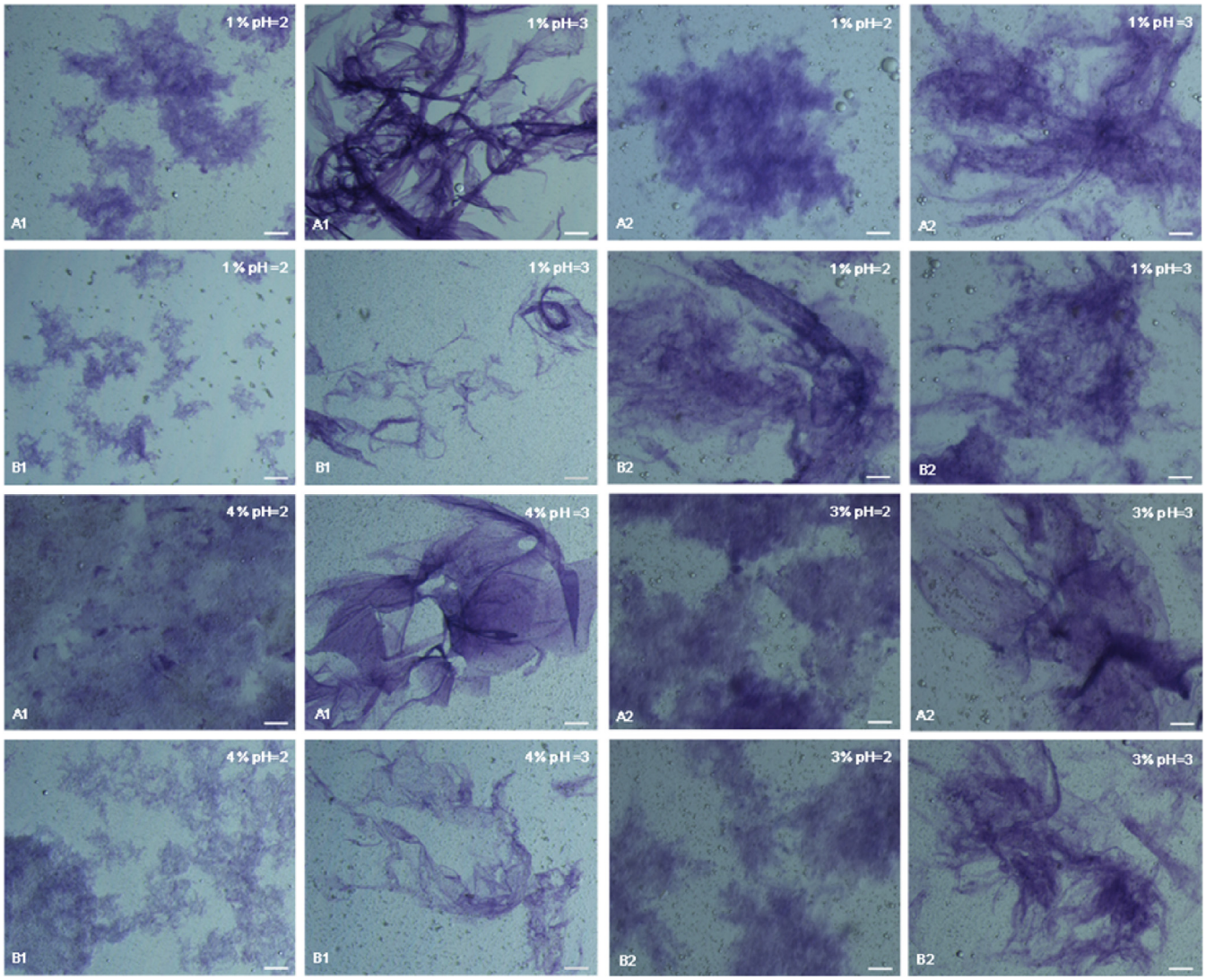

Fig. 3. Optical micrographs illustrating the microstructural aspects of the model gastric chyme systems obtained by mixing (1:4) the initial sodium alginate stabilised (label 1) or ionically structured (label 2) o/w emulsions with either deionised water (A and C) or physiological saliva and gastric fluids (B and D). Gastric chymes were standardised at two different pH values and incubated at 37 °C for 1 h 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: 40×, Scale bar: 20 μm.

counterion composition complexity of saliva and gastric fluids can impact, under specific pH conditions, its ionotropic complexation performance. In addition, the interaction of sodium alginate with other biopolymers via e.g. electrostatic or hydrogen bond binding mechanisms as well as the physical state transformation of the biopolymer matrix throughout pre-absorptive digestion passage due to the action of digestive enzymes may also modify the acid self-assembly of sodium alginate when present in complex food matrices (Brownlee, 2011). Hereby the acid self-assembly of high M/G ratio low viscosity sodium alginate exposed at different pre-absorptive digestion conditions was assessed by means of light microscopy and oscillatory dynamic rheology.

3.2.1. *Morphology*

In Figs. 2 and 3 the morphological changes of o/w emulsions containing sodium alginate, on their exposure to herein assessed pre-absorptive digestion protocol conditions, are shown. It should be noted that due to the compression of the samples between the glass slide and the cover slip the captured images are not necessarily representative of the actual phase volume (Wolf, Scirocco, Frith, & Norton, 2000). As clearly depicted in Fig. 2, the microstructural aspects of the oral phases were affected primarily by the compositional profile of the simulated saliva fluid (physiological vs. deionised water) and the physical state of sodium alginate (dissolved vs. Ca^{2+} sheared gel state). Oral phases containing sodium alginate in the dissolved state exerted an uneven morphology described by either jagged thread-like or compact polymeric aggregates (Fig. 2A and B). Mixing of the o/w emulsions with physiological saliva fluid did not remarkably change their morphological aspects, apart from particle size e.g. thinner polymer cords or finer aggregates were detected. In the case of the Ca^{2+} sheared gel structured emulsions (Fig. 2C and D) mixed with deionised water, the presence of low width-to-length particles was confirmed which could be attributed to the high viscosity of the continuous phase and the high shear forces imposed during their fabrication (Wolf et al., 2000). The increase of sodium alginate concentration from 1 to 4%, w/w did not confer any remarkable modification of the morphology of the detected particles. When the aforementioned structured emulsions were combined with physiological saliva fluids, the detected biopolymer particles exhibited a more swollen – less compact structure which possibly is associated with the rapid exchange of calcium ions by sodium ions, the latter being present in

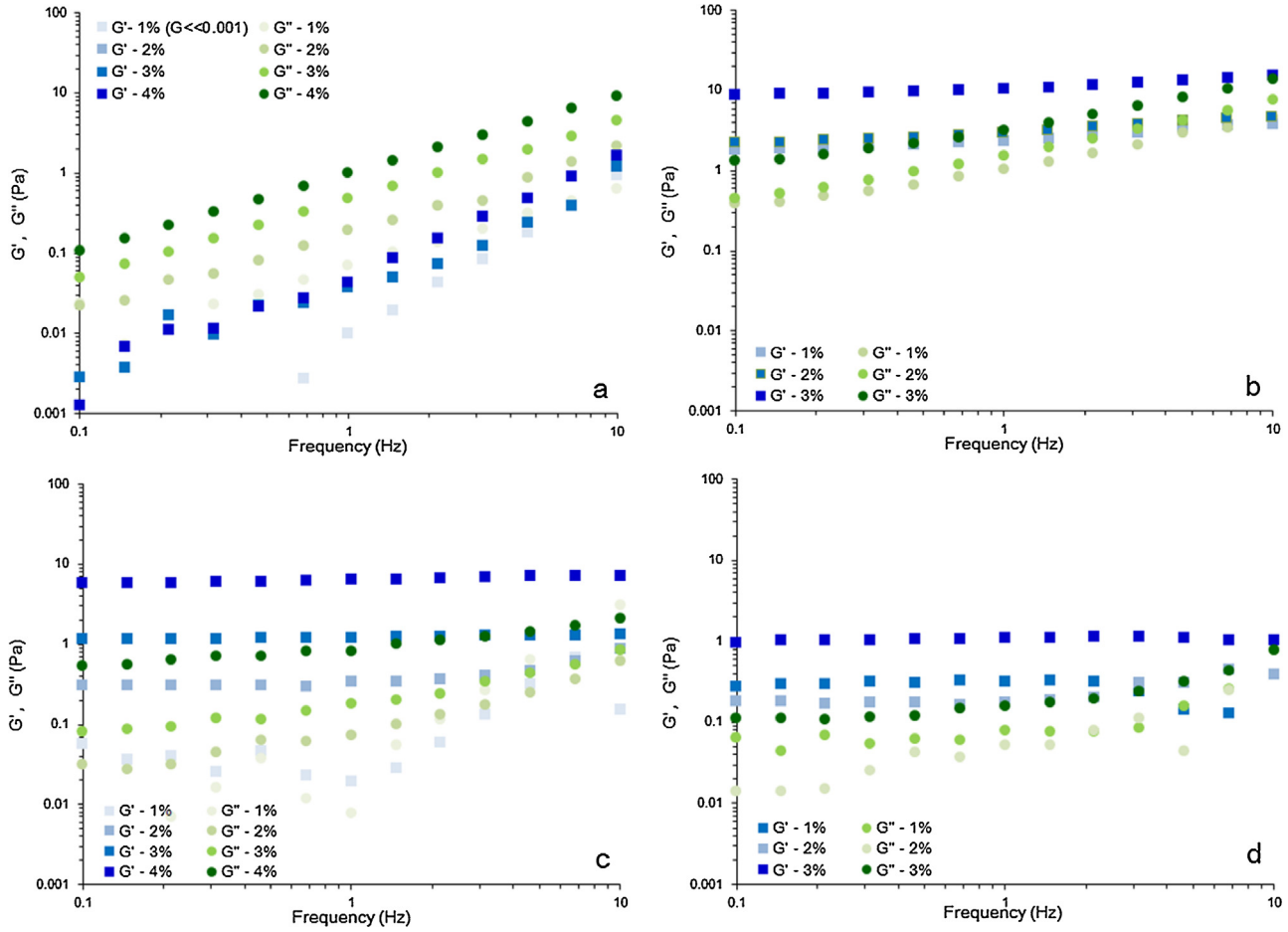

Fig. 4. Dynamic oscillatory frequency sweep spectra of sodium alginate stabilised o/w emulsions (a: dilute state, b: fluid gel) and the resulting model gastric chyme systems adopting physiological saliva and gastric juice patterns (c: dilute state, d: fluid gel).

abundance in the simulated saliva fluids. A similar effect on sodium alginate fibres crosslinked with Ca^{2+} immersed in 0.9%, w/w saline solution, has been reported by Qin (2004). On the increase of the M/G ratio, the fibrous alginate structures underwent a significant decrease in length which is well corroborating to our observations (Qin, 2004).

Exposure of the biopolymer stabilised/structured o/w emulsions to acidic conditions resulted in significant morphological changes as fairly illustrated in Fig. 3. Parameters such as the composition and the pH of the simulated gastric juice, the biopolymer concentration as well as its physical state, e.g. dissolved or Ca^{2+} crosslinked, influenced notably the intragastric structuring performance of the o/w emulsions. Gastric chymes at pH = 3 comprising sodium alginate in the dissolved state, exerted a rather compact filamentous structure with protruding outstretched polymeric sheets of limited swelling degree. When the same systems were exposed to a highly acidic environment (pH = 2), cloudy-like polymer aggregates indicative of the formation of acid gel particulates were detected. Although the implemented conditions for light microscope do not allow an accurate determination of the phase volume, it appears that the hydrodynamic volume of sodium alginate was increased by increasing the biopolymer content and decreasing the pH, as expected. When the same gastric chyme systems were brought to physiological pre-absorptive conditions, a noteworthy size reduction of the structure polymeric elements was detected regardless the pH value (Fig. 3A1 and B1). Therefore, the observed pH depending reduction of hydrodynamic volume of the gel particulates under the in vitro gastric conditions could attributed to a fibre contraction and solvent exclusion associated mechanism (Andriamanantoanina & Rinaudo, 2010; Qin, 2004).

In the case of Ca^{2+} mediated structured emulsions (Fig. 3A2 and B2) the morphological changes found to be strongly dependent on the composition of the gastric chyme. For gastric chymes comprising solely deionised water, only minor changes in the gastric structuring performance of sheared ionic gels at the herein tested sodium alginate content and pH conditions were observed. This is in agreement with the findings of Fernández Farrés and Norton (2014) who demonstrated that ionic sheared gels acid structuring ability shows a limited responsiveness to intragastric pH conditions. In all cases, the gel particulates exerted an agglomerated structure configuration consisting of swollen biopolymer filaments. However, when the Ca^{2+} gel structured emulsions were subjected to physiological *in vitro* pre-absorptive digestion conditions, the biopolymer gel particles underwent a noticeable structure conformational change resembling that of the gastric chymes containing sodium alginate in the non-crosslinked state (particularly at the low pH band). In addition, acid gel particulates sustained a hydrodynamic volume reduction in a similar manner, though less extensive, to that of gastric chymes containing sodium alginate in the non-crosslinked state. Hence, it can be deduced that the prevalence of Na^{+} in both saliva and gastric phases exerts a strong Ca^{2+} exchanging role (dialysis) which leads to the reduction of amount of Ca^{2+} occupied in the GG blocks. This implies that the M/G ratio also plays a critical role on the gastric structuring ability of sodium alginate, as the increase of the percentage of the MM blocks could lead to a high responsiveness

to the ionic composition and pH of the pre-absorptive digestion fluids.

3.2.2. Rheological characterisation

Dynamic oscillatory frequency sweeps were carried out in order to assess the structural changes of the sodium alginate containing o/w emulsions following pre-absorptive digestion conditions (Fig. 4). As illustrated in the rheological spectra (Fig. 4a and b), the physical state of sodium alginate (dissolved or Ca^{2+} mediated gel-like) in the continuous aqueous phase was the governing factor influencing the structure of the o/w emulsions at pH = 7. Specifically, sodium alginate stabilised o/w emulsions (Fig. 4a) exerted a fair viscous behaviour ($G'' > G'$), with moduli being highly dependent on frequency, particularly at the lower concentrations, that is, 1 and 2%, w/w. On the other hand, o/w emulsions structured using sheared Ca^{2+} mediated gels, exhibited a clear viscoelastic behaviour ($\tan\delta$ ranging from 0.43 to 0.52) with the storage modulus (G') being in all cases independent of frequency, which suggests the formation of a loose gel primarily formed via the intermolecular junction of the egg-box dimer structures (Fernández Farrés & Norton, 2014).

Mixing of the emulsions with simulated saliva and gastric fluids inducing a plausible 4-fold increase of the gastric chyme bulk volume, accompanied by a direct acidification at pH = 2, led to a diversified intragastric structuring pattern (Fig. 4c and d). When emulsions containing sodium alginate in the dissolved state underwent physiological pre-absorptive digestion conditions, a sol–gel transition was observed ($G' > G''$) attaining a 3- to 4-order increase of G' values which were comparable to those obtained in the case of initial sheared gel structured emulsions. On the contrary, pre-absorptive digestion of Ca^{2+} sheared gel structured emulsions induced a 10-fold decrease of G' implying that both the dilution factor (4-fold) but also the high monovalent cation (Na^+ and K^+) concentration affected adversely the mechanical properties of the intragastric formed gels. However, it should be noted that the digested Ca^{2+} sheared gel structured emulsions maintained their low frequency independent viscoelastic character as a result of their loose gel-like structure. In both cases, the rheological analysis observations corroborate the fairly depicted acid structuring of sodium alginate occurring on direct acidification.

To elucidate the magnitude of the impact of the simulated pre-absorptive conditions on the acid structuring performance of the sodium alginate containing emulsions, the gastric chyme samples were rheologically characterised over a broad pH range (reflecting both fasted (empty) and fed (full) stomach conditions) and under diversified multivalent counterion composition of the oral and gastric phases (deionised water vs. physiological saliva and gastric fluids; Figs. 5 and 6). Although the absence of multivalent counterions did not alter the viscoelastic behaviour of the gastric chymes, the gastric structuring potential remained higher for emulsions containing sodium alginate in the dissolved state. Interestingly, we observed that the G' modulus of the formed acid gels was further increased achieving an almost 10-fold increase compared to initial emulsions. Similarly, gastric chyme systems structured using Ca^{2+} sheared gels (1 and 2%, w/w in sodium alginate) exhibited a higher G' modulus (36 and 6-fold, respectively) compared to those obtained adopting physiological conditions (Fig. 5b). On the contrary, a 5-fold reduction of the G' modulus was detected in the case of chyme systems stabilised by 3%, w/w fluid gel. Our findings underpin that the adopted pre-absorptive digestion conditions may lead to notable discrepancies (under- or over-estimation) in the acid self-structuring of biopolymers and therefore, the adoption of standardised in vitro digestion protocols purposed for the assessment of biopolymer based satiety enhancing processed food prototypes is recommended. In the case of sodium alginate systems, their structural configuration (ratio of M- to G-residues, distribution of homopolymeric and heteropolymeric blocks) and

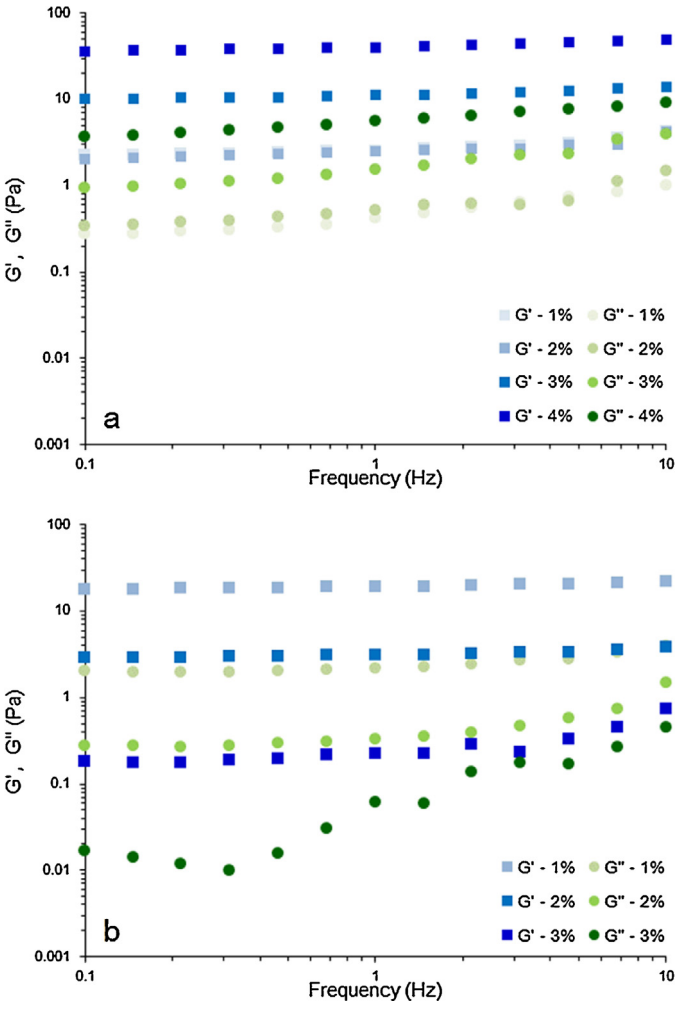

Fig. 5. Dynamic oscillatory frequency sweep spectra of model gastric chyme systems (at pH = 2) of pre-absorptively digested o/w emulsions containing sodium alginate either in the dissolved state (a) or in the Ca^{2+} mediated gel state (b) adopting non-physiological ionic strength conditions (deionised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

their functional properties (thickening, ion-exchange and gelation properties) influence critically their intragastric structuring ability (Draget, 2009). Andriamanantoanina and Rinaudo (2010) analysing the acid gel forming ability of sodium alginate dialysed with saline solution (0.15%, w/w NaCl), reported that in the case of high M/G ratio systems (ca. 1.3), acid gel formation (approx. pH = 2.5) is induced via the GG block junction zones interactions stabilised by a hydrogen bond network. When the same biopolymer systems were pre-treated with Ca^{2+} and subsequently exposed to highly acidic conditions (pH = 1.35) the authors observed a significant loss of the mechanical gel strength (decline of G'), and reduction of the gel hydrodynamic volume compared to the acid gels obtained in the case of Na^+ mediated dialysis, which was mainly attributed to the complete exchange of the Ca^{2+} ions by H^+. This is in agreement with our observations for the o/w emulsions structured via Ca^{2+} sheared gels and therefore, it can be hypothesised that direct acidification of the ionically structured o/w emulsions triggers the replacement of Ca^{2+} ions by H^+ favouring the electrostatic repulsion of same charged biopolymer segments and the formation of intermolecular junction zones due to hydrogen bonding of both polyguluronate (GG) and polymannuronic (MM) sequences (Draget, Skjåk-Bræk, & Stokke, 2006). The decrease of G' modulus observed in the gastric chymes containing 3%, w/w Ca^{2+} sheared gel is most likely associated with the insufficient dialysis of the highly entangled ionically

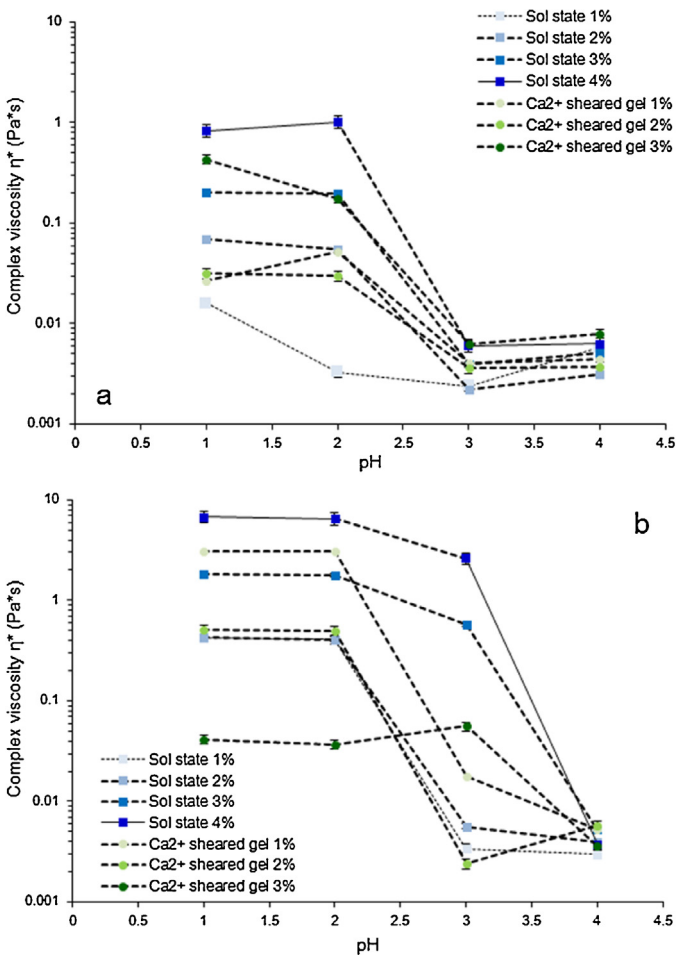

Fig. 6. pH and ionic composition responsiveness of complex viscosity of model gastric chyme systems of pre-absorptively digested o/w emulsions structured with sodium alginate (a: initial system diluted exclusively with deionised water; b: physiological saliva and gastric juice ion composition and concentration).

set alginate gels, which in turn obstructs sterically the formation of intermolecular junction zones (via hydrogen bonding) between the homo-polymeric (GG and MM) blocks under highly acidic conditions (Draget et al., 2006). Thus, it is assumed that although the partial dialysis of the ionic gel is able to induce a reduction of the hydrodynamic volume of alginate molecules lowering the swelling and storage modulus of the ionic gel, at the same time the acid self-structuring of the protonated alginate molecules remains restricted causing eventually the reduction of the G' modulus of the gastric chyme.

Screening the pH response of the gastric structuring ability of the sodium alginate containing emulsions (Fig. 6), it was observed that gastric chymes prepared using physiological saliva and gastric fluids exhibit a quite different pH response pattern of complex viscosity compared to that of deionised water based ones. In the presence of a physiological multivalent counterion environment (Fig. 6a), acid structuring was evidenced only at sufficiently low pH conditions associated mainly with gastric conditions in the fasted state, i.e. pH < 2.5 (Dressman et al., 1990). Increase of pH was accompanied by a steep decrease of the complex viscosity of the chymes suggesting no prominent gastric structuring ability. Inasmuch as model chyme systems in the absence of multivalent counterions (Fig. 6b), it was observed that complex viscosity appears to be more sensitive to pH changes, with most of the tested systems to exerting a fair acid self-structuring ability in the pH range of 1–3. The latter implies that adopting non-physiological gastric conditions (e.g. direct acidification with no pre-adjustment of the ionic composition and strength) may result to an overestimation of the acid self-structuring ability of sodium alginate purposed for satiety promoting applications.

4. Conclusion and perspectives

Overall the present work demonstrated that the adoption of the *in vitro* physiological pre-absorptive conditions affects the acid-self structuring ability of sodium alginate. It was observed that the multivalent counterion composition (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}) of the simulated pre-absorptive digestion fluids, i.e. saliva and gastric phases, critically influences the structuring ability. The structuring responsiveness of sodium alginate was minimised in the presence of multivalent counterions and in the case of the sheared calcium-mediated gels. Dilution of the initial structured/stabilised emulsions with the simulated saliva phase affected pronouncedly the sheared gel containing systems, which however they retained their viscoelastic character. Adoption of a physiological pre-absorptive digestion conditions protocol (dilution factor, pH range and multivalent counterion concentration and composition) appears to be of paramount importance when developing innovative food products with optimal intragastric ability purposed for satiety modulation. Exploiting the acquired knowledge, in future studies is required to demonstrate how, by adopting physiological *in vitro* pre-absorptive digestion conditions, the intragastric structuring and colloidal destabilisation of complex model food systems is affected.

References

Andriamanantoanina, H., & Rinaudo, M. (2010). Relationship between the molecular structure of alginates and their gelation in acidic conditions. *Polymer International*, *59*(11), 1531–1541. http://dx.doi.org/10.1002/pi.2943

Benelam, B. (2009). Satiation, satiety and their effects on eating behaviour. *Nutrition Bulletin*, *34*(2), 126–173. http://dx.doi.org/10.1111/j.1467-3010.2009.01753.x

Bradbeer, J. F., Hancocks, R., Spyropoulos, F., & Norton, I. T. (2014). Self-structuring foods based on acid-sensitive low and high acyl mixed gellan systems to impact on satiety. *Food Hydrocolloids*, *35*, 522–530. http://dx.doi.org/10.1016/j.foodhyd.2013.07.014

Brownlee, I. A. (2011). The physiological roles of dietary fibre. *Food Hydrocolloids*, *25*(2), 238–250. http://dx.doi.org/10.1016/j.foodhyd.2009.11.013

Carmagnola, S., Cantù, P., & Penagini, R. (2005). Mechanoreceptors of the proximal stomach and perception of gastric distension. *The American Journal of Gastroenterology*, *100*(8), 1704–1710. http://dx.doi.org/10.1111/j.1572-0241.2005.41350.x

Chambers, L., McCrickerd, K., & Yeomans, M. R. (2015). Optimising foods for satiety. *Trends in Food Science & Technology*, *41*(2), 149–160. http://dx.doi.org/10.1016/j.tifs.2014.10.007

Draget, K. I. (2009). Alginates. In G. O. Phillips, & P. A. Williams (Eds.), *Handbook of Hydrocolloids* (2nd edition, pp. 807–828). New York: Woodhead Publishing.

Draget, K. I., Skjåk-Bræk, G., & Stokke, B. T. (2006). Similarities and differences between alginic acid gels and ionically crosslinked alginate gels. *Food Hydrocolloids*, *20*(2–3), 170–175, http://doi.org/10.1016/j.foodhyd.2004.03.009.

Dressman, J. B., Berardi, R. R., Dermentzoglou, L. C., Russell, T. L., Schmaltz, S. P., Barnett, J. L., et al. (1990). Upper gastrointestinal (GI) pH in young, healthy men and women. *Pharmaceutical Research*, *7*(7), 756–761. http://dx.doi.org/10.1023/A:1015827908309

Fernández Farrés, I., & Norton, I. T. (2014). Formation kinetics and rheology of alginate fluid gels produced by *in-situ* calcium release. *Food Hydrocolloids*, *40*, 76–84. http://dx.doi.org/10.1016/j.foodhyd.2014.02.005

Fiszman, S., & Varela, P. (2013). The role of gums in satiety/satiation. A review. *Food Hydrocolloids*, *32*(1), 147–154. http://dx.doi.org/10.1016/j.foodhyd.2012.12.010

Hoad, C. L., Rayment, P., Spiller, R. C., Marciani, L., Alonso, B., de, C., et al. (2004). *In vivo* imaging of intragastric gelation and its effect on satiety in humans. *The Journal of Nutrition*, *134*(9), 2293–2300.

Hoad, C., Rayment, P., Cox, E., Wright, P., Butler, M., Spiller, R., et al. (2009). Investigation of alginate beads for gastro-intestinal functionality, Part 2: *In vivo* characterisation. *Food Hydrocolloids*, *23*(3), 833–839. http://dx.doi.org/10.1016/j.foodhyd.2008.04.013

Houghton, D., Wilcox, M. D., Chater, P. I., Brownlee, I. A., Seal, C. J., & Pearson, J. P. (2015). Biological activity of alginate and its effect on pancreatic lipase inhibition as a potential treatment for obesity. *Food Hydrocolloids*, *49*, 18–24. http://dx.doi.org/10.1016/j.foodhyd.2015.02.019

Komaiko, J., & McClements, D. J. (2015). Low-energy formation of edible nanoemulsions by spontaneous emulsification: Factors influencing particle size. *Journal of Food Engineering*, *146*, 122–128. http://dx.doi.org/10.1016/j.jfoodeng.2014.09.003

Lavie, C. J., Milani, R. V., & Ventura, H. O. (2009). Obesity and cardiovascular disease: Risk factor, paradox, and impact of weight loss. *Journal of the American College of Cardiology*, *53*(21), 1925–1932. http://dx.doi.org/10.1016/j.jacc.2008.12.068

Ma, J., Lin, Y., Chen, X., Zhao, B., & Zhang, J. (2014). Flow behavior, thixotropy and dynamical viscoelasticity of sodium alginate aqueous solutions. *Food Hydrocolloids*, *38*, 119–128. http://dx.doi.org/10.1016/j.foodhyd.2013.11.016

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., et al. (2014). A standardised static *in vitro* digestion method suitable for food – An international consensus. *Food & Function*, *5*(6), 1113–1124. http://dx.doi.org/10.1039/C3FO60702J

Morell, P., Fiszman, S. M., Varela, P., & Hernando, I. (2014). Hydrocolloids for enhancing satiety: Relating oral digestion to rheology, structure and sensory perception. *Food Hydrocolloids*, *41*, 343–353. http://dx.doi.org/10.1016/j.foodhyd.2014.04.038

Norton, A. B., Cox, P. W., & Spyropoulos, F. (2011). Acid gelation of low acyl gellan gum relevant to self-structuring in the human stomach. *Food Hydrocolloids*, *25*(5), 1105–1111. http://dx.doi.org/10.1016/j.foodhyd.2010.10.007

Norton, I. T., Frith, W. J., & Ablett, S. (2006). Fluid gels, mixed fluid gels and satiety. *Food Hydrocolloids*, *20*(2–3), 229–239. http://dx.doi.org/10.1016/j.foodhyd.2004.03.011

Qin, Y. (2004). Gel swelling properties of alginate fibers. *Journal of Applied Polymer Science*, *91*(3), 1641–1645. http://dx.doi.org/10.1002/app.13317

Spyropoulos, F., Norton, A. B., & Norton, I. T. (2011). Self-structuring foods based on acid-sensitive mixed biopolymer to impact on satiety. *Procedia Food Science*, *1*, 1487–1493. http://dx.doi.org/10.1016/j.profoo.2011.09.220

Tárrega, A., Martínez, M., Vélez- Ruiz, J. F., & Fiszman, S. (2014). Hydrocolloids as a tool for modulating the expected satiety of milk-based snacks. *Food Hydrocolloids*, *39*, 51–57. http://dx.doi.org/10.1016/j.foodhyd.2013.12.025

Vardakou, M., Mercuri, A., Barker, S. A., Craig, D. Q. M., Faulks, R. M., & Wickham, M. S. J. (2011). Achieving antral grinding forces in biorelevant *in vitro* models: Comparing the USP dissolution apparatus II and the dynamic gastric model with human *in vivo* data. *AAPS PharmSciTech*, *12*(2), 620–626. http://dx.doi.org/10.1208/s12249-011-9616-z

Vigneri, P., Frasca, F., Sciacca, L., Frittitta, L., & Vigneri, R. (2006). Obesity and cancer. *Nutrition, Metabolism and Cardiovascular Diseases*, *16*(1), 1–7. http://dx.doi.org/10.1016/j.numecd.2005.10.013

Wolf, B., Scirocco, R., Frith, W. J., & Norton, I. T. (2000). Shear-induced anisotropic microstructure in phase-separated biopolymer mixtures. *Food Hydrocolloids*, *14*(3), 217–225. http://dx.doi.org/10.1016/S0268-005X(99)00062-4