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Intragastric structuring of anionic polysaccharide kappa-carrageenan filled gels under physiological *in vitro* digestion conditions

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ABSTRACT

In the present work, sodium alginate (SA), low methoxyl pectin (PEC) and κ-carrageenan (κ-CAR) were evaluated for their intragastric structuring ability by means of light microscopy and dynamic oscillatory rheology. SA and PEC solutions, their Ca^{2+} complexed gel analogues as well as their binary blends with ionically or thermally set sheared κ-CAR gels, were subjected to *in vitro* orogastric conditions. SA and PEC – Ca^{2+} complexed sheared gels exerted the highest vulnerability to digestive fluid exposure due to the dialysis of egg-box dimer structures via proton-calcium exchange. Incorporation of SA and PEC systems to κ-CAR gels prevented the loss of mechanical strength of the gastric gels due to the ability of κ-CAR to undergo spontaneous gelation in the presence of Na^{+} and K^{+} ions. Binary blends of SA and PEC – Ca^{2+} complexed sheared gels with κ-CAR-Ca^{2+} gels exerted a significantly lower mechanical strength loss sensitivity against pH and counterion composition of the gastric fluids.

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1. Introduction

Over the last decades the prevalence of obesogenic lifestyle associated with the consumption of highly calorific food products, limited uptake of essential micronutrients and dietary fibre, as well as restricted physical activity have led to an alarming increase of obesity and obesity related health complications (Lake and Townshend, 2006). As result, health disease such as type II diabetes, metabolic syndrome, hypertension, coronary artery disease, stroke, osteoarthritis, liver and gall bladder disease, and obstructive sleep apnoea have been evidenced (Kopelman, 2007). In addition, the association of obesity to several forms of cancer such as endometrial, kidney, postmenopausal breast and colocteral adenoma has been reported (Kopelman, 2007; Vigneri et al., 2006). Modulation of eating behaviour via suppressing appetite and controlling the dietary and calorific value of food are widely recognised as effective strategies to counteract obesity. As for appetite suppression, it has been demonstrated that satiety is a responsive construct of the combination of environmental, physiological and neurobiological signalling which involves food choice and intake based on the cross-modal orosensory perception response, as well as pre-absorptive (gastric stretching and emptying, suppression of digestive enzymes activity) and post absorptive (macro- and micronutrients absorption, regulation of the gut microbiota) parameters (Bellisle, 2008; Chambers et al., 2015; Fiszman and Varela, 2013a, 2013b; Llewellyn and Wardle, 2013).

Food macromolecules including proteins, dietary fibre and lipids are known as having a pivotal impact on satiety signalling; however, this is generally attained via different mechanistic physiological and neurobiological pathways (Fiszman and Varela, 2013b). As concerns to dietary fibre, their satiety suppression effectiveness stems from their chemical and functional aspects such as thickening and gelling ability, water and oil holding capacity, fermentability/digestibility, absorption and mucoadhesivity (Brownlee, 2011; Fiszman and Varela, 2013a, 2013b; Kristensen and Jensen, 2011). In a pre-absorptive digestion context, dietary fibre can induce a plausible suppression of appetite via their ability to prolong orosensory exposure (oral processing/mastication, secretion of saliva and gastric juice) and to modulate the gastric response to food ingestion, e.g. activation of stomach mechanoreceptors

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triggering stomach distension due to intragastric structuring, reduction of gastric enzymes activity and delay of gastric emptying. Therefore, soluble dietary fibre exerting a fair thickening ability and/or self- or co-structuring (in the presence of other macronutrients) ability under acidic conditions, such as pectins, seaweed extracts (alginates and carrageenans), root extracts (konjac gum), microbial synthesised gums (curdlan and gellan) and cellulose derivatives (HPMC, CMC), have been scrutinised as potential intragastric structuring materials (Borreani et al., 2016; Bradbeer et al., 2014; Fiszman and Varela, 2013a; Garrec et al., 2013; Logan et al., 2015; Morell et al., 2014; Soukoulis et al., 2016; Spyropoulos et al., 2011; Zhang et al., 2014). In addition, biopolymer assisted structural and interfacial engineering methods have also been developed to reduce the energy density and promote satiety response of staple processed food (Norton et al., 2006, 2015). For example, crosslinked gel networks (hydrogels) and ionotropically gelled microparticulates (fluid gels), protein-polysaccharide assembled structures, as well as highly viscosified aqueous systems (w/w or o/w emulsions) are only some of the structurally bespoke food models promoting satiation response via their intragastric structuring ability (Norton et al., 2015). When it comes to scrutinising the intragastric ability of biopolymer structure engineered food models, adopting physiological pre-absorptive digestion conditions is of paramount importance. Soukoulis et al. (2016) have demonstrated that the adoption of a harmonised *in vitro* digestion protocol (INFOGEST) was associated with an evidently diversified structuring performance of sodium alginate based o/w emulsions throughout gastrointestinal passage. Therefore, parameters such as the pH fluctuation due to human host physiological diversity and stomach fullness state, as well as the counterions complexity of the individual simulating pre-absorptive digestive fluids (including the oral phase) should be considered as validating criteria of the foreseen intragastric structuring performance of food biopolymers.

In the present work we aimed to investigate the intragastric structuring ability of gel composites comprising anionic polysaccharides as the responsive construct of intrinsic (ionotropic, random to ordered coil and acid self-induced gelling ability) and extrinsic (pH of the gastric chymes, concentration and ionic strength of the simulating pre-absorptive digestive fluids) parameters. The morphological and mechanical characteristics of the gastric chymes were assessed by means of optical microscopy and dynamic oscillatory rheology respectively.

2. Materials and methods

2.1. Materials

Low viscosity sodium alginate (250 mPa s, 2% w/w in water at 25 °C, M/G ratio = 1.6, mannuronic to guluronic acid content 61-31, $M_w = 1.43 \times 10^5$ g mol^{-1}), κ-carrageenan (5–25 mPa s, 0.3% w/w in water at 25 °C), anhydrous calcium carbonate, δ-glucono-lactone, and porcine pepsin (ca. 474 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. Low calcium reactivity apple pectin (45% degree of esterification, 80% galacturonic acid content, $M_w = 2 \times 10^5$ g mol^{-1}) was kindly provided as a gift from Herbstreith and Fox GmbH (Neunbürg, Germany). All biopolymers listed were used without any further purification.

2.2. Preparation of the biopolymer based solutions and Ca^{2+} mediated gel systems

Two grams of biopolymer (sodium alginate, κ-carrageenan, or pectin) were dispersed into 100 mL of deionised 18 MΩ water (Millipore, USA), heated at 80 °C, kept at the same temperature for 1 h to allow complete dissolution and then the obtained aliquots were cooled at 50 °C and left to fully hydrate overnight under constant magnetic stirring. To prevent microbial spoilage, a small amount of sodium azide (0.002% w/w) was added. One hundred mL aliquots of sodium alginate, pectin and κ-carrageenan solutions (2% w/w) were mixed with anhydrous calcium carbonate in order to achieve a final concentration of 40 mM. The biopolymer solutions were successively ultrasonicated (5 min, 90% amplitude, UP200S, Hielscher GmbH, Teltow, Germany) to ensure uniform distribution of $CaCO_3$. Finally, the biopolymer solutions were mixed with δ-glucono-lactone (at a 2:1 GDL to $CaCO_3$ ratio), covered with aluminium foil and kept under magnetic agitation (1000 rpm) at 50 °C for 6 h to allow ionotropic gelation of the biopolymers triggered via the *in situ* release of Ca^{2+} ions for 6 h. Evaporated water was added back and the gels were cooled at ambient temperature under stirring (20 ± 2 °C). A similar approach was also used in the case of thermally set κ-carrageenan systems, i.e. the heated solutions were left to cool down (ca. 2 °C/min) to ambient temperature under constant magnetic agitation as previously described. Binary blends (1:1) of the Ca^{2+} complexed sodium alginate and pectin with either ionically or thermally set κ-carrageenan were also prepared. All biopolymer comprising systems were stored overnight at ambient temperature before carrying out the *in vitro* pre-absorptive digestion experiments.

2.3. In vitro pre-absorptive digestion of the biopolymer solutions and Ca^{2+} mediated gels

The gastric structuring ability under simulated physiological conditions was studied adopting the INFOGEST static standardised *in vitro* model as previously described by Minekus et al. (2014). In brief, 5 g of the biopolymer system (solution or gel), preconditioned at 37 ± 1 °C, were transferred into 50 mL plastic centrifuge tubes and mixed with 5 mL of simulated salivary fluid (SSF) (pH = 7, $K^+ = 18.8$, $Na^+ = 13.6$, $Mg^{2+} = 0.15$, $Ca^{2+} = 1.5$ mM). The obtained oral phase was successively mixed with 10 mL 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 by means of dynamic oscillatory rheology. *In vitro* digestion experiments were carried out in triplicate.

In addition, a duplicate batch 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, was also prepared adopting either physiological (systems diluted with SSF & SGF) or non-physiological (systems diluted exclusively with Millipore water) pre-absorptive digestion conditions.

2.4. Dynamic oscillatory rheological measurements

Dynamic oscillatory rheological measurements of the initial biopolymer aqueous systems as well as of the obtained gastric chymes were carried out in an Anton-Paar rheometer (MCR 302, WESP, Graz, Austria). Initial biopolymer aliquots were measured using a cone plate geometry (CP50-1) whilst the gastric chyme suspensions were analysed by means of double gap concentric cylinder geometry (DG 26.7). All measurements were performed at 25 ± 0.03 °C.

Strain-sweep (0.001–1000%) measurements of the biopolymer

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