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In-vitro starch hydrolysis of chitosan incorporating whey protein and wheat starch composite gels

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Abstract

The study examined the influence of chitosan, incorporated into whey protein and wheat starch thermo gels, on the *in-vitro* hydrolysis of the polysaccharide. Gels were subjected to the following external conditions containing α-amylase at constant incubation temperature of 37 °C: In the first procedure, they were immersed in phosphate buffer (0.05 M) and maintained at pH 6.9 throughout the entire digestion. In the second instance, they were introduced into a salt solution, with pH and total volume adjusted at times in sync with the human gastrointestinal tract. Results indicate that low and medium molecular weight chitosan, in combination with whey protein, were effective at enhancing the protective barrier against starch degradation. Less maltose was liberated from gels containing medium molecular weight chitosan, as opposed to the low molecular weight counterpart, and results compare favorably with the outcome of the *in-vitro* digestion of binary whey protein and wheat starch composites.

Keywords: Food science

1. Introduction

Chitosan is a linear heteropolysaccharide derived from the partial alkaline N-deacetylation of chitin. The molecule is comprised of glucosamine (2-amino-2-

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deoxy-β-D-glucopyranose) and N-acetylglucosamine [β-(1–4)-2-acetamido-2-deoxy-β-D-glucopyranose] polymers (Helgason et al., 2008; Huang et al., 2012). In acidic aqueous solutions below the pKa value (~6.3), chitosan is soluble due to the protonation of amine groups (Thongngam and McClements, 2004). The dissociated form adopts an extended conformation as a result of electrostatic repulsion and stabilisation by inter and intra molecular hydrogen bonds (Franca et al., 2011).

A great deal of research has been fueled towards the use of chitosan in many food and pharmaceutical applications based on the ability to interact with anionic components *via* electrostatic forces (Yuan et al., 2013; Zhang et al., 2017). Chitosan was used successfully to precipitate β-lactoglobulin from liquid cheese whey through complex formation with pH manipulation (Casal et al., 2006). It exhibits antibacterial activity by direct interaction with lipopolysaccharides on the surface of Gram-negative bacteria (Kong et al., 2010). Microencapsulation of drugs and lipids using chitosan in polymer blends has also been investigated, with a study by Honary et al. (2009) examining the *in vitro* release of prednisolone from chitosan-alginate systems with varying the molecular weight of the former. Helgason et al. (2008) aimed to enhance the understanding of chitosan interactions with oil-in-water emulsion droplets by simulating the conditions of the human gastrointestinal tract to provide a realistic perspective of the influence of the heteropolysaccharide on the bioavailability of lipids.

The subject of interest in this study is starch hydrolysis and digestion, i.e. a complex physicochemical process involving three key organs: mouth (pH ~6.5), stomach (pH ~1.5) and small intestine (pH ~6-7) (Chen et al., 2017). Dhital et al. (2017) examined the structural aspects of the mechanism of starch digestion whereas, the *in-vitro* digestion of starch has been studied by others (Edwards et al., 2015; Koh et al., 2009; Yousefi et al., 2015). The pH, food retention time, peristaltic motion and external environment, i.e. digestive enzymes, secretion of bile salts and total volume, vary vastly between and within these compartments. Each system plays a distinct role, but it is the site of the small intestine that facilitates absorption of most nutrients across the mucosa into the bloodstream (Kong and Singh, 2008).

Chitosan has been used extensively in the development of food products that reduce fat absorption in the gastrointestinal tract in response to pressing global concerns of obesity (Helgason et al., 2008). This is associated with Type 2 diabetes, the prevalence of which is also on a dramatic rise (Xu et al., 2017). Statistics estimate that in excess of a million Australians suffer from obesity and Type 2 diabetes (Health Insite, 2008). The prevention and management of the latter involves adhering to a low GI diet where carbohydrate consumption sees glucose release and absorption in a slow and steady manner (Jenkins et al., 2002; Jenkins et al., 1982).

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Significantly, even at a low concentration, alginates in the presence of calcium ions can form good networks (Koh et al., 2009). This is also the case for kappa-carrageenan and agarose. By contrast, chitosan cannot form gels by itself but instead it creates a slimy non-cohesive weak structure. Therefore, for chitosan to create an effective barrier, milk proteins are needed. Previously, heated whey protein and wheat starch dispersions were found to form micro phase separated gels with a continuous protein phase supporting discontinuous polysaccharide inclusions (Yang et al., 2013). When subject to *in-vitro* starch hydrolysis, the rate and degree of reducing sugar liberated from the co-gels of whey protein and wheat starch were noticeably lower than single polysaccharide systems of equal concentration. It was concluded that the binary gel matrix provided a promising basis for further development of low GI foods. In the same study (Yang et al., 2013), it was shown that whey protein isolate (WPI) was an effective barrier to enzymatic digestion as the presence of 15% WPI decreased enzymatic digestion of 15% wheat starch by 70%. There is the potential to further reduce the starch digestion and consequently have a beneficial barrier effect with the introduction of chitosan to the binary whey protein isolate – wheat starch gel.

In a separate forum, it was noted that whey protein was capable of stimulating the release of insulin, which is beneficial in the control of glycemic index (Belobrajdic et al., 2009; Luhovyy et al., 2007). Since the chitosan molecule is non-toxic and biodegradable (Tan et al., 2013), there is opportunity for its inclusion in the whey protein based matrix with potentially beneficial effects on starch digestion. Therefore, chitosan was incorporated into whey protein-wheat starch gels and its protective effect during *in-vitro* hydrolysis of the polysaccharide following gelatinisation was investigated.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) was purchased from Fonterra Co-operative Group Ltd., New Zealand. The fine powder consisted of 90.31% protein, 0.93% fat, 4.83% moisture and 3.3% ash (w/w). Native wheat starch (WS) was supplied from National Starch (National Starch and Chemical Co., Thailand), with the white powder containing 94.1% starch, 0.3% ash, 0.1% fat and 5.5% moisture (w/w). Low molecular weight chitosan (LCT), medium molecular weight chitosan (MCT) and α-amylase from *Aspergillus oryzae* was supplied from Sigma-Aldrich, Co. (Castle Hill, Australia). As specified by the manufacturer, the degree of deacetylation for both low and medium molecular weight chitosan, supplied in the form of a pale yellow powder, was about 80% and the viscosity of 1.0% (w/w) solution at ambient temperature was about 150 and 800 cP, respectively. According to the supplier, the

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molecular weight of LCT and MCT was about 70 kDa and 250 kDa, respectively. All other chemicals utilised were of analytical grade.

2.2. Methods

2.2.1. Sample preparation

Required amounts of WPI and WS were weighed and dissolved in deionised water to produce single or binary mixtures. Both chitosans were solubilised in 1% (v/v) acetic acid prior to incorporating with the WPI-WS dispersions. The focus was on low and intermediate solid preparations, which included the following concentrations (%, w/w): 15 WPI, 7.5 WS, 15 WS, 2 LCT, 2 MCT, 15 WPI-7.5 WS, 15 WPI-15 WS, 15 WPI-2 LCT, 15 WPI-2 MCT, 7.5 WS-2 LCT, 7.5 WS-2 MCT, 15 WS-2 LCT, 15 WS-2 MCT, 15 WPI-7.5 WS-2 LCT, 15 WPI-7.5 WS-2 MCT, 15 WPI-15 WS-2 LCT and 15 WPI-15 WS-2 MCT.

The fixed amounts of 15% WPI, 7.5% WS and 15% WS were chosen based on their physicochemical and structural characterisation in Yang et al. (2013), and the intention presently is to utilise those research outcomes, i.e. the level of fundamental understanding achieved, in the optimization of digestion properties in mixtures. In addition, we incorporate 2% chitosan in the WPI-WS sample and adjust the pH to 5.5 with the use of 0.1 M HCl or NaOH, hence creating a regime where whey protein is above its pI of ~5.1 and chitosan below its pKa value of ~6.3. Adjusting the pH to 5.5 targets maximum electrostatic bonding between the predominantly negatively charged molecules of the protein and the cationic chitosan chains, as discussed in Yang et al., (2015), in an effort to further manipulate starch hydrolysis in the tertiary gels.

To avoid variability in the functional properties of chitosan solutions with extensive storage following preparation (Tan et al., 2013), all dispersions in the present study were freshly made prior to immediate experimentation. Huang et al. (2012) reported that temperature also affects the nature of interactions between soy protein isolate and chitosan and, in the present study, all samples were subjected to the same temperature-time regime to eliminate unforeseen variability in the hydrolysis results. Our materials were heated in an 85 ± 1 °C waterbath for 75 min to form thermogels ready for use in the hydrolysis protocol. Gelatinised starch is contained in most processed foods, and we mimic production of real food systems by heat application to transform polymer dispersions into viscosity enhancing gels.

2.2.2. In vitro starch hydrolysis

Thermogels were pushed through a screen sieve (opening size of 0.5 mm) and 3 g of the sample was subjected to α-amylase hydrolysis in two experimental set-ups. In the first condition, gels were hydrolysed in “benign systems” with an external

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medium consisting of anhydrous monobasic sodium phosphate buffer (0.05 M) adjusted to pH 6.9. This environment remained consistent throughout the period of experimentation. The second layout of amylolysis encompasses a more complex setting by factoring in time and pH adjustments of the external medium to reflect to a certain extent that of the human gastrointestinal tract. In this scenario, gels were immersed in a NaCl solution of 0.75 g/l adapted from the artificial-saliva work of Rabe et al. (2004). The medium was adjusted to pH 6.5 for 5 min followed by a decrease to pH 1.5 for 30 min. Subsequently, the pH was raised to 6.4 for 1 h, pH 6.9 for 1 h and pH 7.3 for 1 h.

All enzyme hydrolysis experiments were conducted at 37 ± 1 °C for 215 min. The total volumes were also taken into account to reflect those likely to be found in the human digestive tract for both set-up protocols. Aliquots were taken at the initial 5 min mark and then for every 30 min until the end of experimentation. The enzyme was deactivated by heating at 100 °C for 5 min (Koh et al., 2009) and samples were centrifuged at 4,000 rpm for 15 min. Hydrolytic activity was measured by quantifying the liberated maltose equivalent from the supernatant using a 3,5-dinitrosalicyclic acid (DNSA) assay according to Kumar et al. (2016). The absorbance was measured at 540 nm and compared against a good quality maltose standard curve ($r^2 = 0.991$). All samples were replicated three times and the mean with standard error were calculated and reported.

2.2.3. Statistical analysis

The data obtained were subjected to a one-way analysis of variance (ANOVA) and Tukey's Post-Hoc tests were applied to determine statistical significance between treatments. P values ≤ 0.05 were considered to be statistically significant. Data were obtained in triplicate and presented as the mean ± standard error (SE), expressed as percentages.

3. Results and discussion

3.1. The effect of chitosan on the retardation of starch hydrolysis in benign systems

Earlier work examined the microstructure of gels formed by whey protein and wheat starch at concentrations applicable to the current study (Yang et al., 2013). The multidisciplinary approach of analysis pointed to the formation of a micro phase separated gel that was predominantly WPI continuous and WS discontinuous. Findings from the *in-vitro* hydrolysis of gelatinized starch showed a decreased amount of maltose equivalent liberated from the binary WPI-WS matrix in comparison to single polysaccharide preparations.

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While literature agrees that protein can hinder starch hydrolysis by the formation of disulphide bonds and physically blocking enzymes from accessing starch (Singh et al., 2010), there is further complexity to consider with the introduction of chitosan in these systems. Following pH adjustment to 5.5 and heating at 78 °C for 10 min, beta-lactoglobulin in the presence of chitosan forms aqueous solutions, where about half of the polymeric material is in the form of soluble complexes (Mounsey et al., 2008). It was further established that in the presence of chitosan and by heating above the protein's denaturation temperature, extensive aggregation of the latter can be eliminated.

Recently, we followed the thermal transformation of whey protein and wheat starch in the presence of chitosan as a function of pH (Yang et al., 2015). Values of small-deformation storage modulus in 15 WPI-2 MCT gels were comparable to those of single protein preparations following denaturation. Large-deformation texture profile analysis of the binary system also demonstrated the absence of enhanced mechanical strength commonly attributable to phase separation between two polymeric components. These findings, in conjunction with electron microscopy and infrared spectroscopy evidence, suggest the formation of a single phase and heterologous network whose functionality in retarding enzymatic starch hydrolysis will be presently examined.

In the benign set up of pH 6.9, a significant reduction in the amount of starch hydrolysed from 15 WPI-7.5 WS gels containing 2 MCT was seen throughout all

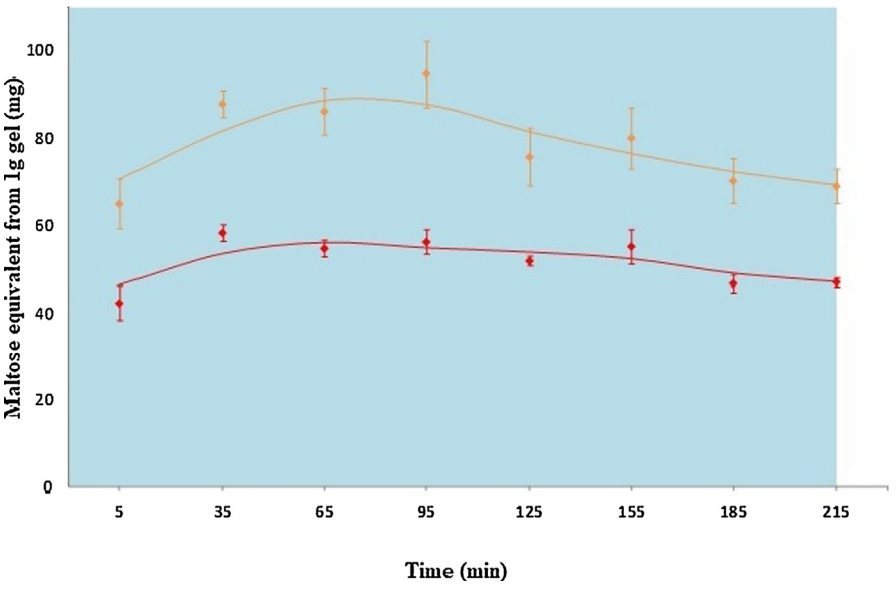

Fig. 1. Total maltose equivalent liberated from gels of 15% whey protein with 7.5% wheat starch (orange symbol), and 15% whey protein with 7.5% wheat starch and 2% medium molecular weight chitosan gel (red symbol), during in vitro digestion at constant pH 6.9 condition. The error bars are ± 5% SE in relation to the mean of the set of data.

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time points of sampling. Fig. 1 optimizes the presentation of this part of the work by illustrating only one set of results with error bars. The total maltose liberated from the gel with and without chitosan at 5 min is 42.2 and 64.9 mg/g, respectively. By the end of the experimental routine (215 min), maltose liberation in the presence and absence of chitosan corresponded to 47.0 and 69.0 mg/g. Next, the effect of chitosan addition on the composite matrix of whey protein suspending a phase of 15% wheat starch was examined.

It appears that the presence of 2 MCT in the 15 WPI-15 WS system effected a greater degree of starch retardation from enzymatic action than in the absence of the heteropolysaccharide. That was again true for all time points of analysis with the same quality of result variation (error bars are not shown in this and subsequent figures to avoid clutter). To be specific, the total maltose liberated from the whey protein-starch gel with and without chitosan is 36.3 and 53.5 mg/g, respectively, in Fig. 2. At the 215 min mark, the binary gel with and without the heteropolysaccharide has been hydrolysed to a maltose equivalent of 72.3 and 88.0 mg/g. Qualitatively, an overall reduction in the hydrolysis of gelatinized starch was also recorded when the formulation was changed by adding low molecular weight chitosan. At 215 min, for example, 82.7 mg/g of maltose has been liberated from the 15 WPI-15 WS-2 LCT combination in Fig. 3, as opposed

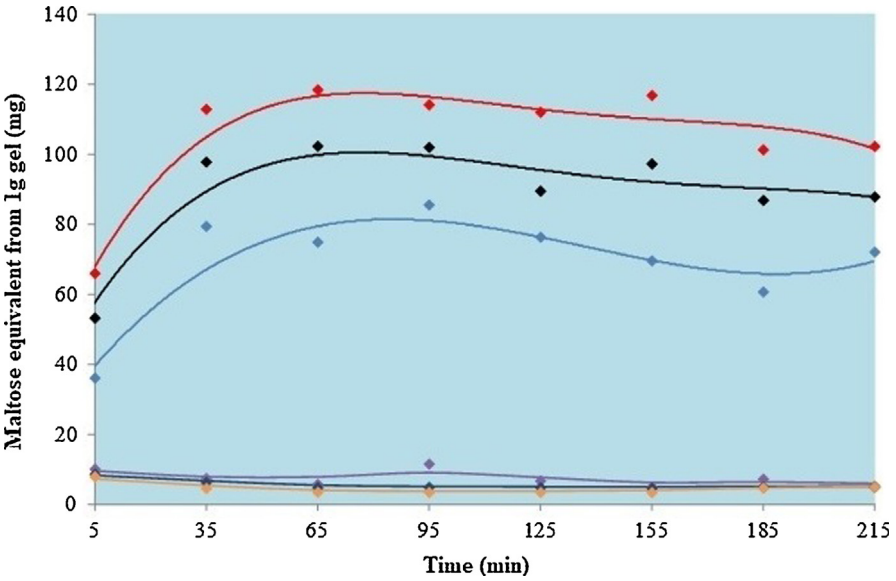

Fig. 2. Total maltose equivalent liberated from gels of 2% medium molecular weight chitosan (orange symbol), 15% wheat starch (red symbol), 15% whey protein (violet symbol), 15% whey protein with 2% medium molecular weight chitosan (blue symbol), 15% whey protein with 15% wheat starch (black symbol), and 15% whey protein with 15% wheat starch and 2% medium molecular weight chitosan (light blue symbol), during *in vitro* digestion at constant pH 6.9 condition. The error bars are ± 3% SE in relation to the mean of the set of data.

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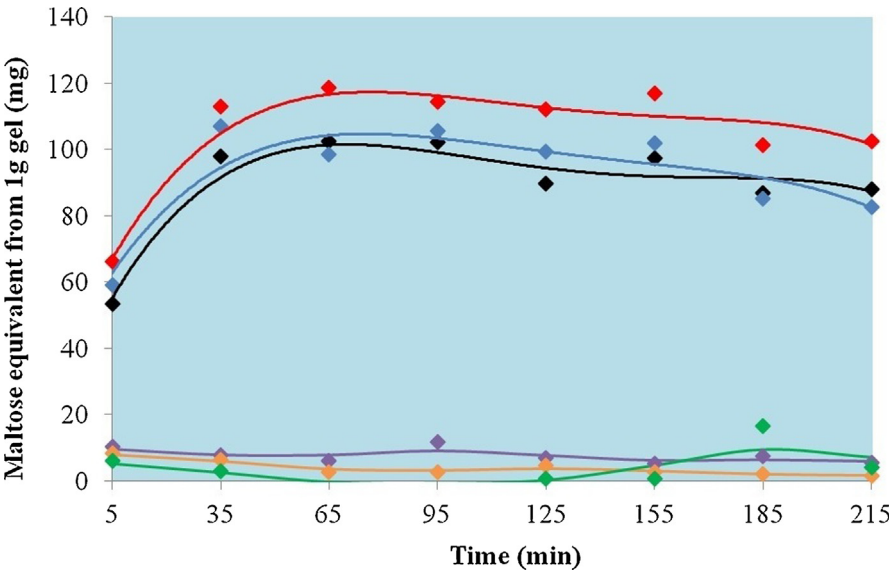

Fig. 3. Total maltose equivalent liberated from gels of 2% low molecular weight chitosan (orange symbol), 15% wheat starch (red symbol), 15% whey protein (violet symbol), 15% whey protein with 2% low molecular weight chitosan (green symbol), 15% whey protein with 15% wheat starch (black symbol), and 15% whey protein with 15% wheat starch and 2% low molecular weight chitosan (light blue symbol), during *in vitro* digestion at constant pH 6.9 condition. The error bars are ± 2.5% SE in relation to the mean of the set of data.

to the single starch preparation with high levels of generated disaccharide (102.4 mg/g).

The rate of hydrolysis in the benign set up for 15 WPI-15 WS with the two chitosan variants is presented in Fig. 4. For all gels, the majority of starch degradation is recorded within the first 65 min, with a dramatic decrease occurring thereafter. Rapid hydrolytic behavior is the outcome of the polymeric sites being exposed to enzyme activity, as instigated by the protocol of this study. The concentration corresponding to the highest rate of hydrolysis throughout the entire experiment is 15 WS due to its full exposure as substrate to the enzyme in the absence of non-digestible polymer barriers. Thus, at the beginning of hydrolysis, 13.2 mg maltose was liberated per min from each gram of 15 WS, as compared to only 7.3 mg per min in 15 WPI-15 WS-2MCT.

The main reason for the relatively low amount and rate of maltose release in the tertiary system should relate to intermolecular associations between protonated amino groups of chitosan and negatively charged surface patches of the globular molecules in whey protein. Through this interaction (Lee and Hong, 2009; Yuan et al., 2013), the WPI continuous phase, which protects gelatinized starch from hydrolysis, is strengthened considerably. Moreover, Huang et al. (2012) observed noticeable vacuoles in the complex network of soybean protein isolate and chitosan

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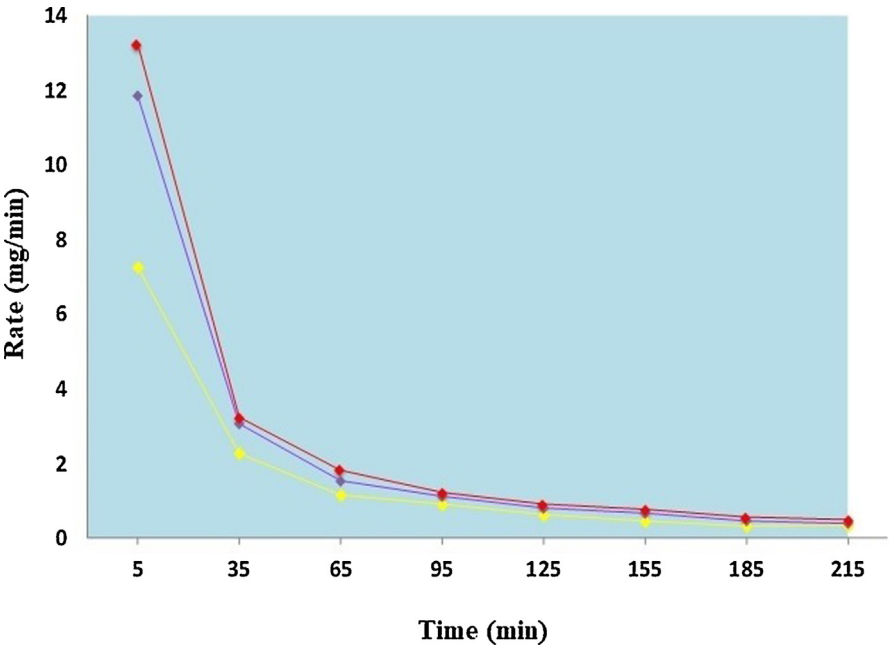

Fig. 4. Rate of total maltose equivalent liberated from gels of 15% wheat starch (red symbol), 15% whey protein with 15% wheat starch and 2% medium molecular weight chitosan (yellow symbol), and15% whey protein with 15% wheat starch and 2% low molecular weight chitosan (violet symbol), during *in vitro* digestion at constant pH 6.9 condition. The error bars are ± 2% SE in relation to the mean of the set of data.

coacervates using SEM micrographs; these pores would be equivalent to the location of wheat starch molecules in our system. The hydrolytic activity may also be hindered indirectly due to interference with starch gelatinisation. As noted by Tester and Sommerville (2003), a non-starch polysaccharide like chitosan could decrease the free volume and mobility of water molecules necessary in the gelatinisation process. This "antiplasticising" effect of chitosan means that starch is not fully gelatinised with a subsequent decrease in the amount of available sites for enzymatic hydrolysis.

The disparity in structural functionality due to varying the molecular weight distribution of chitosan has been highlighted in literature (Tsai and Chen, 2017). Addition of the high viscosity counterpart immobilises water efficiently to delay starch gelatinisation and forms enhanced associations with whey protein at pH around its pKa value. Thus, a significant difference does exist between the amount of maltose liberated from the medium and low molecular weight variants. In the first 5 min of hydrolysis, 15 WPI-15 WS-2 MCT yielded 36.3 mg/g, as opposed to 59.2 mg/g in 15 WPI-15 WS-2 LCT (Figs. 2 and 3, respectively). Further, the amount of wheat starch that has been hydrolysed by 215 min from 15 WPI-15 WS-2 MCT (72.3 mg/g) was also lower than for 15 WPI-15 WS-2 LCT (82.7 mg/g) in the same two illustrations.

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Honary et al. (2009) studied the impact on the release rate of prednisolone when chitosan of varying molecular weights complexed with alginate. It was found that limited contact (3 h) was sufficient in systems with the high molecular weight polymer to effectively reduce the rate of prednisolone release due to extensive electrostatic bonding between $-NH_3^+$ groups of chitosan and $-COO^-$ of alginate. Low molecular weight chitosan was able to diffuse but required extra time to react efficiently with alginate (24 h), as opposed to maximum binding of the high molecular weight counterpart that occurred within 4 h.

3.2. The effect of chitosan on the retardation of starch hydrolysis in systems of changing pH

By tweaking the pH of the dissolution medium, the amount of maltose liberated from the gels for each experimental polymer concentration can be examined. As depicted in Fig. 5, the amount of reducing sugar generated in the first 5 min from mixtures of whey protein and wheat starch with low or medium molecular weight chitosan is confined within 68.0 and 85.3 mg/g. In the benign set up, i.e. corresponding to the same treatment time and material concentration, these values vary from 36.3 to 59.2 mg/g from data in Figs. 2 and 3. Maltose liberated in the

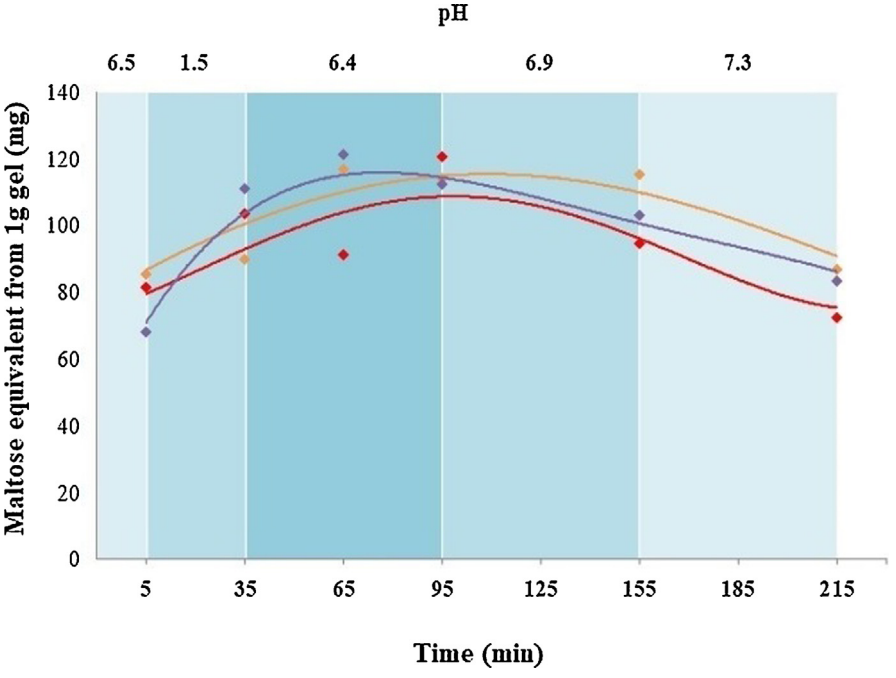

Fig. 5. Total maltose equivalent liberated from gels of 15% whey protein with 15% wheat starch (orange symbol), 15% whey protein with 15% wheat starch and 2% medium molecular weight chitosan (red symbol), and 15% whey protein with 15% wheat starch and 2% low molecular weight chitosan (violet symbol), during *in vitro* digestion with pH values mimicking those found in the human gastrointestinal tract. The error bars are ± 2.5% SE in relation to the mean of the set of data.

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changing pH system was considerably higher in all gel preparations. Since its value is near neutral at the beginning of the experimentation for both cases, the disparity in results of the two set-up protocols could have another origin.

The two experimental preparations vary in the composition of the external medium, with a NaCl solution of artificial saliva (and a phosphate buffer) being the electrolytic environment of choice in Fig. 5. Researchers have previously highlighted that the presence of NaCl can interfere with the formation of electrostatically charged complexes. For example, Thongngam and McClements (2004) noted that complexation of chitosan with the anionic surfactant sodium dodecyl sulfate (SDS) was noticeably weaker in the presence of 100 mM NaCl. It was concluded that salt shielded charges on both polyelectrolytes, hence screening electrostatic interactions. Similarly, the extent of soy protein isolate-chitosan coacervate formation was reduced within a salt solution (Huang et al., 2012). It appears, therefore, that the competitive binding of sodium chloride ions reduces the strength of the protective barrier for WS by discouraging close proximity and direct electrostatic associations between whey protein and chitosan in our mixtures.

Following the first 5 min of hydrolysis, the pH is reduced to 1.5 to mimic the acidity of the stomach and, at this interval, whey protein is well below its pI and similarly with the pKa of chitosan. Both polymers assume a predominantly positive net charge and electrostatic association is reduced. It is also important to note that as a response to low pH, the structure and active sites of the protein and α-amylase are prone to alteration. Indeed, the manufacturer has specified that α-amylase activity exists in a pH range of 5.5 to 10, which implies that at pH 1.5, α-amylase activity is minimal at most. This assumption is justified by comparing the relatively low increments of maltose liberation from 5 to 35 min in Fig. 5 with the same timescale in the benign system, where the corresponding increase is consistently higher (Figs. 2 and 3).

Specifically, the incremental difference between the first two recordings in the 15 WPI-15 WS gel, where the pH has dropped dramatically, is only 4.5 mg/g (Fig. 6). By comparison, the corresponding difference in the same system, but at constant pH, is 44.5 mg/g in Fig. 2. Addition of chitosan in the binary whey protein-wheat starch mixture follows qualitatively the same trend, with the additional amount of starch hydrolysed from 5 to 35 min in the 15 WPI-15 WS-2 MCT gel is 22.1 mg/g in Fig. 6, as opposed to the benign system that records a value of 43.3 mg/g (Fig. 2). Since the maltose equivalent values between these two measurements are still increasing, the protective effect of whey protein-chitosan mixture should be seen in the context of reduced α-amylase activity in the pH dropping set-up.

Following the 35 min mark and at every hour, the pH is adjusted to simulate conditions applicable to the duodenum (pH 6.4), jejunum (pH 6.9) and ileum (pH 7.3). In this part of the work, dropwise addition of sodium hydroxide or

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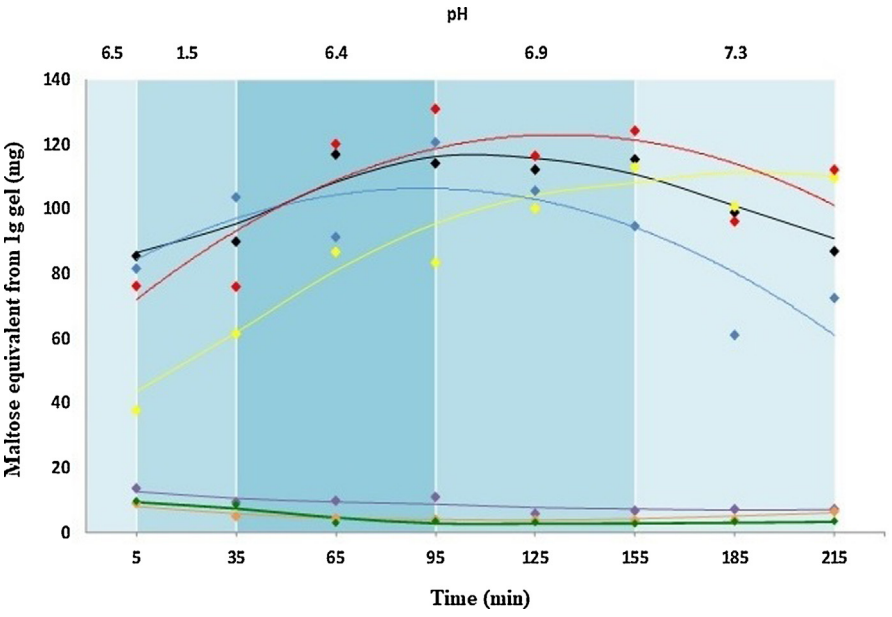

Fig. 6. Total maltose equivalent liberated from gels of 2% medium molecular weight chitosan (orange symbol), 15% wheat starch (red symbol), 15% whey protein (violet symbol), 15% wheat starch with 2% medium molecular weight chitosan (yellow symbol), 15% whey protein with 2% medium molecular weight chitosan (green symbol), 15% whey protein with 15% wheat starch (black symbol), and 15% whey protein with 15% wheat starch and 2% medium molecular weight chitosan (light blue symbol), during *in vitro* digestion with pH values mimicking those found in the human gastrointestinal tract. The error bars are ± 4% SE in relation to the mean of the set of data.

hydrochloric acid solutions is used to adjust the pH at every hour interval. HCl reacts with NaOH and the outcome of this reaction is to alter the aquatic environment, i.e. below pH 7, the two reactants can increase the pH to about neutral, whereas when the pH of the medium is above 7, HCl addition increases acidity. The reaction causes pH mediated changes in charge density affecting the overall protection afforded from the whey protein-chitosan barrier against starch hydrolysis.

Environmental conditions related to small intestine dictate that the pH of the present medium deviates from the pKa value of chitosan as digestion progresses. This is a critical issue documented in the literature by Hong and McClements (2007) when examining the stability of protein-coated lipid droplets with chitosan addition. Modulation of pH from 5 to 7 led to a steep decrease in zeta potential, which translates to increasing deprotonation of the charged amino groups. Huang et al. (2017) also stressed the particular sensitivity of chitosan to changing acidity, and in our case it means that the effect of adjusting the pH from 6.4 through 6.9 to 7.3 would reduce the overall positive charge of chitosan.

Finally, the maltose equivalent liberated at the end of the experimental routine from the 15 WPI-15 WS-2 LCT gel in the benign and changing pH systems of

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Figs. 3 and 5, respectively, is about 83 mg/g. The corresponding amount of the disaccharide generated from 15 WPI-15 WS-2 MCT at 215 min in the benign and changing pH set up of Figs. 2 and 6, respectively, is about 72 mg/g. Results indicate that medium molecular weight chitosan is a more efficient barrier to enzymatic hydrolysis of gelatinized starch, as compared to the low molecular weight counterpart. By the end of the starch hydrolysis experiments, liberated maltose for both types of the heteropolysaccharide is congruent in the benign and changing pH preparations. This outcome argues that the electrostatic interactions of chitosan and whey protein are reestablished to a good extent in the slightly acidic to alkaline environment of the small intestine following imitation of the highly acidic condition in the stomach.

4. Conclusions

Low to intermediate-solid thermogels consisting of single, binary and tertiary systems of whey protein, wheat starch and chitosan were prepared and subjected to *in-vitro* hydrolysis of the gelatinised starch. A marked decrease in the total amount and rate of maltose equivalent liberated from chitosan incorporating WPI-WS gels was evident. While the protection of wheat starch by the chitosan-whey protein mixture was more consistent throughout the experiment in systems with constant near-neutral pH, the trend could still be established in preparations with changing pH of the dissolution medium to imitate conditions from the mouth through the stomach to the small intestine in the human gastrointestinal tract.

Fluctuations of liberated maltose measured in the changing pH system argue that the interactions between chitosan and whey protein are electrostatic in origin. In both hydrolysis scenarios, the medium molecular weight chitosan afforded greater starch protection than the low molecular weight counterpart due to its enhanced techno-functionality in creating long three-dimensional structures supporting electrostatic associations with whey protein. Reduction in starch hydrolysis by incorporating medium molecular weight chitosan into WPI-WS gels of changing pH from 1.5 to 7.3, to mimic conditions from stomach to ileum, provides a good basis for future research in the development of low GI food products. This will require consideration of a more complete digestive environment including pepsin and bile salts present in the human gastrointestinal tract.

Declarations

Author contribution statement

Stefan Kasapis, Natasha Yang, John Ashton, Elisabeth Gorczyca: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

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Additional information

No additional information is available for this paper.

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