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Adding neutral or anionic hydrocolloids to dairy proteins under *in vitro* gastric digestion conditions

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ABSTRACT

The effect of adding uncharged polysaccharides such as konjac glucomannan (KGM) or negatively charged polysaccharides such as alginate to dairy protein ingredients – milk, whey proteins and calcium caseinate – was investigated through simulated *in vitro* gastric digestion. The apparent viscosity, microstructure (light microscopy), particle size distribution and degradation (SDS-PAGE) of the proteins were monitored after different *in vitro* gastric digestion times (0, 30, 60 and 120 min). The addition of KGM increased the viscosity values of the samples during gastric digestion, which probably would increase gastric distention affecting satiety. The microstructure and particle size distribution results showed that the aggregates formed in the dairy protein-konjac glucomannan mixtures at the start of gastric digestion were broken down into smaller ones over time. However, the aggregates formed with the addition of alginate were larger and remained almost unchanged throughout gastric digestion, due to the strong interaction between the opposite charges of the protein and alginate. The SDS-PAGE results showed that whey proteins were more resistant to pepsin digestion than caseins and that the alginate slowed down protein degradation. These findings suggest that a combination of whey proteins and alginate could be used to delay gastric emptying and promote satiety.

1. Introduction

Over recent decades, the problems of overweight and obesity have increased and, therefore, the interest in formulating satiating foods has grown. The concept of appetite control comprises two components: satiation (the processes that induce meal termination) and satiety (which determines the intervals between meals) (Geraedts, Troost, & Saris, 2011; Solah et al., 2010). Ingested food evokes satiety in the gastrointestinal (GI) tract by mechanical and humoral stimulation. Post-gastric factors seem to play a key role in satiety through secretion of various peptides by the walls of the small and large intestine in response to ingested food (Geraedts et al., 2011). Satiety signals differ as the meal moves through the gut but include oral (taste and texture), gastric (distension and emptying), and intestinal (distension and nutrient absorption) factors (Hoad et al., 2004). Fibres (carbohydrates resistant to digestion) and various proteins have commonly been used as ingredients in foods and beverages to enhance satiety (Halford & Harrold, 2012).

Proteins suppress food intake, make a stronger contribution to satiety and delay the return of hunger more than fats and carbohydrates (Anderson & Moore, 2004; Geraedts et al., 2011; Solah et al., 2010). The mechanisms by which the peptide products of protein digestion exert their effect on food intake via the gut include slowing stomach emptying and direct or indirect stimulation of gut hormone receptors (Anderson & Moore, 2004). As dairy products contain high levels of protein, they are good for designing satiating food products. Casein is the most abundant milk protein, accounting for 80% of total protein, with whey proteins constituting the remaining 20% (Chen, Chen, & Hsieh, 2016). Hall, Millward, Long, and Morgan (2003) and Veldhorst et al. (2009) found that whey proved more satiating than casein. The digestion and absorption of whey and casein differ in that casein, unlike whey, coagulates in the stomach due to its precipitation by gastric acid. Furthermore, casein is considered a “slow” protein, whereas whey protein is a relatively “fast” protein (Boirie et al., 1997; Veldhorst et al., 2009), so whey consumption leads to higher plasma concentrations of factors known to contribute to satiety, such as amino

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acids, glucose-dependent insulinotropic polypeptide, glucagon-like peptide-1 and cholecystokinin (Anderson & Moore, 2004; Hall et al., 2003).

A number of studies shows that fibre-rich foods can increase the feeling of satiety and decrease short-term food intake. Certain fibre types bind water and swell, causing increased viscosity, which is associated with delayed gastric emptying and increased satiety (Halford & Harrold, 2012; Hoad et al., 2004; Peters et al., 2011).

Konjac glucomannan (*Amorphophallus konjac* K. Koch) and alginate are often used to formulate satiating food. Their mechanisms to induce satiation are different due to differences in their charge and structure: konjac glucomannan (KGM) is a neutral polysaccharide while alginate is a negatively-charged polysaccharide.

KGM is a soluble (Fang & Wu, 2004), fermentable and highly viscous dietary fibre (Keithley & Swanson, 2005), due to its high water-absorption capacity (Chua, Baldwin, Hocking, & Chan, 2010). It has a mechanical function in slowing food intake and reducing appetite (Chen, Cheng, Liu, Liu, & Wu, 2006). KGM promotes satiety through the induction of cephalic and gastric-phase signals, delayed gastric emptying and slowed bowel transit time due to the increased viscosity of the gastrointestinal content, and a reduced rate of food absorption in the small intestine leading to attenuated postprandial glucose and insulin surges (Chua et al., 2010).

Alginate gel formation can be triggered by low pH or the presence of divalent cations such as Ca^{2+}. Once the alginate comes into contact with acids in the stomach it can become a gel, leading to prolonged gastric emptying and a considerably slower rate of intestinal absorption (Brownlee et al., 2005). Torsdottir, Alpsten, Holm, Sandberg, and Tolli (1991) found that a small dose of alginate induce delayed gastric emptying. Peters et al. (2011) showed that a specific alginate that gelled strongly in the presence of Ca^{2+} increased satiety more than an alginate that formed a weak gel. Hoad et al. (2004) suggested that a sense of fullness can be obtained by using a palatable, relatively low-viscosity meal (low-G alginate) which forms solids in the stomach, due to distension of the gastric antrum and/or transport of nutrients to the small intestine in the lumps.

Because alginate, KGM and milk proteins are negatively, neutrally and positively charged, respectively, it may be expected that the interaction between the milk proteins and the two hydrocolloids will be different, and therefore, that the protein digestion will also differ. Many interactions can occur between proteins and polysaccharides, depending on the pH and ionic strength of the environment, the ionisation and charge density and the structure and concentration of the different biopolymers. Protein-polysaccharide complexes form due to strong interactions such as covalent bonding, or to several weak interactions (electrostatic, van der Waals', hydrogen or hydrophobic bonding) (Dickinson, 1998; Mouécoucou, Villaume, Sanchez, & Méjean, 2004).

Several authors (El Kossori et al., 2000; Mouécoucou et al., 2004; Polovic et al., 2007; Shah, Atallah, Mahoney, & Pellett, 1982) have reported that the protein digestion rate depends on the chemical composition of the fibres and their physical properties, such as viscosity and protein-polysaccharide complex formation, which provide a physical obstacle at the protein to pepsin enzymatic cleavage site and reduce its activity (Larsen, Wilson, & Moughan, 1994). Therefore, both KGM, due to its viscosity when mixed with water, and alginate, due to its electrostatic interactions with proteins, may be expected to decrease pepsin activity. The rate of proteolysis will be higher or lower depending on the accessibility of peptide bonds to pepsin, so more or fewer peptides will be broken down into smaller ones or even into amino acids, influencing satiety.

The present study aimed to evaluate the effect of adding neutral or charged hydrocolloids on the *in vitro* gastric digestibility of different milk proteins when formulating satiating dairy products. The digested samples were characterised by apparent viscosity, light microscopy (LM), particle size distribution and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

2. Materials and methods

2.1. Ingredients

The ingredients used to prepare the samples were skimmed milk powder (Central Lechera Asturiana, Siero, Spain), whey protein concentrate (AVONLAC 482, Glanbia Nutritionals Ltd., Kilkenny, Ireland), calcium caseinate (Fonterra Co-operative Group Ltd, Reference 385, Palmerston North, New Zealand), konjac glucomannan (GLUCOMANNAN 86 TDF, 120 MESH M202, Trades S.A., Barcelona, Spain), sodium alginate (MANUCOL DMF, FMC Biopolymer, Philadelphia, United States) and distilled water.

2.2. Sample preparation

Three protein solutions were prepared by dissolving skimmed milk powder, whey powder or casein powder, respectively, in distilled water. While slowly adding the powder, the water was stirred and heated (50 °C) for 1 h. Two polysaccharide solutions, konjac glucomannan and alginate, were prepared in the same way. Each protein solution was mixed with each polysaccharide solution to obtain six different samples: MK (10% w/w of skimmed milk powder + 0.5% w/w of konjac glucomannan), MA (10% w/w of skimmed milk powder + 0.55% w/w of alginate), WK (10% w/w of whey protein concentrate + 0.5% w/w of konjac glucomannan), WA (10% w/w of whey protein concentrate + 0.55% w/w of alginate), CK (10% w/w of calcium caseinate + 0.5% w/w of konjac glucomannan) and CA (10% w/w of calcium caseinate + 0.55% w/w of alginate). The percentage of KGM was calculated on the basis of the manufacturer's recommendations and the percentage of alginate was selected through a preliminary study to obtain a similar apparent viscosity to that of KGM systems at low shear rates at 37 °C. Three control samples (M, W and C, all without any polysaccharide) were also analysed.

2.3. In vitro gastric digestion

The simulation of gastric digestion was performed in a jacketed glass reactor (1 L capacity) maintained at 37 °C in a temperature-controlled circulating water bath with continuous magnetic stirring throughout the test.

The simulated gastric fluid (SGF) consisted of 0.034 M NaCl, with the pH adjusted to 1.2 using HCl 10 N. The SGF (200 mL) was pre-incubated for 5 min with continuous stirring (Zhang & Vardhanabhuti, 2014b) at 300 rpm.

Each sample (200 g) was mixed with simulated gastric fluid. The pH value was reduced to 1.9 (Abdel-Aal, 2008) with HCl 10 N. Pepsin (P7125, pepsin from porcine gastric mucosa, ≥400 units/mg protein, Sigma–Aldrich) was added at a pepsin to protein ratio of 1:250 on a weight basis, in accordance with Zhang and Vardhanabhuti (2014b). The mix was maintained at 37 °C with continuous stirring (650 rpm) for a recommended time of 120 min, which corresponds to a half-gastric emptying (Minekus et al., 2014). Aliquots (28 mL) were withdrawn into a glass beaker containing 22 mL NaOH (0.1 N) to inactivate the enzyme after 0, 30, 60 and 120 min of incubation. The samples were centrifuged at 5000 g for 15 min at 4 °C. The hydrolysed protein content in the supernatant was measured at 280 nm using a UV–visible spectrophotometer (Cecil Instruments Limited, Cambridge, UK).