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Study of effect of sodium alginate on potato starch digestibility during *in vitro* digestion

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ABSTRACT

Foods are complex matrices formed by many compounds whose nutritional properties vary when they are mixed. Therefore, the study of ingredients interactions that occur during food formulation is necessary to understand the final behavior of food during digestion. This study examined the effects of sodium alginate mixed with a starch solution (3.0 g/100 g) on glucose release during *in vitro* digestion. To evaluate the effect of alginate, three concentrations were used (0.5, 1.0 and 2.0 g/100 g), and the *in vitro* digestion was carried out in two stages: gastric and intestinal digestion. The glucose release was evaluated by colorimetric. In order to understand the behavior of this mixture, complex index (CI), viscosity analysis and differential scanning calorimetry were performed to study the alginate–starch interaction. The results showed that starch hydrolysis began during intestinal digestion, reaching a hydrolysis percentage of 72% during the first 15 min. A significant reduction in starch hydrolysis was observed when 1.0 and 2.0 g/100 g of sodium alginate was incorporated, with hydrolysis percentages around 55% during the first 15 min. The CI varied from 34.34% to 57.11% when alginate was increased from 0.5 to 2.0 g/100 g. Also, the sodium alginate affected the viscosity which can be attributed to a protector role of alginate on the starch granules that diminishes the swelling and amylose leaching. Through this study it was possible to show the importance of molecular interaction between different ingredients used in food formulation and how these interactions can be relevant in the regulation of the glycemic response in carbohydrate based food.

1. Introduction

The structures of food at different levels (nano, micro, meso and macro scale) have always presented a challenge for food engineers. Structure is a fundamental variable that influences the transport and physical properties of food (Aguilera, 2005). The perceived quality of food is impacted by its microstructure that contributes to attributes such as mouthfeel and creaminess. Food structure engineering has therefore become important to food scientists and engineers as a controlled effort to preserve modify or create structures through processing techniques and matrix composition (Kulozik, Tolkach, Bulca, & Hinrichs, 2003). These food processes determine the structural, physical (color, size and shape), mechanical (texture, stability and flavor release) and nutritional (satiation and digestion) properties of food products (Lundin, Golding, & Wooster, 2008). There is evidence that the effect in the post-prandial elevation of blood glucose during carbohydrates consumption can cause physiological complications related to obesity and diabetes, and these complications could be managed by structure control during food design (Mishra & Monro, 2009).

Furthermore, the glycemic response to different starchy products, such as pasta or dough, varies according to the integrity of the polymeric network in the food material. Riccardi, Clemente, and Giacco (2003) found that the differences in the glycemic response to different types of pasta and dough were due to differences in the matrix microstructure, which enclosed the starch and prevent enzymatic access during digestion (Parada & Aguilera, 2011).

These studies have suggested that designing microstructures to control and tune the physiological responses that are triggered during the digestive process could be useful (Kaufmann & Palzer, 2011). For example, emulsions can trigger different responses during digestion. Emulsions with a droplet size of approximately

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160 nm present faster lipolysis *in vitro* than emulsions with a droplet size of 200 μm; therefore, the droplet size affects the digestion rate and bioavailability (Lundin et al., 2008). Similar results were obtained by Troncoso, Aguilera, and McClements (2012), in which fatty acid content increased from 61% to 71% when the oil droplet radius was decreased from 86 to 30 nm.

Food design examines ingredient interactions that can improve or delay food digestion. The different biopolymer interactions that can occur on the nanoscale level are determinant in food development. Biopolymer properties can be modified when interactions occur between biopolymers. For example, Weber, Clerici, Collares-Queiroz, and Chang (2009) studied the interactions of guar and xanthan gums with starch, and this study found that the pasting properties of starch were affected by the gum type and concentration. Guar gum significantly increased the viscosity of the starch gel, and xanthan gum significantly decreased the starch viscosity. These interactions can be attributed to hydrogen bonding because covalent bonding was not observed by infrared spectra analysis. Galactomannan-based gums can affect the water molecule availability, reducing the starch swelling and gelatinization and affecting the paste properties (Kaur & Singh, 2009).

If the rheological and textural properties are modified by the presence of other biopolymers, it is possible that glucose can be released from the starch molecules during digestion. Dartois, Sing, Kaur, and Singh (2010), studied the influence of guar gum on the *in vitro* starch digestibility, and the results of this study showed that the starch digestibility was affected by the presence of guar gum. This change in the starch availability was attributed to the increase of viscosity due to the presence of guar gum, which inhibited the enzymatic action on the amylose chains.

The main objective of this work was to study the effect of sodium alginate on starch solutions and to evaluate the effect of sodium alginate on glucose release during the *in vitro* digestibility of starch gel.

2. Methodology

2.1. Materials

Potato starch (Chuño delicado, Santiago, Chile) and food grade sodium alginate (Loba Chemie Ltda., Mumbai, India) were acquired from a local market and Vimaroni S.A (Quilpué, Chile), respectively. Pepsin from porcine gastric mucosa, (3200–4500 U/mg of protein), pancreatin from porcine pancreas and invertase from baker's yeast (*Saccharomyces cerevisiae*), grade VII (>300 U/mg solid), were supplied by Sigma-Aldrich (Santiago, Chile), and amyloglucosidase (Megazyme, 3260 U/mL) was obtained from Granotec (Santiago, Chile).

2.2. Preparation of potato starch and starch–alginate mixture

Potato starch gel was prepared by dissolving 3.0 g of powder in 100 mL of distilled water, and cooking the mixture at 80 °C for 15 min with mechanical stirring at 300 rpm until complete gelatinization was achieved. To evaluate the effect of sodium alginate, 3 g of starch was mixed with 0.5, 1.0 and 2.0 g of alginate dissolved in 100 mL of distilled water, and the mixture was cooked at 80 °C for 15 min with mechanical stirring at 300 rpm.

2.3. The in vitro digestibility of potato starch and starch–alginate mixtures

The model used to simulate the digestion process was based on the model described by Dartois et al. (2010). This digestion is divided in two stages: gastric and intestinal digestion. First, 170 g of cooked and cooled starch and starch–alginate gels were placed in 500 mL vessels in a thermoregulated bath at 37 °C and stirred at 300 rpm. Gastric digestion was initiated by adding 1 N HCl until pH 1.2 was reached. During the 30 min of gastric digestion, the pH was maintained at pH 1.2 with 0.5 M NaOH using a pH meter. Gastric juice containing pepsin (enzyme:starch 1.765:100 w/w) was added to the starch solution. Samples were withdrawn at 0, 15 and 30 min and were analyzed for reducing sugars. After 30 min, 1 M NaOH was added to increase the pH to 6.8 to inactivate the pepsin. To initiate intestinal digestion, intestinal fluid (35.3 mL) containing pancreatin (enzyme/starch (d.w.b) ratio, 1.3:100 w/w), amyloglucosidase (enzyme/starch (d.w.b) ratio, 0.26:1 w/w), and invertase (enzyme/starch (d.w.b) ratio, 1:1000 w/w) were added to the solution. The digestion was conducted at 37 °C for 2 h at 300 rpm, and the pH was maintained at 6.8 with 0.5 M HCl. Samples of 0.5 mL were collected at 31, 35, 40, 45, 60, 75, 90, 105, 120, 135 and 150 min during digestion and were immediately analyzed for reducing sugars using 3,5-dinitrosalicylic acid (DNS).

2.4. Reducing sugar analysis by DNS

The samples obtained during digestion were analyzed for reducing sugar using the DNS method according to Bello, carrera, and Diaz (2006). For this analysis, the 0.5 mL samples were mixed with 2 mL of absolute ethanol for 30 min to stop hydrolysis. Then, 0.1 mL of the ethanolic solution was mixed with amyloglucosidase/invertase in acetate buffer (4 mg invertase/0.1 mL amyloglucosidase in 10 mL of acetate buffer, pH 5.2) for 10 min at 37 °C to convert all of the remaining small sugars after digestion into glucose.

The glucose content was analyzed using the 3,5-dinitrosalicylic acid (DNS) method. The DNS was prepared by weighing 5 g of DNS, 150 g of Na–K tartrate and 8 g of NaOH. The NaOH was dissolved into 200 mL of distilled water, and then the Na–K tartrate was slowly added to the solution. The solution was then brought to 400 mL with distilled water. The DNS was then slowly added with constant stirring for 12 h, and the volume was brought to 500 mL with distilled water. The solution was then filtered to remove impurities.

The glucose content was measured by mixing 0.5 mL of each sample with 0.5 mL of DNS in a 10 mL glass tube at 100 °C for 5 min. The tubes were then cooled, and 5 mL of distilled water were added. The measurement was conducted in a spectrophotometer at 540 nm. To determine the glucose concentration during digestion, a calibration curve using different glucose concentrations was created using the following equation:

$$A = 0.561 * \left(\text{Glucose}\left(\text{g}/\text{L}\right)\right) + 0.0032 \quad R^2 = 0.985 \tag{1}$$

2.5. Complex index of gelatinized starch–alginate mixtures

Like was explained by Guraya, Kadan, and Champagne (1997) the measurement of the complex index (CI) involves formation of starch–iodine complex, which implies that the absorbance is related to the portion of starch that was complexed to the iodine. Therefore, the absorbance will be changed according to amount of free starch to be complexed by iodine. In order to determine the reduction in the iodine binding capacity of starch the method proposed by Kawai, Takato, Sasaki, and Kajiwara (2012) was considered. Deionized water (25 mL) was added into a test tube of 50 mL that contain starch–alginates mixtures, which were stirred for 2 min. The dispersion was centrifugated at 10,000 *g* and 0.4 mL of supernatant was mixed with 8.6 mL of deionized water and 1 mL of iodine solution (2.0% (w/w) KI and 1.3% (w/w) I_2 in deionized