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Physicochemical properties of different thickeners used in infant foods and their relationship with mineral availability during *in vitro* digestion process

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ABSTRACT

Locust bean gum (LBG) and modified starches are commonly used as thickeners in food products for infants. However, there is no consensus on their possible effects on infant nutrition, especially on mineral availability. The aim of the present work was to characterize the effect of LBG, cross-linked, hydroxypropylated maize starch (Mhdp) and pre-gelatinized rice starch (gRS) on Ca, Fe and Zn availability during a gastric and intestinal *in vitro* digestion assay in relation to their physicochemical properties in solution (apparent viscosity, solubility, molar mass (M) and conformational properties) through the simulated digestion process. LBG gave the highest decrease in Ca and Fe gastric (17.96% and 17.6% respectively) and intestinal (19.5% and 13.5%) solubility with respect to the reference without thickeners. Ca (11.1% ± 1.1), Fe (2.77% ± 0.3) and Zn (7.78% ± 0.6) dialyzability was also lower than for the reference (23.4% ± 2.9; 19.65% ± 3.53 and 27.74% ± 3.3 respectively). LBG solubility remained stable during gastric digestion, decreasing significantly from a range of 65–69% to 61.1% after intestinal digestion. LBG viscosity remained stable during the digestion process, being these findings attributable to its resistance to enzymes. On the other hand, the addition to Mhdp or gRS slightly affected Ca and Fe solubility or Ca dialyzability, decreasing after gastric digestion and then increasing after intestinal digestion with respect to the reference. These results correlated to the changes in their viscosity enhancing properties, which increased during gastric digestion and decreased after intestinal digestion, being attributable to their digestion by pancreatic enzymes. Gastric digestion resulted in an increase in M for the modified starches (more pronounced for gRS). The increase in mineral solubility and dialyzability after intestinal digestion with respect to the gastric stage was explained by the degradation of starches by intestinal enzymes, which resulted in a decrease in apparent shear viscosity (from 1.2 to 1 Pa s, measured in a shear rate range 0.00–50 s^{-1}) and an increase in solubility (from 3 to 6% to approximately 70%) after intestinal digestion. In conclusion, LBG could be more effective than Mhdp and gRS as thickener, providing higher viscosity and resistance to digestive process. However, its negative effect on mineral solubility and dialyzability should be taken into account. On the contrary, Mhdp and gRS showed to be degraded after intestinal digestion.

1. Introduction

Thickeners are commonly used in infant nutrition in a wide range of foods, such as special infant formulas (anti-reflux (AR) infant formulas), baby purees or cereal based foods. Some of these products are specifically intended for infants with swallowing issues, such as dysphagia or gastroesophageal reflux. As thickening agents, locust bean gum (LBG) and modified corn and rice starches, alone or combined, are the most frequently used in Europe (Aggett et al., 2002). Their addition to infant foods is legally allowed in Europe, where different maximum legal limits have been established for each ingredient (Commission Directive 2006/141; European Parliament and Council Directive No 95/2/EC). According to this legislation, modified starches can be added up to a maximum legal limit of 2 g/100 mL, and 30% of the total carbohydrate content. Regarding LBG, the maximum legal limit established for weaning foods for infants and young children in good health is 10 g/kg individually or in combination.

LBG is a neutral galactomannan derived from the endosperm of the seeds of Carob tree (*Ceratonia siliqua*). It is widely used as an industrial thickener (E410) due to its ability to form very viscous solutions at

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relatively low concentration, being only slightly affected by pH or heat treatment (Dakia, Blecker, Robert, Wathelet, & Paquot, 2008; Haddarah et al., 2014; Prajapati, Jani, Moradiya, Randeria, & Nagar, 2013). LBG is a highly polydisperse polysaccharide, consisting of a linear chain of (1 → 4)-linked β-D-mannopyranosyl units with randomly distributed (1 → 6)-linked-D-galactopyranosyl residues as side chains (McCleary, 1980). The average degree of branching is approximately 25% and its molar mass (M) is in a range of 0.3–2.0 · 10^6 g/mol. LBG physicochemical properties are strongly influenced by the galactose content and distribution of the galactose units along the β-D-mannopyranosyl backbone, which in turn, is determined by different factors such as plant source, growing conditions and manufacturing processes (Barak & Mudgil, 2014; Coviello, Alhaique, Dorigo, Matricardi, & Grassi, 2007; Dakia et al., 2008; Haddarah et al., 2014; Prajapati et al., 2013). LBG has been classified as a non-digestible carbohydrate, remaining quite resistant to hydrolysis under a wide range of pH (from 3 to 11), and to digestive enzyme treatment (Fabek, Messerschmidt, Brulport, & Goff, 2014; Prajapati et al., 2013).

Starches are carbohydrate polymers consisting of two polysaccharides, amylose and amylopectin. Amylose is a mainly linear molecule of α-(1 → 4)-linked-D-glucopyranosyl units with relatively low M, whereas amylopectin has high M and highly branched structures consisting of a-(1 → 4)-linked-D-glucopyranosyl chains linked with 5–6% non-randomly distributed α-(1 → 6)-D-glucopyranosyl branch points (Fernandez, Rojas, & Nilsson, 2011). The ratio of amylose to amylopectin depends on the botanical source and is commonly 10–30% amylose (Hizukuri, 1985; Vandeputte, Vermeylen, Geeroms, & Delcour, 2003). Starches from different plant sources (maize, rice or potato) are commonly used as texturizing ingredients, added for improving food rheological characteristics as thickness and viscosity. Non-modified or native starches have been used as good texturizers. However, many limitations to their uses have been described, such as low shear resistance, low thermal resistance, or low resistance to the digestion process and enzymes. In order to improve their technological properties, different physico-chemical modifications are applied. Common types of starch modifications are derivatization (etherification, esterification or cross-linking), acid or enzymatic hydrolysis, and physical treatment using heat or moisture (Chung, Liu, Lee, & Wei, 2011; Singh, Kaurb, & McCarthy, 2007). In contrast to LBG, starches are sensitive to degradation by intestinal enzymes, being more or less sensitive depending on the modification (Han & BeMiller, 2007; Tester, Karkalas, & Qi, 2004).

In vitro studies have shown that the use of these ingredients could affect mineral availability (Gonzalez-Bermudez, Frontela-Saseta, López-Nicolás, Ros-Berruezo, & Martínez-Graciá, 2014; Vandenplas, Hauser, Devreker, & Salvatore, 2013; Bosscher, Van Caillie-Bertrand & Deelstra, 2003; Bosscher, Van Caillie-Bertrand, Van Cauwenberg & Deelstra, 2003). According to these works, the effect on mineral availability could be due to the combination of different factors, such as the viscosity increase during the *in vitro* digestion, the interaction between minerals and polar groups in the ingredients or the presence of antinutrients as phytates. However, no studies about the molecular and physicochemical properties of these ingredients during the *in vitro* digestion process and their possible relationship with *in vitro* mineral availability have been found.

With this background, the aim of this study is to analyze if apparent viscosity, solubility and molecular properties of locust bean gum and modified corn and rice starches could give an insight into the mechanism of the previously described effect on *in vitro* mineral availability during an *in vitro* gastro-intestinal digestion process. The molecular properties were characterized using asymmetric flow field-flow fractionation (AF4) coupled to multiangle light scattering (MALS) and differential refractive index (dRI) detection. The principles of AF4 are described elsewhere (Wahlund & Giddings, 1987; Wahlund & Nilsson, 2012). AF4–MALS–RI has been shown to be a versatile tool for the characterization of food macromolecules (Nilsson, 2013). In order to avoid the possible influence of food matrix on the properties of thickeners and mineral availability during the digestion process, the isolated ingredients were studied.

2. Materials & methods

2.1. Ingredients and samples

Locust bean gum (Grindsted LBG 860, Danisco, Portugal), cross-linked maize hydroxypropyldistarch phosphate (Mhdp) (MCS; Multi-Thick®, Abbott Nutrition, Spain) and pre-gelatinized rice starch (gRS) (MRS; Beneo-Remy Industries, Belgium) were used. Samples were obtained by mixing ingredients with a previously prepared mineral stock, containing $CaCl_2$, $FeSO_4$ and $ZnSO_4$. All the minerals were purchased from Panreac (Barcelona, Spain).

These mineral compounds were chosen as they are widely used for commercial infant formula fortification. Both ingredients and mineral concentration in each sample were chosen according to the maximum legal limit established by the European legislation, or composition declared by the manufacturers of commercial infant formulas available in the Spanish market respectively. Mineral composition of each sample is being shown in Table 1. As reference, minerals without thickeners added were considered.

2.2. Sample reconstitution

Each sample was dispersed with rapid stirring in pre-heated deionized water (37 °C) at a concentration of 0.13 g/100 mL of mineral stock (reference) and 0.63 g/100 mL of thickeners with minerals added.

2.3. In vitro digestion for calcium, iron and zinc in vitro availability determinations

Ca, Fe and Zn *in vitro* availability was analyzed through the mineral solubility and dialyzability determination after an *in vitro* digestion process. With this aim, the *in vitro* method described by Boato, Wortley, Liu, and Glahn (2002), adapted to the gastrointestinal conditions in infants younger than 3 months of age (Frontela, Haro, Ros, & Martinez, 2008; Frontela, Ros, & Martinez, 2009) was used. These modifications consisted in a modification of the amount of enzymes added, as well as a modification in the pH values for each stage.

The *in vitro* digestion process, which consisted in a gastric and intestinal stage, was performed at 37 °C. For the gastric stage, a solution of 2.57 g of pepsin dissolved in HCl 0.1 N was added per 100 mL of sample prepared. Samples were then maintained in a shaking water bath for 1.5 h. For the intestinal stage, a solution of 0.64 mg of pancreatin (P-1750, from porcine pancreas) and 3.85 mg of bile salt (B-8756), dissolved in 0.1 M $NaHCO_3$, was added per 100 mL of sample. All the enzymes were purchased from Sigma Aldrich. After adding enzymes, samples were maintained in a shaking water bath for 2 h. As detailed by Frontela et al. (2009) and in order to mimic gastrointestinal conditions in infants younger than 6 months of age, the pH values for gastric and intestinal stages were 4 and 5 respectively. At the end of both stages, aliquots of 20 g of sample were transferred to 50 mL polypropylene centrifuge tubes (Costar

Table 1
Calcium, iron and zinc content in each sample (data reported as average ± standard deviation; n = 6).

Sample	Calcium (mg/100 g)	Iron (μg/100 g)	Zinc (μg/100 g)
Reference[a]	34.00 ± 1.61	708.72 ± 11.82	405.51 ± 60.14
Minerals + LBG	7.08 ± 1.00	138.57 ± 5.33	99.68 ± 3.39
Minerals + Mhdp	6.96 ± 1.73	150.23 ± 2.29	116.75 ± 11.28
Minerals + gRS	8.41 ± 0.44	149.26 ± 7.00	94.16 ± 9.30

[a] Mineral stock without thickeners added.