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Effect of adding different thickening agents on the viscosity properties and *in vitro* mineral availability of infant formula

Carlos A. González-Bermúdez *, Carmen Frontela-Saseta, Rubén López-Nicolás, Gaspar Ros-Berruezo, Carmen Martínez-Graciá

Department of Food Science and Nutrition, Faculty of Veterinary Sciences, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Spain

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ABSTRACT

The effect of adding different thickening agents (locust bean gum (LBG), modified corn and rice starches (MCS, MRS)) to an infant formula on both *in vitro* mineral availability (Ca, Fe and Zn), quantified by atomic absorption spectrophotometry (AAS), and formula viscosity, after *in vitro* gastrointestinal digestion, was investigated. LBG was the most effective agent to increase formula thickness. However, it showed a negative effect on Ca, Fe and Zn *in vitro* solubility and dialysability. MCS and MRS only affected calcium solubility and dialysability when they were used at $\geqslant$50% of the maximum legal limit. No negative effect was observed for Fe and Zn when modified starches were added at the different concentrations assessed. The phytate content in the thickening ingredients was also analysed. Despite finding a considerable amount of phytic acid in the raw ingredients, its final concentration in the infant formula was insufficient to decrease *in vitro* mineral availability.

1. Introduction

Reflux episodes are frequent in infants, although most of them are mild and brief. However, when this passage of gastric content into the oesophagus is accompanied by abdominal pain, oesophagitis or inspiratory disorders, as well as others pathological consequences, therapeutic intervention becomes necessary (Horvath, Dziechciarz, & Szajewska, 2008). In this regard, a variety of approaches have been proposed, including pharmacological and non-pharmacological therapies. In the treatment of non-complicated gastroesophageal reflux, thickening of infant formulas has commonly been recommended (Vandenplas, Hauser, Devreker, Mahler, Degreef & Veereman-Wauters, 2013). Thickening agents, such as locust bean gum (LBG) or modified starches, have frequently been added to infant formulas with the aim of increasing their viscosity. The efficacy of thickening agents depends on their ability to increase gastric retention time, avoiding a return to the oesophagus during the first digestion phase, and reducing almost consistently the frequency and volume of regurgitation (Corvaglia, Martini, Aceti, Arcuri, Rossini, & Faldella, 2013). Nevertheless, the number of studies on the effect of thickening agents on rheological properties of infant formulas is very limited, and those that do exist have not made reference to the ideal viscosity value that will lead to a positive effect on children (Bosscher, Van Caillie-Bertrand, & Deelstra, 2003a; Miyazawa, Tomomasa, Kaneko, Arakawa, & Morikawa, 2007; Miyazawa, Tomomasa, Kaneko, & Morikawa, 2004; Vanderhooh, Moran, Harris, Merkel, & Orenstein, 2003). These types of products are commercialised under the name of antireflux or antiregurgitation (AR) infant formulas, and are promoted with the claim that they benefit infants who have gastroesophageal reflux or who spit up regularly (Pina, Llach, Ariño-Armengol, & Iglesias, 2008; Vandenplas, 2008; Vanderhooh et al., 2003). The use of AR formulas has been recommended by the North American and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN and ESPGHAN, respectively) (Vandenplas et al., 2009) as far as it has been demonstrated that these products significantly reduce regurgitation in infants with recurrent vomiting. (Agget et al., 2002; Chao & Vandenplas, 2007; Vandeplas, 2008; Vanderhooh et al., 2003)

Locust bean gum and modified starches, as ingredients for AR formulas, are legally allowed in Europe, where different maximum concentrations have been established for each group (European Parliament and Council, 1995; European Parliament and Council, 2006). According to this legislation, modified starches may be added to infant formulas up to either 30% of total carbohydrates or 2 g/100 mL. In the case of LBG, it may be added up to a maximum level of 10 g/L from birth onwards. These maximum levels are similar to those recommended by ESPGHAN in its Global Standard for the Composition of Infant Formula (Koletzko et al., 2005) and by

* Corresponding author. Tel.: +34 868 88 47 98; fax: +34 868 88 84 97.
E-mail address: cagb1@um.es (C.A. González-Bermúdez).

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the European Commission Scientific Committee for Food (1997). Moreover, several authors have indicated the need to explore further the effect of these ingredients on the nutrition and health of infants, as some studies using an *in vitro* model have suggested that the bioavailability of calcium, iron and zinc may be affected by thickening agents and probably also by the presence of certain antinutrients, such as phytic acid (Bosscher et al., 2000, 2003a; Vandenplas et al., 2013). In particular, this antinutrient has been reported as a strong chelator of multivalent metal ions, specifically iron, zinc and calcium (Frontela, Haro, Ros, & Martinez, 2008).

With this background, the aim of this study was to evaluate the effect of different concentrations of LBG and pregelatinised corn and rice starches added to a commercial infant formula on both viscosity and mineral (calcium, iron and zinc) availability *in vitro*.

2. Materials and methods

2.1. Samples

Standard infant formula (Hero Baby® 1) was provided by Hero España SA (Alcantarilla, Murcia, Spain). As thickening agents, locust bean gum (Grinsted LBG 860, Danisco, Portugal), modified corn starch (MCS; Multi-Thick®, Abbott Nutrition, Spain) and modified rice starch (MRS; Beneo-Remy Industries, Belgium) were selected. For the infant formula reconstitution, 200 mL of deionised water were mixed with 30 g of powder according to the manufacturer's instructions.

2.2. Materials and reagents

Deionised water (MilliQ; Millipore, Bedford, MA) was used throughout the study. Pepsin (P-7000, from porcine stomach mucosa), bile salts (B-8756) and pancreatin (P-1750, from porcine pancreas) were purchased from Sigma (St. Louis, MO). To simulate the gastrointestinal conditions of children less than 6 months of age, pepsin solution was prepared by dissolving 1.6 g of pepsin in 10 mL of 0.1 N HCl. The pancreatin-bile extract solution was prepared by dissolving 0.2 g of pancreatin and 1.25 g of bile in 50 mL of 0.1 M $NaHCO_3$. The working solutions of these enzymes were prepared immediately before use. For mineral dialysis assays, dialysis membranes with molecular mass cut-offs (MMCO) of 12,000 Da were purchased from Medicell Intl Ltd., London, UK). Ca, Fe and Zn contents were determined by flame atomic absorption spectroscopy (AAS) according to the AOAC method (Jorhem 2000). The glass material was washed with detergent, soaked in concentrated nitric acid (SG 1.41) and rinsed three times with distilled deionised water before use. In the case of calcium determination, a lanthanum chloride 1% (w/v) solution (Fluka Analytical, Buchs, Switzerland) was used to suppress phosphate interferences.

2.3. Sample preparation

Different concentrations of each thickening agent were added to the standard infant formula (Hero Baby® 1), and then samples were homogenised using a VH-5 high-efficiency mixer (Comecta SA, Barcelona, Spain). As can be seen in Table 1, for each thickener, the selected concentrations were 7.5%, 15%, 50% and 100% of their respective maximum legal limit (European Parliament and Council, 2006, 1995).

2.4. Inositol phosphate (IPs) extraction and measurement

Inositol phosphates (IPs), including phytic acid (myo-inositol hexaphosphoric acid), were extracted from the different samples with 0.5 N HCl at room temperature for 2 h. Each extract was then centrifuged and the supernatant frozen overnight, followed by thawing and centrifugation. An aliquot of supernatant was poured onto an anion exchange (SAX) column (500 mg; Supelco, Bellefonte, PA) connected to a vacuum manifold set at 20 mmHg. The resin-bound inositol polyphosphates were eluted with 2 mL of 2 M HCl. Eluted samples were evaporated to dryness *in vacuo* at 40 °C and dissolved in 1 mL of deionised water.

Inositol phosphates were determined by LC–MS (Liu, Villalta, & Sturla, 2009) using reverse-phase chromatography on an Agilent 1100 series (Agilent Technologies, Santa Clara, CA, USA) HPLC system equipped with a thermostated micro-well plate autosampler and a quaternary pump, and connected to an Agilent Ion Trap XCT Plus mass spectrometer (Agilent Technologies) using an electrospray interface (ESI).

Samples and standards (40 μL) were injected into a C18 reverse-phase HPLC column (Agilent Technologies), thermostated at 40 °C, and eluted at a flow rate of 200 μL/min throughout the separation. Samples were passed through 0.22-μm HPLC filters before injection. The mobile phase consisted of two solvents: solvent **A**, 0.1% formic acid in water; and solvent **B**, 0.1% formic acid in acetonitrile. Inositol phosphates were eluted as follows: from 10% to 100% **B** in 30 min; from 100% to 10% **B** in 15 min; an isocratic elution of 10% was maintained from 45 to 60 min to equilibrate the column under the initial conditions.

The mass spectrometer was operated in negative ion mode with a capillary spray voltage of 3500 V, and a scan speed of 2200 amu/s from *m/z* 50–750. The nebuliser gas pressure, drying gas flow rate and drying gas temperature were set at 30 psi, 8 L/min and 350 °C. Control and data acquisition of the HPLC–MS equipment was performed with Agilent Chemstation Rev B.01.03.SR2. Data were

Table 1
Composition (g of thickening agent/100 g of infant formula) and mineral content (mg/100 g of infant formula) per sample.

Sample	Thickening agent (%)	LBG (g/100 g)	MCS (g/100 g)	MRS (g/100 g)	Ca (mg/100 g)	Fe (mg/100 g)	Zn (mg/100 g)
1	0	–	–	–	398 ± 27.9	4.98 ± 0.27	3.81 ± 0.14
2	7.5	0.5	–	–	374 ± 24.4	4.80 ± 0.15	3.81 ± 0.15
3	7.5	–	1	–	294 ± 17.2	4.97 ± 0.28	3.92 ± 0.18
4	7.5	–	–	1	303 ± 10.6	5.22 ± 0.11	4.59 ± 0.02
5	15	1	–	–	338 ± 6.36	5.34 ± 0.13	4.56 ± 0.03
6	15	–	2	–	296 ± 24.4	4.62 ± 0.12	4.01 ± 0.08
7	15	–	–	2	301 ± 5.11	4.76 ± 0.15	4.19 ± 0.14
8	50	3.36	–	–	342 ± 6.19	4.85 ± 0.36	3.98 ± 0.07
9	50	–	6.66	–	394 ± 2.37	5.11 ± 0.07	4.04 ± 0.08
10	50	–	–	6.66	301 ± 22.0	4.48 ± 0.13	3.99 ± 0.05
11	100	6.67	–	–	279 ± 5.37	5.11 ± 0.06	4.22 ± 0.11
12	100	–	13.33	–	381 ± 12.8	4.82 ± 0.04	4.23 ± 0.06
13	100	–	–	13.33	362 ± 30.5	5.18 ± 0.11	4.18 ± 0.05

LBG: locust bean gum; MCS: modified corn starch; MRS: modified rice starch.