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

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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, Martinini, Aceti, Arcuri, Rossini, & Falclella, 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, Tomomasu, Kaneko, Arakawa, & Morikawa, 2007; Miyazawa, Tomomasu, Kaneko, & Morikawa, 2004; Vanderhoof, 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-Arnegol, & Iglesias, 2008; Vandenplas, 2008; Vanderhoof 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 (NASCPLAN 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; Vanderplas, 2008; Vanderhoof 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

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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 40 mL of 0.1 M 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 NaHCO3. 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 hydrochloric acid (37%, 37% v/v) 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), thermostatted 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 presented as a peak area.

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

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

LBG: locust bean gum; MCS: modified corn starch; MRS: modified rice starch.
processed using the data analysis software for LC/MSD Trap version 3.3 (BrukerDaltonik, GmbH, Bremen, Germany) provided by the manufacturer.

2.5. In vitro digestion

To measure the solubility and dialysability of iron, calcium, and zinc, each sample was reconstituted in rapidly stirring and preheated (37 °C) deionised water according to the manufacturer's recommendations. After reconstitution, samples were digested using the widespread in vitro method described by Boato, Wortley, Liu, and Glahn (2002), with modifications aimed at reducing the amounts of enzymes used, since the gastrointestinal tract in the early stages of life is not yet fully developed (Frontela, Ros, & Martinez, 2009; Frontela et al., 2008). The in vitro digestion process, which consists of gastric and intestinal stages, was performed at 37 °C. At the end of the intestinal stage, aliquots of 20 g of sample were transferred to 50-mL polypropylene centrifuge tubes (Costar Corning Europe, Badhoevedorp, Netherlands) and then centrifuged (Eppendorf 5804-R Centrifuge, Hamburg, Germany) at 3500g for 1 h at 4 °C. The supernatant (soluble fraction) was used to determine the mineral content. Dialysis comprised the gastric stage, followed by an intestinal step in which a dialysis bag containing 50 mL of deionised distilled water and an amount of NaHCO₃ equivalent to the titratable acidity (previously measured) was placed in flasks containing 20-g aliquots of the pepsin digest. The iron, calcium and zinc dialysed through the semipermeable membrane represent the bio-available fraction (expressed as a percentage) of the total minerals present in the sample (Etcheyverry, Grusak, & Fleige, 2012; Frontela, Ros, & Martinez, 2011).

2.6. Determination of mineral content

The Fe, Ca and Zn concentrations in samples and the mineral soluble and dialysable fractions were determined by AAS (Thermo Scientific AA Spectrometer S Series; Thermo, Waltham, MA). Prior to analyses, the organic matter was destroyed in an ashing oven (Nabertherm, Lilienthal, Germany) at 525 °C for 32 h. Three millilitres of HNO₃ were then added to the ashes and the samples were heated to dryness. After cooling, the residue was dissolved with 1 mL of HCl (SG 1.9), and the solution was transferred to a 10-mL volumetric flask and made to volume with water. The mineral content in the diluted, acidified samples was determined against Fe, Ca and Zn standard solutions (Merck, Germany). The calibration curves obtained were between 1 and 15 ppm for Ca, 0.25 and 5 ppm for Fe and 0.25 and 2 for Zn, and showed an acceptable linearity with correlation coefficients greater than 0.995. Mineral solubility and dialysability (%) were calculated as follows (Frontela et al., 2011):

Soluble (%) = \(\frac{\text{Soluble mineral content (mg/100 g)}}{\text{Total mineral content of the sample (mg/100 g)}} \times 100\)

Dialysable (%) = \(\frac{\text{Dialysable mineral content (mg/100 g)}}{\text{Total mineral content of the sample (mg/100 g)}} \times 100\)

2.7. Validation criteria for the AAS technique

The reference material obtained from the International Atomic Energy Agency (IAEA) 153–Milk Powder (Vienna, Austria) was used as a control to test the method for accuracy. Fe, Ca and Zn were analysed in the reference material. The measured mean values \((n = 3)\) for Fe, Ca and Zn were 2.04, 13125 and 37.98 µg/g respectively, which were in accordance with the certified range of 2.56 ± 1.28 µg/g for Fe, 12855 ± 445 µg/g for Ca, and 39.45 ± 2.52 µg/g for Zn. The precision of the method was calculated from the results obtained in the analysis of the soluble mineral fraction from six aliquots of a sample. The values expressed as coefficient of variation (%), were 1.19 for iron, 0.99 for calcium and 1.00 for zinc.

2.8. Viscosity measurement during in vitro gastric digestion

Viscosity, understood as a quantitative rheological measurement of frictional resistance to shear in a fluid, was measured in each sample, as well as in the standard formula (Hero Baby® 1). This was done using a rotational viscometer (Haake Viscotester VT6L plus, Thermo Electron Corporation, Germany) with a number 1 spindle at 60 rpm and 37 °C. With the aim of characterising the rheology of each agent added to the standard formula, four measurements were made for each sample during the first stage of in vitro digestion: immediately after reconstitution \((0)\), just after pH adjustment and enzyme addition \{(beginning of digestion) \((1)\), after 30 min \((2)\), after 60 min \((3)\), and after 120 min \((4)\) of in vitro gastric digestion.

2.9. Statistical analysis

All experiments were carried out six times, and the results were reported as means ± SD. After confirming the data normality using the Kolmogorov–Smirnov test and homoscedasticity by the Levene test, solubility and dialysability of the samples were compared by one-way analysis of variance (ANOVA) and a Tukey post-test for multiple comparisons to determine the significance of the effects of different thickening agents used at the same legal concentration level \((p < 0.05)\). For each level, the results were compared with the standard formula. Pearson's correlation test was performed to investigate the relationship between the concentration of each thickening agent's viscosity values, phytate content and Ca, Fe and Zn solubility and dialysability. Values of \(p < 0.05\) (two-tailed) were considered significant. All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS version 14.0; SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Effect of different concentrations of thickening agents on in vitro mineral availability in infant formulas

The three measured minerals (Ca, Fe and Zn) assessed in infant formula were aligned to the levels recommended by infant formula regulation (Commission Directive, 2006). Differences observed in mineral content between samples can be considered negligible and are attributed to the different proportions of thickening agents used (Table 1). The effect of the concentration of each thickening agent on Ca, Fe and Zn solubility and dialysability percentages of infant formulas are shown in Figs. 1 and 2, respectively.

Regarding mineral solubility, calcium was the only mineral negatively affected by the three thickening agents when they were added in high concentrations (>50% of maximum legal limit) compared with the standard formula (Hero Baby® 1 without thickening agents). This negative effect was significantly higher \((p < 0.05)\) for LBG than for modified starches at the same concentration \((100\%). This could be explained by the higher amount of IP6 (myoinositol hexaphosphoric acid) in LBG (47.3 mg/100 g) compared with amounts observed in modified starches (19.2 mg/100 g in MCS and 17.5 mg/100 g in MRS). In this regard, it must also be noted...
that during the gastrointestinal process, optimal conditions for α-amylase were achieved (Frontela et al., 2009).

Based on this, total starch loss is probably responsible for breaking modified starches into oligosaccharides. Moreover, these conditions might favour endogenous phytase activity, which has an optimal temperature of around 55°C and which depends on the phytate content. When iron solubility was studied, a significant negative effect was observed when LBG was added in high concentrations (>50% of maximum legal limit), whereas the solubility of zinc was reduced only by LBG at 100% of maximum legal limit). No negative effect occurred when modified starches were used as thickeners. Moreover, at the different concentrations of MCS, a significant improvement of zinc solubility with respect to standard formula was found. In this context, an explanation could be found relating to the content of the hexa (IP₆) and penta (IP₅) forms of IP detected in the thickening agents added to the infant formula (Hurrell, 2004). However, we observed that the phytate (IP₃ + IP₆) concentrations measured in LBG, MCS and MRS provided infant formula with a phytate/mineral molar ratio that was lower than values reported to be critical in all the proportions studied (Table 2). Although the critical phytate/iron molar ratio has not been well established, according to Hurrell (2004), for an optimal mineral absorption, it should be reduced to below 0.4:1. In the case of calcium, it should not be higher than 0.24 (Gibson, Bailey, Gibbs, & Ferguson, 2010) and in the case of zinc, ratios above 1.5:1 may inhibit zinc availability (Ma, Li, Jin, Zhai, Kok & Yang, 2007).

As can be seen on Table 2, LBG showed the highest amount of phytate (109 mg/100 g) when compared to MCS and MRS (36.2 and 41.4 mg/100 g, respectively). However, as a result of the legal limit of addition for each thickening agent (European Parliament and Council, 2006, 1995), the final content of phytate in infant formula did not compromise the mineral availability.

Concerning mineral dialysability, calcium was affected by the addition of LBG at concentration levels of 50% and 100%, and by MCS/MRS at all the tested concentration levels. When iron and zinc dialysability were analysed, only formula added with a 50% and

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**Fig. 1.** Effect of different concentration levels for each thickening agent, on Ca, Fe and Zn solubility. Different superscripts (a–c) indicate significant differences (p < 0.05) between Ca, Fe or Zn solubility within the same concentration level for each thickening agent, locust bean gum (LBG), modified corn starch (MCS) and modified rice starch (MRS). Data expressed as mean ± SD.
100% concentration of LBG showed a significantly negative effect, which was more important for iron (2.224% and 1.857% for LBG concentrations of 50% and 100%, respectively) than for zinc (7.656% and 7.541% for LBG concentrations of 50% and 100%, respectively). Meanwhile, MCS and MRS did not seem to affect iron or zinc dialysability when compared with standard formula. These results are in agreement with those observed by Bosscher, Van Caillie-Bertrand, Van Cauwenberg, and Deelstra (2003b), who studied dairy infant formulas with pregelatinised starches added. They reported that LBG brought about reduced availability of both Fe and Zn. Meanwhile, pregelatinised starches used as thickeners increased mineral availability compared to non-thickened infant formulas.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>LBG</th>
<th>MCS</th>
<th>MRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP₆</td>
<td>47.3 ± 1.05</td>
<td>19.2 ± 0.50</td>
<td>17.5 ± 0.39</td>
</tr>
<tr>
<td>IP₅</td>
<td>61.7 ± 1.21</td>
<td>17.0 ± 0.52</td>
<td>23.9 ± 0.91</td>
</tr>
<tr>
<td>IP₄</td>
<td>13.2 ± 0.63</td>
<td>12.8 ± 0.34</td>
<td>20.0 ± 0.73</td>
</tr>
<tr>
<td>IP₃</td>
<td>77.5 ± 0.95</td>
<td>11.7 ± 0.25</td>
<td>21.3 ± 0.72</td>
</tr>
<tr>
<td>IP₂</td>
<td>13.4 ± 0.31</td>
<td>13.5 ± 0.44</td>
<td>18.4 ± 0.50</td>
</tr>
<tr>
<td>IP₁</td>
<td>9.98 ± 0.20</td>
<td>4.27 ± 0.52</td>
<td>14.1 ± 0.61</td>
</tr>
<tr>
<td>Inositol</td>
<td>3.61 ± 0.43</td>
<td>3.62 ± 0.31</td>
<td>3.53 ± 0.53</td>
</tr>
</tbody>
</table>

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

Table 3

<table>
<thead>
<tr>
<th></th>
<th>LBG</th>
<th>MCS</th>
<th>MRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>-0.909</td>
<td>-0.793</td>
<td>-0.866</td>
</tr>
<tr>
<td>Fe</td>
<td>-0.912</td>
<td>0.483</td>
<td>0.437</td>
</tr>
<tr>
<td>Zn</td>
<td>-0.834</td>
<td>-0.187</td>
<td>0.710</td>
</tr>
</tbody>
</table>

LBG: locust bean gum; MCS: modified corn starch; MRS: modified rice starch. *p < 0.05.
The possibility, during digestion process, bound minerals could be rendered them unavailable for absorption. Suggested that LBG could form strong complexes with metal ions, digesting of food components. Related to this, Bosscher et al. (2003b) suggested that LBG could form strong complexes with metal ions, rendering them unavailable for absorption.

Regarding modified or pregelatinised starches, MCS and MRS might be considered digestible carbohydrates. According to this possibility, during digestion process, bound minerals could be released to the intestinal lumen, increasing mineral availability in comparison with LBG. Nevertheless, the in vitro availability of calcium seems to be decreased, which could be due to the formation of unabsorbable complexes (Agget et al., 2002; Commission Directive, 2006). The results of similar published in vitro studies also suggest that the bioavailability of Ca, Fe and Zn in infant formula may be decreased by thickened formulas with non-absorbable complexes (Agget et al., 2002; Commission Directive, 2006). The results of similar published assays, no minimum viscosity limits for infant regurgitation management in children can be fixed. Nevertheless, a viscosity higher than 100 cps could exert a negative effect, since oesophageal clearance of refluxed material would be reduced (Infante-Pina, Lara-Villoslada, López-Ginés, & Morales-Hernández, 2010). This would affect the oesophageal acid exposure time, increasing the risk of oesophagitis secondary to gastric content reflux (Corvaglia et al., 2013). Moreover, if the viscosity of infant formula is too high, infants could reject it, since they may have trouble sucking such a formula through a standard nipple.

3.2. Effect of thickening agent concentrations on in vitro viscosity

The viscosity values of infant formulas mixed with different concentrations of each thickening agent are presented in Table 4. The results are grouped according to the time of measurement and concentration of the thickening agent. Overall, after pH adjustment to 4 with 6 N HCL, the viscosity of formulas increased in relation to their respective viscosities just after reconstitution, being more evident at a higher concentration of thickening agents (15% or greater). This finding has been also described by other authors (Vanderhoof et al., 2003). Although the viscosity-increasing mechanism does not seem to be well-known, it might have to do with the physical interaction between the thickening agents and protein components in the assayed product.

Comparing viscosity values, standard infant formula with a LBG concentration of 15% or greater, showed a significantly higher (p < 0.05) viscosity value than that for the standard infant formula with MCS and MRS added. Focusing on the viscosity provided by LBG, it reached values higher than 60 cps and 100 cps for a 50% and 100% concentration, respectively, under the maximum legal limit (3.36 g or 6.67 g of LBG per 100 g of infant formula). In contrast with these values, MCS and MRS provided viscosity values higher than 20 cps and 27 cps for a 50% and 100% concentration, respectively, under the maximum legal limit (6.66 g or 13.33 g of starches per 100 g of formula).

Due to the lack of clinical trials or in vivo assays, no minimum viscosity limits for infant regurgitation management in children can be fixed. Nevertheless, a viscosity higher than 100 cps could exert a negative effect, since oesophageal clearance of refluxed material would be reduced (Infante-Pina, Lara-Villoslada, López-Ginés, & Morales-Hernández, 2010). This would affect the oesophageal acid exposure time, increasing the risk of oesophagitis secondary to gastric content reflux (Corvaglia et al., 2013). Moreover, if the viscosity of infant formula is too high, infants could reject it, since they may have trouble sucking such a formula through a standard nipple.

4. Conclusions

LBG, MCS and MRS are frequently used as thickening ingredients in AR-infant formulas. In the present study, the effect of their addition to a standard infant formula on both, mineral availability (solubility and dialysability percentages measured by AAS) and viscosity has been determined. Analysing the viscosity results, it can be concluded that LBG is more effective as a thickening agent than MCS or MRS when added to infant formula under the legal limits. However, LBG negatively affects the availability of calcium, iron and zinc in vitro, decreasing mineral solubility and dialysability, whereas MCS and MRS only affect calcium solubility and dialysability in a negative way. These findings should be taken into account when developing an antiregurgitation infant formula, since the acceptance of foods by infants and mineral requirements could be potentially compromised. A possible solution could be the adequate combination of these thickening agents to minimise the negative effect on mineral availability while maximising the effect on formula viscosity.

With the aim of characterising the in vivo effects of these ingredients, it would be necessary to conduct clinical trials or in vivo
assays in infants. This would lead not only to a better understanding of the nutritional management of infant regurgitation, but also to the establishment of an effective thickening agent concentration which leads to a reduction in regurgitation episodes. In these types of studies, an interesting and innovative focus will be to analyse the effect of thickening agents on colonic microbiota.

Acknowledgements

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References


