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Determination of the phytic acid levels in infant foods using different analytical methods

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Abstract

The analytical methods of phytic acid determination in infant foods were evaluated, and then, the method suggested in this study was applied to determine the phytic acid level in commercially available infant foods for both the flour and paste types. The spectrophotometric (AOAC and Wade reagent) and chromatographic (GC-FID and HPLC) methods were compared, and the spectrophotometrically determined value showed higher phytic acid levels than that of the chromatographic methods ($p < 0.05$). The AOAC method showed a complete recovery for the infant foods after spiking the phytic acid, while a poor recovery was observed by GC-FID and HPLC-RI. The average levels of phytic acid in the infant foods determined by the AOAC methods were 363 mg/100 g for the flour type and 46.3 mg/100 g for the paste type on a wet basis. When the phytic acid level was converted into a dry basis, the phytic acid levels of the paste types were much higher than that of the flour type. Thus, the amount of phytic acid intake per meal was calculated, and the phytic acid level per meal was high in the paste type as wells as the flour type. Therefore, a problem with phytic acid might be considered for the infant foods of both the flour and paste types.

Keywords: Phytic acid; Infant foods; AOAC; Wade reagent; GC-FID; HPLC

1. Introduction

Recently, according to the increased number of working couples, commercial infant foods have been developed in many countries. The main components of infant foods are cereals, vegetables and legumes. They are a good source of protein, minerals and vitamins for babies, but most of these components may contain phytic acid as an antinutritional factor. During the pre-weaning period, the diet of an infant is based upon cereal flours as well as upon baby milk formulas. Therefore, the negative properties of phytic acid, particularly on the bioavailability of minerals, may have an effect on a baby's health during this first period. Phytic acid (myo-inositol hexaphosphate, IP6) is a widely found in cereals, nuts, legumes, oil seeds, spores, and pollen constituting 1–5% (Graf & Eaton, 1990). The phytic acid acts as an antinutrient due to its chelation of various metals and its binding of protein, therefore, diminishing

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the bioavailability of protein and nutritionally important minerals (Liu, Rafiq, Tzeng, & Rob, 1998).

Structure of phytic acid in a solution is the chair conformation of hexaphosphorylated myoinositol and it usually occurs as a mixed calcium–magnesium–potassium salt in discrete regions of the seeds, such as the aleurone layer of wheat and rice (Oberleas, 1983). In vitro studies show that the phytic acid–protein complexes are formed by electrostatic interactions. Many of these complexes are insoluble and are not biologically available for humans under normal physiological conditions (Cheryan, 1980).

Recently, phytic acid has been reported to be an antioxidant (Graf & Eaton, 1990), anticarcinogenic (Shamsuddin, Vucenik, & Cole, 1997) and hydroglycemic or hypolipidemic (Rickard & Thompson, 1997). Phytic acid is considered to be an antioxidant agent, because it is a potent inhibitor of the iron-catalyzed hydroxy radical formation by chelating the free iron and then blocking it's coordination site (Graf & Eaton, 1990). However, the mechanism of this action is still unclear (Shamsuddin et al., 1997). Furthermore, lower inositol phosphate, such as IP4 and IP3 may play the roles of mediating the cellular responses and have been noted as having a function in the second messenger transduction system (Berridge & Irvine, 1989).

Many analytical methods for phytic acid in foods have been suggested, but their applicability is very different, regarding the type of the samples. Therefore, it is important that a method can be selected according to the samples. So far, the researches related to phytic acid levels in infant foods have focused on the flour types including various cereals (Brooks & Lampi, 2001; Febles, Arias, Hardisson, Rodriguez-Alvarez, & Sierra, 2001). No information on the paste type contained in fruits and vegetables is available as yet. The data on the phytic acid levels of the paste type will also be important, because phytic acid is contained in fruits and vegetables as well as cereals or legumes.

The purpose of this study was to evaluate an appropriate method for determining the phytic acid level in infant foods, and also to provide available information for the phytic acid levels in various infant foods using the method suggested.

2. Materials and methods

2.1. Sample preparation

A total of 27 samples of infant foods from different companies were purchased from retail stores in Seoul, Korea. Most ingredients labeled in infant foods were cereals, vegetables, legumes, and fruits. The labeled information of the samples is shown in Table 1.

2.2. Phytic acid analysis

According to Harland and Oberleas (1986), fat influences the extractability of phytic acid from food or foodstuffs and should be kept low (<5%) or reduced before extraction. The fats of these infant foods were removed. A sample (5 g) was treated twice with chloroform–methanol (2:1, v:v). Defatted samples were extracted with 2.4 M HCl (15 mL) except for the HPLC analysis, which was extracted with 0.5 M HCl (15 mL). The extracted solution was centrifuged at 3500 rpm for 10 min. The supernatant was stored in a 4 °C refrigerator before analysis.

A recovery test was carried out in order to evaluate the validity of the analytical method by spiking the phytic acid sodium salt (Sigma Chemical Co., St. Louis, MO, USA) at 1000 ppm levels.

2.2.1. Spectrophotometric methods

Phytic acid levels in the infant foods were determined by the AOAC method modified by Harland and Oberleas (1986). A chromatographic column (0.7 cm × 15 cm) containing 0.5 g of a anion-exchange resin (100–200 mesh, chloride form; AG1-X4, Bio-Rad Co., CA, USA) was equilibrated with 0.7 M NaCl. The column was washed with distilled water. Then, the mixture of the sample supernatant and the Na_2EDTA–NaOH solution was passed onto an anion exchange column. The column was washed with distilled water and 0.1 M NaCl in order to remove the inorganic phosphate. Then the retained phytic acid was eluted with 0.7 M NaCl, which was collected in a 100-mL Kjeldahl flask. This flask was added to with 0.5 mL H_2SO_4, 3.0 mL HNO_3, and then digested. When the flask is cool, add 10 mL H_2O, swirl, or heat flask at a low temperature setting if necessary to dissolve salt. Let solution cool. Quantitatively transfer the solution to a 50-mL volumetric flask. Added 2.0 mL of a Molybdate solution and 1.0 mL of a sulfonic acid solution to this flask, and make to 50 mL with distilled water. After 15 min, the sample solution was measured for its wavelength at 640 nm with a UV-spectrophotometer (model UV-1601PC; Shimadzu Co., Tokyo, Japan).

Phytic acid levels were determined by the method of Latta and Eskin (1980). This analysis was done with a chromatographic column (0.7 cm × 15 cm) containing 0.5 g of an anion-exchange resin (100–200 mesh, chloride form; AG1-X8, Bio-Rad Co.). The process was the same as the AOAC method, and only the digest step was omitted. The Wade reagent (1 mL, 0.03% $FeCl_3 \cdot 6H_2O$ and 0.3% sulfosalicylic acid in distilled water) was added into the extract (3 mL), and centrifuged at 3500 rpm at 5 min. The absorbance of the supernatant was measured at 500 nm with a UV-spectrophotometer.