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Phytic acid level in infant flours

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Abstract

This study determined the phytic acid content in the infant flour commonly consumed in the Canary Islands. A total of 400 samples from different cereals was analyzed. The method proposed by García-Villanova et al. (1982) [García-Villanova, R., García-Villanova, R. J., Ruiz de Lope, C. (1982). Determination of phytic acid by complexometric titration of excess of iron (III). *Analyst* *107*, 1503–1506] was the one used for determination of the phytic acid content in cereal flours. Phytic acid concentrations are within the range <1–$\geqslant 36$ mg/g. The arithmetic mean obtained from all the samples studied is 24.6 mg/g. Most of the samples studied show a phytic acid content higher than 20 mg/g, and much lower values are observed in gluten-free flours ($<$ 5 mg/g). Significant differences are observed for the different *flour* types. *Gluten-free flour* has a content lower than the rest; 9-cereal flour has a phytate concentration lower than the other flours tested but higher than gluten-free flour. Among wheat samples, phytate values are lower than in the varieties muesli-chocolate, 7-cereal, 8-cereal, multicereal and cereal-biscuit. Multicereal flour has a lower content than muesli-chocolate.

1. Introduction

Phytic acid is widely distributed in commonly consumed foods, particularly in vegetables. It is found in high concentrations in seeds of grains, pulses and oleaginous products, and in lesser amounts in tubers and garden produce (Alabaster, Tang, & Shivapurkar, 1996). In general, its presence does not imply the existence of acute toxicity problems. However, as it interferes with the function of essential nutrients, phytic acid may be considered a natural antinutrient substance (Ko & Gold, 1990; Patearroyo, Fernández, & Cid, 1995). Vegetarians are most affected by the high content of phytic acid in food because, although the content of some minerals such as iron and zinc is similar to that of full diets, bioavailability of these minerals may be compromised.

Phytic acid is the hexaphosphoric ester of cyclohexane (inositol hexaphosphoric acid, IP6). It is usually found as a complex with essential minerals and/or proteins (Cheryan, 1980; Fox & Tao, 1989; McCance et al. 1943; Mitjavilla, 1990; Widdowson & McCance, 1942).

The true mechanisms of the interactions between phytic acid and minerals are not yet understood, although it is possible that it could form a complex with a cation on the same or different molecules within a simple phosphate group or between two phosphate groups (Fox & Tao, 1989).

The acid groups present in this molecule facilitate the formation of several salts (Thompson, 1993), those of the alkaline metals being soluble in water, while divalent metals salts are almost insoluble.

As the phytate cannot be absorbed and humans have a limited ability to hydrolyze this molecule, an adverse effect of the phytic acid on the bioavailability of minerals is predicted (Pawar & Ingle, 1988). In addition, the phosphorus of the phytate is not nutritionally available.

In vitro studies show that the phytate–protein complexes are formed by electrostatic interactions. Many of these complexes are insoluble and are not biologically available for humans under normal physiological conditions. In addition, these proteins are less prone to attack by proteolytic enzymes than are free proteins (Cheryan, 1980; Mitjavilla, 1990).

There is a series of processes in which phytic acid is involved in a positive way due to its antioxidant (Graf & Eaton, 1990) and anticarcinogenic effects (Alabaster et. al, 1996; Thompson, 1993; Thompson & Zhang, 1991;

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Vucenic, Yang, & Shansuddin, 1995; Zhou & Erdman, 1995) prevention of coronary disease (Ko & Gold, 1990; Sing, Gupta, Mittal, Niaz, Ghosh, & Rastogi, 1997; Trout, Behall, & Osilesi, 1993). A great controversy exists as to the desirability of eliminating it from food.

It is necessary to point out the relevance of an adequate combination of food in order to have a correct intake of all the nutrients that are essential to the organism, especially in those population groups where the impact of higher phytate concentrations can be more serious. This is the case with children, whose mineral requirements are particularly critical.

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 phytates, particularly on the bioavalability of minerals, may have an effect on health during this first period.

The diet of an infant is determined by different factors: metabolic and growth needs and the development of the nervous system, the kidneys and the digestive apparatus. Thus, it is always necessary to assess both premises so as to provide the infant with adequate supplies at every stage (Morán, 1988). The most important thing is to offer an adequate intake of energy to cover the requirements and to stimulate growth and neoformation of tissues, in order to maintain the correct velocity of growth (FAO/WHO/UNO, 1985).

The main aim of complementary diet is to supplement the maternal milk with essential nutrients (particularly carbohydrates) that propitiate adequate growth and development of the infant. Furthermore, complementary diet stimulates gastrointestinal function and favours dietetic habits.

Cereals are the commonly recommended food in commencing a complementary diet because they have a high energy content (80 kcal/100 g). This new food has to be introduced in small amounts, and it is advisable to do so separately and progressively. Cereals are carbohydrate-rich foods, which also provide proteins, minerals and vitamins (particularly thiamin). Moreover, their fat contents are low but they are rich in essential fatty acids.

The iron content in cereal is a widely debated subject. The tendency nowadays is to make cereals richer by using electrolytic iron, which is absorbed in approximately 4%. In Spain, the iron content in cereals is relatively low (2–18 mg/100 g), much lower than that indicated by ESGAN (1982) Enriched Products, which recommends 0.5 mg/g of dry product. The infant, after 4 months of age, is at risk of developing an iron deficit as a result of a decrease of his organic deposits and the increase of his needs, determined by the higher growth velocity. This shortage may also be influenced by the presence of phytates in these cereal derivatives.

All kinds of cereal grains and other products, such as roots (tapioca) and seeds (peanuts, soy), may be used to prepare the cereals for children. Some of these foods are composed of only one kind of cereal; others contain a combination of them or are enriched with milk, vegetables or fruit.

According to their gluten contents, we can distinguish two types: gluten-free cereals (corn, soy, rice, tapioca) and cereals with gluten (wheat, oats, barley, rye). According to the Codex Alimentarius, cereals can be classified as gluten-free when the residual concentration of proteins from wheat, rye, barley or oats is lower than 0.3 g/100 g of cereal.

Our study determines the phytic acid content in infant flours because of the growing interest in the phytic acid levels of this kind of food and because, in infants, the negative effect on the bioavailability of the minerals may be of great relevance.

2. Material and methods

2.1. Samples

A total of 400 samples of *infant flours* from different cereals were analyzed. These samples were provided by a supplier of pharmaceutical products in Santa Cruz de Tenerife and they correspond to eight different varieties widely consumed among the infant population (Table 1).

2.2. Technique

The method proposed by García-Villanova, García-Villanova, and Ruiz de Lope (1982) was used for determination of phytic acid content in cereal flours. Based

Table 1
Samples of infant flour according to type

Type	Combination of cereals	No.
Wheat-honey	Wheat	50
Gluten-free cereal	Rice, corn	50
7-Cereal	Wheat, corn, rice, barley, rye, oats, millet	50
8-Cereal honey	Wheat, corn, rice, oats, barley, rye, millet, tapioca	50
9-Cereal	Wheat, corn, rice, rye, barley, oats, millet, sorghum, triticale	50
Multicereal	Wheat, corn, rice, barley, rye	50
Muesli-chocolate	Muesli	50
Cereal-biscuits	Wheat, corn, rice, barley, rye, millet, tapioca	50

Table 2
The distribution in intervals of the phytic acid content, arithmetic mean and percentiles 50 and 90, in the samples of infant flour by type

Concentration mg/g	Number of samples							
	Wheat	Gluten-Free	7-Cereal	8-Cereal	9-Cereal	Multicereal	Muesli-Chocolate	Cereal-biscuit
>0–$\geqslant 1$	–	17	–	–	–	–	–	–
>1–$\geqslant 5$	–	18	–	–	–	–	–	–
>5–$\geqslant 10$	–	15	–	–	–	–	–	–
>10–$\geqslant 15$	2	–	–	–	9	–	–	–
>15–$\geqslant 20$	7	–	3	2	8	–	–	–
>20–$\geqslant 25$	10	–	6	2	21	39	10	5
>25–$\geqslant 30$	19	–	16	27	12	11	11	22
>30	12	–	25	19	–	10	29	23
Arithmetic mean	25.5	3.3	29.7	28.7	20.6	28.6	30.4	29.6
Percentile 50	25.7	2.0	29.7	29.0	21.8	29.0	31.0	29.7
Percentile 90	32.3	7.3	36.3	33.0	26.4	31.7	34.9	33.7
Range	13.9–33.0	0.7–9.2	19.8–39.6	18.5–33.0	10.6–26.4	25.1–31.7	21.8–38.9	24.4–43.9

on indirect iron (III) complexometry, it uses sulfosalicylic acid as an indicator of the titration end-point.

2.3. Solutions

Solutions were: 2×10^{-2} M Fe (III) solution in 0.16 M HCl; 10^{-2} M EDTA standard solution; 4×10^{-1} M HCl solution with 5% of Na_2SO_4; and 20% Sulfosalicylic acid solution.

2.4. Method

In a 50–100-ml flask, 40 ml of HCl- Na_2SO_4 solution were added to a 2-g dried sample, and it was left for 90 min with intermittent agitation.

Twenty millilitres of the transparent floating liquid of the previous extraction (filtered, if necessary), 20 ml of HCl- Na_2SO_4 solution, 20 ml of Fe (III) solution and 20 ml of 20% sulfosalicylic acid solution were placed in a neutral glass tube. This was then closed with a gum cap pierced by a narrow glass tube about 30 cm in length, and heated for 15 min in a boiling water bath. It was cooled under tap water and left standing. (The appearance of ferric phytate precipitate should be checked). Twenty millilitres of the clean floating material were separated and placed in a 250 ml precipitation beaker, filling it up to around 200 ml with deionized water and increasing pH to 2.5. This is heated to about 701°C and the Fe (III) titrated, while hot, with 0.010 M EDTA solution until bright yellow.

3. Calculations

The percentage of phytic acid in the sample was deduced from the following equation after adhering precisely to the instructions detailed in Section 2.4.:

Table 3
Kruskal–Wallis test in total infant flour samples by type

	Average range	*P*- value
Wheat-honey	186	≈ 0
Gluten-free cereal	25.5	
7 Cereal	263	
8 Cereal	245	
9 Cereal	104	
Multicereal	240	
Müesli-chocolate	276	
Cereal-biscuit	260	

Phytic acid (%) = 1.32 $(10-V)/P$; V = EDTA solution volume, ml; P = Sample weight (g).

A recovery study was carried out in order to check the validity of the technique by adding to the sample a fixed quantity of phytic acid standard (Sigma), considered to be 50 mg/g (phytic standard). The recovery percentage obtained was 98.5%.

3.1. Statistical analysis of the data

Statistical analysis of the data obtained was carried out using the Kruskal–Wallis tests and the nonparametric multiple comparisons test (Martin & Luna, 1993).

4. Results and discussion

Phytic acid concentration are within the range <1–>36. The average of phytic acid content (mg/g) in all the infant flours analyzed was 24.6 mg/g.

Table 2 shows the distribution, in intervals, of the phytic acid content (mg/g), arithmetic mean and percentiles 50 and 90, in the samples of infant flour, by type. The flours pertaining to the gluten-free variety show values much lower than the rest . The gluten-free

Table 4
Multiple comparison test: total infant flour by cereal (95%)[a]

	Gluten-free		7-Cereal		8-Cereal		9-Cereal		Multicereal		Muesli-Chocolate		Cereal-biscuits	
	ARD	SV	ARD	SV	ARD	SV	ARD	SV	ARD	SV	ARD	SV	ARD	SV
Wheat	161	45.2	77.2	45.2	58.9	45.2	81.7	45.2	54.6	45.2	90.2	45.2	73.8	45.4
Gluten-free	–	–	238	45.2	220	45.2	78.9	45.2	215	45.2	251	45.2	235	45.4
7-Cereal	–	–	–	–	18.3	45.2	158	45.2	22.6	45.2	12.9	45.2	3.4	45.4
8-Cereal	–	–	–	–	–	–	141	45.2	4.3	45.2	31.2	45.2	14.9	45.4
9-Cereal	–	–	–	–	–	–	–	–	136	45.2	172	45.2	155	45.4
Multicereal	–	–	–	–	–	–	–	–	–	–	155	45.2	35.5	45.4
Muesli-chocolate	–	–	–	–	–	–	–	–	–	–	–	–	16.3	45.4

[a] ARD, average range differences; SV, statistical value.

Table 5
Multiple comparison test: total infant flour by cereal (99%)[a]

	Gluten-free		7-Cereal		8-Cereal		9-Cereal		Multicereal		Muesli-Chocolate		Cereal-biscuits	
	ARD	SV	ARD	SV	ARD	SV	ARD	SV	ARD	SV	ARD	SV	ARD	SV
Wheat	161	82.4	77.2	82.4	58.9	82.4	81.7	82.4	54.6	82.4	90.2	82.4	73.8	82.8
Gluten-free	–	–	238	82.4	220	82.4	78.9	82.4	215	82.4	251	82.4	235	82.8
7-Cereal	–	–	–	–	18.3	82.4	158	82.4	22.6	82.4	12.9	82.4	3.4	82.8
8-Cereal	–	–	–	–	–	–	141	82.4	4.3	82.4	31.2	82.4	14.9	82.8
9-Cereal	–	–	–	–	–	–	–	–	136	82.4	172	82.4	155	82.8
Multicereal	–	–	–	–	–	–	–	–	–	–	155	82.4	35.5	82.8
Muesli-chocolate	–	–	–	–	–	–	–	–	–	–	–	–	16.3	82.8

[a] ARD, average range differences; SV, statistical value.

variety had the lowest mean (3.3 mg/g) and the muesli-chocolate variety, the highest (30.4 mg/g). However, most of the samples of the other varieties analyzed show phytic acid contents higher than 20 mg/g.

In order to analyze the phytate content (mg/g) of the eight varieties of infant flour, wheat, gluten free, 7-cereal, 8-cereal, 9-cereal, multicereal, muesli-chocolate and cereal-biscuits and (after discarding the variance equality by means of the Barlett statistic), the Kruskal–Wallis test was applied, which detected significant differences ($P=0.01$; Table 3). Afterwards, and to determine where these differences appear, the multiple comparison non-parametric test was applied; this manifested that the phytate content in gluten-free flour was lower than the rest with a significance of $P=0.01$ (Table 4) and lower than 9-cereal with a significance of $P=0.05$ (Table 5).

The 9-cereal variety has a phytate content lower than the rest of the flours analyzed ($P=0.01$), with the exception, as mentioned above, of the gluten-free variety.

In wheat samples, the phytate values are lower than those in the muesli-chocolate variety ($P=0.01$), 7-cereal, 8-cereal, multicereal and cereal-biscuits ($P=0.05$). Multicereal has a phytate content lower than that in muesli-chocolate ($P=0.01$).

We have not found any study in the literature that deals with the content of phytic acid in infant flours except for the publication by Ruiz de Lope, García-Villanova, and García-Villanova (1983). Here, there is a determination of phytic acid (mg/g) by means of the method used in our study (García Villanova et al., 1982) in 10 samples of infant flours of different compositions. The phytic acid values obtained were within a range of 4.4–7.6 mg/g, i.e. lower than those obtained in our study.

In other cereal derivatives, Francois (1988), in wheat flour and ourselves (Febles, Arias, Hardisson, Rodiguez-Alvarez, & Sierra, 2000), in gofio samples (Canary Island gofio consists of the roasted flours, by themselves or mixed in variable proportions, of wheat, corn, barley, rye), have found slightly lower values.

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