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Phytic acid content in milled cereal products and breads

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Abstract

Phytic acid was determined in cereal (brans, flours and milled wheat-products) and breads. The method was based on complexometric titration of residual iron (III) after phytic acid precipitation. The cereal flours showed values ranged between 3–4 mg/g for soft wheats, 9 mg/g for hard wheat and 22 mg/g for whole wheat. Corn, millet and sorghum flours reported a mean of 10 mg/g and oat, rice, rye and barley between 4 and 7 mg/g. Wheat brans had wide ranges (25–58 mg/g). The phytic acid for oat brans was half that of wheat bran (20 mg/g) and higher value (58 mg/g) than that for rice bran. The milling products (semolinas) from hard wheat exhibited 10 mg/g and soft wheat a mean of 23 mg/g. The breads made with single or mixture cereal flours exhibited ranges between 1.5 and 7.5 mg/g. The loss of phytic acid relative to unprocessed flours was between 20% for oat bread and 50% for white bread.

Keywords: Phytic acid; Milled cereal-products; Breads

1. Introduction

Phytic acid (myoinositol hexa-phosphoric acid, IP6) is the major phosphorus storage compound of most seeds and cereal grains, it may account for more than 70% of the total phosphorus. Phytic acid has a strong ability to chelate multivalent metal ions, specially zinc, calcium and iron. The binding can result in very insoluble salts with poor bioavailability of minerals (Rhou & Erdman, 1995). Besides its well-known negative properties IP6, by complexing iron, may bring about a favorable reduction in the formation of hydroxyl radicals in the colon (Graf & Eaton, 1993), also positive effect against carcinogenesis have been shown with in vitro cell culture systems, mice, rats and guinea pigs, but the mechanism of action is not understood (Harland & Morris, 1995).

Because of the numerous health benefits of dietary fiber, the consumption of brans from various cereal grains is increasing. The phytic acid is associated with brans; some brans can contain over 5% phytic acid. As a consequence, greater consumption of phytic acid has resulted from increased consumption of high fibre foods. If the consumer is eating a marginal diet in essential minerals, the phytic acid may lead to a nutritional deficiency (Lehrfeld, 1994).

Phytic acid is hydrolyzed, enzymatically by phytases, or chemically to lower inositol phosphates such as inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), inositol triphosphate (IP3) and possibly the inositol di- and monophosphate during storage, fermentation, germination, food processing and digestion in the human gut (Burbano, Muzquiz, Osagie, Ayet & Cuadrado, 1995). Only IP6 and IP5 have a negative effect on a bioavailability of minerals, the other hydrolytic products formed have a poor capacity to bind minerals, or the complexes formed are more soluble (Sandberg, Carlsson & Svanberg, 1989).

Many methods of phytic acid determination have been developed. The precipitation and ion-exchange method's are not specific as they do not separate inositol hexaphosphate from lower inositol phosphates and thus overestimate the phytate content in processed foods (Sandberg, 1995). The HPLC method determines the inositols in processed foods. The phytic acid in unprocessed products mainly appear as inositol hexaphosphate (IP6); since the precipitation methods are useful to measure the phytic acid content in unprocessed products. Besides, they may also be appropriate to evaluate the complexing capacity from a nutritional

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standpoint (Phillippy, Johnston, Tao & Fox, 1988). Thus, the goal of this work was to assess total phytate content of cereal samples by a complexometric measure of residual iron after phytic acid precipitation and know the complexing capacity in processed foods (breads). So, according to our results to estimate the phytic acid intake from some products in Spain.

2. Materials and methods

2.1. Flours

Barley (*Hordeum vulgare*); corn (*Zea mays*); millet (*Panicum miliaceum*); oat (*Avena sativa*); rice (*Oryza sativa*); rye (*Secale cereale*); sorghum (*Sorghum vulgare*); wheat (*Triticum aestivum*) and whole wheat samples were analyzed.

2.2. Brans

Nine wheat, five oat and one rice commercial samples were investigated.

2.3. Wheat milling products

Soft wheat (*Triticum aestivum*) cv. Astral, cv. Inglés and cv. Yecora and Hard wheat (*Triticum durum*) were ground by the milling industry. The bran, semolina, and flour products from these varieties were analyzed. Brans and flours of three varieties (unknown cultivars), were also studied.

2.4. Breads

White, whole, bran, mixed-grains, oat, and soy breads were obtained from a bakery in Granada, Spain. The flours used to manufacture the breads were also supplied by the industry. The different types of breads were made with baking flour mixed with other cereal flours. The composition and proportion of cereal are reported in Table 3.

The brans were purchased from the different local markets in Granada, Spain. The cereal flours and milling products were obtained from Spanish companies.

The samples were ground in a Moulinex 320 grinder to pass a 0.60-mm sieve. The breads were dried at room temperature. All the samples were stored at −40°C prior to analysis.

2.5. Determination of phytic acid

The original method (García-Villanova, García-Villanova & Ruiz de Lope, 1982) was developed to analyze cereal samples. The same methodology was used in this study with modifications (García-Estepa, García-Villanova & Guerra-Harnández, 1998). The ground samples (0.5–5.0 g) were extracted under magnetic agitation with 40.0 ml of extraction solution (10 g/100 g Na_2SO_4 in 0.4 mol/l HCl) for 3 h at room temperature. The suspension was centrifuged at 5000 rpm for 30 min and the supernatant was filtered.

Ten millilitres of supernatant (containing between 3.3 and 9.0 mg of phytic acid) were pipetted into a 100 ml centrifuge tube together with 10.0 ml of 0.4 mol/l HCl, 10.0 ml of 0.02 mol/l $FeCl_3$ and 10.0 ml of 20 g/100 g sulphosalicylic acid, shaked gently and the tube used was sealed with a rubber cork through which passes a narrow 30-cm long glass tube, to prevent evaporation. The tube was placed in a boiling water bath for 15 min, then allowed to cool. The sample was centrifuged at 5000 rpm for 10 min, decanted, filtered and the residue was washed several times with small volumes of distilled water. The supernatant and washed fractions were diluted (100.0 ml). One aliquot (20.0 ml) adjusted to pH 2.5 ± 0.5 by addition of glycine was diluted to 200 ml. The solution was heated at 70–80°C and, whilst still warm, titrated with 50 mmol/l EDTA solution. The 4:6 Fe/P atomic ratio was used to calculate phytic acid content.

The moisture was determined by air oven method (AOAC, 1990).

3. Results and discussion

3.1. Precision

The precision, expressed as coefficient of variation, obtained in 11 samples of wheat bran (A) was 1.62%. The determinations were carried out in different days to consider possible day-to-day variations.

3.2. Phytic acid in cereals

3.2.1. Flours

The phytic acid content in commercial flours of different cereals is presented in Table 1. The values for wheat were 4 mg/g in white and 22.2 mg/g for whole wheat flours. The phytic acid content reported in other studies shows a wide variability depending on flour yield, extraction method and flour types. The values reported for white wheat flour were between 1.54 and 3.2 mg/g (Graf & Dintzis, 1982; Harland, 1993; Oberleas & Harland, 1981). Higher values (9.6 and 17.5 mg/g) were reported for whole wheat flours (Harland, 1993; March, Villacampa & Grases, 1995). The phytic acid content in other cereal flours (Table 1) ranged between 4.5 and 7.5 mg/g for rye, rice, barley and oat. Corn, millet and sorghum flours contained approximately 10 mg/g of phytic acid. These values are slightly lower than those reported for whole grain samples (Blatny, Kvasnicka &

Kenndler, 1995; Harland, 1993; Marfo, Simpson, Idowu & Oke, 1990; Ockenden, Falk & Lott, 1997).

3.2.2. Brans

The phytic acid content in brans are summarized in Table 1; this compound is found in outer layers of the kernel (De Boland, Garner & O'Dell, 1975; Ravindran, Ravindran & Sivalogan, 1994) and therefore, present in higher amounts in bran products.

Wheat bran samples from nine commercial brands consumed in Spain, were analyzed. The wheat bran is very commonly consumed in Spain. Wide differences of phytic acid were found. The values were between 25 and 59 mg/g. However, the 80% of samples showed values between 34 and 47 mg/g. It was observed that the brans with greater size particle (visual observation) exhibited higher phytic acid content (48.2 mg/g). The brans with medium size particle showed an average value of 39.8 mg/g and those with smaller size exhibited a content of 29.5 mg/g. It could be due to the fact that the commercial brans with smaller size particle contained higher proportion of endosperm than the brans with bigger particle size. On the other hand, this wide range could also easily be due to the wheat varieties analyzed which are unknown for these samples. Differences between the phytic acid contents of wheat brans was also reported by the following researchers. A collaborative study (Harland & Oberleas, 1986) reported values between 34 and 47 mg/g by AOAC method. The values found for hard and soft wheat bran by the colorimetric method were 42–54 mg/g and 46–67 mg/g by HPLC method (Camire & Clydesdale, 1982; Knuckles, Kuzmicky & Betschart, 1982). Other researchers applied the Latta and Eskin's method (1980), reported values of 36–48 mg/g (Lee & Abendroth, 1983; Bos, Uerbeck, van Eeden, Slump & Wolters, 1991; Blaney, Zee, Mangenau & Marin, 1996). Recently, Kasim and Edwards (1998) by the HPLC method reported values of 39.5 mg/g of IP6. The labeling information requests two or three tablespoon for a serving. It may suppose a variable intake of phytic acid ranged between 200 and 300 mg per serving.

Oat bran samples from five commercial brands were analysed. The phytic acid content was lower than wheat brans. The values ranged between 19.0 and 24.0 mg/g. According to the labeling information (2–3 tablespoons), the intake of phytic acid from oat bran is similar to wheat bran so the weight of each serving of oat is often double that of wheat bran.

Rice bran is not commonly available in our country and only one commercial sample was studied. The phytic acid content was 57.7 mg/g. The values obtained by colorimetric and HPLC's methods were 54, 73 and 78 mg/g respectively (Knuckles et al., 1982). Values lower (36.5 mg/g) were found by the colorimetric method (Ravindran et al., 1994). Kasim and Edwards (1998) reported values of 60 mg/g of IP6, determined by the HPLC method.

3.2.3. Milled wheat products

Table 2 summarized the phytic acid contents in different milled wheat fractions (bran, semolina and flour) and their mixtures. Hard wheat and three soft wheat varieties (Astral, Inglés and Yecora) are common in Spain. The mixture of varieties is often carried out to improve the quality of flours. The brans are commercialized by a milling company primarily to animal feed industries and to a lesser extent as for human food. The values range between 24.6 and 45.4 mg/g. The hard wheat and fraction (B)-mixture 2 showed the lowest and the fraction (A)-mixture 3 brans have the highest values. The fraction (A) of mixtures 2 and 3 corresponds to the

Table 1
Phytic acid content[a] (mg/g) of commercial flours and brans of cereals

Flours			
Barley	6.32 ± 0.22	Rye	4.52 ± 0.22
Corn	10.78 ± 0.13	Sorghum	10.12 ± 0.29
Millet	10.64 ± 0.22	Wheat	4.04 ± 0.41
Oat	7.44 ± 0.14	Whole wheat	22.20 ± 0.90
Rice	5.52 ± 0.42		
Brans			
Wheat			
A	47.08 ± 0.76	F	25.32 ± 0.77
B	40.39 ± 1.76	G	38.57 ± 1.51
C	46.97 ± 0.75	H	58.39 ± 1.97
D	33.68 ± 1.07	I	40.75 ± 1.73
E	40.49 ± 1.45		
Oat			
J	21.51 ± 0.11	M	18.97 ± 0.43
K	22.11 ± 0.83	N	20.16 ± 0.57
L	24.06 ± 0.45		
Rice	57.71 ± 0.80		

[a] Mean and standard deviation of four determinations, expressed on a dry weight basis.

Table 2
Phytic acid content[a] (mg/g) of milling wheat products

	Brans	Semolinas	Flours
Soft wheat			
cv. Astral	34.50 ± 1.22	25.49 ± 0.21	2.97 ± 0.33
cv. Ingles	30.34 ± 0.91	26.73 ± 0.41	2.94 ± 0.30
cv. Yecora	36.42 ± 1.43	20.18 ± 0.40	4.04 ± 0.50
Hard wheat	24.96 ± 0.90	9.87 ± 0.46	9.41 ± 0.24
Wheat mixtures			
mix. 1	34.82 ± 1.41	–	3.04 ± 0.12
mix. 2	–	–	3.34 ± 0.22
fraction A[b]	40.45 ± 1.35	–	–
fraction B[c]	24.63 ± 0.91	–	–
mix. 3	–	–	5.49 ± 0.59
fraction A[b]	45.44 ± 0.95	–	–
fraction B[c]	35.24 ± 1.07	–	–

[a] Mean and standard deviation of four deteminations, expressed on a dry weight basis.
[b] Bran obtained in first steps milling.
[c] Bran obtained in later steps milling.

Table 3
Phytic acid and moisture content of breads[a] and the flours utilized to breadmaking

	Phytic acid (mg/g)[b]	Reduction (%)	Moisture (%)
White bread			
Baking flour (a) (wheat flour)	2.98 ± 0.20		15.0
Bread	1.48 ± 0.09	50	28.6
Oat bread			
Oat flour (b) (oat fiber; wheat and oat flakes; wheat flour)	0.36 ± 0.23		10.7
Mix. flours (a:b, 1:1)	6.43 ± 0.20		12.2
Bread	5.16 ± 0.29	20	24.5
Bran bread			
Bran flour (c) (wheat bran; wheat, soy and malt flours; germ wheat; whey)	20.00 ± 0.79		10.2
Mix. flours (a:c, 1:1)	11.30 ± 0.22		12.7
Bread	7.53 ± 0.19	33	31.1
Soy bread			
Soy flour (d) (soy granulated; whole wheat and rye flours)	11.71 ± 0.35		10.8
Mix. flours (a:d,1:1)	7.54 ± 0.24		13.0
Bread	5.51 ± 0.23	27	27.4
Mixed-grains bread			
Mix. flour (e) (wheat, corn, sesame, flax, oat, barley, millet, whole soy and whole rye flours)	9.81 ± 0.24		13.1
Mix. flours (a:e, 1:1)	5.35 ± 0.23		13.2
Bread	3.81 ± 0.08	29	23.2
Whole bread			
Whole white flour (f) (wheat and whole wheat flours)	11.55 ± 0.35		14.6
Mix. flours (a:f,1:2)	7.49 ± 0.10		13.0
Bread	4.74 ± 0.19	37	30.2

[a] Fermentation: time (25–30 min), T^a (30–35°C) baking time (30 min), T^a (200–225°C).
[b] Mean and standard deviation of four determinations, expressed on a dry weight basis.

first steps of the milling process for which the outer layers predominate and the particle size is greater. The fraction (A) is traditionally commercialized for animal feed but recently it has been used in whole flours and dietetic products. The fraction (B) obtained from the subsequent steps contents more proportion of internal layers and endosperm and, consequently smaller size particle. Blatny et al. (1995), analyzed the different fractions obtained in wheat milling; the phytic acid decreased about 45% for the 2nd fraction. We found less differences, 22 and 39% for mixture 3 and 2 respectively. The semolina contained a mean of 24.4 mg/g except for hard wheat which had 9.9 mg/g. The soft flours had 3.0–5.5 mg/g phytic acid which are approximately 1/10 times than the lowest of the respective brans. Similar results were reported by Blatny et al. (1995). However, the phytic acid in hard wheat flour (9.4 mg/g) is only 1/4 times that of the bran.

3.2.4. Breads

The wheat bread is the main cereal product consumed in Spain. Because of the numerous health benefits of dietary fiber, the consumption of whole wheat breads and breads added to other cereals has been increased. During bread-making, the content of phytic acid decreases due to the action of phytases as well as the high temperature (Plaami, 1997). Table 3 shows the phytic acid content of flours used for breadmaking, as well as the values found in the bread. The bread-making flour revealed the lowest value of phytic acid (2.98 mg/g) and the whole flours of different cereals showed the highest contents (10.0–20 mg/g).

The flours used in bread-making had 5.4–11.3 mg/g of phytic acid. The values of phytic acid in breads ranged between 1.48 mg/g (white wheat-bread) and 7.53 mg/g (bran bread). Lower values were reported for white bread (Harland, 1993; Harland & Harland, 1980; Phillippy et al., 1988). However, similar values were reported for whole wheat breads (Harland, 1993; Harland & Harland, 1980; Phillippy & Johnston, 1985).

The breads analyzed had similar form, size and processed conditions. The reduction of chelating capacity (expressed as phytic acid content) in these breads is found between 20% for oat bread and 50% for white bread. Nayini and Markakis (1983a,b) compare the inositols contents in breads made with different extraction grade (70–100%) flours and also found a minor reduction of phytate for whole breads.

3.2.5. Intake of phytic acid

The intake of cereals in Spain is estimated as 224 g/day of which, 151 g/day correspond to bread (Saura-Calixto & Goñi, 1993).

According to our results, the phytic acid supplied from white bread would be 159 mg/day. Considering other types of breads, the intake from mix-cereal bread would be 442 mg/day, whole bread 500 mg/day, oat bread 589 mg/day, soy bread 604 mg/day and bran bread 784 mg/g. The intake would be increased 3–5 times with respect to white bread. The recomendation of bran intake according to labeling information request 20 and 10 g/day for oat and wheat brans respectively. The phytic acid supplied by brans would be close to 375 mg/day (wheat brans 367 mg/day and oat brans 389 mg/day). The intake of whole bread instead of white bread or brans would cause a daily increase of phytic acid of approximately 350 mg.

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