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Content of phytic acid and its mole ratio to zinc in flour and breads consumed in Tabriz, Iran ☆

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Abstract

Zinc deficiency is one of the most prevalent nutritional problems. In a food, phytic acid to zinc mole ratio is the main factor for determination of zinc bioavailability, therefore we measured phytic acid and its mole ratio to zinc in flour and various breads – including: Lavash, Mashini, Sangak, Oskoo, Barbari, Roghani and Baggett – which are consumed in Tabriz, Iran. The study was a descriptive, cross-sectional investigation. Mean of phytic acid and its mole ratio to zinc in flour was 262.75 (CI with 95%: 210.38–315.11 mg/100) and 19.36 (CI with 95%: 14.93–23.79), respectively. For the breads above indices were 108.53 (CI with 95%: 93.75–123.31 mg/100) and 8.91 (CI with 95%: 7.76–10.06), respectively. The differences between breads' phytic acid and its mole ratio to zinc with those of flours were significant ($p < 0.001$). From breads Baggett had the lowest and Lavash had the highest amount of phytic acid and phytic acid to zinc mole ratio. According to WHO cut-offs, zinc in Baggett and Roghani had good bioavailability (50–55%), while other breads had moderate (30–35%) and flour had low (10–15%) zinc bioavailability.

Keywords: Bread; Flour; Phytic acid; Zinc; Zinc bioavailability

1. Introduction

Phytic acid is hexaphosphate ester of inositol. It is fund in highest levels in legumes, nuts, and cereal grains (Harland & Oberleas, 1987). The amount of phytic acid in a food depends on various processes, which are used (Harland & Oberleas, 1987; Reddy, Sathe, & Salunkhe, 1982). Phytic acid can decrease absorption of minerals, such as zinc (Lonnerdal, 2000), iron (Hallberg, Brune, & Rossander, 1989), calcium (Weaver, Heaney, Martin, & Fitzsimmons, 1991), and manganese (Davidsson, Almgren, Jullerat, & Hurrell, 1995), so its high intake can cause mineral deficiency. Zinc deficiency is among nutritional problems in the world (Prasad, 2003). Zinc deficiency has been reported in different age groups in Iran (Ahrari & Kimiagar, 1997; Mahmoodi & Kimiagar, 2001; Montazerifar, Karajibani, & Kimiagar, 2000). For the first time, clinical signs of zinc deficiency were reported from Iran and Egypt (Anderson, 2004). High phytate intake with staple foods is one possible cause for zinc deficiency (Reinhold, 1971).

In a food phytic acid to zinc mole ratio is an important factor for determining potency of zinc bioavailability (Morris & Ellis, 1989). This index has been used widely (Bosscher et al., 2001; Brown et al., 2001; Fitzgerald et al., 1993; Gibson et al., 2003; International Zinc Nutrition Consultative Group-IZiNCG, 2004; Lo et al., 1981; Navert and Sandstrom, 1985; Sandstrom et al., 1987; Sandstrom, Kivisto et al., 1987), and it is considered as a good index for zinc bioavailability by world health organization (World Health Organization, 1996) and International Zinc Nutrition Consultative Group (IZiNCG,

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2004). Bread is a staple food in Iran and its per capita consumption in urbanized, rural, and the entire country has been estimated as 286, 443, and 351 g/day, respectively (Ghassemi, 1997).

With attention to the above issues, and to scarcity of data on phytate content and zinc bioavailability from various breads in Iran, we decided to determine phytic acid and its mole ratio to zinc, as an index of zinc bioavailability, in flour and different breads which are consumed in Tabriz, centre of East Azerbaijan province, Iran, and compare them with WHO zinc bioavailability cut-offs.

2. Materials and methods

The study was a descriptive, cross-sectional one. All materials used for the experiments were purchased from Merck Co. (Darmstadt, Germany), unless otherwise specified. All experiments were carried out in the Soil and Water Research Institute (Tehran – Iran).

2.1. Collection and procedures of samples

List of all bakeries in Tabriz were collected from flour and bread council. By using simple systematic random sampling, 10 samples of various breads, including Lavash, Mashini, Sangak, Oskoo Bread, Barbari, Roghani and Baggett, were purchased from different bakeries in Tabriz. In addition, three samples of flour from each kind of bread were procured. Each bread flour sample was mixed completely, so for each kind of bread one flour sample was assayed. All samples was completely dried in 70 °C for 5 h, and then transferred into a descicator. All bread samples were powdered using an acid washed glass mortar. Completely powdered samples were used for the experiments. Phytic acid and zinc content of samples were determined using from AOAC (Association of Official Analytical Chemist) methods (Ihnat, 1995, Chap. 32; Lane, 1995, Chap. 9). In addition to phytic acid and phytic acid to zinc mole ratio, iron content of all samples were also measured by the AOAC (1995) methods.

2.2. Phytic acid measurement

The amount of phytic acid in original samples was measured by using ion-exchange method, and calculated as hexaphosphate equivalents. Briefly, 2.0000 g of dried and powdered sample were mixed with 40 ml HCl (2.4%) for 3 h at room temperature. The solution was filtered with vacuum through Whattman No. 1 filter paper. The filtrate (1.0 ml) was pipetted into a 25 ml flask, 1.0 ml of NaOH (0.75 M)/Na_2EDTA (0.11 M) solution was added, and then diluted to 25 ml with distilled water. The solution was poured completely into the ion-exchange column. The column was made from AG1-X4, 100-200 mesh, chloride form resin (Bio-Rad Laboratories, Hercules, CA, USA). Phytic acid was washed with 15 ml of NaCl (0.7 M). Phosphorus in the resulting solution was released with a mixture of concentrated HNO_3/H_2SO_4 (3.0 and 0.5 ml, respectively), and then mixed with molybdate (2.5% ammonium molybdate in 1 N H_2SO_4) solution. Absorbance was read at 640 nm (Spectrophotometer model 320, Spectra UV, Sherwood Scientific Ltd., Cambridge, UK). Phosphorus concentration was calculated by using phosphate standards (80, 240, 400 μg/ml) curve. Because 28.2% of phytic acid is phosphorus, so the amount of phytic acid was calculated using the following equation.

$$\text{Phytic acid (mg/g of dried sample)} = (\text{mean } k * A * 20)/(0.282 * 1000),$$

where A = absorbance, K = standard phosphorus concentration (μg)/A, mean $k = \sum k/n$, n = number of standards (Lane, 1995, Chap. 9).

2.3. Zinc and iron measurements

One gram of samples was wet-digested with heat and concentrated HNO_3/H_2SO_4 (2.5 and 0.5 ml, respectively). After material begins to char, digestion was continued by HNO_3 until a colorless or very pale yellow liquid was obtained. In the resulting solution, iron was measured by using *O*-phenanthroline at 510 nm (Lane, 1995, Chap. 9) and zinc was measured by atomic absorption spectrophotometry at 213.9 nm (Chem. Tech Analytical 2000, Bedford, UK) (Ihnat, 1995, Chap. 32). All results were reported as mg/100 g of dried sample.

2.4. Phytic acid to zinc mole ratio

Phytic acid to zinc mole ratio was calculated as follows (International Zinc Nutrition Consultative Group – IZiNCG, 2004):

$$\text{Phytic acid to zinc mole ratio} = \frac{\text{Phytic acid (mg/100g)}/660}{\text{Zinc (mg/100g)}/65.4}$$

2.5. Statistical analysis

For statistical analysis, ANOVA, posthoc Tukey test and t test were used by employing SPSS 11.5 for windows XP. For control of the study, all experiments were carried out in duplicate and mean of two experiments considered.

3. Results and discussion

Mean and standard deviation of phytic acid in flours was 262.75 ± 75.57 (CI with 95%: 210.38–315.11 mg/100). Payan (1998) showed that various wheats cultivated in Iran have about 1.0% (1000 mg) phytic acid and in processed wheat, depending on flour extraction rate, different amounts of phytic acid remain. In flours with high extraction rate (percentage by weight of flour milled from the cleaned grain more than 80%) 600–700 mg of phytic acid remains, while in low extraction rate flours (percentage by weight of flour milled from the cleaned grain less than 60%), its amount decreases to less than 30 mg. Oberleas and Harland (1981) have shown that flour with extraction rate more than 72% and whole-wheat flour have 136–320 and 845–960 mg/100 g phytic acid, respectively. Studies in the UK (Committee on Medical Aspects of food policy, 1981) have shown that phytic acid in 72, 85 and 100% extracted flour is 100, 527 and 806 mg/100 g, respectively.

The present study showed a significant decrease (mean $\sim$ 59%) in phytic acid content of flour during bread making. Mean and standard deviation of phytate in all studied breads was 108.53 ± 67.45 (CI with 95%: 93.75–123.31 mg/100). Among different breads, Lavash had the highest, while Baggett and Roghani had the lowest content of phytic acid. Lavash, Mashini and Oskoo breads all are flat and thin, while Sangak, Barbari and Roghani are flat and thick breads. Baggett is cylindrical or round bread, which is usually used in fast food services in Iran.

High phytic acid can decrease zinc absorption, so Reinhold (1971) attributed zinc deficiency in rural communities of Iran to high content of phytic acid in consumed staple foods, such as bread. He showed 326–684 mg of phytic acid in three different breads, which were made from high extracted flour (75–100%). In another study, Mameesh and Tomar (1993) showed 300 mg per 100 g of dried weight, phytic acid in the Iranian bread.

Our study showed that a noticeable amount of zinc and iron remains in consumed breads. Zinc and iron content of flour was 1.40 ± 0.32 and 1.82 ± 0.72 mg/100 g of dried weight, respectively. In the studied breads mean and standard deviation for zinc and iron was 1.18 ± 0.34 and 1.57 ± 0.61 mg/100 g, respectively. Our results are in the range of those reported by Malakouti, Sawaghebi, and Balali (1999) on flour, which showed 0.9–2.4 mg/100 g zinc and 1.5–5.1 mg/100 g iron in various flours consumed in Iran. These researchers showed 1.9 and 1.3 mg/100 g of iron and zinc in flour consumed in East Azerbaijan, Iran.

Phytic acid mole ratio to zinc of flour in our study was 19.36 ± 6.39 (CI with 95%: 14.93–23.79), while for the studied breads it was 8.91 ± 5.23 (CI with 95%: 7.76–10.06) ($p < 0.005$). The studies by Fallahi and Mohtadinia (2003) and Malakouti et al. (1999) in whole-wheat flour have shown phytic acid to zinc mole ratio of 39.92 and 25.4, respectively. The main reasons for these differences are variety, climatic and irrigation conditions, locations, type of soil and extraction rate (Reddy et al., 1982). Our studied samples were not from whole-wheat flour.

In Table 1, mean, standard deviation and confidence interval of mean with 95% (CI with 95%), for phytic acid, zinc, iron and phytic acid to zinc mole ratio in the different breads are shown. In Table 2, mean, standard deviation and CI of mean with 95%, for the above indices in flour and all kinds of breads are shown.

There was a significant decrease in phytic acid to zinc mole ratio during bread making (54%) ($p < 0.001$). In different breads, there was a significant difference in phytic acid to zinc mole ratio ($p < 0.001$). The Baggett and Roghani had the lowest, while Lavash and Mashini had the highest amounts of phytic acid to zinc mole ratio ($p < 0.001$). Zinc and iron content of breads were 0.73–1.40 and 0.72–1.87 mg/100 g, respectively.

Phytic acid to zinc mole ratio for different breads in our study was 2.59–13.24. Phytic acid to zinc mole ratio has been used in many studies (Bosscher et al., 2001; Brown et al., 2001; Fitzgerald et al., 1993; Gibson et al., 2003; Lo et al., 1981; Navert & Sandstrom, 1985; Sandstrom et al., 1987; Sandstrom, Kivisto, & Cederblad, 1987) and it was mentioned as an index of zinc bioavailability potency by WHO (1996) and International Zinc Nutrition

Table 1
Mean, standard deviation and confidence interval (CI) of mean with 95%, for phytic acid, zinc, iron and phytic acid to zinc mole ratio in different breads in Tabriz, Iran

Bread types ($n = 10$)	Phytic acid (mg/100 g)	Zinc (mg/100 g)	Iron (mg/100 g)	Phytic acid to zinc mole ratio
Baggett	$18.41 \pm 10.03^{(a)}$ (12.19–24.63)	$0.73 \pm 0.16^{(a)}$ (0.63–0.83)	$0.72 \pm 0.38^{(a)}$ (0.48–0.96)	$2.56 \pm 1.93^{(a)}$ (1.36–3.76)
Roghani	$35.77 \pm 20.89^{(a)}$ (22.82–48.33)	$1.01 \pm 0.53^{(a,b)}$ (0.68–1.34)	$1.06 \pm 0.46^{(a,b)}$ (0.78–1.34)	$3.79 \pm 2.89^{(a)}$ (2.0–5.58)
Barbari	$57.38 \pm 29.69^{(a)}$ (38.81–75.95)	$1.02 \pm 0.23^{(a,b)}$ (0.88–1.16)	$1.76 \pm 0.61^{(c)}$ (1.38–2.14)	$6.11 \pm 3.94^{(a,b)}$ (3.67–8.55)
Sangak	$127.65 \pm 27.70^{(b)}$ (110.48–144.82)	$1.35 \pm 0.19^{(b)}$ (1.24–1.46)	$1.87 \pm 0.35^{(c)}$ (1.65–2.09)	$9.90 \pm 2.47^{(b,c)}$ (8.37–11.43)
Oskoo	$122.05 \pm 39.11^{(b)}$ (97.81–146.29)	$1.22 \pm 0.39^{(b)}$ (0.98–1.46)	$1.77 \pm 0.57^{(c)}$ (1.42–2.12)	$10.52 \pm 3.44^{(b,c)}$ (8.4–12.64)
Mashini	$170.00 \pm 36.86^{(b)}$ (147.19–215.85)	$1.36 \pm 0.20^{(b)}$ (1.24–1.48)	$1.79 \pm 0.25^{(c)}$ (1.64–1.94)	$12.72 \pm 3.63^{(c)}$ (10.47–14.97)
Lavash	$170.00 \pm 30.33^{(b)}$ (151.21–188.79)	$1.29 \pm 0.07^{(b)}$ (1.25–1.33)	$1.67 \pm 0.51^{(b)}$ (1.35–1.99)	$13.24 \pm 2.86^{(c)}$ (11.47–15.01)

In each column, values with different superscripts are different ($p < 0.05$).

Table 2
Mean, standard deviation and confidence interval (CI) of mean with 95%, for phytic acid, zinc, iron and phytic acid to zinc mole ratio in flour and studied breads in Tabriz, Iran

	Phytic acid (mg/100 g)	Zinc (mg/100 g)	Iron (mg/100 g)	Phytic acid to zinc mole ratio
Flour ($n = 7$)	262.75 ± 75.57 (210.38–315.11)	1.40 ± 0.32 (1.18–1.62)	1.82 ± 0.72 (1.32–2.32)	19.36 ± 6.39 (14.93–23.79)
All breads ($n = 70$)	108.53 ± 67.45 (93.75–123.31)	1.16 ± 0.33 (1.09–1.23)	1.55 ± 0.58 (1.42–1.68)	8.91 ± 5.23 (7.76–10.06)
t test	$p < 0.001$	NS	NS	$p < 0.001$

Consultative Group (IZINCG) (2004). According to WHO cut-offs in a special food, phytic acid to zinc mole ratio $\geqslant 15$, 5–15 and <5 is equal to zinc bioavailability as low (10–15%), moderate (30–35%) and high (50–55%), respectively. According to WHO cut-offs, flour had phytate to zinc mole ratio more than 15, while Lavash, Mashini, Barbari, Sangak and Oskoo breads had phytate to zinc mole ratio of 5–15. Remaining breads (Roghani and Bagget) had phytate to zinc mole ratio of less than 5. Albeit some samples of Lavash, Oskoo, and Sangak breads had phytic acid to zinc mole ratio of more than 15. Therefore, zinc bioavailability in Iranian flours is low (10–15%), while breads, such as Baggett and Roghani have good zinc bioavailability (50–55%). Zinc bioavailability in other remaining breads was moderate (30–35%).

High zinc bioavailability in Baggett and Roghani breads can be because of sufficient fermentation process or using low extracted flour. The less extraction rate of flour the more phytic acid is deleted (Reddy et al., 1982). In Iran, Roghani and Baggett are usually made from low extracted flour (percentage by weight of flour milled from the cleaned grain less than 72%), Sangak and Barbari from high extracted flour (percentage by weight of flour milled from the cleaned grain more than 78%), and remaining breads from 72–78% extracted flour.

4. Conclusions

Bread is an important source of minerals, such as zinc and iron, but its high phytic acid and phytic acid to zinc mole ratio can decrease absorption of these minerals. Since low amount of phytic acid, as low as 10 mg, can decrease mineral absorption (Fairbanks, 1999) and all studied breads in our study have higher phytic acid level than 10 mg, so decreasing of phytic acid during bread making in our community bakeries is not sufficient. With attention to high consumption of bread in Iran, it is essential to use efficient procedures to further decrease phytic acid in breads. This approach can be considered as an important way for combating mineral, especially zinc and iron, deficiencies.

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