



## Rapid Communication

## Effects of phytate and minerals on the bioavailability of oxalate from food



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## ABSTRACT

Phytate and mineral cations are both considered as important dietary factors for inhibiting the crystallisation of calcium oxalate kidney stones in susceptible individuals. In this paper, the phytate and mineral composition of whole bran cereals (wheat, barley and oat) and legumes were determined together with their soluble and insoluble oxalate concentrations in order to investigate the effects on oxalate solubility. The oat bran sample had the highest soluble oxalate concentration at  $79 \pm 1.3$  mg/100 g, while total and soluble oxalate concentrations in the food samples studied range from 33 to 199 mg/100 g and 14 to 79 mg/100 g, respectively. The phytate concentration was in the range from 227 to 4393 mg/100 g and the concentrations of cations were in the range 54–70 mg/100 g for calcium, 75–398 mg/100 g for magnesium, 244–1529 mg/100 g for potassium and 4–11 mg/100 g for iron. Soluble oxalate concentration did not increase in proportion to total oxalate, and the phytate concentration in all foods was sufficient to contribute to an increase in soluble oxalate concentration by binding calcium.

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## 1. Introduction

The availability of soluble oxalate from food has been considered to be one of the main contributors to the development of hyperoxaluria, which is the excessive urinary excretion of oxalate (Holmes, Goodman, Assimos, & Winston-Salem, 1996). Hyperoxaluria can lead to deposition of calcium oxalate (oxalosis) in kidney tissue or crystallisation as calcium oxalate kidney stones (nephrolithiasis) in the urinary tract (Sanz & Reig, 1992). Foods with oxalate levels greater than 50 mg/100 g are categorised as high oxalate foods, and these include whole bran cereals and legumes (Boontaganon, Jéhanno, & Savage, 2009; Chai & Liebman, 2005). Oxalate absorption usually depends on the presence of free or soluble oxalate in the intestine (Brinkley, McGuire, Gregory, & Pak, 1981). It has been reported that soluble oxalate is totally released from bran at gastrointestinal pH, but it can combine with calcium already available in the bran sample to form the insoluble salt (Siener, Heynck, & Hesse, 2001). It is therefore important when assessing intake of oxalate to consider the balance of soluble to insoluble forms of oxalate available from foods.

A main factor that regulates soluble oxalate is the concentration of divalent cation minerals, including calcium and magnesium (Reddy, Sathe, & Salunkhe, 1982). The presence of cations in the gut has been found to interfere with oxalate absorption. Higher concentrations of cations, e.g. calcium and, to a lesser extent,

magnesium, have been found to decrease oxalate absorption, and their concentration in simultaneously ingested foods has therefore been considered as important with respect to kidney stone formation (Asplin, 2002). The solubility of calcium oxalate is strongly pH-dependent with solubility increasing strongly below pH 4 (Jaeger & Robertson, 2004). Magnesium oxalate is more soluble than is calcium oxalate, 0.07 g/100 ml versus 0.0007 g/100 ml, respectively, but it still contributes to insoluble oxalate in the gut, when its concentration exceeds the solubility limit (Tiselius, 1991). The solubility product constant for magnesium oxalate at pH 7 has been reported as  $8.5 \times 10^{-5} \text{ mol}^2 \text{ dm}^{-6}$ , compared to  $2.7 \times 10^{-9} \text{ mol}^2 \text{ dm}^{-6}$  for calcium oxalate (University of Rhode Island, 2001), although the solubility in urine is more complex, since calcium oxalate crystals can occur as mixtures differing in the degree of hydration (Streit, Tran-Ho, & Königsberger, 1998). It has been suggested that magnesium may have a small effect on oxalate uptake by complexing oxalate and making it less available for absorption (Jaeger & Robertson, 2004). However, magnesium supplementation also has been reported to have no effect on urinary oxalate level (Allie & Rodgers, 2003). Phytate is also considered as beneficial with respect to nephrolithiasis due to its antioxidant properties (Graf & Eaton, 1990), although, more recently, phytate was found to increase soluble oxalate available for absorption as well as recurrence of kidney stones as a consequence of its combination with calcium in the human gut (Al-Wahsh, 2005). Cereals and legumes have been found to contain high concentrations of phytate (Reddy et al., 1982), which makes it an important factor to consider when evaluating these foods for oxalate.

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The molar ratio of oxalate to concurrent minerals has been used as a measure of the availability of oxalate for absorption. Molar ratios of oxalate to minerals greater than 2 and phytate to minerals greater than 0.24 have been reported as hazardous (Fassett, 1973; Reddy & Sathe, 2002). This study aimed to investigate the molar ratio of oxalate and phytate to concurrent minerals in common plant materials in order to assess the availability of oxalate for absorption. Few studies on the effect of a combination of oxalate and phytate on the availability of oxalate and its influence on kidney stones have been reported. Although bran and beans are common dietary components, the concentrations of phytate and oxalate in the same samples of these foods have not been reported. The aim of this study was to investigate the effects of oxalate, phytate and mineral concentrations on oxalate solubility, in order to predict its bioavailability. These findings would allow conclusions to be drawn about the influence of these foods on the risk of hyperoxaluria in susceptible subjects.

## 2. Materials and methods

### 2.1. Food samples

Whole bran cereals (wheat bran, barley bran and oat bran) were obtained from Premier Foods, UK. Legumes (red beans and white beans) imported from Spain were purchased at a local market. One batch of each foodstuff was purchased for analysis.

### 2.2. Oxalate analysis

Oxalate was extracted by a method based on that described by Savage, Vanhanen, Mason, and Ross (2000).

Samples (1 g) were extracted with 50 ml of 1.0 M H<sub>2</sub>SO<sub>4</sub> at 21 °C for 15 min in a shaking water bath. The extracts were transferred into a 100 ml volumetric flask, and made to volume with 1.0 M H<sub>2</sub>SO<sub>4</sub> for total oxalate and with distilled water for soluble oxalate. The dissolved oxalate solution was separated by centrifugation at 3000 rpm for 15 min and passed through a 0.45 µm nylon syringe filter. The oxalate concentration in each sample was determined by HPLC, using an Agilent 1100 series chromatograph with autosampler, isocratic pump and UV–Vis detector set at 210 nm. Data capture and analysis were done by using Chemstation software Version A-7.1. A 5 µl injection volume was used with an Aminex Ion exclusion HPX-87H 300 × 7.8 mm analytical column fitted with an Aminex Cation-H guard column. Isocratic elution was used with 0.0125 M H<sub>2</sub>SO<sub>4</sub> (Sigma Aldrich, UK) as mobile phase and a flow of 0.5 ml/min. The analytical column was held at 65 °C, and the column was equilibrated with a flow rate of 0.2 ml/min prior to use.

### 2.3. Phytate analysis

Phytate was extracted by the method described by (Oberleas & Harland, 2007). Finely ground dried sample (1 g) was extracted with 10 ml of 0.66 M HCl with gentle agitation for 3 h on a shaking mixer. The sample was centrifuged at 3500 rpm for 10 min, and the supernatant was filtered through a 0.45 µm syringe filter into an HPLC vial.

The sample was analysed by HPLC, using an Agilent 1050 series chromatograph consisting of two pumps, UV–Vis detector, set at 500 nm and Chemstation software Version A-8.3. The column was a strong anion-exchange type, Polymer Laboratories PL-Sax 5 × 0.46 cm, particle size 8 µm and 100 nm pore size (Varian, Inc. Shropshire, UK). A flow rate of 1 ml/min was used for the mobile phase and 0.5 ml/min was used for Wade's reagent. The injection volume was 5 µl. The analytical column was kept at room

temperature and equilibrated with 0.01 M methyl piperazine at pH 4 as mobile phase with a flow rate of 0.2 ml/min before analysis of the sample. The gradient buffer was 0.6 M sodium nitrate in 0.01 M methyl piperazine at pH 4. Phytate concentration was calculated using 660 g mol<sup>-1</sup> as the hexaphosphate molecular weight, as recommended by (Oberleas & Harland, 2001).

### 2.4. Mineral analysis

Calcium, magnesium, potassium and iron were analysed by atomic absorption spectrophotometry at 422.7, 285.2, 766.5 and 248.3 nm, respectively (Analytik Jena AG, Germany Model NovAA® 350) (Analysis of agriculture materials, 1986).

### 2.5. Statistical analysis

Results are presented as means of triplicate determinations ± SEM. Significant differences between samples ( $p < 0.05$ ) were identified by Analysis of Variance (ANOVA) with the Tukey HSD test. The analysis was carried out with SPSS version 18.

## 3. Results and discussion

### 3.1. Oxalate

The total oxalate content of wheat bran, oat bran and red beans is shown in Table 1. The oxalate content for intake of 100 g of test food samples is high compared with the maximum recommended daily intake of oxalate from food which is 40–50 mg/day (American dietetics association, 2005). The total oxalate content was in the order wheat bran > oat bran > red bean >> barley bran > white bean. However, only soluble oxalate is absorbed, and the soluble oxalate fell in the order oat bran > wheat bran > barley bran > red bean > white bean. Thus, it is clear that the cereal bran samples had a higher concentration of soluble oxalate than had the legume samples. The oxalate content for these foods was within the range reported in the literature (Siener, Hönow, Seidler, Voss, & Hesse, 2006; Chai & Liebman, 2005; Boontaganon et al., 2009).

### 3.2. Cations

Oxalate absorption is highly dependent on the availability of the soluble form. Potassium oxalate is an important soluble form for absorption (Brinkley et al., 1981). The proximal small intestine is a major site for absorption of oxalate (Hanes, Weaver, Heaney, & Wastney, 1999), but changes of pH throughout the gastrointestinal tract also have an effect on the absorption of oxalate. Oxalate is more soluble under the acid conditions of the stomach, which ranges from pH 1.5 to 2, than at higher pH, so insoluble oxalate forms again after passing into the alkaline environment of the small intestine. Thus oxalate which has been solubilised in the

**Table 1**

Phytate, and total, soluble and insoluble oxalate in food samples (mg/100 g dry weight ± SEM).

| Sample          | Total oxalate          | Soluble oxalate       | Insoluble oxalate      | Phytate                  |
|-----------------|------------------------|-----------------------|------------------------|--------------------------|
| Wheat bran      | 199 ± 3.5 <sup>c</sup> | 56 ± 4.2 <sup>c</sup> | 146 ± 1.8 <sup>d</sup> | 4393 ± 1.4 <sup>d</sup>  |
| Oat bran        | 159 ± 1.6 <sup>b</sup> | 79 ± 1.3 <sup>d</sup> | 80 ± 4.3 <sup>b</sup>  | 992 ± 1.2 <sup>c</sup>   |
| Barley bran     | 47 ± 1.4 <sup>a</sup>  | 21 ± 1.4 <sup>b</sup> | 26 ± 1.0 <sup>a</sup>  | 227 ± 0.4 <sup>a</sup>   |
| Red kidney bean | 146 ± 1.6 <sup>b</sup> | 25 ± 1.2 <sup>b</sup> | 121 ± 1.2 <sup>c</sup> | 616 ± 0.3 <sup>b</sup>   |
| White bean      | 33 ± 2.8 <sup>a</sup>  | nd <sup>a</sup>       | 33 ± 2.0 <sup>a</sup>  | 671 ± 1.3 <sup>b,c</sup> |

Results are presented as means ± SEM of triplicate determinations.

Nd = not detected; concentration <0.01 mg/100 g.

<sup>a–d</sup>Numbers with different superscripts in the same column are significantly different ( $p < 0.05$ ).

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