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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 ± 1.3 mg/100g, while total and soluble oxalate concentrations in the food samples studied range from 33-199 mg/100 g and 14-79 mg/100 g, respectively. The phytate concentration was in the range from 227-4393 mg/100 g and the concentrations of cations were in the range 54-70 mg/100g for calcium, 75-398 mg/100g for magnesium, 244-1529 mg/100g for potassium and 4-11 mg/100g 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.

Key words: Bioavailability, calcium, minerals, oxalate and phytate

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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 categorized 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, MgGuire, 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 like 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 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 $8.5x10^{-5}$ mol^2. $dm^{-6,}$ compared to $2.7x10^{-9}$ mol^2. 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.

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, & Ross, 2000).

Samples (1 g) were extracted with 50 ml 1.0 M H_2SO_4 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_2SO_4 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_2SO_4 (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 analyzed 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^{-1} as the hexaphosphate molecular weight as recommended by (Oberleas & Harland, 2001).

2.4. Mineral analysis

Calcium, magnesium, potassium and iron were analyzed 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 ± S.E M. 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 to 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 contained a higher concentration of soluble oxalate than 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); and (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 1.5-2, 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 stomach will form a sparingly soluble complex again with calcium, magnesium and iron in the intestine. Soluble oxalate is available for absorption from the intestine through the mucosa (Savage & Catherwood, 2007).

Calcium is the main cation that forms an insoluble complex with oxalate, and thereby reduces absorption from the gut (Benitez, Grijalva, & Valencia, 1994). Ferrous oxalate is similar in solubility to calcium oxalate, so ferrous ions may contribute to a reduction in soluble oxalate, and iron was identified as a metal that may promote the formation of calcium oxalate stones, whereas magnesium was considered as an inhibitor (Atakan et al., 2007). The range of mineral concentrations in the food samples tested was 23-70 mg/100 g for calcium, 75-398 mg/100 g for magnesium, 244-1382 mg/100 g for potassium and 4-11 mg/100 g for iron (Table 2).

3.3. Molar ratio of oxalate and minerals

The presence of cations in foods eaten at the same time as sources of oxalate is highly important for determining the relative concentrations of soluble and insoluble oxalate (Asplin, 2002). Therefore, the potential of foods for contributing to soluble oxalate is best assessed in terms of the oxalate: mineral ratio for minerals that form insoluble oxalate complexes. A ratio greater than 2 indicates that a food contains excess oxalate that is bioavailable, whereas, foods having a ratio of 1 or less contain enough calcium, or similar minerals, to minimise formation of soluble oxalate (Gontzea & Sutzescu, 1968). The solubility product constant for calcium oxalate was reported as 2.7×10^{-9} mol^2 . dm^{-6} (URI(Chemistry;University of Rhode Island), 2001). The whole wheat and oat bran samples studied have a molar ratio of oxalate: calcium greater than 2 as shown in Table 3 and the soluble oxalate content is quite high. In contrast, the molar ratio of oxalate: calcium for red kidney beans was 0.91, and the soluble oxalate content was relatively low compared to the cereal brans. The soluble oxalate content of white beans and barley bran was also low. Magnesium oxalate is more soluble than calcium oxalate with a solubility product constant of 8.5×10^{-5} mol^2. dm^{-6} (URI(Chemistry;University of Rhode Island), 2001), and it does not form stones at physiological urine concentrations. However, the solubility of the magnesium

155 salt is sufficiently low to reduce dietary oxalate absorption (Liebman & Costa, 2000) (Massey,
156 2005) . The molar ratio of oxalate: magnesium was low for all foods studied, but magnesium is
157 less effective than calcium in reducing oxalate bioavailability (Brinkley et al., 1981). Potassium
158 oxalate is a soluble form, but the potassium concentration was very low in all the samples
159 analyzed.

160 ***3.4. Phytate***

161 The wheat bran sample contained a high concentration of phytate compared to the other food
162 samples i.e 4393 ± 1.4 mg/100 g. The barley bran sample contained the lowest concentration of
163 phytate, and the phytate concentration in the beans ranged from 610-670 mg/100 g. The phytate
164 concentrations were comparable to the values reported in the literature (Kirby & Nelson, 1988);
165 (Harland, Oke, & Felix-Phipps, 1988).

166 ***3.5. Molar ratio of phytate and mineral***

167 Phytate has been considered as beneficial for kidney disease due to its ability to chelate metal
168 ions which reduces oxidative reactions (Graf & Eaton, 1990). However, the ability of phytate to
169 form insoluble complexes with divalent cations in the human gut has the consequence of
170 increasing the availability of soluble oxalate for absorption and urinary excretion (Al-Wahsh,
171 2005). A molar ratio of phytate:calcium > 0.24 has been found to be associated with reduced
172 calcium bioavailability (Morris & Ellis, 1985). The solubility product constants for calcium
173 phytate and calcium phosphate were reported as 10^{-22} and 2.07×10^{-33} mol^2. dm^{-6} respectively
174 (Evans & Pierce, 1981; KTF (Chemical Technology Faculty; University of Split), 2003). A high
175 molar ratio of phytate: Ca was present in the whole bran samples, so this would increase the

176 soluble oxalate concentration by reducing the availability of minerals for forming insoluble
177 oxalate in the test food samples.

178 The molar ratio of Mg: phytate was very low in the test samples ranging from 0.15 to 0.41, and
179 this would further reduce any effect of magnesium on soluble oxalate content.

180 ***3.6. Correlation of molar ratio of oxalate, phytate and minerals***

181 Phytate is known to be effective in chelating minerals. It reduces the availability of complex-
182 forming minerals in the body and makes oxalate more bioavailable (Brinkley, Gregory, & Pak,
183 1990; Harland & Morris, 1995). Ca binding by fibre is low in wheat and oat brans at gastric pH
184 (Siener et al., 2001), but calcium absorption from the small intestine after intake of wheat bran
185 has been reported to decrease slightly in ileostomy patients, who have had a surgical procedure
186 to allow them to excrete waste from the small intestine into an external bag, where it is collected
187 (Sandberg, Hasselblad, Hasselblad, & Hultén, 1982). Phytate complexes with Mg are soluble at
188 low pH (Grynspan & Cheryan, 1983), but complexes with Ca and Fe are less soluble. In soy
189 foods, the content of phytate increased with an increase in oxalate content, so soy foods with a
190 low oxalate content were recommended for kidney stone patients (Al-Wahsh, Horner, Palmer,
191 Reddy, & Massey, 2005). The wheat bran sample had relatively high insoluble oxalate content
192 despite a high concentration of phytate and a low concentration of calcium. The high magnesium
193 content of the wheat bran sample may contribute to the high insoluble oxalate content. In the oat
194 bran sample, the insoluble oxalate concentration was much reduced compared to the wheat bran
195 which is consistent with the low calcium and magnesium concentrations. The molar ratio of
196 soluble: insoluble oxalate of oat bran was much higher than for the wheat bran as shown in table
197 4. The barley bran sample had a low calcium concentration but a relatively high magnesium

198 concentration, which is consistent with the values for barley bran reported previously (Dendy &
199 Bogdan, 2001). The phytate concentration showed a moderate correlation with the insoluble
200 oxalate concentration with an R^2 value of 0.46, but the correlation with the soluble: insoluble
201 oxalate ratio was poor with R^2 <0.01. The beans had lower soluble: insoluble oxalate ratios than
202 the brans.

203 **Conclusion**

204 High total oxalate and phytate as well as low calcium and magnesium contents contributed to the
205 high soluble oxalate content in the oat bran sample. The soluble oxalate concentration was higher
206 for the oat bran sample than for the wheat bran sample despite a reverse order for total oxalate,
207 and this can be ascribed to the lower concentration of minerals in the oat bran sample, with the
208 minerals in the wheat bran contributing to a reduction of soluble oxalate in the wheat bran. All
209 the food samples analysed had a phytate: calcium ratio >0.24, so this indicates that the phytate
210 concentration is sufficient to reduce the calcium available for binding to oxalate, and thereby
211 contributes to an increase in soluble oxalate Soluble oxalate concentration was relatively low in
212 the barley bran and red kidney bean samples and was not detected in the white bean sample.

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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^{c}	56 ± 4.2^{c}	146 ± 1.8^{d}	4393 ± 1.4^{d}
Oat Bran	159 ± 1.6^{b}	79 ± 1.3^{d}	80 ± 4.3^{b}	992 ± 1.2^{c}
Barley Bran	47 ± 1.4^{a}	21 ± 1.4^{b}	26 ± 1.0^{a}	227 ± 0.4^{a}
Red Kidney Bean	146 ± 1.6^{b}	25 ± 1.2^{b}	121 ± 1.2^{c}	616 ± 0.3^{b}
White Bean	33 ± 2.8^{a}	nd^{a}	33 ± 2.0^{a}	671 ± 1.3^{bc}

Results are presented as Mean ± SEM of triplicate determinations

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

$^{a-d}$ Numbers with different superscripts in the same column are significantly different ($p<0.05$)

316 **Table 2**

317 **Calcium, magnesium, potassium and iron from test food samples (mg/100 g dry weight ±**
318 **SEM).**

Sample	Calcium	Magnesium	Potassium	Iron
Wheat Bran	30 ±1.6[b]	398±2.5[b]	1529±1.9[c]	11±4.2[c]
Oat Bran	23±2.1[a]	118 ±3.6[a]	377 ±1.6[a]	4 ±0.4[a]
Barley Bran	55 ±2.1[c]	75 ±1.9[a]	244±0.2[a]	7±0.6[b]
Red Bean	70±1.7[d]	114 ±1.5[a]	984±1.5[b]	6 ±0.2[ab]
White Bean	54±0.3[c]	166 ±2.5[a]	914±3.0[b]	8±0.2[b]

319 Results are presented as Means±SEM and each sample was analyzed as triplicate

320 [a-d] Numbers with different superscripts in the same column are significantly different ($p<0.05$)

321

Table 3

Molar ratio of oxalate: minerals and phytate: minerals.

Test Samples					
Parameters	Wheat Bran	Oat Bran	Barley Bran	Red Kidney Bean	White Bean
Oxalate: Calcium	3.02	3.14	0.40	0.95	0.28
Oxalate: Magnesium	0.14	0.37	0.17	0.34	0.05
Oxalate: Potassium	0.06	0.19	0.09	0.06	0.02
Oxalate: Iron	11.13	23.89	4.23	12.04	3.10
Phytate: Calcium	8.89	2.61	0.26	0.53	0.76
Phytate: Magnesium	0.41	0.31	0.11	0.20	0.15
Phytate: Potassium	0.17	0.16	0.06	0.04	0.04
Phytate: Iron	32.77	19.92	2.73	7.05	8.40

Table 4

Molar ratios of phytate with calcium and magnesium and its correlation with soluble: insoluble oxalate

Sr. No	Test Samples	Phytate ratio	Calcium ratio	Magnesium ratio	Ca+Mg ratio	Phytate: Ca+Mg	Soluble: Insoluble ratio
1	Wheat Bran	6.65	0.74	16.36	17.1	0.40	0.38
2	Oat Bran	1.5	0.57	4.87	5.44	0.28	0.99
3	Barley Bran	0.34	1.34	3.07	4.41	0.08	0.81
4	Red Bean	0.93	1.74	4.7	6.44	0.14	0.21
5	White Bean	1.01	1.34	6.84	8.18	0.12	0*

*Soluble oxalate < 0.01mg/100g, so the ratio is < 0.001.