

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

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20 **1. Introduction**

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

34 A main factor that regulates soluble oxalate is the concentration of divalent cation minerals,
35 including calcium and magnesium (Reddy, Sathe, & Salunkhe, 1982). The presence of cations in
36 the gut has been found to interfere with oxalate absorption. Higher concentrations of cations like
37 calcium and, to a lesser extent, magnesium have been found to decrease oxalate absorption, and
38 their concentration in simultaneously ingested foods has therefore been considered as important
39 with respect to kidney stone formation (Asplin, 2002). The solubility of calcium oxalate is
40 strongly pH dependent with solubility increasing strongly below pH 4 (Jaeger & Robertson,
41 2004). Magnesium oxalate is more soluble than calcium oxalate, 0.07 g / 100 ml versus 0.0007
42 g/100 ml respectively, but it still contributes to insoluble oxalate in the gut, when its

43 concentration exceeds the solubility limit (Tiselius, 1991). The solubility product constant for
44 magnesium oxalate at pH 7 has been reported as $8.5 \times 10^{-5} \text{ mol}^2 \cdot \text{dm}^{-6}$, compared to $2.7 \times 10^{-9} \text{ mol}^2 \cdot$
45 dm^{-6} for calcium oxalate (University of Rhode Island, 2001), although the solubility in urine is
46 more complex, since calcium oxalate crystals can occur as mixtures differing in the degree of
47 hydration (Streit, Tran-Ho, & Königsberger, 1998). It has been suggested that magnesium may
48 have a small effect on oxalate uptake by complexing oxalate and making it less available for
49 absorption (Jaeger & Robertson, 2004). However, magnesium supplementation also has been
50 reported to have no effect on urinary oxalate level (Allie & Rodgers, 2003). Phytate is also
51 considered as beneficial with respect to nephrolithiasis due to its antioxidant properties (Graf &
52 Eaton, 1990), although more recently phytate was found to increase soluble oxalate available for
53 absorption as well as recurrence of kidney stones as a consequence of its combination with
54 calcium in the human gut (Al-Wahsh, 2005). Cereals and legumes have been found to contain
55 high concentrations of phytate (Reddy et al., 1982), which makes it an important factor to
56 consider when evaluating these foods for oxalate.

57 The molar ratio of oxalate to concurrent minerals has been used as a measure of the availability
58 of oxalate for absorption. Molar ratios of oxalate to minerals greater than 2 and phytate to
59 minerals greater than 0.24 have been reported as hazardous (Fassett, 1973; Reddy & Sathe,
60 2002). This study aimed to investigate the molar ratio of oxalate and phytate to concurrent
61 minerals in common plant materials in order to assess the availability of oxalate for absorption.
62 Few studies on the effect of a combination of oxalate and phytate on the availability of oxalate
63 and its influence on kidney stones have been reported. Although bran and beans are common
64 dietary components, the concentrations of phytate and oxalate in the same samples of these foods
65 have not been reported. The aim of this study was to investigate the effects of oxalate, phytate

66 and mineral concentrations on oxalate solubility in order to predict its bioavailability. These
67 findings would allow conclusions to be drawn about the influence of these foods on the risk of
68 hyperoxaluria in susceptible subjects.

69 **2. Materials and methods**

70 ***2.1. Food samples***

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

74 ***2.2. Oxalate analysis***

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

77 Samples (1 g) were extracted with 50 ml 1.0 M H₂SO₄ at 21°C for 15 min in a shaking water
78 bath. The extracts were transferred into a 100 ml volumetric flask, and made to volume with 1.0
79 M H₂SO₄ for total oxalate and with distilled water for soluble oxalate. The dissolved oxalate
80 solution was separated by centrifugation at 3000 rpm for 15 min and passed through a 0.45 µm
81 nylon syringe filter. The oxalate concentration in each sample was determined by HPLC using an
82 Agilent 1100 series chromatograph with autosampler, isocratic pump and UV/VIS detector set at
83 210 nm. Data capture and analysis were done by using Chemstation software Version A-7.1. A 5
84 µl injection volume was used with an Aminex Ion exclusion HPX-87H 300 × 7.8 mm analytical
85 column fitted with an Aminex Cation-H guard column. Isocratic elution was used with 0.0125 M
86 H₂SO₄ (Sigma Aldrich, UK) as mobile phase and a flow of 0.5 ml/min. The analytical column
87 was held at 65°C, and the column was equilibrated with a flow rate of 0.2 ml/min prior to use.

88 **2.3. Phytate analysis**

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

93 The sample was analyzed by HPLC using an Agilent 1050 series chromatograph consisting of
94 two pumps, UV-Vis detector, set at 500 nm and Chemstation software Version A-8.3. The
95 column was a strong anion-exchange type, Polymer Laboratories PL-Sax 5 × 0.46 cm, particle
96 size 8 µm and 100 nm pore size (Varian, Inc. Shropshire, UK). A flow rate of 1 ml/min was used
97 for the mobile phase and 0.5 ml/min was used for Wade's reagent. The injection volume was 5
98 µl. The analytical column was kept at room temperature and equilibrated with 0.01 M methyl
99 piperazine at pH 4 as mobile phase with a flow rate of 0.2 ml/min before analysis of the sample.

100 The gradient buffer was 0.6 M sodium nitrate in 0.01 M methyl piperazine at pH 4. Phytate
101 concentration was calculated using 660 g mol⁻¹ as the hexaphosphate molecular weight as
102 recommended by (Oberleas & Harland, 2001).

103 **2.4. Mineral analysis**

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

107 **2.5. Statistical analysis**

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

111 **3. Results and discussion**

112 **3.1. Oxalate**

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

122 **3.2. Cations**

123 Oxalate absorption is highly dependent on the availability of the soluble form. Potassium oxalate
124 is an important soluble form for absorption (Brinkley et al., 1981).The proximal small intestine is
125 a major site for absorption of oxalate (Hanes, Weaver, Heaney, & Wastney, 1999), but changes
126 of pH throughout the gastrointestinal tract also have an effect on the absorption of oxalate.
127 Oxalate is more soluble under the acid conditions of the stomach, which ranges from pH 1.5-2,
128 than at higher pH, so insoluble oxalate forms again after passing into the alkaline environment of
129 the small intestine. Thus oxalate which has been solubilised in the stomach will form a sparingly
130 soluble complex again with calcium, magnesium and iron in the intestine. Soluble oxalate is
131 available for absorption from the intestine through the mucosa (Savage & Catherwood, 2007).

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

139 ***3.3. Molar ratio of oxalate and minerals***

140 The presence of cations in foods eaten at the same time as sources of oxalate is highly important
141 for determining the relative concentrations of soluble and insoluble oxalate (Asplin, 2002).
142 Therefore, the potential of foods for contributing to soluble oxalate is best assessed in terms of
143 the oxalate: mineral ratio for minerals that form insoluble oxalate complexes. A ratio greater than
144 2 indicates that a food contains excess oxalate that is bioavailable, whereas, foods having a ratio
145 of 1 or less contain enough calcium, or similar minerals, to minimise formation of soluble
146 oxalate (Gontzea & Sutzescu, 1968). The solubility product constant for calcium oxalate was
147 reported as $2.7 \times 10^{-9} \text{ mol}^2 \cdot \text{dm}^{-6}$ (URI(Chemistry;University of Rhode Island), 2001). The whole
148 wheat and oat bran samples studied have a molar ratio of oxalate: calcium greater than 2 as
149 shown in Table 3 and the soluble oxalate content is quite high. In contrast, the molar ratio of
150 oxalate: calcium for red kidney beans was 0.91, and the soluble oxalate content was relatively
151 low compared to the cereal brans. The soluble oxalate content of white beans and barley bran
152 was also low. Magnesium oxalate is more soluble than calcium oxalate with a solubility product
153 constant of $8.5 \times 10^{-5} \text{ mol}^2 \cdot \text{dm}^{-6}$ (URI(Chemistry;University of Rhode Island), 2001), and it does
154 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} mol². 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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310 **Table 1**

311 **Phytate, and total, soluble and insoluble oxalate in food samples (mg/100 g dry weight ±**
 312 **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}

313 Results are presented as Mean ± SEM of triplicate determinations

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

315 ^{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 \pm**
318 **SEM).**

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

319 Results are presented as Means \pm 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

322 **Table 3**

323 **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

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333 **Table 4**

334 **Molar ratios of phytate with calcium and magnesium and its correlation with soluble:**
335 **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*

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

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