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SOLUBILITY OF OXALATE AND ITS ASSOCIATION WITH METAL IONS ASSESSED BY A SIMULATED *IN VITRO* DIGESTION MODEL

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Bioavailability of oxalate is highly influenced by the presence of metal cations in the gastrointestinal tract as a result of the different solubility of oxalate salts. Bioavailability is further influenced by pH effects on oxalate solubility at various stages of digestion, particularly at gastric pH at which insoluble oxalate has been found to solubilize. The aim of the present study was to assess the effect of gastrointestinal pH and different metal ions on the relative solubility of oxalate after treatment with relevant gastric and intestinal enzymes in simulated gastrointestinal digestion conditions. Whole bran cereals and beans were used as test samples. Oxalate solubility increased along with reproducible recovery such as 97±4 at gastric pH-2 compared to higher pH. High amount of soluble oxalate (32±1.2) was found in wheat bran after simulated in vitro digestion under gastric conditions as compared to intestinal conditions i.e. 11±0.5. The availability of calcium for absorption was found to be very low compared to that of magnesium and potassium i.e., 15±0.3, 13±0.5 to 10±0.42 and 4±1 in wheat bran sample at gastric and intestinal conditions simultaneously. The formation of a complex between calcium and oxalate reduced solubility and hence made calcium less available for absorption. Presence of metals and oxalate not only reduces oxalate availability, but it also reduces availability of metal cations.
Keywords: Organic acid, calcium regulation, metal detoxification, soluble salts, soluble oxalate, gastric pH.

INTRODUCTION

Oxalate, being an organic acid, occurs widely in plant and plant products. Its presence in foods has been found to contribute several protective roles *in vivo* e.g., ion balance, calcium regulation and heavy metal detoxification (Franceschi and Nakata, 2005). Oxalate is found as soluble salts of potassium and sodium, which are termed soluble oxalates as well as mono and divalent cations and insoluble salts including calcium, magnesium and iron known as insoluble oxalates (Nooan and Savage, 1999). Consumption of foods containing large amounts of soluble oxalate has been considered deleterious for human health, since soluble oxalates are eliminated in urine, as they do not have any metabolic function. As a consequence, urinary oxalate levels are raised, which can cause the formation of kidney stones (Albihn and Savage, 2000).

However, ingested soluble oxalate or divalent cations combines with certain metal cations in the small intestine, leading to reduce oxalate availability for absorption and subsequent excretion via urine. Calcium is available as soluble salts in the stomach and small intestine. However, it may bind to soluble organic molecules to form insoluble salts before it can pass through the intestinal wall (Guéguen and Pointillart, 2000). Thus, the formation of insoluble calcium oxalate in the form of divalent may make both oxalate and calcium unavailable for absorption.

Insoluble oxalate i.e., formed after combination of oxalate with calcium, magnesium or iron, dissolves at gastric pH-2. But change of pH greatly influences oxalate solubility. Oxalate absorption may occur in the stomach, where the pH is 2, but also in the duodenum, where the pH increases to 6.5 (Hautmann, 1993). However, absorption of oxalate from the small intestine has been reported to be greatly influenced by co-ingestion of calcium, magnesium and fibre (Holmes *et al*., 1995). Metal cations form complexes with oxalate by chelation and this may make them unavailable for absorption if the complexes are insoluble. Absorption of oxalate along with available metal cations may occur if the complex is soluble.

The present study determined solubilized oxalate by modelling the natural digestion process after taking account of changes in pH occurring in the gastrointestinal tract. Effects of metal cations that could affect oxalate absorption were checked by *in vitro* methods. *In vitro* digestion processes that can be used as a model of human gastric and intestinal digestion (Radek and Savage, 2008). The method proposed by Versantvoort *et al*. (2005) was a static gastrointestinal model which was based on consideration of transit time, temperature, constituents, concentration and pH of normal physiological conditions. It used four solutions representing saliva, gastric, duodenal and bile solutions at 37°C. However, this method was modified by (Catherwood, 2005) to better reproduce gastric pH to provide a better model for

determining solubilized oxalate at pH values relevant to gastric and intestinal digestion. The latter method has been used in the present study in order to check availability of oxalate for absorption after combination with metal ions as well as its estimation as total oxalate. Moreover, availability of metal ions was also determined with a few modifications introduced into the simulated *in vitro* digestion model.

MATERIALS AND METHODS

***Food samples*:** Whole bran cereals including wheat, barley and oat bran with scientific name *T. aestivum*, *H. vulgare* and *A. sativa*, respectively were obtained from Premier Foods, UK. Legumes including red beans and white beans with scientific name *P. vulgaris* imported from Spain were purchased at a local market. One batch of each foodstuff was purchased for analysis.

***Dry matter determination*:** Dry matter of fresh food test samples was determined by heating at 105°C in an oven (Memmert-UN 110) for 24 h. Samples were reweighed 2 h later to check for the complete removal of moisture. The dry weight was calculated according to method 925.10 described in AOAC (AOAC, 2000).

Simulated in vitro extraction:

Soluble oxalate under simulated gastric and intestinal conditions: Soluble oxalate under gastric and intestinal conditions was measured by the method of Savage and Catherwood (Fig. 1) (Savage and Catherwood, 2007).

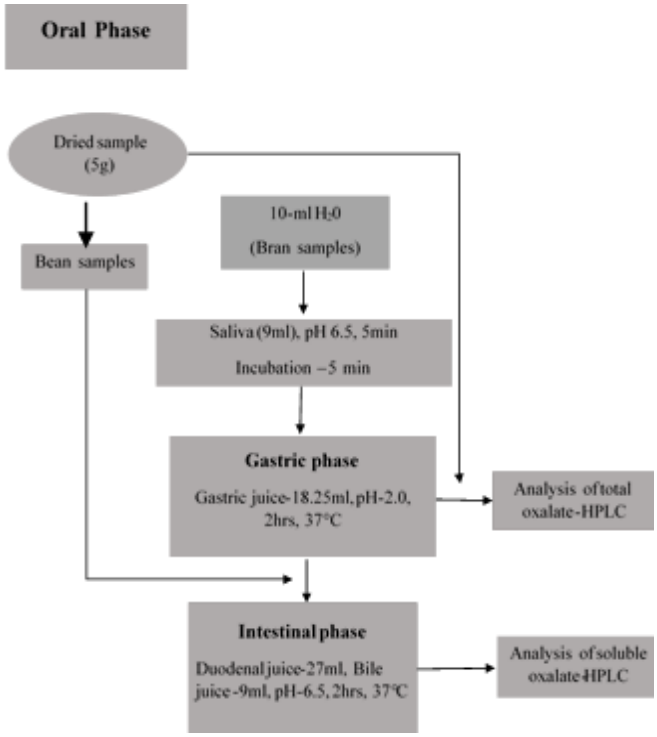

Figure 1. Simulated *in vitro* digestion method for oxalate.

Composition of solutions for simulating digestion: All chemicals were purchased from Fisher (Fisher Scientific Ltd., Loughborough, UK) and Sigma (Sigma–Aldrich Co. Gillingham, UK). Each digestive solution was prepared freshly each day by dissolving the following chemicals and reagents in distilled water. Each solution was warmed to 37°C before use. Details of the digestive solutions are as follows.

***Saliva solution (pH 6.5 ± 0.2)*:** Saliva solution was prepared by mixing an inorganic and organic solution. The inorganic solution contained 10 ml of 1.2M potassium chloride (8.96g/100ml), 10 ml of 0.2M potassium thiocyanate (2g/100ml), 10 ml of 0.7M sodium dihydrogen phosphate (8.88g/100ml), 10ml of 0.4M disodium hydrogen phosphate (5.7g/100 ml), 1.7ml of 3M sodium chloride (17.53g/100 ml), and 1.8ml of 1M sodium hydroxide (4g/100 ml) solution in a 500 ml volumetric flask, which was diluted to volume with water.8ml of 0.4M of urea (2.5g/100 ml) was added into a 500 ml volumetric flask and the solution was diluted to volume with distilled water. Model saliva solution was prepared by mixing 50 ml of organic and 50 ml of inorganic solution and heating at 37°C before addition of 14.5 mg α-amylase, 1.5 mg uric acid and 5 mg mucin. Finally, the pH was adjusted to 6.5 with 1 M sodium hydroxide or 1 M hydrochloric acid before addition to the test sample.

***Model gastric solution (pH 2.0± 0.07)*:** The model gastric solution contained an inorganic solution prepared from 15.7 ml of 3M sodium chloride (17.53g/100ml), 3.0 ml of 0.7M sodium dihydrogen phosphate (8.88g/100ml), 9.2 ml of 1.2M potassium chloride (8.96g/100ml), 18.0 ml of 0.1M calcium chloride dihydrate (2.22g/100ml), 0.83 ml hydrochloric acid (37% g/g) and 10 ml of 0.6M ammonium chloride (3.06g/100ml). The solution was made up to 500 ml with distilled water. A mixture of organic compounds was prepared in a 500 ml volumetric flask from 10 ml of 0.4M glucose (6.5g/100ml), 0.01M glucuronic acid (0.2g/100ml), 0.18M glucosamine hydrochloride (3.3g/100ml) and 3.4 ml of 0.4M urea (2.5g/100ml) along with distilled water. The two solutions (60 ml) were mixed and warmed to 37°C.Then 0.12 g bovine serum albumin (BSA), 0.12 g pepsin and 0.36 g mucin were added. The pH was maintained at 2.0 before addition to the test samples.

***Model duodenal solution (pH 7.8 ± 0.2)*:** The model duodenal solution was prepared from a mixture of inorganic solutions namely 40 ml of 3M sodium chloride (17.53g/100 ml), 40 ml of 1M sodium bicarbonate (8.47g/100 ml), 10 ml of 0.05M potassium dihydrogen phosphate (0.8g/100 ml), 6.3 ml of 1.2M potassium chloride (8.96g/100 ml), 10 ml of 0.05M magnesium chloride (0.5g/100 ml) and 180 µl hydrochloric acid (37% m/m) in a 500 ml volumetric flask along with distilled water. A solution of 4 ml of 0.4M urea (2.5g/100 ml) was added into a 500 ml volumetric flask along with distilled water. The duodenal solution was prepared by mixing 120 ml inorganic and urea solutions and was warmed to 37°C. Finally; a solution was prepared by mixing 2.16 ml

of 0.2M calcium chloride dihydrate (2.22g/100 ml), 0.24 g BSA and 0.72 g pancreatin, and the pH was adjusted to 7.8 before the solution was added to the test samples.

***Bile solution (pH 8.0 ± 0.2)*:** The bile solution was prepared by diluting a solution containing 10 ml of 0.4M urea (2.5g/100 ml) to volume in a 500 ml volumetric flask with distilled water. An inorganic solution was prepared from 4.2 ml of 1.2M potassium chloride (8.96 g/100 ml), 30 ml of 3M sodium chloride (17.53 g/100 ml), 68.3 ml of 1M sodium bicarbonate (8.47 g/100 ml) and 200 µl hydrochloric acid (37% m/m) and diluted to 500 ml in a volumetric flask along with distilled water. The inorganic and urea solutions (50 ml) were mixed and warmed to 37°C, and then 1 ml of 0.2M calcium chloride dihydrate (2.22 g/100 ml), 0.18 g BSA and 0.6 g bile salts were added, and the pH was adjusted to 8.0 before the solution was added to the test samples.

***Soluble oxalate at gastric pH (pH-2)*:** Five grams of dried test food sample was added into a 125 ml conical flask and 9 ml saliva solution was added. The pH was checked and maintained at 6.5. The sample was incubated on a shaking water bath at 37°C for 5-min. Model gastric solution (13.5 ml) was added and the pH was again checked and maintained at 2. The sample was then maintained for 2 h at 37°C, and then it was transferred into a 250 ml volumetric flask and made up to volume with 1M sulfuric acid. An aliquot (40 ml) was centrifuged at 2900 rpm at 4°C for 15 min. The supernatant was filtered through a 0.45µm cellulose acetate filter prior to injection into the HPLC.

***Soluble oxalate at intestinal pH (pH 6.5±0.5)*:** Soluble oxalate at intestinal pH was determined by following the procedure that was described for the gastric solution. However; gastric solution having pH values 2.0 was added with 27 ml model duodenal and 9 ml bile salt solution to prepare the intestinal phase at pH 6.5±0.5. After these solutions were added, the solution was stored for 2 h at 37°C. The solution was then transferred into a 250 ml volumetric flask and diluted to volume with distilled water. A 40 ml aliquot was centrifuged at 2900 rpm at 4°C for 15 min. The supernatant was filtered through a 0.45µm cellulose acetate filter prior to injection into the HPLC.

***HPLC analysis*:** 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 (Savage *et al.* 2000). Data capture and analysis were performed with Chemstation software Version A-7.1. A 5 µl injection volume was used with an Aminex Ion exclusion HPX-87H 300 × 7.8mm 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.

Recovery was measured by determining the increase in oxalate concentration assessed by HPLC after addition of 10 mg oxalic acid into the test food samples before adding the digestive solutions.

Determination of soluble metal ion concentrations under simulated digestion conditions: Soluble metal ion concentrations under simulated digestion conditions (Fig. 2) were determined according to (Miller et al., 1981) and (Luten et al., 1996).

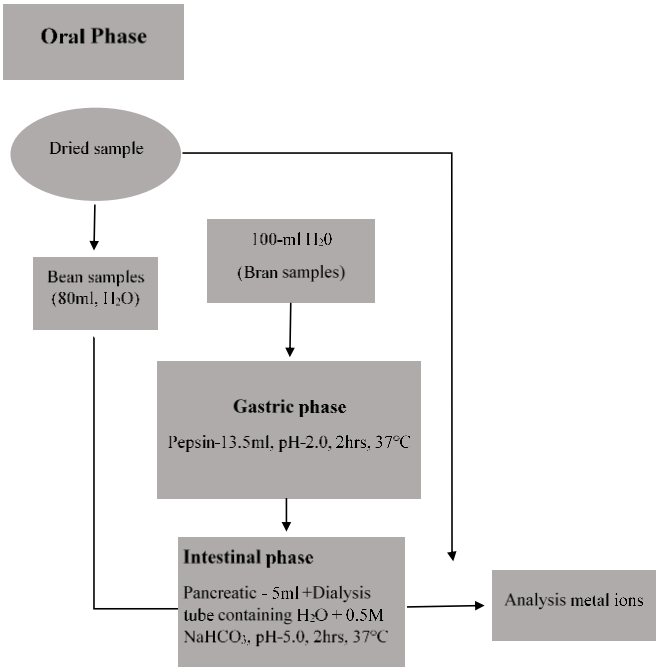

Figure 2. Simulated in vitro digestion method for metal ions.

Chemicals: All chemicals were purchased from Fisher (Fisher Scientific Ltd., Loughborough, UK) and Sigma (Sigma–Aldrich Co. Gillingham, UK). The following solutions were prepared

to simulate gastric and intestinal conditions. Pepsin (16 g/100 ml 0.1M hydrochloric acid), Pancreatic-bile extract mixture (0.4 g pancreatin and 2.5 g bile extract/100 ml 0.1M sodium bicarbonate), 0.5M sodium hydroxide. Dialysis tubes with 10, 000kDa cut off (MMCO) were purchased from Fisher Scientific and VWR, respectively.

***Gastric conditions*:** Sample (10 g) was mixed with 80 g water in a 250 ml Erlenmeyer flask. The pH was adjusted to 2.0 by adding 6 M hydrochloric acid. Pepsin solution (18.75 ml) was added. The sample was diluted to100 ml with distilled water and then incubated in a shaking water bath at 37°C for 2 h. Finally, the gastric digest was stored in ice for 90 min and the titratable acidity was measured. The titratable acidity was measured after adding 20 g pepsin digest at 20°C. Freshly prepared pancreatin mixture (5 g) was added and the pH was

adjusted to 7.5 with 0.5 M sodium hydroxide. The pH was checked after an equilibrium period of 30 min and was re adjusted.

***Intestinal conditions*:** Homogenised pepsin digest (20 g) was placed in a 250 Erlenmeyer flask at 37°C for 5 min. Then 250 mm dialysis tube containing 25g water and 0.5 M sodium bicarbonate was added. The flask was sealed with parafilm in order to avoid loss of carbon dioxide. Then the sample was maintained at 37°C in a shaking water bath until the pH reached 5. After the pH reached 5, 5ml pancreatin mixture was added and the sample was maintained at 37°C for 2 h. At the end of the incubation, the dialysis tube was removed and washed with water. The content was then weighed and analysed.

Analysis of metal ions: The sample was digested by the AOAC wet method 985.35 (AOAC, 2000). Calcium, magnesium and potassium were analysed by atomic absorption spectrophotometry at 422.7, 285.2 and 766.5 nm, respectively (Analytik Jena AG, Germany Model NovAA® 350).

***Statistical analysis*:** The data was first plotted using a scatter plot to check the relationship between variables. Linear regression analysis was used to investigate the correlation of oxalate absorption with pH. ANOVA was used to check comparison of means.

RESULTS AND DISCUSSION

***Oxalate content after extraction at different pH values*:** The availability of oxalate for absorption is affected by the change of pH along the gastrointestinal tract. The value of the stomach pH has been found to influence the absorption of oxalate, which increases under more acid conditions due to increased solubility, such that insoluble oxalate has been reported to solubilize at gastric pH (Savage and Catherwood, 2007). Thus, different pH values representing different part of gastrointestinal tract were studied, namely pH-2 to mimic stomach conditions for studying solubility and effect of metal ions on soluble oxalate and pH 6.5 was used to determine soluble oxalate under intestinal conditions.

Oxalate content after extraction at pH-2.0 and pH-6.5: Oxalate can be generated during extraction with hot acid, so oxalate content is more reliable after extraction at mild temperatures (Hönow and Hesse, 2002). For that reason oxalate content was analyzed after extraction at pH 2 and 37°C, which is comparable to the conditions in the stomach (Daugherty and Mrsny, 1999). Soluble oxalate was less than total oxalate in all tested food samples with concentrations ranging from 2.4 to 134 mg/100 g compared to total oxalate ranging from 43 to 164 mg/100 g (Table 1). Soluble oxalate was extracted after an additional 2 h incubation at pH 6.5±0.5. Oxalate occurs as divalent oxalate anions $C_2O_4^{2-}$ at higher pH and would react to form salts with cations. This reduces oxalate availability due its low solubility product constant with certain metal ions (Savage and Mårtensson, 2010). That could cause a reduction in soluble oxalate concentration under intestinal conditions. Red and white beans were found to contain soluble oxalate concentrations below the detection limit.

Table 1. Extraction of oxalate at pH-2.0 after using *in vitro* static digestion model (mg/100g dry wt ± SEM).

Sample	% dry matter	Total	Soluble	Insoluble
Wheat Bran	91	164±3	134 ±6	30±2
Barley Bran	92	43±3	2.4±0.2	40.6±3
Oat Bran	91	82±12	7±2	75±8
Red Bean	92	52±2	ND	52±2
White Bean	91	44±4	ND	44±4

Results are presented as Means±SEM; Each sample was analyzed as triplicate; ND = not detected

***Oxalate concentration after extraction with a simulated in vitro model*:** Oxalate concentration under simulated *in vitro* digestion conditions was less than total oxalate after treatment with conditions like both gastric and intestinal sites. Oxalate extracted from wheat bran under conditions similar to those of the gastric juices at pH 2 was determined as 32 mg/100g, which is about 20% of the concentration that was obtained by the static *in vitro* digestion model (Table 1). The oxalate concentration released from food is highly influenced by the use of different digestion models and applied conditions (Oomen *et al*., 2002).

Low concentrations of oxalate were detected in the rest of the samples i.e. 15, 3, 3 and 2 mg/100g in barley bran, oat bran, red and white bean respectively after treatment with the model gastric conditions (Table 2). Oxalates soluble in the intestine would be available for absorption at that site. The soluble oxalate concentration was 11 mg/100 g in wheat bran and 3 mg/100 g in barley bran at pH 5.

However, no oxalate could be detected at that pH in the rest of the test samples. The equilibrium constant (*K*a) for loss of the first proton from oxalic acid is 5.37×10^{-2} (p*K*a = 1.27), so metal salts occur as metal hydrogen oxalates under strongly acidic conditions, and an equilibrium exists between metal hydrogen oxalates and metal oxalates above pH 4. During gastric digestion, most of the oxalate would be present as metal hydrogen oxalates. However, as the pH increases to 5-6.5, which is the pH of the small intestine, both metal hydrogen oxalates and metal oxalates are present.

The pH of the medium has a significant effect on soluble oxalate concentration. As the pH decreases, oxalate ions have been found to be highly protonated. It has been reported that pH less than 6 makes divalent oxalate ions ($C_2O_4^{2-}$) less deprotonated. As a result of which, its ability to bind with mineral cations has been found to be reduced, because oxalate is found in the form of semi-dehydro-oxalic acid (HC_2O^{4-}) and oxalic acid ($H_2C_2O_4$) (Simpson *et al*., 2009).

Table 2. Solubility of oxalate and metal ions from test food samples after simulated *in vitro* digestion (mg/100g dry wt ± SEM).

Parameter	Wheat Bran	Barley Bran	Oat Bran	Red Bean	White Bean
		Soluble Oxalate			
Gastric pH	32 ±1.2^{c}	15 ±1.3^{b}	3 ± 0.17^{a}	3 ± 0.01^{a}	2 ± 0.03^{a}
Intestinal pH	11 ± 0.5^{c}	3 ± 0.2^{b}	ND^{a}	ND^{a}	ND^{a}
		Available Calcium			
Gastric pH	3.00 ± 0.03^{ab}	4.00 ± 0.21^{ab}	2.00 ± 0.34^{a}	5.00 ± 0.34^{b}	4.00 ± 0.15^{ab}
Intestinal pH	0.30 ± 0.08^{a}	0.10 ± 0.07^{a}	1.00 ± 0.17^{ab}	1.06 ± 0.16^{ab}	2.00 ± 0.04^{b}
		Available Magnesium			
Gastric pH	15 ± 0.30^{c}	7.00 ± 0.21^{a}	10 ± 0.74^{b}	16 ± 0.09^{c}	16 ± 0.07^{c}
Intestinal pH	10 ± 0.42^{c}	1.00 ± 0.02^{a}	6 ± 0.17^{b}	13 ± 0.47^{d}	15 ± 0.21^{d}
		Available Potassium			
Gastric pH	13 ± 0.5^{c}	4 ± 1^{a}	4 ± 0.5^{a}	11 ± 0.5^{bc}	10 ± 0.25^{b}
Intestinal pH	4 ± 1^{ab}	3 ± 0.5^{a}	2 ± 0.25^{a}	8 ± 0.5^{c}	7 ± 0.5b^{c}

Results are presented as Means±SEM; Each sample was analyzed in triplicate; a-c Numbers with different superscripts in the same column are significantly different ($p<0.05$); ND = not detected

Combination of oxalate with calcium gives metal salts that are very insoluble under the conditions of the small intestine, but the solubility of calcium oxalate is strongly pH dependent with solubility increasing strongly below pH-4 (Jaeger and Robertson, 2004). Formation of metal salts depends on the total concentration of metal cations and the metal: oxalate ratio in the sample. Consumption of foods containing calcium together with oxalate reduces the concentration of oxalate available for absorption in the intestine (Radek and Savage, 2008). Reduction in soluble oxalate has been reported in several studies due to the presence of metal cations (Ritter and Savage, 2007) and (Asplin, 2002).

***Availability of metal ions to combine with oxalate*:** The presence of certain metal ions namely calcium and magnesium reduces the absorption of oxalate. However there are certain other compounds like phosphate and phytate that can bind metal ions and can release oxalate for absorption (Kelsay and Prather, 1983). Whereas, presence of antinutrients in food has been reported to decrease bioavailability of minerals especially diet rich in phytate (Udomkun *et al*., 2019).

***Available calcium in foods*:** Soluble calcium concentration at gastric pH 2 was similar in all test food samples ranging from 2-5 mg/100 g. Solubility of calcium was significant among test food samples (P= 0.02). However, soluble calcium concentration was higher in bean samples than in the wheat bran or oat bran samples, although this difference was non-significant (NS). The lower concentration of total oxalate in the bean samples may contribute to this difference, although the difference is small. Metal cations form complexes with oxalate, and this would reduce the concentration of cations available for absorption (Brinkley *et al*., 1981). However, calcium availability at intestinal pH was also lower in the bran samples than in the beans. Available calcium can be reduced by binding to oxalate (Brinkley *et al*., 1990), or to phytate (Walker, 1951). Both complexes with oxalate and phytate are insoluble and contribute to a reduction in soluble calcium. Phytate has also probability to bind with calcium, making an insoluble complex and increase amount of soluble oxalate (Israr *et al*., 2013).

Several studies have shown that co-ingestion of foods containing calcium, e.g. milk (rich source of calcium), reduces oxalate bioavailability (Brogren and Savage, 2003). Addition of calcium from milk or from other food sources of calcium reduced the concentration of soluble oxalate that was available for absorption in the small intestine (Radek and Savage, 2008). However, combination of calcium with certain antinutrients in food like phytate forms complex that ultimately increases concentration of soluble oxalates (Israr *et al*., 2017).

***Magnesium solubility in the presence of oxalate*:** The concentration of soluble magnesium was higher in bean samples than in bran samples. The concentration of soluble magnesium at gastric pH was 16 mg/100g in red and white bean samples. The bran samples contained lower concentrations of magnesium, which ranged from 7-15 mg/100 g. Magnesium has also been reported to form an insoluble oxalate (Nooan and Savage, 1999) but it is more soluble than calcium oxalate due to its higher solubility product i.e. $8.5x10^{-5}$ $mol^2.dm^{-6}$ compared with 2.7 $x10^{-9}$ $mol^2.dm^{-6}$ for calcium oxalate (URI Chemistry;University of Rhode Island, 2001). The higher solubility of magnesium oxalate helps to make oxalate more available at gastric pH in the presence of magnesium than if calcium is present. Relatively high magnesium concentrations would be required to contribute precipitation of oxalate. Magnesium solubility was not dramatically reduced by increase of pH to intestinal pH. The concentration of soluble magnesium at intestinal pH was in the range from 14-94% of total magnesium in tested food samples. Magnesium has been reported to be less effective for reducing oxalate absorption compared to calcium (Brinkley *et al*., 1981). Moreover, several dietary factors such

as metal ions and compounds like phytate and oxalate has been reported to impair absorption of magnesium (Schuchardt and Hahn, 2017).

Potassium solubility with oxalate: Potassium salts are highly soluble, so the presence of potassium does not contribute to any reduction of available oxalate. The potassium concentration is higher in the wheat bran sample than in the other foods, which is consistent with the literature (Jenkins et al., 2002). However, the concentration is still relatively low, and does not affect oxalate absorption. Potassium salts are well absorbed in the body and eliminated in urine as it has no metabolic function (Albihn and Savage, 2000b).

***Conclusions*:** This study has shown that the soluble oxalate concentration in the presence of metal ions varies with pH and confirms that it would be better to simulate human gastrointestinal conditions as closely as possible for studies of simulated *in vitro* digestion. pH 2 is suitable to simulate gastric conditions. The soluble oxalate concentration is less at intestinal pH 6.5 after simulated digestion. That would be due to the presence of metal cations especially calcium and its combination with oxalate. It has been further noticed that the presence of metals and oxalate not only reduces oxalate availability but it also reduces availability of metal cations too, although the concentrations of calcium and magnesium in the foods studied are very low compared with the dietary reference values for adults of 700 mg per day for calcium and 270-300 mg/day for magnesium.

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