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OPTIMIZATION OF OXALATE EXTRACTION CONDITIONS APPLIED TO LEGUMINOUS AND CEREAL PRODUCTS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Extraction of oxalate has been found to be problematic due its formation from precursors as well as variability in extraction recovery. Exact determination of oxalate is highly important for the provision of dietary advice to subjects that are at a high risk of kidney stone formation. Present study was proposed to optimize oxalate extraction conditions including acid type, time and temperature. Cereal (wheat bran, barley bran and oat bran) and bean (red bean and white bean) samples were selected for analysis. Hot (80°C) and cold acidic extraction (21°C) was performed with 15 and 30 min incubation times. Total and soluble oxalate was analyzed by HPLC. Extraction of total and soluble oxalate increased at higher temperatures along with long incubation times i.e. ranging from 576±5 to 73±1 mg/100g for total oxalate and 255±2 to 75±2 mg/100 g for soluble oxalate respectively. This was attributed to generation of oxalate from its precursors at higher temperatures. Cold extraction with shorter incubation time gave reproducible values along with good recovery in the range 279±4 to 71±2 mg/100 g for total oxalate and 112±3 to 58±3 mg/100 g for soluble oxalate with recovery ranging from 95 to 99%.
Keywords: Bean, cereal, HPLC, soluble oxalate, calcium oxalate

INTRODUCTION

Quantitative extraction of oxalate and its accurate determination are essential for preparing low oxalate diets as well as controlling dietary oxalate related risk factors for recurrence of calcium oxalate kidney stones in susceptible subjects (Massey, 2007). Oxalate extraction and analysis has been found to be challenging due to the complex structure of plants i.e. the presence of pectin or the difficulty of dissolving calcium oxalate crystals as well as artifactual generation of oxalate from ascorbic acid. However, this possibility may be minimised after using hot acid that not only ensures complete dissolution of calcium oxalate crystals but also is reported to avoid oxalate generation due to oxidation of ascorbic acid (Fretzdorff and Betsche, 1998).

On the other hand, use of hot acid has been reported to yield higher values for oxalate due to possible generation of oxalate from its precursors. Possible precursors include sources of carbohydrate including; D-galactose, glyoxylic acid and pectin as well as oxaloacetic acid, mesoxalic acid, ascorbic acid and pyruvate. However, incubation time for extraction of oxalate has been suggested to overcome problems caused by use of hot acid. As small incubation time reduces evolution of oxalate from its precursors. On the other hand, longer incubation ensures complete dissolution of oxalate crystals (Hönow and Hesse, 2002). Use of cold acid gives low oxalate concentrations due to undissolved calcium oxalate crystals as well as incomplete extraction (Ohkawa, 1985). Whereas; use of cold acid with small incubation time for extraction of soluble oxalate has also been reported. It has been observed to minimise oxalate generation from ascorbic acid (Hönow and Hesse, 2002). Therefore, selection of proper time and temperature is considered as crucial for ensuring complete extraction. Optimal conditions of extraction may vary according to the type of plant part. Oxalate distribution varies throughout the plant with large amounts of oxalate in the leaves as compared to the stem (Noonan and Savage, 1999).

Moreover, selection of the acid for extraction of oxalate is also important in order to minimise losses and ensure complete dissolution as well as for compatibility with the analytical column. Acid having a low pH dissolves the oxalate mineral complex, and also dissolves pectin at low temperatures and gives reproducible results for oxalate extraction (Nguyen and Savage, 2013).

Oxalate, which is extractable with water, is termed soluble oxalate and it is predominantly found to be potassium oxalate. Total oxalate is that which is extracted with acid, and insoluble oxalate is calculated as the difference between total and soluble oxalate. Insoluble oxalate includes calcium oxalate crystals and magnesium oxalate (Libert and Franceschi, 1987). The classification of magnesium oxalate as insoluble has been confirmed by the observation of decreasing oxalate absorption with increased intake of magnesium in an oxalate load test meal (Liebman and Costa,

2000). Overall oxalate absorption has been found to depend on the soluble oxalate content of test food samples rather than total oxalate (Chai and Liebman, 2004; Holmes and Assimos, 2004).

Sample preparation for oxalate analysis by different methods may also lead to oxalate generation or loss. Various approaches have been reported in the literature for the quantitative extraction of oxalate from a range of food sample matrices as shown in Table 1. Precipitate formation (CaOx) during the analysis of oxalate by the colorimetric method may also lead to loss of oxalate (Zarembski and Hodgkinson, 1962a).

Table 1. Total and soluble oxalate extraction from raw food samples at different temperature (°C).

Sample/Treatment	Total Oxalate (mg/100 g)	Soluble Oxalate (mg/100 g)	Increase 80°C vs 21°C (%)	
			Total Oxalate	Soluble Oxalate
Wheat Bran				
80°C-15min	361±3^{c}	203±1^{c}	23	52
21°C-15min	279±4^{a}	98±2^{a}		
80°C-30min	576±5^{d}	255±2^{d}	40	59
21°C-30min	345±2^{b}	105±3^{b}		
Wheat Bran Flakes				
80°C-15min	136±4^{c}	80±4^{b}	34	70
21°C-15min	90±3^{a}	24±2^{a}		
80°C-30min	219±3^{d}	140±3^{d}	51	26
21°C-30min	108±1^{b}	104±3^{c}		
Breakfast Cereal Bran				
80°C-15min	192±1^{c}	138±2^{b}	7	23
21°C-15min	186±5^{a}	112±3^{a}		
80°C-30min	201±6^{c}	158±4^{c}	5	37
21°C-30min	199±7^{b}	115±6^{a}		
White Kidney Bean				
80°C-15min	192±5^{b}	66±4^{b}	3	0**
21°C-15min	187±6^{a}	-		
80°C-30min	238±4^{d}	75±2^{c}	8	4
21°C-30min	218±3^{c}	72±8^{c}		

Results are presented as Means ± SEM and each sample was analyzed as triplicate; $^{a-d}$ Numbers with different superscripts in the same column are significantly different ($p<0.05$); *Effect of temperature has been found as significant tested by Paired T test; ** Soluble oxalate < 0.01mg/100g

On the other hand, analysis of oxalate by the enzymatic method using oxalate decarboxylase may lead to elevated values due to incomplete removal of carbon dioxide from the sample solution (Kasidas and Rose, 1980). Thus, to overcome all the problems that might occur due to sample preparation as well as during analysis, the sample preparation procedure and the analytical methodology must be carefully selected. The primary objective was to reduce the chance of errors occurring due to the handling or extraction procedure. Therefore, the present study was planned to overcome all the problems during extraction and analysis that might hamper accurate determination of oxalate content. Therefore, high temperature i.e. 80°C and lower temperature i.e. 21°C has been selected for complete dissolution of calcium oxalate crystals as well as to minimise oxalate generation from ascorbic acid as well as from its precursors respectively. Thus, present study also checks the efficiency of hot and cold extraction method for oxalate analysis

MATERIALS AND METHODS

***Food samples*:** Raw and processed food samples were selected for extraction and analysis of oxalate. Bran samples were supplied by Premier Foods, UK. Bean and processed food samples were bought from local markets. Wheat bran, wheat bran flakes (Kellogg's), breakfast cereal bran (Kellogg's), white kidney bean, wholemeal bread and white soft bread (Hovis) were selected for the analysis of total and soluble oxalate. One batch of each food stuff was purchased for analysis.

***Sample preparation*:** Hot and cold extraction at 80°C and 21°C respectively for 15 and 30 min was performed in order to assess the effects of temperature and time on the extent of oxalate extraction from test food samples. Sulphuric acid was used in place of hydrochloric and phosphoric acid for extraction in order to ensure the longevity of the analytical column. As use of some strong acids e.g. HCl has been reported to cause corrosion as well as decrease column life (Meyer, 2013). Insoluble oxalate was calculated by difference between the total and soluble oxalate (Holloway *et al.*, 1989).

***Hot & cold extraction*:** One gram ground test food sample was weighed into a 200 ml beaker. For the analysis of total oxalate, H_2SO_4 (1M, 50 ml) was added and soluble oxalate 50 ml distilled water was added. The sample was completely covered and kept in a shaking water bath at the following temperatures and times for hot and cold extraction.

- 80°C - 15 and 30 min for hot extraction
- 21°C -15 and 30 min for cold extraction

The extract was diluted and the volume was made up to 100 ml in a volumetric flask with H_2SO_4 (1 M) and distilled water for total and soluble oxalate respectively. After making to volume, the solution was transferred into 50 ml centrifuge tubes and centrifuged at 3500 rpm for 15 min. The supernatant was filtered through a 0.45 µm nylon syringe filter and transferred into HPLC vials for further analysis. Each sample was extracted in triplicate.

***Preparation of standards*:** Oxalate standards were prepared with oxalic acid (Sigma-Aldrich Co., St Louis, USA) in the concentrations of 1, 5, 10, 20, 40, 50, 100 mg/100 ml with 1M H_2SO_4 for total oxalate and distilled water for soluble oxalate. Standards were filtered through a 0.45µm nylon syringe filter and transferred into HPLC sample vials for analysis. Calibration curve was made after using different concentration of standards (Fig. 1).

***Recovery standards*:** Recovery of oxalic acid in the total and soluble oxalate extraction methods described above was

measured by HPLC in samples containing 50 mg added oxalic acid (Sigma-Aldrich Co., St Louis, USA).

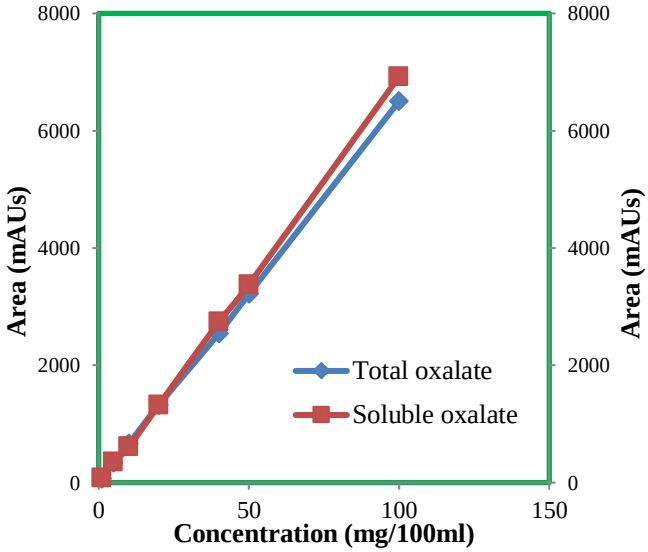

Figure 1. Calibration curve of total and soluble oxalate (mg/100ml).

***Quantification of total and soluble oxalate*:** 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 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.

***Statistical analysis*:** All data were presented as mean ± SD. The significance of differences in each variable was tested by a paired t test and values less than 0.05 were considered as significant. ANOVA was applied to assess whether significant differences existed within the group of samples tested and was further confirmed by post hoc test i.e. Tukey HSD test. (SPSS Inc. 2009, version 18, Chicago.

RESULTS

The occurrence of oxalate in plant tissues either as calcium oxalate crystals or soluble anions contributes to the difficulties of extraction (Holmes and Kennedy, 2000). The effects of acid (1M H_2SO_4) at two temperatures (21°C and 80°C) and times (15 min and 30min) on total oxalate in wheat bran were studied in order to minimise the errors occurred during extraction of oxalate. However, extraction of oxalate at high and low temperature can minimise error during extraction of oxalate (Hönow and Hesse, 2002; Al-Wahsh *et al.*, 2012). Initial validation was done after getting a linear calibration curve for the standards. The concentration of acid used for extraction was finalised after checking the effect of different concentrations (0.2 to 1M) of acid used.

***Linearity response for standards*:** Oxalic acid standards with different concentrations (1-100 mg/100ml) of oxalic acid mixed with sulphuric acid and water were prepared to check the reproducibility of oxalate analysis (Ross *et al.*, 1999). The calibration curve for both solutions was linear with R^2=0.99. The gradient of the calibration curves was slightly higher for the standard dissolved in sulphuric acid for extraction of total oxalate (Fig. 1).

***Accuracy and reproducibility of acid concentration for total oxalate extraction*:** Oxalate extraction can be incomplete due to the insolubility of CaOx crystals (Fretzdorff and Betsche, 1998). Sulphuric acid was used in the present study instead of hydrochloric acid that was used previously (Savage *et al.*, 2000). This change of acid was introduced to extend the column life as well as to minimise corrosion that could be due to use of HCl. Several concentrations of sulphuric acid were investigated to assess the effect on total oxalate extraction and pH. Concentration of total oxalate has been found to decrease with different concentration of sulphuric acid. However, this decrease has been found as non-significant. But the pH decreased as the concentration of sulphuric acid increased. The pH was less sensitive to change with change in sulphuric acid concentration at 1M concentration as shown in Fig. 2. Thus, 1M was selected as the final concentration for dissolution as well as extraction of total oxalate from test food samples.

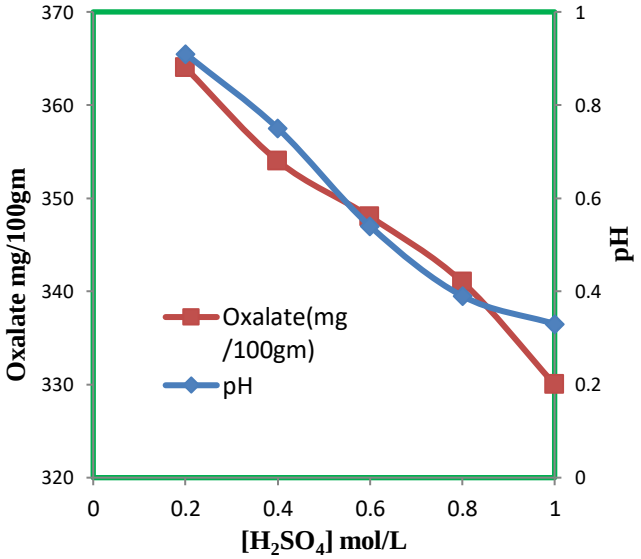

Figure 2. Total oxalate extraction and pH after using different concentrations of H_2SO_4 in extraction of oxalate from the wheat bran sample.

***Extraction of oxalate at different temperatures*:** Selection of temperature for oxalate extraction has been controversial in the literature. High temperature has been found to increase oxalate concentration due to generation of oxalate from different food constituents such as ascorbic acid and other precursors, but low temperature gave decreased values due to incomplete extraction (Chalmers *et al.,* 1985; Zarembski and Hodgkinson, 1962). Thus the effect of temperature for oxalate extraction from test food samples was studied as described below.

***Oxalate generation during extraction at high temperature i.e. 80°C*:** Total oxalate determined by acid extraction at 80°C was higher than soluble oxalate extracted with water at the same temperature. Acid used in total oxalate extraction has been found to have greater oxalate extraction efficiency as compared to water (Al-Wahsh *et al.,* 2012) and that was confirmed in present study. Whole wheat bran showed higher levels of oxalate (total and soluble) as compared to breakfast cereal bran and wheat bran flakes i.e. ranging from 576 ± 5 for total oxalate in whole wheat bran extracted with acid for 30 mins at 80°C to 219 ± 3 mg/100g for total oxalate in breakfast cereal bran extracted with acid for 30 mins at 80°C and 255 ± 2 to 140 ± 3 mg/100g for soluble oxalate in same samples extracted under analogous conditions but with water. However, total oxalate concentration is generally less in processed food samples i.e. whole meal bread 118 ± 3 and soft white bread 73 ± 1 mg/100g under similar condition (Table 2). It is 13-20% of the total oxalate extracted from whole wheat bran under same extraction condition. Thus, this finding is consistent with the suggestion that processing might have some effect on the concentration of total and soluble oxalate (Boontaganon *et al.,* 2009; Liebman and Okombo, 2009; Okombo and Liebman, 2010).

Table 2. Total and soluble oxalate extraction from processed food samples at different temperatures (°C).

Sample/Treatment	Total Oxalate (mg/100gm)	Soluble Oxalate (mg/100gm)	Increase 21°C vs 80°C (%)	
			Total Oxalate	Soluble Oxalate
Whole Meal Bread				
21°C-15 min	120±0.5^{c}	78±3^{ab}	32	9
80°C-15 min	82±2^{a}	71±5^{a}		
21°C-30 min	123±1^{d}	111±4^{c}	4	29
80°C-30 min	118±3^{b}	79±4^{b}		
White Soft Bread				
21°C-15 min	71±2^{a}	58±3^{a}	-3	-8
80°C-15 min	72±3^{a}	63±1^{b}		
21°C-30 min	73±2^{a}	58±5^{a}		
80°C-30 min	73±1^{a}	82±4^{c}	0	-29

Results are presented as Means±SEM and each sample was analyzed in triplicate; $^{a-d}$ Numbers with different superscripts in the same column are significantly different ($p<0.05$); *Effect of temperature has been found as significant tested by Paired T test

***Oxalate generation during extraction at 21°C*:** The values for total oxalate were less when extraction was performed at low temperature as compared to high temperature. However, the use of acid for extraction of oxalate has been found to give a similar trend at lower temperature to that found at higher temperature, i.e. acid gives a higher value for total oxalate extraction as compared to water in the form of soluble oxalate. These higher values demonstrate the greater efficiency of acid for oxalate extraction as compared to water (Table 1). A reduced oxalate concentration following extraction at low temperature has been attributed to incomplete extraction (Al-Wahsh *et al.,* 2012). The total oxalate concentration is higher than soluble oxalate when determined at low temperature and shows a similar order as at high temperature i.e. Wheat bran > Breakfast cereal bran> Whole meal bread > Wheat bran flakes >> White soft bread.

***Comparison of oxalate extraction at high and low temperature*:** Both total and soluble oxalates were higher after extraction at 80°C compared to the values after extraction at 21°C. High temperature extraction has been reported to achieve complete dissolution of calcium oxalate (Zarembski and Hodgkinson, 1962a). Low temperature extraction gave lower values for oxalate, and this has been attributed to minimisation of in vitro oxalate synthesis as well as incomplete dissolution of calcium oxalate crystals (Hönow and Hesse, 2002). However, acid has been found to dissolve oxalate crystals that might form with minerals, fibre/pectin. Acid at lower temperature was reported as an effective strategy for the dissolution of pectin as well as analysis of oxalate (Nguyen and Savage, 2013). In general generation of soluble oxalate increased more as a percentage when the extraction temperature was increased compared to total oxalate, although this was not the case for all foods. The increase of soluble oxalate due to extraction at 80°C compared to 21°C is in the range 0-70% for wheat bran, wheat bran flakes, breakfast cereal bran and white kidney bean (Table 1). The percentage increase due to extraction at high temperature may be partly due to generation of oxalate from precursors, although it is unclear if it happens in these foods. Processed food samples, whole meal bread and soft white bread, did not show a trend for increased oxalate concentrations due to extraction at high temperature.

***Recovery rates*:** It is important to check recovery rate as part of the authentication of the procedure. Recovery rates above 90% have been considered as acceptable and applicable. Thus, total and soluble oxalate content after addition of oxalic acid salt has been analysed to determine the recovery rate. The recovery rate ranged from 92-98% with good reproducibility at 21°C. This demonstrated a complete dissolution and extraction of total and soluble oxalate. However, the recoveries at 80°C ranged from 108-122%. This shows the overestimation of total and soluble oxalate at 80°C. Selection of low temperature for the determination of total and soluble oxalate in these foods seems to be appropriate.

***Extraction of oxalate at different times*:** Different extraction times for determination of total and soluble oxalate have been proposed in order to ensure complete extraction as well as dissolution of CaOx (Hönow and Hesse, 2002). In order to determine the effect of extraction time, 15 and 30 min extraction times were used at high and low temperature.

***Oxalate generation during extraction for 15 and 30 min*:** Total and soluble oxalate in test food samples was higher after extraction for 30 min as compared to 15 min at both 21°C and 80°C (Table 2 & 3). It could be partly due to release of oxalate from precursors due to the increase in incubation period. No significant difference was reported in the literature between total and soluble oxalate in samples following different extraction times (Hönow and Hesse, 2002).

Table 3. Total and soluble oxalate extraction from raw/processed food samples at different times (min).

Sample /Treatment	Total Oxalate (mg/100gm)	Soluble Oxalate (mg/100gm)	Increase 30min vs 15 min (%age)	
			Total Oxalate	Soluble Oxalate
Wheat Bran				
21°C-15min	279±4[a]	98±2[a]	24	7
21°C-30min	345±2[b]	105±3[b]		
80°C-15min	361±3[c]	203±1[c]	60	26
80°C-30min	576±5[d]	255±2[d]		
Wheat Bran Flakes				
21°C-15min	90±3[a]	24±2[a]	20	77
21°C-30min	108±1[b]	104±3[c]		
80°C-15min	136±4[c]	80±4[b]	61	75
80°C-30min	219±3[d]	140±5[d]		
Breakfast Cereal Bran				
21°C-15min	186±5[a]	112±3[a]	3	75
21°C-30min	192±1[b]	115±6[a]		
80°C-15min	199±7[c]	138±2[b]		
80°C-30min	201±6[c]	158±4[c]	1	9
Whole Meal Bread				
21°C-15min	120±0.5b[c]	78±3[ab]	2	42
21°C-30min	123±1[c]	111±4[c]		
80°C-15min	82±2[a]	71±5[a]	44	11
80°C-30min	118±3[b]	79±4[b]		
White Soft Bread				
21°C-15min	71±2[a]	58±3[a]	3	0
21°C-30min	73±2[a]	58±5[a]		
80°C-15min	72±3[a]	63±1[b]	1	30
80°C-30min	73±1[a]	82±4[c]		
White Kidney Bean				
21°C-15min	187±6[a]	0	17	72
21°C-30min	218±3[c]	72±8[c]		
80°C-15min	192±5[b]	66±4[b]	24	12
80°C-30min	238±4[d]	75±2[c]		

Results are presented as Means±SEM and each sample was analyzed in triplicate; [a-d] Numbers with different superscripts in the same column are significantly different ($p<0.05$); *Effect of temperature has been found as significant tested by Paired T test

However, an increase in total and soluble oxalate was observed in most raw and processed food samples after the increase in extraction time. The percentage increase in total and soluble oxalate was higher for raw food samples (i.e. Whole wheat bran, wheat bran flakes and breakfast cereal bran) as compared to processed food samples (whole meal bread and white soft bread).

***Comparison of acid used for extraction of total oxalate*:** Use of sulphuric acid for extraction has been proposed for longevity of the column that was used for oxalate analysis (Blake *et al.*, 1987), so hydrochloric acid was replaced by sulphuric acid for extraction. The concentration of acid used for extraction was selected following preliminary experiments that demonstrated that 0.2M sulphuric acid gave higher values for oxalate than higher concentrations as shown in Figure 2.

Samples analysed at 21°C for 15 min with hydrochloric acid and sulphuric acid demonstrated that the values for oxalate in wheat bran at 21°C were lower using sulphuric acid, but the values were higher with sulphuric acid at 90, 71 and 120 mg/100g in comparison to 76, 12 and 37 mg/100g after extraction with hydrochloric acid from wheat flakes, white bread and whole meal bread respectively (Table 4).

Table 4. Comparison of acid used for extraction of total oxalate in previous and proposed method (mg/100g).

Sample	Previous method (HCl used for extraction)		Proposed method (H_2SO_4 used for extraction)	
	Oxalate (21°C-15min)*	Oxalate (80°C-15min)**	Oxalate (21°C-15min)	Oxalate (80°C-15 min)
Wheat bran	457	346	279	361
Wheat flakes	76	393	90	136
White bread	12	-	71	72
Whole meal bread	37	-	120	82

*(Siener *et al.*, 2006b); **(Boontaganon *et al.*, 2009)

The oxalate concentration after using hydrochloric acid for extraction at 80°C for 15min was 346 and 393 mg/100g in wheat bran and wheat flakes, but the value was lower in wheat flakes when sulphuric acid was used.

DISCUSSION

Accurate analysis of total and soluble oxalate content of food is required for correct dietary assessment of the oxalate content of food, and in order to make recommendations for dietary change to reduce formation of calcium oxalate kidney stones (Siener *et al.*, 2006). Most reported studies have been hampered due to inaccurate analysis and inadequate food composition tables for oxalate content. Usually, the methods that have been proposed for oxalate extraction involve extraction with different acids and concentration of calcium oxalate by precipitation. Calcium oxalate crystals have been

further dissolved and analysed by Gas chromatography, High performance liquid chromatography, Capillary electrophoresis and Ion Chromatography (Ohkawa, 1985; Holloway *et al.,* 1989; Holmes and Kennedy, 2000; Chai and Liebman, 2005).

Selection of temperature and time for extraction of oxalate was considered to be controversial due to incomplete extraction at lower temperature and overestimation at higher temperature (Fretzdorff and Betsche, 1998; Hönow and Hesse, 2002). Higher temperature i.e. 80°C has been found to increase the values for total and soluble oxalate, with the highest values occurring at the longer extraction time i.e. 30 min. Higher incubation time along with hot acid extraction gave the highest values, and this can be attributed to conversion of certain precursors of oxalate. An increase of 1.2 mg/100gm in total oxalate was reported following extraction at higher temperature compared to low temperature in both herbs and fruits (Al-Wahsh *et al.,* 2012). Degradation of carbohydrate has been claimed to elevate oxalate values (Zarembski and Hodgkinson, 1962a). The increase in soluble oxalate at higher temperature has been found to be greater than total oxalate. This is due to the conversion of oxalate precursors e.g. ascorbate or other sources of carbohydrate into oxalate. Ascorbic acid has been found to be a source of oxalate at pH-7 (Chalmers *et al.,* 1985). On the other; ascorbic acid has been found as negligible in all test food samples including raw cereal bran except red kidney bean. It has been reported to contain ascorbic acid as 2mg/100g (Krebs *et al.,* 2002). However, processed food samples i.e. whole meal bread and soft white bread have been found to exhibit higher total and soluble oxalate with good recovery with extraction at lower temperature as compared to higher temperature. As higher temperature has been found to generate oxalate from precursors, extraction of oxalate has been recommended at room temperature, where no new oxalate generation occurs, although incomplete extraction may occur (Hönow and Hesse, 2002).

Selection of appropriate time and temperature is important for exclusion of any error or oxalate generation during sample preparation (Hönow and Hesse, 2002). Total and soluble oxalate has been found to increase as the incubation time for extraction increases due to excessive generation of oxalate with an apparent increased recovery. The trend of increasing total and soluble oxalate with the longer extraction time was found to be similar in raw and processed food samples. Mild conditions for oxalate extraction from food samples were recommended in previous reports (Ross *et al.,* 1999; Hönow and Hesse, 2002; Catherwood *et al.,* 2007). Binding of oxalate to fibre, or poor extraction of calcium or magnesium oxalate can cause incomplete oxalate extraction at lower temperatures. It has been reported that a negligible amount of oxalate in a residual fibre sample of bran flakes after oxalate extraction by using 1 M H_3PO_4 at 55°C for 1 hour (Holmes *et al.,* 1995). This confirms reduced oxalate extraction due to the presence of fibre (Holmes *et al.,* 1995). The reduced yield would be due to complex formation of fibre-mineral-oxalate. This complex has been reported to be scarcely soluble as compared to a fibre-mineral or mineral oxalate complex (Kelsay and Prather, 1983). The pectin-part of fibre has been reported to form a complex with oxalate and this reduces total and soluble oxalate concentrations (Hanson *et al.,* 1989). Oxalate binding is low in fibre free oca tubers. This is consistent with oxalate binding to fibre in fibre rich tubers (Van Soest, 1967).

***Conclusion*:** Use of cold sulphuric acid and water has been proposed for the extraction of total and soluble oxalate. Hot acid and hot water gave higher values in comparison to cold extraction.

Formation of oxalate from oxalate precursors can lead to elevated values at higher temperatures, but generation of oxalate from its precursors at higher temperatures and incomplete extraction at lower temperatures can lead to higher as well as reduced values.

Incomplete extraction was reduced after changing the acid used for extraction from hydrochloric acid to sulphuric acid. The strong acid increases amount of oxalate with increased incubation time at 80ºC for some foods. That indicates increased generation of oxalate from its precursors.

Selection of a mild temperature along with a short incubation period is recommended for extraction of total and soluble oxalate from test raw foods samples.

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