

## Original Article

# Soluble and insoluble oxalate content of nuts

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### Abstract

This study was conducted to determine the oxalate contents in common nuts either locally grown or imported into New Zealand. Samples of imported nuts were purchased from supermarkets in Christchurch while locally grown nuts were obtained directly from the growers. In this experiment gastric soluble and intestinal soluble oxalates were extracted from the nuts using an *in vitro* assay, which involved incubations of the food samples for 2 h at 37 °C in gastric and intestinal juice. The extracted oxalates were then determined by HPLC chromatography. Roasted pistachio nuts and chestnuts contained very low levels (<85 mg/100 g fresh weight (FW)) of gastric soluble oxalate. Peanuts, Spanish peanuts, peanut butter, ginkgo, cashew nuts and pecan nuts all contained relatively low levels of gastric soluble oxalate (147–250 mg gastric soluble oxalate/100 g FW). Almonds, Brazil, pine and candle nuts contained high levels of gastric soluble oxalate (492.0–556.8 mg/100 g FW). The intestinal soluble oxalate is the fraction that will be absorbed in the small intestine. Peanuts, Spanish peanuts, peanut butter, ginkgo and pecan nuts all contained relatively low levels of intestinal soluble oxalate (129–173 mg intestinal soluble oxalate/100 g FW). Almonds, Brazil, cashew and candle nuts contained higher levels of intestinal soluble oxalate (216–305 mg/100 g FW). Pinenuts contained the highest levels of intestinal soluble oxalate (581 mg/100 g FW), while chestnuts and roasted pistachio nuts were low (72 and 77 mg/100 g FW). Overall the mean soluble oxalate contents of nuts was 78% of the gastric soluble oxalate content (41–100%). The results obtained in this study confirm that the intestinal soluble oxalate contents of nuts range widely and people who have a tendency to form kidney stones would be wise to moderate their consumption of certain nuts.

**Keywords:** Gastric soluble oxalate; Intestinal soluble oxalate; Nuts

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### 1. Introduction

Nuts have been part of the human diet for a long time; remains have been found in archaeological sites dating back to before 10,000 BC. Vegetarians have valued nuts as an alternative source of protein but nuts have more than protein to offer. Up to eight different constituents, linolenic acid, folic acid, arginine, fibre, vitamin E, potassium, copper and magnesium contribute to the positive nutritional value of nuts (Hu et al., 1998). These constituents occur at high levels in most nuts. While nuts generally contain high oil levels and are therefore energy dense, they still appear to be an important part of a healthy diet. In a prospective cohort study, regular consumption of nuts has been associated with a reduced risk of both fatal ischaemic

heart disease and non-fatal myocardial infarction (Hu et al., 1998). These results are consistent with an earlier epidemiological study (Fraser et al., 1992) which showed that people who consumed nuts five or more times a week had a 50% reduction in risk of ischaemic heart disease compared with those who never consumed nuts. A similar reduction in relative risk was observed in a cohort of women in the Nurses' Health Study (Hu et al., 1998; Colditz et al., 1997).

Nuts are often recommended as part of a healthy diet but people who have the tendency to form kidney stones are sometimes warned that nuts may contain oxalates. Reliable recent data is sparse (Table 1). It is unfortunate that the oxalate content of nuts quoted in many standard texts refer to data derived using older methods of analysis and rarely make the distinction between soluble and insoluble oxalate contents. Soluble (oxalic acid and soluble salts) and insoluble oxalates (predominantly the calcium

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Table 1  
Total oxalate content of different cultivars of nuts (mg/100 g FW)

	Total oxalate	Reference
Roasted almonds	469.0	Chai and Liebman (2005)
Almonds	383.3	Hönow and Hesse (2002)
Macadamia nuts	42.0	Chai and Liebman (2005)
Cashew nuts	231.0	Noonan and Savage (1999)
Pistachio nuts	56.5	Hönow and Hesse (2002)
Peanuts	142.0	Judprasong et al., (2006)
Peanut butter	70.5	Chai and Liebman (2005)
Pecans	0.1	Brinkley et al. (1981)

salt) can be extracted from foods using hot water to extract soluble oxalates and 0.2 mol/L HCl to extract total oxalates, which includes both soluble and insoluble oxalate fractions (Savage et al., 2000; Holloway et al., 1989). More recently, an *in vitro* method has been proposed to investigate the amounts of oxalates that will become available for absorption in the gastrointestinal tract (Savage and Catherwood, 2007). An *in vitro* assay takes account of the changing pH which occurs in the gastrointestinal digesta and should give a much clearer indication of the amounts of soluble oxalate that may be actually absorbed from the small intestine. Another important feature of the *in vitro* assay is that the extractions are carried out at 37 °C. Savage and Catherwood (2007) showed that the gastric available oxalate method gave similar results to the 0.2 mol/L HCl extraction method at 80 °C that extracts total oxalates (Savage et al., 2000; Holloway et al., 1989). The intestinal available oxalate method tended to extract more soluble oxalates than the hot water (80 °C) extraction method used to measure soluble oxalates (Savage et al., 2000; Holloway et al., 1989). The object of this study was to use the Savage and Catherwood (2007) method to measure the oxalate content in nuts commonly consumed in New Zealand so that reliable advice can be given to people who have to consider the possible adverse effects of consuming foods which may contain high levels of available oxalates.

## 2. Materials and methods

### 2.1. Sample collection and preparation

Bulk samples (1 kg) of 11 different types of imported nuts, peanut butter (Sanitarium smooth peanut butter) and desiccated coconut (Basics, General Distribution Ltd.) were purchased from local supermarkets in Christchurch, New Zealand. Named cultivars of hazelnuts were obtained from a local orchard. Representative sub-samples of nuts were ground in a coffee mill (Sunbeam Multigrinder EMO400). The pellicle was removed from the peanuts, ginkgo nuts, pistachios and hazelnuts.

#### 2.1.1. Dry matter and fat extraction

Dry matter of all samples was determined in duplicate by drying them to constant weight in an oven at 105 °C for 24 h, AOAC method 925.10 (AOAC, 2002). Total fat was extracted from each sample using a Soxhlet extractor and petroleum ether (Shell-X4), AOAC method 948.22 (AOAC, 2002).

#### 2.1.2. Determination of gastric soluble and intestinal soluble oxalate

Gastric available and intestinal available oxalate contents of 2 g of finely ground samples of raw nuts (hazelnuts, almonds, peanuts, Spanish peanuts, pistachios, chestnuts, ginkgo nuts and coconuts) or 1.5 g of fat extracted nuts (Brazil nuts, candle nuts, cashew nuts, pecans and pine nuts) were determined using an *in vitro* method described by Savage and Catherwood (2007). This method was based on the method proposed by Versantvoort et al. (2005).

**2.1.2.1. Gastric soluble oxalate.** Triplicate samples of finely ground nuts were accurately weighed into a 125 mL conical flask and 9 mL of the artificial saliva solution (pH 6.5 ± 0.2) was added. The flask was then incubated at 37 °C for 5 min then 13.5 mL of gastric juice (pH 1.07 ± 0.07) was added and the flask was incubated at 37 °C for a further 2 h. The contents of the flask were then quantitatively transferred to a 250 mL volumetric flask and made up to volume with 0.2 mol/L HCl. An aliquot was centrifuged at 2889 rcf for 15 min. The supernatant was then filtered through a 0.45 µm cellulose acetate filter (Sartorius, Goettingen, Germany) prior to injection into the HPLC.

**2.1.2.2. Intestinal soluble oxalate.** The initial gastric digestion, as outlined above, was followed by the addition of the intestinal digestive enzymes in 27 mL of duodenal juice (pH 7.8 ± 0.2) and 9 mL of bile solution (pH 8.0 ± 0.2) to the flask. The flask was then incubated for a further 2 h at 37 °C. The contents of the flask were then quantitatively transferred to a 250 mL volumetric flask and made up to volume with nanopure water. An aliquot was centrifuged at 2889 rcf for 15 min. The supernatant was then filtered through a 0.45 µm cellulose acetate filter (Sartorius, Goettingen, Germany) prior to injection into the HPLC.

**2.1.2.3. Oxalate quantification.** Chromatographic separation was carried out using a 300 × 7.8 mm ion exchange column (Alltech Associates Inc., Deerfield, IL, USA) attached to a cation H<sup>+</sup> guard column (Bio-Rad, Richmond, CA, USA). The analytical column was run at 25 °C. The equipment consisted of a ternary Spectra-Physics, SP 8800 HPLC pump (Spectra-Physics, San Jose, CA, USA), a Waters, U6K injector (Waters Inc., Marlborough, MA, USA), a UV/VIS detector Spectra-Physics SP8450 (Spectra-Physics, San Jose, CA, USA) set on 210 nm. Data capture and processing were carried out using Chromatopac C-R3A integrator (Shimadzu, Corporation, Kyoto, Japan). The mobile phase used was an

aqueous solution of 25 mM sulphuric acid (BDH, Aristar). Before use the mobile phase was filtered through a 0.45 µm membrane filter and degassed using a vacuum. 20 µL samples were injected onto the column and eluted at a flow rate of 0.6 mL/min. The oxalic acid peak was identified by comparing the retention time of several common plant organic acids. All data are presented as mg oxalate/100 g fresh weight (FW) basis as this is how nuts are normally consumed.

**2.1.2.4. Standard calibration.** A standard curve was prepared in the range 2.5–50 mg oxalate/250 mL using oxalic acid (Analar, BDH, UK). A standard curve was prepared to quantify the samples extracted using gastric soluble oxalate method using the same proportions of saliva and gastric juice solutions as used for the sample extractions. A separate standard curve was prepared to quantify the intestinal soluble oxalate extractions using the duodenal and bile solutions in the same proportions as for the sample extractions.

**2.1.2.5. Oxalate recovery.** A recovery study was carried out where 10 mg oxalic acid was added to triplicate 5 g samples of the finely ground nuts. One set of recovery analysis was carried out on finely ground full fat nuts while the other recovery analysis was carried out on finely ground fat extracted nuts. No difference in recovery was observed between the two sets of recovery analysis. The mean recovery of oxalate ± SD after gastric extraction was 97.7 ± 1.2% and the mean recovery following the intestinal extraction was 96.7 ± 1.3%.

### 3. Results

The identification and quantification of the oxalate peak following gastric soluble and intestinal soluble extractions of finely ground sub-samples of hazelnuts, almonds, peanuts, Spanish peanuts, pistachios, chestnuts, ginkgo nuts and coconuts gave repeatable results. The same extractions of finely ground sub-samples of Brazil nuts, candle nuts, cashew nuts, pecans and pine nuts gave incomplete chromatographic separations which could only be resolved by extracting the fat from the finely ground sub-samples prior to gastric soluble and intestinal soluble extractions.

Data presented in Table 2 for locally grown nuts and nuts imported into New Zealand are the mean values for three separate determinations. The dry matter content (mg/100 g FW) of each sample is also shown in Table 2. Nuts are considered to be dry foods and the dry matter content of all the nuts was 95.5 ± 0.95% except for chestnuts and ginkgo nuts, which had dry matter contents of 70.4% and 76.9%, respectively.

Roasted pistachio nuts and chestnuts contained very low levels (<85 mg/100 g FW) of gastric soluble oxalate. Peanuts, Spanish peanuts, peanut butter, ginkgo, cashew nuts and pecan nuts all contained relatively low levels of gastric soluble oxalate, ranging from 147 to 250 mg gastric soluble oxalate/100 g FW. Almonds, Brazil, pine and candle nuts contained high levels of gastric soluble oxalate contents, ranging from 492.0 to 556.8 mg/100 g FW. Dried coconut contained very low levels of gastric soluble oxalate (6.6 mg/100 g FW). It was not possible to detect any intestinal soluble oxalate in this material.

Table 2  
Mean gastric soluble, intestinal soluble oxalate (mg/100 g FW) (±SE) and dry matter (mg/100 g FW) content of locally grown and imported nuts

Cultivars	Dry matter (mg/100 g FW)	Gastric soluble oxalate (mg/100 g FW)	Intestinal soluble oxalate (mg/100 g FW)
<b>Grown in New Zealand</b>			
Hazelnuts ( <i>Corylus avellana</i> cv. Barcelona)	93.9	212.1 ± 7.2	187.6 ± 6.5
Hazelnuts ( <i>Corylus avellana</i> cv. Tonda Di Giffoni)	95.9	265.6 ± 2.2	195.8 ± 3.4
Hazelnuts ( <i>Corylus avellana</i> cv. Whiteheart)	95.2	272.0 ± 5.2	157.2 ± 6.9
<b>Imported into New Zealand</b>			
Almonds ( <i>Prunus amygdalus</i> )	95.0	538.8 ± 9.2	222.7 ± 5.0
Brazil nuts ( <i>Bertholletia excelsa</i> )	97.9	492.0 ± 16.4	304.5 ± 16.1
Candle nuts ( <i>Aleurites moluccana</i> )	96.4	556.8 ± 20.8	315.9 ± 7.5
Cashew nuts (roasted) ( <i>Anacardium occidentale</i> )	97.8	225.3 ± 6.4	216.8 ± 7.3
Chestnuts ( <i>Castanea vesca</i> )	70.4	83.9 ± 7.3	72.3 ± 3.6
Ginkgo nuts ( <i>Ginkgo biloba</i> )	76.9	205.3 ± 4.1	156.3 ± 10.5
Pecan nuts ( <i>Carya illinoensis</i> )	95.6	162.8 ± 12.5	155.1 ± 32.8
Peanuts (roasted) ( <i>Arachis hypogaea</i> )	95.6	147.1 ± 8.5	115.8 ± 2.6
Spanish peanuts ( <i>Arachis hypogaea</i> )	95.3	200.1 ± 20.1	173.2 ± 13.7
Peanut butter (roasted) ( <i>Arachis hypogaea</i> )	98.7	158.9 ± 5.12	129.1 ± 5.6
Pinenuts ( <i>Pinus pinea</i> )	97.6	496.4 ± 17.8	581.0 ± 62.0
Pistachio nuts (roasted) ( <i>Pistacia vera</i> )	97.9	67.4 ± 3.7	76.5 ± 4.8
Coconut (deshelled) ( <i>Cocos nucifera</i> )	98.0	6.6 ± 0.7	— <sup>a</sup>

<sup>a</sup>Value below the detection limit of the HPLC (5 mg/100 g FW).

The gastric soluble contents of the nuts is equivalent to the total oxalate content of the nuts, while the intestinal soluble oxalate content is equivalent to the soluble oxalate fraction (Savage and Catherwood, 2007). In all cases the intestinal soluble oxalate content of the nuts is lower than the gastric soluble oxalate content of the nuts except for roasted pistachio nuts and chestnuts where the gastric and intestinal values are essentially the same.

The intestinal soluble oxalate content of the nuts ranged from 100% of the total for pinenuts and pistachios down to 41% of the total gastric soluble oxalate content for almonds. Overall, the intestinal soluble oxalate of the nuts constituted 78% of the gastric soluble oxalate content.

The three nuts that contained the highest levels of gastric soluble oxalate (almonds, Brazil and candle nuts) also contained the highest levels of insoluble oxalate (mg/100 g FW) compared with all the other nuts analysed in this experiment (Table 2).

The three different cultivars of hazelnuts grown in the same orchard showed different values for both gastric and intestinal soluble oxalates. A large proportion (73%) of the oxalate in hazelnuts was soluble in intestinal fluid.

#### 4. Discussion

This is the first experiment in which a comprehensive examination of the oxalate contents of all edible nuts available for sale in the supermarket has been undertaken. It is also the first time that an analysis has been undertaken that uses an extraction procedure that takes some account of the digestive processes that occur in the digestive tract.

The accurate determination of oxalates in plants poses some problems during extraction from food materials. Previous methods have used hot (80 °C) water and hot (80 °C) acid extractions (Savage et al., 2000; Holloway et al., 1989). Some conversion of ascorbic acid, pectin, mesoxalic acid and glyoxylic acid to oxalic acid does occur during extraction at 100 °C for 180 min with 35% HCl (Hönow and Hesse, 2002). It is not clear whether extraction of food materials with 0.2 mol/L HCl at 80 °C for 15 min, would have the same effect (Savage et al., 2000). This possibility should not be overlooked as many foods contain reasonable amounts of these constituents.

The *in vitro* gastric extraction method uses a 2 h incubation with saliva, pepsin and gastric juice at physiological concentrations (mean pH of the extraction medium including the ground nut samples was  $2.0 \pm 0.4$ ) and therefore more closely follows the interactions that occur during digestion in the stomach. Earlier studies on the determination of the oxalate content of raw and cooked taro corms (Savage and Catherwood, 2007; Catherwood, 2005) have showed that the total oxalate extraction method (0.2 mol/L HCl at 80 °C for 15 min) gave very similar results to the gastric extraction method.

Savage and Catherwood (2007) and Catherwood (2005) then went on to compare the soluble oxalate extraction method (hot water at 80 °C for 15 min) with the intestinal

available method (incubation of the gastric extraction mixture followed by further 2 h incubation with intestinal juice and bile at 37 °C) content of raw and cooked taro corms. They showed that the intestinal *in vitro* method extracted more soluble oxalate than the hot water method. The mean pH of the intestinal extraction medium of the ground nut samples analysed in this experiment was  $6.1 \pm 0.5$ .

Even so, in this *in vitro* analysis, some analytical problems were encountered due to the very high fat contents of some of the nuts. In these cases, samples of nuts were extracted using petroleum ether in a Soxhlet extractor and the analysis for oxalates carried out on the fat-free residue. The results for oxalate content of each of the nuts are however, presented on the basis of the original sample.

Almonds, Brazil and candle nuts contained the highest levels of total gastric soluble oxalate of all the nuts investigated but it is interesting to note that the intestinal soluble oxalate ranged from 41% to 57% of the gastric soluble oxalate contents of these nuts. Pinenuts also contained high levels of gastric soluble oxalates but analysis showed that all of this oxalate was essentially intestinal soluble oxalate.

The values for total oxalate contents of nuts sourced in the USA range from 469 mg/100 g total oxalates for roasted almonds to 42 mg/100 g total oxalates in raw macadamia nuts (Chai and Liebman, 2005). Nuts grown in New Zealand appear to have higher values for oxalates when compared to nuts sourced in the USA (Chai and Liebman, 2005). The *in vitro* method used in this study may well be more efficient at extracting oxalates from natural products than using hot water or hot acid extractions. However, Noonan and Savage (1999) reported a value of 231 mg/100 g total oxalates for cashew nuts which is very similar to the  $225.3 \pm 6.4$  mg gastric available oxalates/100 g FW obtained with this biological method.

Comparison of the results in this study for almonds, hazelnuts and pistachio nuts with the soluble and insoluble oxalate contents previously reported (Hönow and Hesse, 2002) shows that *in vitro* analysis always gave higher values. It is possible that the physiological method used in this analysis enabled a more complete extraction of oxalates from the nut matrix.

In this study, the mean oxalate content of peanuts including peanut butter was 168.7 for gastric soluble oxalate/100 g FW and 139.4 for intestinal soluble oxalates/100 g FW. This is much higher than the value of 70.5 mg total oxalates/100 g previously reported for peanut butter (Massey et al., 2001). More recently, however, a value of 142 mg total oxalate/100 g has been reported for peanuts grown in Thailand (Judprasong et al., 2006).

A very low value for total oxalates in pecans (0.1 mg/100 g) has been reported (Brinkley et al., 1981) which is in contrast to the values reported in this study (162.8 mg gastric available oxalate/100 g FW). Pecan nuts appear to contain 95% of their oxalate as intestinal soluble oxalates.

While many nuts are consumed raw, it should be noted that some nuts, for instance, almonds, peanuts and hazelnuts, are often roasted before consumption. While roasting is used to improve the taste of the nuts it should be noted that moisture is driven off during this process. This could concentrate oxalates in the roasted nuts. Some concentration of oxalates could occur when the oxalate levels in fresh peanuts are compared to those in peanut butter. This may occur if the peanut butter is 100% peanuts, however, in many cases commercial peanut butter contains a number of additives that would have the tendency to reduce the oxalate content of the final product as the peanut content is reduced. The data in Table 1 show that the moisture content of the peanut butter analysed in this study was reduced but the gastric available oxalate content is similar to the roasted nuts analysed.

It is interesting to note that people suffering from coronary heart disease have been encouraged to consume 60 g walnuts/day (much higher than the recommended standard serving of 28 g) as this has been shown to have a positive effect on the reduction of blood cholesterol levels with a significant reduction in risk from ischaemic heart disease (Sabaté et al., 1993). If these patients chose to consume almonds, cashews or peanuts, which are considered to be equally effective at reducing blood cholesterol levels, then this would entail the regular consumption of a mean of 109 mg intestinal soluble oxalate/day from these nuts. This should be compared with the value of 117.7 mg soluble oxalate/100 g FW in cooked silverbeet (also called Swiss chard) leaves or 90.9 mg soluble oxalate/100 g FW in cooked spinach leaves (Savage et al., 2000). Both are vegetables that are not commonly consumed on a regular daily basis. In contrast, the consumption of 20 g peanut butter on a slice of bread would result in the consumption of 26 mg intestinal soluble oxalate.

The standard management plan for people who have a tendency to form kidney stones is to increase their fluid intake and to modify their diet. Kidney stone formers are advised to reduce or omit the following foods from their diet: rhubarb, spinach, beetroot, strawberries, nuts, chocolate, cocoa and tea because they contain high levels of oxalates (Savage, 2002). Data from this experiment suggest that some nuts (almonds, Brazil, candle, cashews and pine nuts) should be placed in Group 1 and should be consumed in moderation (Noonan and Savage, 1999). While other nuts (hazelnuts, ginkgo, pecan, peanuts and pistachio nuts) should be placed in Groups 2 or 3 because they contain only moderate amounts of intestinal soluble oxalates. While the consumption of a standard serving of 28 g nuts poses no increased risks; a person consuming 60 g nuts/day to achieve a positive reduction of blood cholesterol levels may increase their risk of kidney stone formation.

An investigation on the impact of the addition of a source of calcium (e.g. milk) to the nuts as a way of reducing the amount of intestinal soluble oxalates available when the nuts are consumed is needed, as earlier studies have shown that soluble oxalates and calcium can combine

at the alkaline pH found in the small intestine. The consumption of nuts with a calcium source may be important as some nuts are commonly consumed in chocolate which has whole milk added to it.

## 5. Conclusions

People suffering from or at high risk of coronary heart disease are encouraged to consume 60 g nuts/day and this can have a positive effect on the reduction of blood cholesterol levels with a significant reduction in risk from ischaemic heart disease. The consumption of this amount of some nut species could lead to a modest increase in oxalate intake. While ischaemic heart disease is a serious medical problem it is important to minimise the potential of preventative strategies to cause other medical problems. The recommendation to consume more nuts should include a clear direction about which nuts are being recommended. Nuts can still be recommended as part of a healthy diet but there should be some consideration of the oxalate content of particular nuts.

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