Original Article

Bioavailability of soluble oxalate from spinach eaten with and without milk products

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Leafy vegetables such as spinach (*Spinacia oleracea*) are known to contain moderate amounts of soluble and insoluble oxalates. Frozen commercially available spinach in New Zealand contains 736.6 ± 20.4 mg/100g wet matter (WM) soluble oxalate and 220.1 ± 96.5mg/100g WM insoluble oxalate. The frozen spinach contained 90mg total calcium/100g WM, 76.7% of this calcium was unavailable as it was bound to oxalate as insoluble oxalate. The oxalate/calcium (mEq) ratio of the frozen spinach was 4.73. When frozen convenience food is grilled there is no opportunity for the soluble oxalates to be leached out into the cooking water and discarded. Soluble oxalates, when consumed, have the ability to bind to calcium in the spinach and any calcium in foods consumed with the spinach, reducing the absorption of soluble oxalate. In this experiment 10 volunteers ingested 100g grilled spinach alone or with 100g additions of cottage cheese, sour cream and sour cream with Calci-Trim milk™ (180 g) and finally, with 20g olive oil. The availability of oxalate in the spinach was determined by measuring the oxalate output in the urine over a 6-hour and 24-hour period after intake of the test meal. The mean bioavailability of soluble oxalate in the grilled spinach was 0.75 ± 0.48% over a 6-hour period after intake and was 1.93 ± 0.85% measured over a 24-hour period. Addition of sour cream and Calci-Trim milk reduced the availability of the oxalate in the spinach significantly (P<0.05) in both the 6-hour and 24-hour collection periods.

Key words: soluble and insoluble oxalates, bioavailability, absorption, spinach, grilling, calcium, New Zealand.

Introduction

A high oxalate uptake from the diet is thought to play a role in hyperoxaluria, a documented risk factor in the formation of calcium oxalate kidney stones.[1] Absorptive or "dietary" hyperoxaluria is generally thought to be a direct consequence of oxalate bioavailability. Therefore, people with an increased risk for calcium oxalate stone formation are commonly advised to avoid consuming oxalate rich foods. A number of foods such as spinach, rhubarb, beets, nuts, chocolate, wheat bran, and strawberries are known to contain high oxalate levels.[1] Noonan and Savage[1] in their survey of oxalate containing foods, placed spinach in group 1. Group 1 contains food with an oxalate/calcium mEq ratio of >2.0. These are foodstuffs that have a high ratio of oxalate to calcium and are thought to have a big effect on calcium availability from other foods consumed at the same time. In a review of oxalate contents of common foods it was found that spinach contains between 320-1260mg total oxalate/100g wet matter (WM) and has a mean oxalate/calcium ratio mEq ratio of 4.27.[1] The oxalate content of some cultivars of spinach (Universal, Winter Giant) contain between 400-600mg/100g WM while others range from 700-900 mg/100 g WM.[2] The total oxalate content of raw spinach measured by several techniques ranges from 330 to 992 mg/100 g WM.[1-7] Savage *et al.,*[8] observed that fresh New Zealand grown spinach contained 266.2mg/100g WM soluble oxalate and 90.9mg/100g WM insoluble oxalate. The soluble oxalate was reduced by 66% by leaching into the cooking water when the spinach was boiled, while the insoluble oxalate content remained the same.

Most studies show a peak in oxalate absorption after 1-6 hours in normal subjects following ingestion of oxalate containing food.[4,9-11] This indicates that there is a major uptake of oxalate from the small intestine in healthy humans. The relationship between soluble and insoluble oxalate in the small intestine seems to have a major effect on oxalate bioavailability, since ingestion of calcium together with oxalate rich foods has been shown to lower the uptake of both calcium and oxalates.[7,9,12-15] This indicates that insoluble calcium oxalate has a much lower bioavailability than the

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soluble form of oxalate, and that an oxalate rich/low calcium diet leads to a greater uptake of oxalate.

This effect has been more recently confirmed by Albihn and Savage[16] who showed that the bioavailability of soluble oxalate in oca (locally called yams in New Zealand and Australia; *Oxalis tuberosa*) was 1.44% in a six-hour bioavailability study. This fell to zero when sour cream (a source of available calcium) was fed with the standard serving of baked oca. The availability of soluble oxalate from tea was also significantly reduced when the tea was consumed with milk (a calcium source) when compared to the consumption of tea without milk.[17]

Finch *et al.*,[18] suggested that the higher fat content of oxalate containing foods, like peanuts and chocolates, could explain why the mean absorption of oxalate was relatively high for peanuts and chocolates, 8.5%, and 9.0% respectively compared to spinach, which has a very low fat content and had a mean absorption of oxalate of 1.5%. Binder[19] and Saunders *et al.*,[20] suggested that fats in the diet bind to calcium in the gastrointestinal tracts and hence less calcium is available to bind to soluble oxalate. More oxalate could then be absorbed into the body. This statement has been proven in a number of investigations where patients with steatorrhea form soaps made of gut luminal calcium and fatty acids, leaving less ionised calcium available to combine with soluble oxalate.[21] Rampton *et al.*,[22] reported a 5.8% oxalate absorption rate (as determined by oxalate output after an oral sodium oxalate loading) in normal persons compared to 14.7% in patients suffering from steatorrhea.

However, in contrast Liebman *et al.*,[11] noted that the addition of fat or olestra to a potato diet depressed the oxalate absorption (10.5 and 12.2% respectively) compared to the potato diet (13.2%). The decrease in oxalate absorption was most pronounced in the initial 4-6 hour period post oxalate ingestion confirming earlier observations that soluble oxalate is principally absorbed from the small intestine.

This study was carried out to measure the soluble and insoluble oxalate content of frozen spinach currently available in New Zealand and to determine the bioavailability of the soluble oxalate when consumed both with and without milk products.

Materials and Methods

Sample collection and basic analysis

Frozen spinach (*Spinacia oleracea*), Wattie's Chopped Spinach (Wattie's Frozen Foods, Auckland) was bought from a supermarket in Christchurch in July 2001 and stored at -18°C before sampling and analysis commenced. The same batch of frozen spinach was used in the feeding experiment. Dry matter content of the sample was determined using the AOAC method 925.10[24] and the calcium content was measured using an atomic absorption photospectrometer (Perkin-Elmer, USA).

Oxalate analysis

Extraction

The oxalate determinations of the grilled spinach were analysed in triplicate using the method outlined in detail by Savage *et al.*,[8] and based on an earlier method.[25] Two-gram samples were weighed into a 250mL conical flask. Approximately 75mL of 0.2M HCl (BDH, UK) for total oxalate analyses, or 75mL of nanopure water (NANOpureII, Barnstead, USA) for soluble oxalate, was added. The samples were then homogenized using a top-drive homogeniser for approximately 1 minute (Kinematica, Germany). The flasks were capped with foil and placed in a water bath at 80°C for 15 minutes and shaken periodically. The flasks were than cooled down in a 20°C water bath. After extraction, the samples were quantitatively transferred to 200mL volumetric flasks and made up to volume with water or acid and mixed. Forty mL of each sample were transferred to a 50mL Falcon tube and the samples were centrifuged at 3000 rpm for 15 minutes (Minifuge, Heraeus-Christ GMBH, Germany). 3-5 mL of the supernatant was removed by syringe and filtered through a 0.45μm cellulose nitrate filter (Sartorius, Goettingen, Germany) into a glass storage vial.

HPLC analysis

HPLC of extracted oxalic acid was carried out on a Rezex ROA 300 x 7.8 mm ion exchange column (Phenomenex, CA, USA). Mobile phase was 0.025 M sulphuric acid (BDH, UK). The equipment consisted of a ternary HPLC pump (Spectra-Physics, SP 8800, CA, USA), a UV/VIS detector (Spectra Physics, SP8450, CA, USA) set on 210 nm and a Cromatopac integrator (Shimadzu, C-R3A, Japan). Ten μL samples were injected onto the column and eluted at a flow rate of 0.60 mL/min. The oxalic acid peak eluted after approximately 8.7 minutes. The results were calculated as mg/100 WM.

Bioavailability assay

Ten healthy subjects were recruited from staff and students at Lincoln University, Canterbury, NZ to a randomised double crossover study. After informed consent was obtained, ten healthy volunteers, five women and five men (21-52 years old) participated in the study. Each volunteer was fed a serving of two grilled spinach sandwiches (a total of 100 g FW spinach) on five different occasions during a period of three weeks. Five different spinach sandwiches were prepared, spinach alone (Wattie's Chopped Spinach, Wattie's Frozen Foods, Auckland, 100g), spinach (100g) and sour cream (Meadow Fresh Foods, (Canterbury) Ltd, Christchurch, 100g) spinach (100g) and cottage cheese (Meadow Fresh Foods, (Canterbury), Ltd., Christchurch, 100g), spinach (100g), sour cream (100g) plus Calci-Trim milk™ (Mainland Products Ltd, Wellington, 180g) and finally spinach and olive oil (Lupi, Minerva Australia Ltd., 20g). The spinach sandwiches were prepared by placing the spinach on two pieces of toasted white bread and grilled in an oven for 10 minutes at 225°C.

After consuming the spinach sandwiches a total six-hour urine sample was commenced on each volunteer. A second container was given to each volunteer after six hours to collect the urine for the following eighteen hours. One day during the test period urine samples were collected from each volunteer without prior intake of spinach sandwiches to serve as a reference blank value. During the three weeks of the experiment the volunteers were asked to keep their normal diet, and not to increase or decrease their calcium intake and to avoid oxalate-rich foods like spinach, rhubarb, tea and beets, the day before each experiment and during the day of urine collection. They were also asked to avoid consuming extensive amounts of medium oxalate containing food such as peanuts, chocolate and strawberries.

Urine collection and sampling

Urine was collected into plastic containers and 10mL concentrated HCL was added to each sample immediately. The total volume of each container was measured and 100mL samples were taken and analysed by Canterbury Health Laboratories, Christchurch, N.Z., using a Sigma oxalate kit (No 591-D).

Bioavailability calculation

The bioavailability of oxalate was calculated as follows. For each subject the urinary oxalate output during the reference blank collection period was subtracted from the output following the consumption of the test meals. The result was expressed as a percentage of the oxalate consumed from the spinach over the same time period.

Statistical analysis

The data are presented as the mean of three determinations ± standard error. Significant differences in the mean output of oxalate in the urine were determined using a Generalised Linear Model Procedure (Minitab 13.1).

Results

The mean oxalate and calcium content of the frozen spinach is shown in Table 1. The dry matter content of the frozen spinach was 11.7 ± 0.7 (g/100g wet matter, WM), this rose to 15.5 ± 0.4 (g/100g WM) after grilling. The frozen spinach contained 90mg total calcium 100g WM, 76.7% of this calcium was unavailable as it was bound to oxalate (as insoluble oxalate). The oxalate/calcium (mEq) ratio of the frozen spinach was 4.73.

Six-hour collection

The mean urinary output of oxalate in 6 hours when no spinach was consumed was 6.8 ± 1.9mg (Table 2). The oxalate output increased significantly (*P*<0.01) when spinach and (*P*<0.05) spinach and cottage cheese and spinach and olive oil (*P*<0.001) were consumed. The mean urinary output of oxalate in 6 hours when the spinach was fed with sour cream plus Calci-Trim milk, and when the spinach was fed with sour cream alone, was not significantly different from the reference blank value.

Table 1. Mean oxalate and calcium content (mg/100g WM) of the frozen spinach

Oxalate (mg/100g WM)	
Total	956.7 ± 83.5
Soluble	736.6 ± 20.4
Insoluble	220.1 ± 96.5
Calcium (mg/100g WM)	
Total	90.0 ± 3.9
Available	21.0 ± 4.8
Bound to insoluble oxalate	69.0 ± 4.3

Table 2. Intake, excretion and absorption of oxalate (mg/6 hours) from spinach and spinach with three different calcium-containing ingredients and one containing an addition of only olive oil

Test meal	Mean oxalate intake ± SD (mg)	[1]Mean urinary oxalate ± SD (mg/6 hrs)	Mean urinary oxalate increase ± SD (mg/6 hrs)	[2]Mean bioavailability ± SD (% intake)
Reference blank	-	6.8 ± 1.9	-	-
Spinach	956.7 ± 83.5	14.0 ± 3.7**	7.21 ± 4.63	0.75 ± 0.48[ab]
Spinach and sour cream	956.7 ± 83.5	11.7 ± 4.2 ns	4.96 ± 4.33	0.52 ± 0.45[ab]
Spinach and cottage cheese	956.7 ± 83.5	15.3 ± 7.0*	8.58 ± 6.13	0.90 ± 0.64[a]
Spinach, sour cream and Calci-Trim milk	956.7 ± 83.5	8.1 ± 2.2 ns	1.34 ± 2.24	0.14 ± 0.23[b]
Spinach and olive oil	956.7 ± 83.5	16.0 ± 6.9***	9.25 ± 7.40	0.97 ± 0.77[a]

[1] Significant difference from reference blank value is shown by *P<0.05, **P<0.01 and ***P<0.001, ns = not significant.
[2] Data with different superscript letters (a,b) are significantly different (P<0.05), obtained by Tuckey Simultaneous Tests.

The mean bioavailability of soluble oxalate in the grilled spinach was 0.75 ± 0.48% and this value was not significantly different from the values obtained when spinach was fed with olive oil or the three calcium sources. The availability of the oxalate when spinach and sour cream and Calci-Trim milk was consumed was 0.14 ± 0.23 which was only significantly different (P<0.05) from the biovailability of the oxalate determined from the spinach and olive oil test meal and the spinach and cottage cheese test meal.

Twenty four-hour collection

The mean reference blank urinary output of oxalate in 24 hours was 23.2 ± 3.8mg this was significantly different (P<0.001) from the values obtained when spinach was fed alone, with cottage cheese and with olive oil (Table 3). The mean urinary output of oxalate was lowest when the spinach was fed with sour cream or sour cream and Calci-Trim milk and was not significantly different from the reference 24-hour value.

The mean bioavailability measured over 24 hours of soluble oxalate in the grilled spinach was 1.93 ± 0.85% which was not significantly different (P<0.05) from the spinach and cottage cheese and spinach and olive oil test meals. The mean bioavailability of the oxalate in the spinach fed with sour cream and Calci-Trim milk was significantly reduced (P<0.05) to 0.75 ± 0.57. The consumption of sour cream alone with spinach also reduced the availability (P<0.05) of the oxalate in the spinach.

Effect on calcium in the test meal

A significant proportion of the calcium in the grilled spinach was unavailable as 76.7% of this calcium was bound to insoluble oxalate. Another way to express this is to note the high oxalate/calcium (mEq) ratio 4.73 of the grilled spinach. The calculation of available calcium can be determined in the following way. One hundred g of spinach consumed by the volunteers contained 90mg of calcium and as the spinach contained 220.1mg of insoluble oxalate a total of 69mg of calcium was bound to the oxalate in the spinach, leaving 21mg of calcium available (Table 1).

This assumes that insoluble oxalate in spinach contains only calcium. In the case of the spinach and the spinach and olive oil test meals (Table 4) there was insufficient calcium in the test meal to bind to the soluble oxalate in the spinach (736.6 mg/100g WM). The spinach and sour cream and the spinach and cottage cheese test meals supplied more calcium, which could then bind to the soluble oxalate in the spinach. In the case of the test meal where 480mg calcium was supplied by the sour cream and Calci-Trim milk this supplied an excess of calcium, which could bind to the soluble oxalate in the spinach

Discussion

The total oxalate of the frozen spinach used in this experiment (956.7mg /100g WM) is much higher that that observed for fresh spinach by Savage *et al.*[8] but is within the range of values (330 to 992mg/ 100g WM) reported by a number of authors.[1,3-8, 26]

The mean bioavailability of the soluble oxalate in the grilled spinach in this 6-hour urine collection assay was 0.75 ± 0.48% which is low when compared to the reported value for the bioavailability of 2.4% after consuming 200g chopped raw or cooked spinach.[4] The bioavailability assay used by Brinkley *et al.*,[4] used an 8-hour collection period. In our experiment the bioavailability measured over a 24-hour period was 1.93 ± 0.85%. This finding indicates that even though grilled spinach contains a significant amount of soluble oxalate (736.6mg/ 100g WM) a very small proportion is absorbed into the body.

The most important results shown in this experiment is the effect of adding a calcium containing food to the test diet. Addition of 100g of sour cream and the addition of 100g sour cream plus Calci-Trim milk (180g) both reduced the availability of the soluble oxalate in the spinach significantly (P<0.05) in both the 6-hour and 24-hour collection periods. Addition of 100g sour cream alone also reduced the soluble oxalate availability, but this was only significant in the 24-hour collection period. The effect of adding a calcium source to a test meal containing soluble oxalate has also been recently shown to be very effective in reducing the amount of oxalate absorbed from oca.[16]

It is clear that combining spinach with a calcium-containing dairy product significantly reduces the output of oxalate in the urine in both the 0-6 hour and the 0-24 hour periods (Tables 2 and 3). It is thought that calcium in the dairy products and the free calcium in the spinach binds to the soluble oxalate in the spinach. The free calcium's oxalate binding capacity is shown in Table 4. Figure 1 shows the correlation (R^2 = 0.834) between increase in urinary oxalate excretion (mg/6 hours) and intake of available calcium (mg) in the test-meals.

Brinkley *et al.*,[27] suggested a rating of oxalate rich foods should be defined as increase in urinary oxalate (mg/load). The foods were rated as "high risk food item" (increase in urinary oxalate >20mg/load), "moderate risk food items" (3-5mg/load), "mild risk food item" (1-3mg/load) and "negligible risk food items"(<1mg/load). According to this rating of oxalate rich foods all results in the present study except the spinach and olive oil test meal (Table 3) fall in between the "high risk food item" and "moderate risk food item" groups. The spinach and olive oil test-meal falls into the "high risk food item", which is where Brinkley *et al.*,[27] placed spinach. Brinkley *et al.*,[27] did not have a definition for food items with an increase in urinary oxalate between 5-20mg/load, which seems a little inconsistent.

Consumption of spinach with olive oil did not change the output of urinary oxalate when compared with the spinach test meal in both the 6-hour and the 24-hour collection periods (Tables 2 and 3), which suggests that the fat content of a test meal had no effect on the absorption of oxalate from the digestive tract. These results conflict with the observation of Liebman *et al.*,[23] who showed that addition of fat or

Table 3. Intake, excretion and absorption of oxalate (mg/24 hours) from spinach and spinach with three different calcium containing ingredients and one containing an addition of only olive oil

Test meal	Mean oxalate intake ± SD (mg)	Mean urinary oxalate ± SD (mg/24 hrs)[1]	Mean urinary oxalate increase ± SD (mg/24 hrs)	Mean bioavailability ± SD (%intake)[2]
Reference blank	-	23.2 ± 3.8	-	-
Spinach	956.7 ± 83.5	41.7 ± 7.4 ***	18.4 ± 8.1	1.9 ± 0.8 [a]
Spinach and sour cream	956.7 ± 83.5	33.1 ± 9.7 ns	9.9 ± 10.2	1.0 ± 1.1 [b]
Spinach and cottage cheese	956.7 ± 83.5	38.4 ± 13.1 ***	15.1 ± 11.9	1.6 ± 1.2 [abc]
Spinach, sour cream and Calci-Trim milk	956.7 ± 83.5	30.4 ± 3.9 ns	7.2 ± 5.4	0.7 ± 0.6 [c]
Spinach and olive oil	956.7 ± 83.5	44.0 ± 9.5 ***	20.8 ± 8.0	2.2 ± 0.8 [a]

[1] Significant difference from reference blank value is shown by * for P<0.05, ** for P< 0.01 and *** for P<0.001, ns = not significant.
[2] Data with different superscript letters are significantly different (P<0.05), obtained by Tuckey Simultaneous Tests.

Table 4. Total calcium in the milk products, total calcium available in the test meals and how much of the soluble oxalate the calcium available could theoretically bind to (oxalate binding capacity)

Test meal	Total calcium in the milk product (mg)	Total Ca available (mg) (free calcium in the spinach and Ca from the dairy product)	Amount of oxalate that the total calcium available could bind to (mg)[1]
Reference blank	-	-	-
Spinach	-	21	47.2
Spinach and sour cream	117	138	309.9
Spinach and cottage cheese	80	101	226.8
Spinach, sour cream and Calci-Trim milk	480	501	1125.0
Spinach and olive oil	-	21	47.2

[1] 736.6 mg soluble oxalate in 100g WM of spinach.

olestra to a test meal depressed oxalate absorption. The present results also do not agree with the suggestion made by Finch *et al.*,[18] who observed that higher fat containing foods appeared to have higher relative absorption of oxalate when compared to spinach.

Overall these results imply that it would be difficult to ingest an excessive amount of oxalate from eating grilled spinach in a normal diet and the consumption of a high calcium containing food, such as sour cream, at the same time would cause little oxalate from the spinach to be absorbed. The results have a greater implication for the availability of calcium in the spinach, 76.7% of the total calcium in spinach is bound to oxalate as insoluble oxalate. The remaining calcium in spinach and calcium in any calcium containing foods consumed at the same time have the potential to be made unavailable by binding to the soluble oxalate released from the spinach as it is digested in the small intestine.

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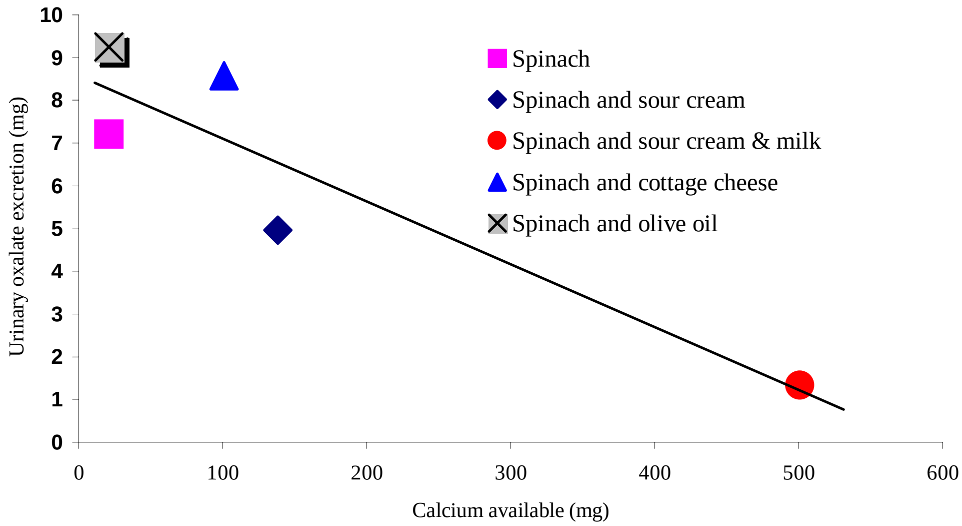

Figure 1. Relationship between mean urinary oxalate increase (mg/6 hours) and the intake of calcium available (mg) in the different test-meals

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