DOI:10.1021/jf902124w

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Oxalate Content of Silver Beet Leaves (*Beta vulgaris* var. *cicla*) at Different Stages of Maturation and the Effect of Cooking with Different Milk Sources

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The work presented here indicates that people who have a tendency to develop kidney stones should avoid consuming regrowth and developed silver beet (*Beta vulgaris* var. *cicla*) leaves. Soluble oxalate contents of leaves range from 58% of the total oxalate for the mature leaves up to 89% for the regrowth tissue, with regrowth tissue containing the highest levels of soluble oxalate at 7267 ± 307 mg/100 g of dry matter (DM). Leaves cooked in milk contained significantly ($p < 0.05$) lower levels of soluble oxalate compared to the leaves that were cooked in water. Leaves cooked in low fat milk contained significantly lower levels ($p < 0.05$) of soluble oxalate (1.9%) than leaves cooked in standard milk (5.3%) or cream (6.3%). To maximize the reduction of soluble oxalate during the cooking of high oxalate foods such as spinach and silver beet, a low fat milk cooking medium with neutral pH should be utilized.

KEYWORDS: Silver beet; soluble and insoluble oxalates; oxalic acid; maturity; baking; milk; cream

INTRODUCTION

Oxalic acid is an organic acid found in many higher plants, including a large variety of commonly consumed food plants (*1*). The anions of oxalic acid, as well as its salts and esters, are known collectively as oxalates. After consumption, insoluble oxalate salts largely pass through the digestive tract of humans without being absorbed and are either degraded by oxalate-degrading bacteria or excreted via the feces. Insoluble oxalates are therefore not so biologically significant. Soluble oxalates, however, are biologically significant and are absorbed in the digestive tract, and the oxalate anion ($C_2O_4^{2-}$) can bind to divalent minerals such as calcium, rendering the mineral unavailable. There are many foods known to contain naturally high levels of oxalates including spinach, rhubarb, beets, nuts, wheat bran and kiwifruit (*1*−*3*).

A diet high in soluble oxalates is widely known to be associated with an increased risk of developing kidney stones. Kidney stones are made up mainly of crystals of calcium oxalate, with a number of other cations identified as possible minor insoluble oxalate constituents. As foods of plant origin are the main source of dietary oxalate (*4*), an increase in fruit and vegetable consumption could be considered as a dietary risk factor for kidney stone formation. The amount of calcium oxalate excreted in the urine is a risk factor in the formation of kidney stones and it is thought that small increases in urinary oxalate concentration have a much greater effect on the supersaturation of urine with calcium oxalate than comparable changes in calcium (*5*). Therefore, people predisposed to forming kidney stones are recommended to minimize their intake of foods high in oxalates (*6*).

While there has been substantial research into the levels of oxalates in many foods, there has been only limited study of oxalates in silver beet (Swiss chard) (*Beta vulgaris* var. *cicla*). Silver beet is a common vegetable plant consumed throughout the temperate world. It is a native of the Mediterranean region and is commonly harvested in autumn in New Zealand, where this current study was conducted. As with many vegetables, silver beet leaf tissue contains significantly greater levels of oxalate than the leaf petioles (*3*). Awadalla (*7*) reported values of 756 mg total oxalate/100 g fresh weight (FW) (532 mg soluble oxalate/100 g FW), Santamaria et al. (*8*) found levels of 332 mg soluble oxalate/100 g FW, while Hönow and Hesse (*9*) reported a concentration range of 436−1614 mg total oxalate/100 g FW. Savage et al. (*3*) found that silver beet leaves grown in New Zealand contained 526 mg total oxalate/100 g FW (252 mg soluble oxalate/100 g FW). Subsequent work by Savage et al. (*10*) revealed that there were no significant differences in oxalate levels for three different colored varieties of silver beet, with overall means of 792 and 350 mg total and soluble oxalate/100 g FW, respectively. It is clear that the oxalate levels in silver beet are highly variable, which is consistent with other plants (*1*).

As mentioned above, the soluble oxalate content of food is biologically important and a diet high in soluble oxalate is correlated to an increased likelihood of developing kidney stones in susceptible individuals. Boiling high oxalate foods decreases soluble oxalate content by leaching (the cooking water is not usually consumed) (*3*, *7*, *10*). Furthermore, the amount of soluble oxalate was greatly decreased in spinach (*Spinacia oleracea*) when consumed with dairy products such as sour cream or milk (*11*). Similarly, when tea (*Camellia sinensis*) was consumed with milk, the level of urinary oxalate excretion in the period after consumption was shown to be markedly reduced in comparison to tea consumed without milk (*12*). Taro baked in cow's milk or coconut milk also showed significantly reduced levels of soluble oxalate compared to conventionally baked taro (*13*).

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 Published on Web 10/30/2009

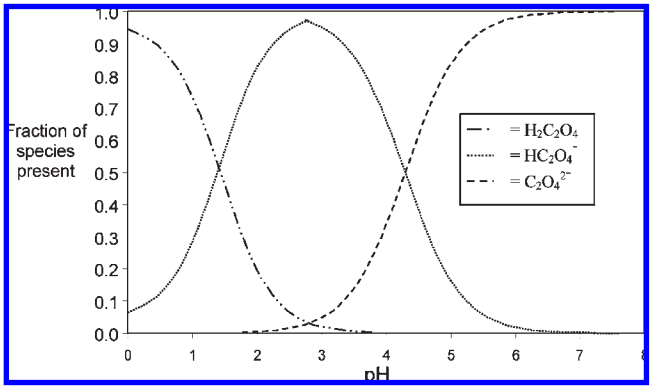

Figure 1. Speciation of oxalate at different pH values.

These observations are thought to be due to the binding of soluble oxalate to calcium, Ca^{2+}, forming insoluble calcium oxalate, CaC_2O_4, that results in the mineral becoming unavailable and inhibiting the absorption of the oxalate ion (*14*). The pH of the cooking media will also have a significant effect on the amount of soluble oxalate that will bind to any available cationic minerals. This is due to the oxalate ion becoming increasingly protonated as pH decreases. From the pH speciation diagram for oxalic acid (**Figure 1**), it is clear that at pH less than six the proportion of fully deprotonated divalent oxalate ion ($C_2O_4^{2-}$) decreases markedly with a correspondingly reduced potential for binding with divalent mineral cations (especially Ca^{2+}) to form insoluble oxalates. Therefore, cooking media containing divalent mineral cations such as Ca^{2+}, intended to decrease soluble oxalate, should have a relatively neutral pH to ensure maximum $C_2O_4^{2-}$ availability. Interestingly, it has been shown that not all soluble oxalate is bound even when high oxalate foods are cooked in media that contain sufficient Ca^{2+} to bind all the available soluble oxalate. Radek and Savage (*15*) determined the levels of soluble oxalate in spinach, purple and green amaranth and colocasia leaves using an *in vitro* digestion method (*16*). Although sufficient Ca^{2+} was added to bind all the soluble oxalate present in the different food materials investigated, there were still relatively large (although significantly reduced) quantities of intestinal soluble oxalate remaining after the addition of the Ca^{2+}. These results are difficult to interpret although it was suggested that the composition of each sample may affect the ability of soluble oxalate to bind to mineral cations such as Ca^{2+} (*15*). The effect of pH clearly cannot be ruled out as a significant factor in this process.

It has been reported that the proximal small intestine is the main site of oxalate absorption in humans (*17*, *18*), as it has been conclusively shown that oxalate absorption peaks 1−6 h after the consumption of oxalate-containing foods in healthy subjects (*19*). The acidic pH of the stomach will result in the solubilization of insoluble oxalates as they pass through into the small intestine. However, the solubilized oxalate will potentially rebind to Ca^{2+} ions (and possibly others such as Mg^{2+} and Fe^{2+}) to form insoluble salts in the alkaline pH of the small intestine. This suggests that some cationic minerals consumed with oxalate-containing foods may benefit from being able to bind to oxalate in the digestive tract, render the oxalate insoluble, and prevent its absorption in the small intestine.

This current work had several objectives. The first objective was to quantify the total and soluble oxalate levels in silver beet during four stages of growth and to determine whether harvest date had an effect on the oxalate content of the regrowth tissue. The second objective was to determine the content of various mineral cations (especially Ca^{2+}) in the silver beet during the four growth stages. The final objective was to assess the ability of milk products to reduce the soluble oxalate content of cooked silver beet and to compare this with cooking in water. To the best of our knowledge this is the first study investigating how oxalate concentration varies in plant tissue and how harvesting affects subsequent oxalate synthesis in the regrowing leaves.

This study will follow the total, soluble and insoluble oxalate content of silver beet leaves as the leaves mature and will provide information about the risk of eating silver beet leaves harvested at different growth stages with and without the addition of different milk sources.

MATERIALS AND METHODS

Plant Growth. Silver beet seeds (*Beta vulgaris* var. *cicla*) (cultivar Fordhook giant, Egmont Seed Company Ltd., New Plymouth, New Zealand) were sown in seed trays in a medium comprising 30 L of peat (Kiwipeat Sphagnum peat moss, Ravensdown Growing Media, Ltd., Christchurch, New Zealand) and 20 L of sterilized pumice (Egmont Seed Company, Ltd., New Plymouth, New Zealand, 1−3 mm grade) on the 18th of October, 2006. The growing medium was fertilized with 75 g of Osmocote Exact (Scotts Miracle-Gro Company, Marysville, OH, N:P:K = 16:3:9), 200 g of Dolomite (Ravensdown Fertiliser Co-operative, Ltd., Christchurch, New Zealand) and 50 g of Hydraflo (Scotts Miracle-Gro Company, Marysville, OH). The plants were grown for five weeks in a glasshouse, and water was applied to the seed trays daily. The seedlings were planted out in the field on the 23rd of November, 2006 (plant age 36 days), in six rows, with the plants at the end of each row and the outside rows serving as guard plants. The plants were grown in Wakanui silt loam soil at the Horticulture Research Area, Lincoln University, Canterbury, New Zealand (43°38′S, 172°27′E) at 19 m above sea level. The plots were irrigated as required throughout the growing period.

Harvest and Sample Preparation. In this experiment there were eight randomized plots of silver beet plants containing 10 plants each. On the 20th of February the plants were harvested from four plots by cutting the plants at ground level and the leaves were divided into two groups, young leaves (≤200 mm long) and developed leaves (≥200 mm long). On the 17th of April regrowth leaves (≤200 mm long) from the plots previously harvested on the 20th of February were harvested. Mature leaves were harvested on the 19th June from the four plots that had not been previously harvested. Following each harvest, the leaf petioles were removed and dry weights determined by drying to constant weight in an oven at 105 °C for 24 h (AOAC, official method 925.10) (*20*). The dried samples were then ground to a fine powder in a multigrinder (Sunbeam coffee mill, L.V. Martin & Son, Newtown, Wellington, New Zealand) prior to HPLC analysis. The pH of the homogenized leaf tissues was determined using a Metrohm 670 Titroprocessor (Metrohm, Herisau, Switzerland).

Additional samples of mature leaves harvested from each of the four plots on the 19th June were divided into 100 g portions and placed into Pyrex bowls (Corning, Reston, VA) with either 300 mL of tap water, low fat milk, standard milk or cream (Meadow Fresh, Goodman, Fielder New Zealand Ltd., Ellerslie, Auckland, New Zealand) (each contained 115 mg of Ca^{2+}/100 mL besides the tap water that contained no added Ca^{2+}). The Pyrex bowls were covered with aluminum foil and fan baked in a preheated oven for 60 min at 220 °C (domestic oven, Simpson Gemini, Australia). The samples were drained in a sieve for one minute to remove excess cooking medium. The samples were then dried at 105 °C and homogenized (as above) prior to analysis.

Total and Soluble Oxalate Extraction and Analysis. To determine total oxalate, one gram samples of finely ground silver beet were weighed into 125 mL flasks and 40 mL of 0.2 M HCl was added. The beakers were placed in a water bath at 80 °C for 15 min. The extract was allowed to cool and then transferred quantitatively to a 100 mL volumetric flask and made up to volume with 0.2 M HCl. A 45 mL aliquot of each extract was centrifuged at 2889 RCF (Varifuge 3.0R, Heraeus, Hanau, Germany) for 15 min before the supernatant was filtered through a 0.45 μm cellulose nitrate filter. To determine the soluble oxalate content the process was repeated, as above, with Nanopure II water (Barnstead International, Dubuque, IA) used instead of 0.2 M HCl. Insoluble oxalate was calculated by subtraction of the soluble from the total (*21*), and bound calcium was calculated assuming that all the insoluble oxalate was calcium oxalate. The chromatographic separation was carried out at room temperature

Table 1. Total and Soluble Oxalate with the Total and Bound Calcium Contents of Silver Beet Leaves of Different Ages (mg/100 g DM ± SE), $n = 4$[a]

			oxalate			calcium	
leaf age	pH	dry matter (%)	total	soluble (% of total)	insoluble	total	bound (% of total)
young	6.0 ± 0.3	10.7 ± 0.2	2993 ± 26 a	2071 ± 31 a (69%)	921 ± 28 a	1103 ± 239 ac	288 ± 8 a (26 abc)
developed	5.7 ± 0.1	10.5 ± 0.6	10408 ± 354 c	6754 ± 361 b (65%)	3654 ± 221 b	2148 ± 39 b	1143 ± 100 b (53 b)
regrowth	4.7 ± 0.2	10.7 ± 0.3	8193 ± 281 b	7267 ± 307 b (89%)	1048 ± 184 a	557 ± 78 a	289 ± 41 a (59 b)
mature	5.2 ± 0.1	10.1 ± 0.2	2441 ± 253 a	1416 ± 103 c (58%)	1025 ± 166 a	1616 ± 106 c	321 ± 94 a (20 c)

[a] Means sharing the same letter in each column are not significantly different ($P < 0.05$).

using a 300 mm × 7.8 mm Rezex ion exclusion column (Phenomenex Inc., Torrance, CA) attached to a cation H^+ guard column (Bio-Rad, Richmond, CA) and a ternary Spectra-Physics, SP 8800 HPLC pump (Spectra-Physics, San Jose, CA). The equipment consisted of an autosampler (Hitachi AS-2000, Hitachi Ltd., Kyoto, Japan) and a UV/vis detector Spectra-Physics SP8450 (Spectra- Physics, San Jose, CA) set on 210 nm. Data capture was facilitated via a PeakSimple chromatography data system (SRI model 203, SRI Instruments, CA) and data was processed using PeakSimple version 3.54 (SRI Instruments, Torrance, CA). The column mobile phase was an aqueous solution of 25 mM H_2SO_4. Samples (20 μL) were injected onto the column and eluted at a flow rate of 0.6 mL/min. The extraction and analysis were carried out in triplicate for each treatment/sample.

Preparation of Standards. Standards of oxalic acid (Sigma-Aldrich Co., St Louis, MO) were made up in the following concentrations: 1.0, 5.0, 10.0, 20.0, 40.0, 50.0, and 100.0 mg/100 mL and made up in either 0.2 M HCl (Aristar, BDH Chemicals, Ltd., Poole, Dorset, U.K.) or Nanopure II water (Barnstead International, Dubuque, IA) and then filtered through a 0.45 μm cellulose nitrate filter (Sartorius AG, Gottingen, Germany) into a 1 mL HPLC auto sample vial.

Recovery Study. The recoveries of pure oxalic acid (Sigma-Aldrich Co., St Louis, MO) were studied by adding 50 mg of oxalate to each of the water and acid extractions. The samples were extracted and measured using the method described above, and the results were compared with samples with no oxalic acid added. The analysis was carried out in quadruplicate. The mean recovery for the water and acid extractions was 94.8 ± 0.4 and 93.6 ± 0.7% respectively.

Mineral Determination. Finely ground freeze-dried silver beet leaves (0.5 g) were weighed into 100 mL Teflon microwave digestion vessels. Five milliliters of 69% nitric acid (Aristar, BDH Chemicals, Ltd., Poole, Dorset, U.K.) and 2 mL of 30% hydrogen peroxide (AnalaR, BDH Chemicals, Ltd., Poole, Dorset, U.K.) were added, and the digestion vessels were allowed to stand for 12 h at room temperature. The samples were then digested for 40 min in a microwave digester (Milestone Ethos Sel microwave oven, Sorisole, Italy) with the temperature rising to 200 °C at the end of the digestion cycle. The digestion vessels were then cooled and the digested solution was made up to 25 mL with Nanopure II water. The mineral content was determined by aspirating the diluted sample into an inductively coupled plasma optical emission spectrometer (ICP-OES, Varian Inc., Mulgrave, Victoria, Australia) through an ultrasonic nebulizer (Cetac 5000, Varian Inc., Mulgrave, Victoria, Australia). The Varian ICP was calibrated using an ICP multielement standard solution (Merck & Co. Inc., Whitehouse Station, NJ).

Oxalate Speciation Analyses. Solubility products (K_{sp}) were obtained (*22*) for Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} oxalate salts and compared with the total concentrations of these cations, as measured by ICP-OES analysis, to determine the likely constituents of the insoluble oxalate present in the leaf tissue.

Statistical Analyses. Statistical analyses were performed using the Genstat Tenth Edition software (Lawes Agricultural Trust, Rothamstead, Harpenden, U.K.). To assess the effect of age and cooking treatments on oxalate concentration, the data were analyzed as a four treatment randomized complete block design. Treatment means were compared using a *t* test, and differences were concluded as significant when $p < 0.05$.

RESULTS AND DISCUSSION

The total oxalate contents of the silver beet leaves in this study ranged from 2441 to 10408 mg/100 g dry weight (DW), while the soluble and insoluble contents ranged from 2071 to 7267 and from 921 to 3654 mg/100 g DW, respectively (**Table 1**). The results from this study are within the range of previous data for silver beet reported elsewhere (*3*, *7*−*10*).

The young and mature leaves contained significantly ($p < 0.05$) lower levels of total oxalate than the regrowth and developed leaves, while the developed leaves contained significantly ($p < 0.05$) higher levels of total oxalate than the regrowth (**Table 1**). The regrowth and developed leaves also contained the highest quantities of soluble oxalate, and while the young leaves had significantly less soluble oxalate than both regrowth and developed leaves, the mature leaves contained the least soluble oxalate of all the leaf ages. It is noteworthy that the total oxalate content of the mature leaves was significantly ($p < 0.05$) lower (2441 ± 253 mg oxalate/100 g DM) than in the developed leaves (10408 ± 354 mg oxalate/100 g DM), suggesting that oxalate could have degraded as the leaves matured or that an increase in plant tissue reduced the overall oxalate content of the leaves. Active cycling of oxalates has been proposed by Keates et al. (*23*), whereby oxalate crystal idioblasts were observed in the meristematic tissues of *Pistia stratiotes*. Meristematic tissue is embryonic growth tissue at the tips of roots and stems that can divide into new cells. It was proposed that the presence of oxalate crystal idioblasts in meristematic tissues indicates that calcium oxalate may not be a metabolic end product as previously thought (*23*), although this has not yet been substantiated. It has also been proposed that the degradation of oxalates to carbon dioxide and hydrogen peroxide may be catalyzed by oxalate oxidase in rice bran (*24*), although the presence of this enzyme in silver beet leaves has not been established.

The high total and soluble oxalate content of the regrowth leaves may have been due to the stress exerted on the plant as a result of the preceding harvest. It has been suggested that one of the primary roles of oxalate in plants is as a defense mechanism against herbivory, in response to environmental stresses (*25*). The results presented here suggest that developed silver beet leaves and those that have regrown after harvest pose more of a risk to people who have a tendency to form kidney stones. These people would be safer consuming either young or mature silver beet leaves. The insoluble oxalate contents of the young, regrowth and mature leaves were not significantly different from each other, while the developed leaves contained significantly higher levels (**Table 1**). The calculated % calcium bound in the insoluble oxalate fraction ranged from 20 to 59% of the total oxalate content of the leaf tissue. Overall this suggests that silver beet leaves are not a good source of calcium in the diet.

The Ca^{2+} contents of the young and mature leaves were not significantly different, while the developed leaves contained significantly more Ca^{2+} than the other three leaf ages. In addition, although the regrowth leaves did not contain significantly less Ca^{2+} than the young leaves, their Ca^{2+} content was significantly less than for the developed and mature leaves. The Ca^{2+} content may relate to the level of soluble oxalate in the leaves. The soluble oxalate content of the developed leaves was slightly lower than for the regrowth (although not significantly different), even though the total oxalate of the developed leaves was relatively higher than

Table 2. Total, Soluble and Insoluble Oxalates and Bound Calcium Contents of Silver Beet Leaves Cooked in Four Different Media (mg/100 g DM ± SE), $n = 4$[a]

			oxalate			calcium	
cooking media	pH	dry matter (%)	total	soluble (% of total)	insoluble	total	bound (% of total)
tap water	4.9 ± 0.1	10.2 ± 0.07	1783 ± 187 a	342 ± 48 a (19.2%)	1370 ± 205 a	1360 ± 106	429 ± 69 (32)
low fat milk	4.8 ± 0.1	12.3 ± 0.12	1801 ± 91 a	35 ± 7 c (1.9%)	1753 ± 95 a	1310 ± 171	549 ± 29 (42)
standard milk	5.5 ± 0.2	15.5 ± 0.24	1912 ± 36 a	102 ± 13 b (5.3%)	1810 ± 31 a	1380 ± 173	566 ± 9 (41)
cream	5.2 ± 0.1	26.6 ± 0.16	1890 ± 125 a	119 ± 41 b (6.3%)	1771 ± 139 a	1120 ± 162	554 ± 46 (49)

[a] Means sharing the same letter in each column are not significantly different ($P < 0.05$).

Table 3. Selected Cations in Silver Beet Leaf Tissue (mmol/100 g DM ± SE), $n = 4$

leaf age	Ca	Mg	Fe	Mn	Zn	Cu
young	27.6 ± 6.0	11.9 ± 2.7	0.22 ± 0.02	0.09 ± 0.02	0.05 ± 0.01	0.16 ± 0.02
developed	53.7 ± 1.0	26.5 ± 3.9	0.23 ± 0.08	0.18 ± 0.02	0.06 ± 0.01	0.18 ± 0.03
regrowth	13.9 ± 2.0	11.5 ± 1.0	0.20 ± 0.04	0.07 ± 0.01	0.07 ± 0.01	0.29 ± 0.02
mature	40.4 ± 2.7	21.2 ± 1.2	0.18 ± 0.02	0.15 ± 0.02	0.05 ± 0.01	0.20 ± 0.01

for the regrowth leaves. The low level of Ca^{2+} in the regrowth leaves, that could potentially form insoluble calcium oxalate, could account for the relatively high concentration of soluble oxalate in these tissues. The pH of the regrowth leaves (pH 4.7) (**Table 1**) may also have added to this effect by reducing the divalent oxalate ion ($C_2O_4^{2-}$), concentration and, hence, ability to form insoluble CaC_2O_4 in the leaf tissue. From **Figure 1**, it can be seen that the proportion of the total oxalate present as the divalent $C_2O_4^{2-}$ ion at pH 4.7 is approximately 40% lower than at pH 6.

Baking the silver beet leaves in tap water for 60 min at 220 °C resulted in a reduction of soluble oxalate from 58% of the total oxalate in the raw leaves to 19.2% of the total oxalate in the cooked leaves (**Table 2**) as a proportion of the soluble oxalates were leached into the cooking water and discarded. The total oxalate contents in leaves cooked in water, low fat and standard milks and cream were not significantly different from each other. The pH values of these three treatments ranged from 4.8 to 5.6, but they were not significantly different from each other. However, the soluble oxalate contents of leaves cooked in the three milk media were significantly lower relative to the leaves cooked in water. Low fat milk had the greatest lowering effect on the soluble oxalate of the leaves, followed by standard milk and then cream. This suggests that low fat milk is a more effective cooking medium for lowering soluble oxalate content than milk products with higher fat content such as standard milk and cream. The Ca^{2+} contents of the low fat, standard milk and cream were equal at 115 mg/100 mL. It is known that dietary fat may bind enteric Ca^{2+} in the small intestine and limit its ability to form CaC_2O_4 (*26*). This effect may be responsible for the slightly lower soluble oxalate binding capacities of the cream and standard milk (35% and 3.3% fat) relative to low fat milk (0.5%). During cooking, Ca^{2+} could have bound to the triacylglycerides which were relatively more abundant in the cream and standard milk. This would reduce the amount of Ca^{2+} that could potentially bind to soluble oxalate and would result in a greater quantity of soluble oxalate remaining in the leaf tissue after cooking.

The results of these cooking trials agree with previous work showing that cooking high oxalate foods in water (when the cooking water was discarded), cow's milk, coconut milk, sour cream and various forms of inorganic Ca^{2+} was an effective method of decreasing soluble oxalate (*3*, *7*, *10*, *12*, *13*, *15*). However, this is the first study that has shown that milk fat can inhibit the formation of calcium oxalate during cooking of high oxalate foods. Low fat milk products are therefore a superior option for reducing the soluble oxalate contents of foods during cooking.

Interestingly, not all the soluble oxalate was bound to Ca^{2+}, even when the leaves were cooked in media containing excess levels of Ca^{2+} (**Table 2**). This effect was also observed by Radek and Savage (*15*), who showed that not all soluble oxalate was bound even when excess Ca^{2+} was added to spinach, purple and green amaranth and colocasia. This effect has not yet been explained in any previous literature, but it may be possible that compartmentalization of soluble oxalate occurred allowing some oxalate to remain in the soluble form. **Table 2** also shows that 32 to 49% of the total Ca^{2+} was bound as calcium oxalate in the cooked leaves compared to 20% in the original raw leaves. This suggests that even when the leaves were cooked, some of the soluble oxalate remained compartmentalized from the available Ca^{2+}, therefore, further work needs to be done to identify ways of allowing more soluble oxalate to bind to Ca^{2+} during cooking. This could include such practices as maceration of leaf tissue prior to cooking and mixing of the milk media and leaf tissue during the cooking process.

In evaluating the dominant oxalate salt making up the insoluble oxalate content of the four leaf ages, calcium (Ca^{2+}), magnesium (Mg^{2+}), iron (Fe^{2+}), manganese (Mn^{2+}), zinc (Zn^{2+}), and copper (Cu^{2+}) oxalates were considered as possible constituents. However, metals that form highly insoluble oxalate salts such as Zn^{2+} and Cu^{2+} were not considered relevant for oxalate salt formation here due to the very low concentration of these metals in the leaf tissue (**Table 3**). The same applies to the slightly more soluble Fe^{2+} and Mn^{2+} oxalates since these metals were also present in very low concentrations in the leaf tissue (**Table 3**). Even if all the Fe in the plant tissue had been present as Fe^{3+} and was chelated with oxalate as the very stable $[Fe(C_2O_4)_3]^{4-}$ complex, the reduction in the concentration of oxalate available to precipitate with Ca^{2+} would have been inconsequential. In theory, when the molar ion product (IP) of the cation and divalent ($C_2O_4^{2-}$) oxalate ion concentrations (e.g., $[Ca^{2+}] \times [C_2O_4^{2-}]$) exceeds the K_{sp} of the oxalate salt, the oxalate salt should precipitate. Precipitation will continue until the actual IP $\leq K_{sp}$. The solubility constants (K_{sp}) shown in **Table 4** indicate that Ca^{2+} oxalate is the least soluble of the six salts ($K_{sp} = 2.7 \times 10^{-9}$). This means that in an oxalate solution containing all six of these cations, the IP for CaC_2O_4 will more likely exceed its K_{sp} than is the case for the other sparingly soluble oxalate salts. Consequently, calcium oxalate, CaC_2O_4, would precipitate from the solution more readily than other possible oxalate species. Zinc oxalate, ZnC_2O_4, has the next lowest K_{sp} (1.7×10^{-9}), and, therefore in the event that the available oxalate concentration exceeded the level of free Ca^{2+}, zinc oxalate would be the next oxalate species to precipitate. From the total ion ICP-OES

Table 4. The Solubility Product Constants (K_{sp}) of the Oxalate Salts Considered as Possible Constituents of the Insoluble Oxalate Portion of Silver Beet

oxalate species	K_{sp}
calcium oxalate	2.7×10^{-9}
magnesium oxalate	2.2×10^{-5}
iron(II) oxalate	1.5×10^{-6}
manganese oxalate	3.4×10^{-5}
zinc oxalate	1.7×10^{-9}
copper oxalate	1.7×10^{-7}

analyses available (**Table 3**), only the calcium concentrations were judged high enough within the leaf tissue for the IP with total oxalate to exceed the K_{sp} of the salt. CaC_2O_4 was the only insoluble oxalate salt likely to be formed in any appreciable amount. **Table 3** shows that the total level of Ca^{2+} in all four leaf ages was always greater than the levels of calcium present as "bound" CaC_2O_4 (bound calcium ranged from 20 to 59% of the total (**Table 1**). The relatively lower K_{sp} of calcium oxalate (compared to the other five oxalate species considered) and the high concentration of calcium relative to the other mineral cations confirm that the insoluble oxalate was predominantly calcium oxalate.

This study has shown that the harvesting of high oxalate species such as silver beet results in increased biosynthesis of oxalates in the regrowth leaf tissue. Therefore, it would be appropriate to recommend that people who have a tendency to form kidney stones should avoid eating the leaf tissue of recently pruned silver beet. Further work needs to be carried out to determine if this effect is also observed in other high oxalate food plants such as spinach and rhubarb. Developed leaves contained the second highest level of soluble oxalate of all the leaf groups, and therefore kidney stone formers should also avoid consuming mature silver beet leaves. Interestingly, the oxalate levels in the mature leaves decreased; this suggests that oxalates may not be an end product of plant metabolism as has been proposed previously. When cooking silver beet and other high oxalate foods to reduce soluble oxalate content, a cooking medium with a neutral pH and minimal milk fat content should be used. This is the first study demonstrating that the soluble oxalate binding capacity of milk products is affected by milk fat content.

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Received for review June 20, 2009. Revised manuscript received September 17, 2009. Accepted October 01, 2009.