# Oxalate Content of Stir Fried Silver Beet Leaves (*Beta Vulgaris* Var. Cicla) with and without Additions of Yoghurt

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**Abstract**

Total and soluble oxalic acids were extracted and analysed by HPLC chromatography following Asian cooking methods, which involved soaking, boiling and stir frying of silver beet (*Beta vulgaris* var. cicla) leaves. Autumn-grown silver beet leaves contained 1658 ± 114 mg/100 g dry matter (DM) of total oxalates, 954 ± 49 mg/100 g DM of soluble oxalates and 704 ± 98 mg/ 100 g DM insoluble oxalates. Soaking and boiling before stir frying reduced the soluble oxalate contents to a mean of 455 mg/100 g DM. Addition of standard or low fat yoghurt following the pre-treatments of soaking, boiling, stir frying and soaking, boiling and stir frying further reduced the soluble oxalate content to a mean of 190.8 ± 49.8 and 227.5. ± 47.0, respectively, for the standard and low fat yoghurt mixes.

**Keywords:** Silver beet, Swiss chard, Total, Soluble and insoluble oxalates, Calcium, Yoghurt

## 1. Introduction

A number of commonly eaten foods such as silver beet, spinach, rhubarb, nuts, multi-grain flours, chocolate, black tea and parsley contain high levels of total and soluble oxalate (Zarembski & Hodgkinson, 1962; Fasett, 1973; Brinkely, McGuire, Gregory, & Pak 1981; Noonan & Savage, 1999: Savage, Vanhanen, Mason, & Bush, 2000; Hönow & Hesse, 2002; Chai & Liebman, 2005; Siener, Hönow, Seidler, Voss, & Hesse, 2006). Brogen and Savage, (2003) showed that many green leafy vegetables commonly grown in the Indian sub-continent such as spinach, purple and green amaranth and colocasia, also contain very high levels of oxalates, while a range of other commonly eaten vegetables and spices such as coriander, curry leaves, dill and fenugreek contain moderate levels of oxalates. Savage et al. (2000) reported values for a range of other common European vegetables that were usually cooked before eating. In contrast, salad mixes, which included young leaves of green beet, spinach and red chard, also contained high levels of oxalates but were eaten raw (Jèhanno and Savage, 2009). It was fortunate that many mature leafy plant foods which contained high levels of oxalates require cooking to improve flavour, taste and palatability before serving. Oxalate levels in these vegetables were considerably reduced when these foods were boiled (Savage et al. 2000). Pre-treatment, such as soaking in cold water, can reduce the oxalate content of raw leaves. Soaking taro leaves for different time periods showed a 6% reduction in soluble oxalate content after 30 min and a 26% reduction following an 18 hour soak (Savage and Dubois, 2006). Soaking vegetables before cooking is a common practice in Asian style cooking. Pacific Islanders also soaked taro corms and leaves prior to cooking (Mårtensson and Savage, 2008).

Cooking treatments can significantly alter the oxalate concentration in the final product (Savage et al.2000; Savage and Dubois, 2006). Chai and Liebman (2005) clearly demonstrated that the soluble oxalate contents of a range of common vegetables could be effectively reduced by boiling and steaming. Soluble oxalate contents of silver beet and spinach leaves were reduced by 84-87% after boiling and 42-46% after steaming. Essentially, soluble oxalate leached into the boiling water (Savage et al., 2000) but oxalate can also be lost during steaming in the hot water dripping from the food. In contrast, baking and drying tended to concentrate the oxalate content in the plant tissues due to moisture loss (Noonan and Savage, 1999).

There was some variation in the parts of plants cooked and eaten by people in different countries. For example, silver beet (*Beta vulgaris* var. cicla) (also known as Swiss chard) leaves were more commonly eaten in the Mediterranean region while the stems are favoured by people in Northern Europe (Kiple and Kreimhild, 2000). New Zealanders preferred to boil the leaves while the stems were boiled and served in a white sauce that contained dairy products (Savage et al. 2004). Silver beet leaves contain higher soluble oxalates, 117 mg/100 g fresh weight (FW) than the stems (19 mg/100 g FW), even after considerable amount*s* of oxalates had been lost during boiling (Savage et al. 2000). The oxalate content of the leaves may depend on the growing conditions, type of soil, season and time of harvesting (Hodgkinson, 1977). Recently published values (Simpson et al., 2009) for total oxalate content of silver beet leaves ranged from 436 to 1614 mg total oxalate/100 g FW. Young leaves contained significantly lower levels of oxalates than mature leaves. Re-growth tissue contained higher levels of soluble oxalate than mature leaves and ranged from 58% of total oxalate for the mature leaves up to 89% for re-growth tissue (Simpson et al., 2009). Raw silver beet leaves contained 792.7 ± 22.9 mg oxalate/100 g FW and 350.0 ± 24.1 mg of soluble oxalate/100 g FW (Savage et al*.* 2004). Siener et al. (2006) reported the oxalate content in raw silver beet leaves grown in Europe to be 874 mg/100 g total oxalate FW and 327 mg/100 g FW for total and soluble oxalate, respectively.

The soluble oxalate content of foods can also be reduced by the addition of foods containing high levels of soluble calcium. Examples include adding sour cream to baked yams, adding milk to cooked taro leaves and serving cooked spinach with milk, cream or cottage cheese and consuming ice cream with rhubarb (Savage, 2002). Albihn and Savage (2001) showed that 100 g baked oca (containing 403.4 mg total oxalate) consumed with 100 g of sour cream effectively reduced the urinary oxalate to zero which strongly suggests that no soluble oxalate was absorbed from the baked oca. Oscarsson and Savage (2007) showed that baking taro leaves with milk reduced the soluble oxalate three-fold compared to the raw leaves (72.4 to 23.7%). More recent studies carried out by Simpson, Savage, Sherlock and Vanhanen*,* (2009) confirmed that addition of standard milk, low fat milk and cream to boiled silver beet leaves were each very effective at reducing the soluble oxalate content of the final mix. It was interesting to note that low fat milk (0.5% fat) had the same levels of calcium as the other two milk sources but was more effective at reducing the total soluble oxalate content. In contrast, there appeared to be little or no correlation between fat addition in a diet and oxalate absorption in the experiments carried out by Brogen and Savage (2003) or Mårtensson and Savage (2008).

This experiment sets out to investigate the effects of an Asian method of cooking silver beet which involves soaking, boiling and then stir-frying in a wok followed by the addition of standard and low fat yoghurt to identify the most effective way to reduce the soluble oxalate content of silver beet leaves.

## 2. Materials and Methods

### *2.1 Harvesting and cooking*

Silver beet seeds (*Beta vulgaris* var. cicla), cultivar Fordhook giant, were sown in the early summer (November, 2009) in Wakanui silt loam in plots in the Horticulture Research Area at Lincoln University, Canterbury, New Zealand (43°38'52.88"S, 172°27'31.40"E), 19 m above sea level. The soil was fertilised with chicken manure prior to sowing the seeds and the plots were irrigated as required throughout the growing period. In late autumn, early May 2009, mature silver beet plants (height between 200-300 mm) were harvested and the mid-veins and petioles were removed. The leaves were shredded into small pieces then divided into one of four processes, soaking, boiling, stir frying, stir frying after soaking and boiling, each process was carried out in quadruplicate.

Shredded silver beet leaves were soaked in tap water at 12˚C for 30 minutes or boiled in tap water for 2 minutes, allowed to stand for 1 minute, then drained and subdivided into 115 g serving sizes (the amount that would fit into a 200 ml cup). Single servings of raw, soaked or boiled silver beet leaves were then stir-fried for 2 minutes in a wok (model: EW 30 Breville Health Smart Wok, Australia) at 200°C together with 28 ml of canola oil (Pam's Salad and Cooking Oil, Pam's Products Ltd., Mt. Roskill, Auckland, NZ) with constant stirring. The cooked silver beet leaves were allowed to cool in the wok for 1 minute and then transferred into individual plastic containers and stored deep frozen at -20°C. Four separate samples were prepared for each pre-treatment method and standard and low fat yoghurt additions.

*2.2 Preparation of yoghurt*

Both standard yoghurt (EasiYo´s Real Base & Culture, Natural, 3.6% oil; EasiYo Products Ltd., Albany, North Shore, Auckland, NZ) and low fat yoghurt powders (EasiYo´s Real Base & Culture, Low Fat Greek, unsweetened, 1.3% oil; EasiYio Products Ltd., Albany, North Shore, Auckland, NZ) were prepared following the manufacturer's instructions.

*2.3 Preparation of silver beet and yoghurt mixes*

Pre-treated (soaked, boiled, stir fried and soaked, boiled and stir fried) silver beet leaves (115 g) were mixed with 115 g of either standard or low fat yoghurt and allowed to cool. All the cooked and processed silver beet leaves were stored frozen until freeze drying in a Cuddon freeze dryer (W.G. Cuddon Ltd., Blenheim, Marlborough, NZ) and then were subsequently ground to a fine powder in a coffee mill (Sunbeam, model: EM0400, China).

*2.3 Proximate analysis*

Dry matter of all samples was determined, in quadruplet (AOAC, 2002), by drying the processed silver beet leaves to constant weight in an oven at105°C for 24 hours. Total fat of each sample was determined by the Soxhlet method (AOAC, 2002) using petroleum ether (Shell X4) in a Foss Tecator Soxtec extraction unit HT6 (Foss Pacific Ltd., Hamilton, NZ).

*2.4 Oxalate determination*

The total and soluble oxalate contents of each fat-extracted sample were determined using the method outlined by Savage et al., (2000). Four separate 0.5 g samples of dried ground cooked silver beet leaves leaves were placed in a 100 ml flask, 40 ml Nanopure water added and incubated in a water bath at 80°C for 15 min with agitation to extract soluble oxalates. Total oxalates were extracted using 40 ml 0.2 M HCL at 80°C for 15 min. The extracts were allowed to cool and then transferred quantitatively into 100 ml volumetric flasks and made up to volume. The extracts were centrifuged at 2889 rcf for 15 min. The supernatant was filtered through a 0.45 mm cellulose nitrate filter. The chromatographic separation was carried out using a300 x 7.8 mm Rezex ROA ion exclusion organic acid column (Phenomenex, Torrance, CA, USA) attached to a cation H+ guard column (BioRad, Richmond, California, USA). The analytical column was held at 25°C. The equipment consisted of an auto sampler (Hitachi AS-2000, Hitachi Ltd., Kyoto, Japan), a ternary Spectra-Physics, SP 8800 HPLC pump (Spectra-Physics, San Jose, California,USA), a Waters, U6K injector (Waters Inc., Marlborough, Massachusetts, USA), a UV/VIS detector Spectra-Physics SP8450 (Spectra-Physics, San Jose, California, USA) set on 210 nm. Data capture and processing were carried out using a peak simple chromatography data system (SSI Scientific Systems Inc, State College, PA, USA). The mobile phase used was an aqueous solution of 25 mM sulphuric acid. Samples (20 ml) were injected onto the column and eluted at a flow rate of 0.6 ml/min. Insoluble oxalate content (calcium oxalate) was calculated by difference (Holloway, Argall, Jealous, Lee and Bradbury, 1989). The final oxalate values were converted to mg/100 g DM of the original test meal, taking into account the fat percentage of each sample.

*2.5 Statistical analysis*

The results are presented as mean values ± standard error. Statistical analysis of the oxalate data for the pre-treatments and cooking experiment was performed using one-way analysis of variance (Minitab version 15.1, Coventry, UK). Statistical analysis of the soluble oxalate data resulting from the pre-treatment and cooking with additions of yoghurt were performed using a general linear model using Minitab version 15.1 (Minitab Ltd., Brandon Court, Progress Way, Coventry, UK).

**3. Results**

Table 1 contains a summary of the dry matter, total fat and oxalate contents of the mixes with additions of either standard or low fat yoghurt. The four processing and cooking methods increased the dry matter content of the cooked silver beet leaves by a mean of 16%. Stir frying the raw silver beet leaves led to a 40% reduction in soluble oxalate content while soaking the leaves in cold tap water for 30 minutes followed by stir frying reduced the soluble oxalate content by 49%. When the leaves were boiled for 2 minutes and allowed to drain for a further minute a 56% reduction in soluble oxalates occurred; this was the most effective way to reduce the soluble oxalate content. Stir frying and stir frying soaked and boiled silver beet leaves showed the lowest reduction in soluble oxalates of 40 and 49%, respectively. The mean soluble oxalate content of the four treatment diets was 492 ± 30 mg/100 g DM which confirmed that these treatments were effective in reducing the soluble oxalate content of the raw leaves when compared to the soluble oxalate content of the raw leaves. In all cases, the four treatments of the silver beet leaves led to a mean reduction in total oxalate of 42% and a mean reduction of 48% in soluble oxalate content.

Overall, the addition of 115 g of standard or low fat yoghurt to 115 g samples of each of the processed and stir fried silver beet leaves changed the overall composition of the yoghurt-based test mixes and reduced the overall total oxalate contents on a DM basis. After the yoghurt additions the mean soluble oxalate content of the stir fried mixes were 334 and 340 mg/100 g DM, respectively, significantly higher than the mean of the other three cooking treatments of 143 mg/100 g DM for standard yogurt and 190 mg/100 g DM for the low fat yogurt additions. Overall, the addition of both standard and low fat yoghurt reduced the mean soluble oxalate content for the four pre-treatments to 190.8 mg/100 g DM on the addition of standard yogurt and 227.5 mg/100 g DM on addition of low fat yoghurt.

## 4. Discussion

Silver beet leaves have little taste after steaming or boiling. Traditional Asian cooking involves rapid, high temperature frying in a wok with a small amount of oil, known as stir frying. Stir frying improves the flavour of many foods. However, Asian cooking normally involves pre-treatments such as soaking and/or boiling before stir frying. It was, therefore, interesting to determine whether Asian cooking methods could also effectively reduce the soluble oxalate content of the leaves. Stir frying alone, without prior soaking and boiling reduced the total oxalate content by 37% and reduced the soluble oxalate content by 40% when compared to the raw leaves (Table 1). All four methods showed an overall mean reduction of 42% of total oxalate and 49% of soluble oxalate when compared to the levels in the raw leaves. Boiling for 2 minutes was the most effective method, resulting in a 56% reduction in soluble oxalate content compared to the level in the raw leaves. In contrast, soaking, boiling and stir frying the leaves only led to a mean 49% reduction in soluble oxalate content.

The total, soluble and insoluble oxalate content of the silver beet leaves used in this study were much lower than those reported by Savage et al. (2004), Savage et al. (2000) and Simpson et al. 2009) for plants grown in a similar location. Simpson et al. (2009) noted that the maturity of the plants and the time of harvest had a considerable effect on the levels of oxalates in the leaves. Franceschi and Nakata (2005) have suggested that many environmental affects, which are difficult to control from year to year, may all affect plant growth and oxalate synthesis in plants. The addition of the two yoghurts to the leaves showed a reduction in the oxalate content of the mixes, by dilution, with the addition of dry matter from the yoghurt. This was not the only effect, as the data shows that the mean soluble oxalate content of the silver beet leaf and yoghurt mixes were significantly ($p < 0.01$) reduced when combined with the calcium in the yoghurt mix. The mean soluble oxalate content of the stir fried and pretreated silver beet leaves was 492.0 ± 29.9 mg/100 g DM, which fell to190.8 ± 49.8 mg/100 g DM for standard yoghurt addition and 227.5 ± 47.0 mg/100 g DM for low fat yoghurt addition, respectively a 61.3% and 53.8% reduction compared to the mean soluble oxalate content of the four pre-treatment values. Brogren and Savage (2003) showed that the addition of milk products were effective to reduce the absorption of oxalate contained in baked spinach in a short term a feeding experiment. The most effective treatment in their experiment was the addition of sour cream and Calci-Trim milk (Meadow Fresh, Goodman Fielder, Auckland, NZ). The effectiveness of the addition of standard and low fat yoghurt to stir fried silver beet leaves has been confirmed in a short term feeding experiment (Johansson and Savage, 2011). The mean absorption of oxalate from stir-fried silver beet leaves was 2.41%, which reduced to 1.10 and 0.89% when consumed with standard yoghurt and low fat yoghurt, respectively

In this experiment all samples of silver beet leaves were stir fried with 28 ml of canola oil resulting in a mean total oil content of 38.5 ± 1.0 g/100 g DM for the four pre-treatments. The mean oil contents of the silver beet leaves with standard yoghurt and low fat yoghurt were 28.3 ± 0.6 and 22.8 ± 1.7 g/100 g DM, respectively. This reduction of oil content in the mixes occurred because the standard and low fat yoghurt contained relatively low levels of oil, 3.6 and 1.3%, respectively. Some reports have stated that fat may increase the oxalate absorption from such foods as chocolate and peanuts which contain about 30% of fat (Finch, Kasidas, and Rose, 1981). It was suggested that dietary fat can bind to free calcium and so more soluble oxalate was available for absorption (Chadwick, Modha, and Dowling, 1973; Earnest, 1974). In this experiment the fat contents of the processed silver beet leaves were relatively high but reductions in soluble oxalate occurred following additions of standard and low fat yoghurt even after the dry mater dilution effect had been taken into account

Addition of standard or low fat yoghurt to the silver beet leaves provided enough calcium to potentially bind to all the soluble oxalate in the leaves. The resulting increased levels of insoluble oxalate (principally calcium oxalate) do not pose any health concerns because it would be not absorbed in the intestine and would eventually be eliminated in the faeces. Advice to patients suffering from kidney stone disease who might eat silver beet as part of their diet should include the importance of cooking methods and the consumption of calcium rich sources along with high oxalate containing foods.

## 5. Conclusions

Pre-treatment of silver beet leaves by soaking and boiling effectively reduced the soluble oxalate in the stir fried leaves. Further reduction in soluble oxalate content can be enhanced through the mixing of either standard or low fat yoghurt into the cooked silver beet leaves.

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Table 1. Mean dry matter, total fat (g/100 g DM ± SE) and total, soluble oxalate and insoluble contents (mg/100 g DM ± SE) of processed silver beet leaves with and without addition of standard and low fat yoghurt

| Treatments | Silver beet leaves | | | | | Silver beet leaves with added standard yoghurt | | | | | Silver beet leaves with added low fat yoghurt | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cooking | Dry matter | Total fat | Total oxalate | Soluble oxalate | Insoluble oxalate | Dry matter | Total fat | Total oxalate | Soluble oxalate | Insoluble oxalate | Dry matter | Total fat | Total oxalate | Soluble oxalate | Insoluble oxalate |
| Raw silver beet | 10.6 ± 0.4 | 0.6 ± 0.4 | 1658 ± 114 | 954 ± 49 | 704 ± 98 | - | - | - | - | - | - | - | - | - | - |
| Stir fried | 11.2 ± 0.5 | 36 ± 1 | 1040 ± 67 | 569 ± 23 | 471 ± 74 | 22.9 ± 1.37 | 28 ± 2 | 866 ± 37 | 334 ± 42 | 531 ± 79 | 20.6 ± 0.28 | 19 ± 2 | 785 ± 42 | 340 ± 17 | 445 ± 47 |
| Soaked and stir fried | 17.2 ± 0.8 | 41 ±1 | 1096 ± 34 | 487 ± 44 | 609 ± 59 | 22.5 ± 0.62 | 27 ± 1 | 578 ± 19 | 135 ± 16 | 443 ± 26 | 25.7 ± 0.39 | 22 ± 2 | 904 ± 39 | 263 ± 28 | 640 ± 40 |
| Boiled and stir fried | 18.6 ± 1.2 | 39 ± 2 | 915 ± 62 | 423 ± 29 | 492 ± 64 | 19.4 ± 0.32 | 28 ± 2 | 835 ± 62 | 179 ± 33 | 656 ± 73 | 23.3 ± 0.71 | 23 ± 2 | 478 ± 80 | 127 ± 14 | 351 ± 79 |
| Soaked, boiled and stir fried | 22.4 ± 0.7 | 38 ± 2 | 801 ± 34 | 489 ± 53 | 312 ± 87 | 21.5 ± 0.16 | 30 ± 1 | 704 ± 90 | 115 ± 13 | 615 ± 80 | 24.3 ± 0.50 | 27 ± 3 | 575 ± 44 | 180 ± 26 | 395 ± 63 |

| | | Significance | | |
|---|---|---|---|---|
| Analysis of variance, | d.f | Total oxalate | Soluble oxalate | Insoluble oxalate |
| Cooking | 3 | ** | ** | NS |
| Treatments | 2 | ** | ** | NS |
| Cooking x treatments | 6 | ** | NS | ** |
| LSD (5%) within cooking | | 90.5 | 50.9 | 110.2 |
| LSD (5%) within treatments | | 78.4 | 44.1 | 95.4 |
| LSD (5%) between cooking | | 156.8 | 88.1 | 190.8 |