

Calcium and Oxalate Contents of Curly Leaf (*Petroselinum crispum*) and Flat Leaf (*P. crispum* var. *neapolitanum*) Parsley Cultivars

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Abstract

The total, soluble and insoluble oxalate contents of the leaves and stems of curly leaf (*Petroselinum crispum*) and flat leaf (*P. crispum* var. *neapolitanum*) parsley cultivars were extracted from fresh tissue and measured using HPLC chromatography. There were no significant differences between the total and insoluble oxalate contents of the leaves between the flat leaf and curly leaf cultivars. There was a small difference ($P < 0.05$) between the soluble oxalate contents of the leaves of the two cultivars. The mean total, soluble and insoluble oxalates of the leaves of the two cultivars were 1137.0, 177.9 and 959.3 mg/100 g dry matter (DM), respectively. The mean total, soluble and insoluble oxalate contents of the stems were 1680.7, 386.2 and 1294.5 mg/100 g DM, respectively, and these were significantly higher than the mean values for the leaves of the two cultivars. Insoluble oxalate made up a mean of 77.0% of the curly leaf stems and leaves compared to a mean of 84.4% found in the flat-leaved cultivar. Unavailable calcium, that is, calcium bound to oxalate as insoluble oxalate, made up a mean of 26.9% of the total calcium in the leaves of both cultivars while the unavailable calcium made up 45.0% of the total calcium in the stems of the two cultivars. Overall, the oxalate contents of both parsley cultivars are relatively high, on a dry matter basis, but their overall contribution to dietary intake is likely to be quite small as parsley is an herb that is only used in small amounts to garnish foods.

Keywords

Total, Soluble and Insoluble Oxalates, Total Calcium, Parsley Leaves, Calcium Availability

1. Introduction

Parsley is native to the Mediterranean region and has since become naturalised in most areas worldwide. There are two main types of leaf parsley: curly leaf (*Petroselinum crispum*) and flat leaf (*P. crispum* var. *neapolitanum*). The unique taste of parsley means that it is commonly used in cooking as a garnish and as a tasty herb in many dishes. In European cooking, curly leaf parsley is often sprinkled as a garnish on many fish, potato and chicken dishes. It is also included in roux-based sauces on, or with, fish or gammon. The Italian dish, *salsa verde*, contains parsley as a main ingredient, while in French cuisine, large amounts of parsley are mixed with garlic to make *persillade*. Curly leaf parsley is used as a minor addition in many dishes but some chefs mention that the flat leaf cultivar has a more intense flavour. Parsley is reported to be a good source of antioxidants, flavonoids, folic acid, vitamins A, K and C and carotenoids, but their possible anti-nutritive constituents have not yet been addressed. Parsley may be a source of potentially harmful compounds such as oxalates. An early report on the oxalate content of a range of vegetables [1] indicated that moss curled parsley contained 166.0 mg/100 g fresh material, while a later report [2] indicated that parsley grown in the USA contained 170 mg total oxalates/100 g wet matter (WM). A review of a wide range of vegetables [3] reported that parsley contained between 140 - 200 mg of total oxalates/100 g WM and placed it in Group 3 (where the ratio of oxalate to calcium is <1) because it was reported to contain high levels of calcium (180 - 290 mg/100 g WM). These results suggested that much of the oxalate found in parsley may be bound to calcium, making both of them unavailable for absorption. More recently [4], parsley grown in Egypt was shown to contain 156 ± 1.02 mg total oxalate/100 g WM and 33.0 ± 0.76 mg soluble oxalate/100 g WM, when measured by an enzyme kit method that used oxalate oxidase. These results were comparable to the values measured using the AOAC method [5] (150 ± 1.30 mg total oxalates/100 g WM and 30.0 ± 0.70 mg soluble oxalates/100 g WM) on the same samples. In the AOAC method [5], the extracted oxalates were measured using titration with KMnO_4 . In contrast, parsley grown in Italy [6] contained up to 160.5 mg soluble oxalate/100 g dry matter (DM) in the leaves while the petioles contained a mean of 200.9 mg soluble oxalate/100 g DM. It was interesting to note that some of the leaves and petioles sampled in this report [6] contained no detectable soluble oxalate.

Although parsley is not consumed in large amounts in the diet, it is recognised to contain high levels of calcium. A value of 105.8 mg/100 g fresh material [1] and Food Standards Australia New Zealand [7] record values for plain and curly parsley as 190 and 245 mg calcium/100 g WM, respectively. The possibility that a large portion of the calcium in parsley leaves might be bound up as insoluble oxalate has not previously been considered.

Oxalate is not an essential nutrient for humans and, if possible, it should not be consumed in large amounts. However, oxalates are found in many kinds of edible plants in varying concentrations [3] [8] and, if consumed in large amounts, they may be harmful to human health [3]. In addition, oxalates occur in two forms in plants, water soluble oxalate (bound to Na^+ or K^+), or water insoluble oxalate (bound to divalent ions such as Ca^{2+} and Mg^{2+}). An intake of large amounts of soluble oxalates can increase the risk of kidney stone development in susceptible people because of the increased concentration of oxalate in the urine. As consumption of additional oxalate in the diet will increase the risk of kidney stone development, it is important to identify high oxalate containing foods and, if possible, reduce these levels by processing [8]. In addition, in mammalian metabolism, endogenous oxalate is produced by the breakdown of dehydroascorbic acid, glyoxylate, serine and glycine in the liver and is excreted in the urine [9]. A moderate level of ascorbic acid (vitamin C) intake about 40% of the total oxalate excreted in the urine comes from the breakdown of ascorbic acid in the liver [9]. As oxalate is the end product of ascorbic acid metabolism in mammals [10], this will add to the burden of oxalates that need to be excreted by the kidney and, potentially, increase the risk of kidney stone development. Any reduction in dietary oxalate intake for at-risk people would, therefore, be of value.

The objective of this study was to investigate the oxalate content of two commonly consumed cultivars of parsley to assess their potential to supply soluble oxalates when used as garnishes in European and Asian cooking. A further objective was to measure the calcium content of the leaves and stems of the parsley cultivars to measure the potential effect of the oxalate content on the availability of calcium in these tissues.

2. Materials and Methods

2.1. Sample Materials

Curly leaf (*P. crispum*) and flat leaf (*P. crispum* var. *neapolitanum*) parsley were grown in a standard three month growing potting mix (80% bark, 20% pumice, Scotts Osmocote® controlled release NPK fertiliser con-

taining trace elements and lime as soil nutrients) in a greenhouse maintained between 15°C and 25°C at the Horticulture Research Area, Lincoln University, Canterbury, NZ (43°38'43"S, 172°27'43"E), 10 m above sea level. The seeds were sown in the potting mix in a greenhouse on 27th April 2015 and the seedlings were planted out on 8th June. The plants were irrigated on demand throughout the growing season. Approximately 100 g of leaves and stems were harvested from 25 plants on the 7th August 2015 when the plants had reached a height of 150 mm. The leaves and stems (petioles) were cut into 1 - 2 mm pieces with a stainless steel knife prior to sampling.

2.2. Dry Matter

The dry matter (DM) contents of each sample of leaves and stems were determined by drying in an oven (Watvic, Watson Victor Ltd., New Zealand) to a constant weight at 105°C [11].

2.3. Extraction of Total and Soluble Oxalic Acids

The measurement of total and soluble oxalates was performed following the method outlined by Savage *et al.* [8]. Four replicates of each stem and leaf sample (5 g) were extracted to measure total oxalate content and four replicates were extracted to measure the soluble oxalate contents. Forty mL of 0.2 M HCl (Aristar, BDH Chemicals, Ltd., Poole, Dorset, UK) was added to flasks for the total oxalate extraction and 40 mL of high purity water (Barnstead International, Dubuque, Iowa, USA, 18 M Ω -cm) was added for the extraction of soluble oxalates. All flasks were placed in a 80°C shaking water bath for 20 minutes. The solutions were quantitatively transferred into volumetric flasks, allowed to cool and then made up to 100 mL with 0.2 M HCl and high purity water, respectively.

2.4. Sample Analysis

The extracts in the volumetric flasks were filtered through a cellulose acetate syringe filter with a pore size of 0.45 μ m (dismic-25cs, Advantec, California, USA) into 1 mL glass vials. The samples were analysed with a high performance liquid chromatography (HPLC) system, using a 300 mm \times 7.8 mm Rezex ion exclusion column (Phenomenex Inc., Torrance, CA, USA) attached to a Cation-H guard column (Bio-Rad, Richmond, CA, USA) held at 25°C. Analysis was performed by injecting 20 μ L of sample or standard onto the column using an aqueous solution of 25 mM sulphuric acid (HPLC grade Baker Chemicals, Phillipsburg, NJ, USA) as the mobile phase, pumped isocratically at 0.6 ml/min, with peaks detected at 210 nm. The HPLC equipment consisted of a Shimadzu LC-10AD pump, CTO-10A column oven, SPD-10Avp UV-Vis detector (Shimadzu, Kyoto, Japan) and a Waters 717 plus auto-sampler (Waters, Milford MA, USA). Data acquisition and processing was undertaken using a Peak Simple Chromatography Data System (model 203) and Peak Simple software version 4.37 (SRI Instruments, Torrance CA, USA). The oxalic acid peak was identified by comparing the retention time to a standard solution and by spiking an already-filtered sample containing a known amount of oxalic acid standard. The insoluble oxalate content of each sample was calculated by difference between the total and the soluble oxalate contents [12].

2.5. Standard Calibration

Two standard curves of oxalic acid (99.99% oxalic acid, Sigma-Aldrich Co., St. Louis, USA) were analysed, with standards in the following concentrations: 1, 2, 5, 10, 15 and 25 mg/100 ml. One batch of standards was prepared in 0.2 M HCl while the other was prepared in high purity water. The acid standard curve was used for identifying and calculating the total oxalate content, while the water standard curve was used for the soluble oxalate content. All blank and standard solutions were passed through a 0.45 μ m cellulose acetate filter prior to analysis.

2.6. Mineral Analysis

Four replicates of each stem and leaf samples were weighed into a tared Teflon microwave vessel and the weight recorded. Five mL of 69% nitric acid (BDH Aristar, BDH Chemicals, Ltd., Poole, Dorset, UK) and 1 mL of 30% hydrogen peroxide (BDH Aristar, BDH Chemicals, Ltd., Poole, Dorset, UK) was then added. The Teflon PFA and Kevlar shielded vessels were then capped and digested using a CEM Mars Express microwave

(CEM Corporation, Matthews, North Carolina, USA). The heating programme was as follows: ramp 10 min to 90°C; hold for 5 min; ramp for 10 min to 150°C; then hold for 5 min. The cooled digest was made up to 25 mL with high purity water (Barnstead International, Dubuque, Iowa, USA, 18 MΩ·cm).

The concentration of total calcium in each sample was carried out on a Varian Axial 720 Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES, Varian, Palo Alto, CA, USA) with a SP3 auto-sampler. Minerals were identified and quantitated using an ICP multi-element standard solution (Certipur, Merck, KGaA, Darmstadt, Germany) containing 23 elements. Data and standard curves were processed using ICP-Expert™ II (Varian, Palo Alto, CA, USA). A high purity water test standard and the ICP multi-element standard solution, containing 0.50 µg/g of each element, were run in triplicate to calibrate the ICP-OES instrument and to determine the standard error of the analysis. The overall standard error was ±0.013 mg/kg. A limit of quantitation (LOQ) test was performed using 10 times the standard deviation of the blank (5% v/v nitric acid) for each mineral. Individual mineral LOQ values ranged from 0.12 to 12.24 µg/L, with a mean of 1.81 µg/L.

2.7. Statistical Analysis

All calculations were performed using Excel 2010, Genstat, Release 12.2 for Windows 7 (VSN International Ltd., Hemel Hempstead, Hertfordshire, UK) to determine the accumulated analysis of variance. The mean values were compared using the LSD method ($P < 0.05$).

3. Results and Discussion

Both the curly and flat leaf parsleys are consumed as a garnish on a relatively few dishes although this may vary between countries. The amounts of garnish added are small as the sprigs of parsley weigh in the region of 2 g/WM. In this experiment the mean dry matter contents of the leaves and stems were 15.8 and 9.0 mg/100 g WM, respectively (Table 1).

There were no significant differences between the total and insoluble oxalate contents of the leaves between the flat leaf and curly leaf cultivars (mean total and insoluble oxalates were 1137.0 and 959.3 mg/100 g DM, respectively). The soluble oxalate content of the curly leaf cultivar was significantly ($P < 0.05$) higher than for the flat leaf cultivar. In contrast, the mean total, soluble and insoluble oxalate contents of the stems were 1680.7, 386.2 and 1294.5 mg/100 g DM, respectively, and these were significantly higher than the mean values for the leaves of the two cultivars. Previous research has suggested that the total oxalate content of parsley ranged from 140 to 170 mg/100 g WM while the soluble oxalate of the leaves ranged from 33 to 160 mg/100 g WM for the leaves and 200 mg/100 g WM for the petioles [2]-[4] [6]. These publications did not clearly state whether the oxalate values were reported on a wet matter or dry matter basis. In addition, no reference was made in these publications to the identity of the cultivar analysed. The present study confirmed that there was a significant difference ($P < 0.05$) in the soluble oxalate content between the flat leaf and the curly leaf cultivars. The main significant differences in oxalate content occurred between the stems (petioles) and the leaves of both cultivars.

Table 1. Dry matter (mg/100 g WM), total, soluble and insoluble oxalate (mg/100 g DM) in the stems and leaves of flat leaf and curly leaf parsleys and, in brackets, % soluble oxalates of total oxalates in the leaves and the stems of both cultivars.

	Dry matter (mg/100 g WM)		Total		Soluble		Insoluble	
	Curly	Flat	Curly	Flat	Curly	Flat	Curly	Flat
Leaf	14.64 ± 0.12	16.96 ± 16.96	1061.0 ± 13.0	1213.0 ± 158.0	220.3 ± 30.1 (20.8)	135.4 ± 7.34 (11.2)	840.6 ± 35.8	1078.0 ± 161.0
Stem	8.33 ± 0.30	9.66 ± 0.14	1630.3 ± 58.6	1731.0 ± 185.0	484.4 ± 81.8 (27.7)	288.0 ± 13.4 (16.6)	1146.0 ± 115.0	1443.0 ± 185.0
Analysis of variance		d.f.		Total		Soluble		Insoluble
Cultivar		1		ns		*		ns
Plant part		1		***		**		*
Cultivar x plant part		1		ns		ns		ns
LSD				266.9		108.4		277.4

Significance: ns = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; LSD least significant difference.

The curly leaf cultivar contained the highest % of soluble oxalate in both the leaves and stems when compared to the flat leaved cultivar (Table 1). This study confirmed the earlier observation [4] that parsley stems contained significantly more oxalates than the leaves (total oxalates $P < 0.001$, soluble $P < 0.01$ and insoluble oxalates $P < 0.05$).

Table 2 shows the total calcium and calcium bound in insoluble oxalate (mg/100 g DM) of the leaves and stems of flat leaf and curly leaf parsleys. The calcium content of insoluble oxalate was calculated assuming that insoluble oxalate was solely made up of calcium bound to the oxalate molecule and was, therefore, the calcium oxalate which contains 31.3% calcium. It is already known that the dominant oxalate salt that makes up the insoluble oxalate content of leafy vegetable leaves is calcium (Ca^{2+}) [13]. The very high solubility product (K_{sp}) of calcium means that it more readily forms a highly insoluble salt with soluble oxalate than Mg^{2+} , Fe^{2+} , Mn^{2+} , Zn^{2+} and Cu^{2+} ions, which also occurs in leaf tissue but has lower solubility products [13]. Calcium oxalate was also more likely to be the dominant insoluble oxalate as calcium was found in a considerable excess compared to the other potential divalent minerals [7] that can form insoluble salts with oxalate.

Calcium bound in the insoluble fraction of the leaves and stems of the two cultivars were very similar but the amounts of calcium bound as insoluble oxalate were significantly different ($P < 0.001$) between the leaves and stems of both cultivars (mean value for the leaves 300.1, mean value for the stems 404.0 mg/100 g DM). Unavailable calcium made up a mean of 26.9% of the total calcium in the leaves of the two cultivars and a mean of 45.0% of the total calcium content of the stems of the two cultivars. Significantly ($P < 0.05$), more unavailable calcium was found in the stems of the two cultivars compared to the amounts found in the leaves.

The calcium content of parsley leaves was reported to be 105.8 mg/100 g fresh material [1], and Food Standards Australia New Zealand [7] recorded values for plain leaf and curly leaf parsleys as 190 and 245 mg calcium/100 g WM, respectively. If a moisture content of 15% was assumed for these samples, this represented between 1267 and 1633 mg calcium/100 g DM. These values were comparable to the mean total calcium value for the leaves of the two cultivars 1111.9 mg/100 g DM and 896.8 mg/100 g DM for the stems in this study. The possibility that a portion of the total calcium in parsley might be bound up as insoluble oxalate had not been previously considered.

4. Conclusion

Overall, this experiment has shown that the leaves and stems of flat leaf and curly leaf parsleys contain reasonably high levels of total oxalates. However, a large portion of the oxalates in both the stems and leaves were insoluble oxalates, which resulted in a large portion of the total calcium and oxalate in the leaves and stems being unavailable for absorption. This suggested that as parsley is only consumed in relatively small amounts, it cannot be regarded as a risk factor for people prone to kidney-stone formation.

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Table 2. Total calcium and calcium bound in insoluble oxalate (mg/100 g DM) in the leaves and stems of flat and curly leaf parsley cultivars.

	Total calcium		Calcium in insoluble oxalate		% Unavailable calcium	
	Curly	Flat	Curly	Flat	Curly	Flat
Leaf	1029.4 ± 122.6	1194.3 ± 96.6	263.0 ± 11.2	337.2 ± 50.5	25.6 ± 1.1	28.2 ± 4.2
Stem	842.1 ± 84.3	951.5 ± 104.8	358.4 ± 36.1	451.34 ± 57.9	42.6 ± 4.3	47.4 ± 6.1
Analysis of variance	d.f.		Calcium insoluble oxalate		% unavailable calcium	
Cultivar	1		ns		ns	
Plant part	1		***		*	
Cultivar x plant part	1		ns		ns	
LSD			108.4		277.4	

Significance: ns = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; LSD least significant difference.

Conflicts of Interest

The authors declare no conflict of interest.

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