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Oxalate content of raw and cooked purslane

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Abstract

Purslane (*Portulaca oleracea* L.) is a rich source of important nutrients such as minerals and antioxidants. In addition, its edible tissues contain high levels of omega-3 fatty acids which are recommenced for a healthy diet. Raw leaves, stems and buds have been reported to contain high levels of oxalate and, therefore, they are not recommended for regular consumption for people who have a tendency to form kidney stones. In this study the fresh leaves, stems and buds contained respectively 23.45±0.45, 5.58±0.18 and 9.09±0.12 g total oxalates kg^{-1} fresh weight. The stems and buds contained a mean of 75.0% soluble oxalates while the leaves contained only 27.5% soluble oxalates. Boiling the leaves, stems and buds resulted in a loss of soluble oxalates from the tissue which resulted an overall 27% reduction in total oxalate in the tissues. Pickling the whole plant resulted in a loss of soluble oxalates from the tissue by leaching into the vinegar, resulting in a reduction of total oxalate content of the pickled tissue by 16%. Larger leaves contained 40% more total oxalates than the small leaves while the oxalate content of the stems ranged between 4.9 and 6.2 g total oxalates kg^{-1} fresh weight. The leaves contained 33% soluble oxalate while in contrast the stems contained a mean of 67% soluble oxalates. Overall, the results of this experiment confirm that cooking and pickling purslane reduces the soluble oxalate content of the processed tissue. Reduction in the soluble oxalate concentration of the tissue will reduce the potential of this high oxalate containing plant to increase urinary oxalate output which could then lead to an increased incidence of kidney stones. This is particularly important as purslane has a number of positive nutritional attributes which suggest that it should be part of a healthy diet.

Key words: Purslane, total, soluble, insoluble oxalate, boiling, pickling.

Introduction

Common purslane (*Portulaca oleracea* L.), an annual succulent plant, is native to India and the Middle East and also grows in the United States, Europe, Australia and China. It has been ranked as the eighth most common plant in the world [1]. This leafy vegetable is eaten throughout Europe and Asia either raw in a salad or cooked. It is appreciated for its tangy or acid taste. Its texture and appearance is similar to spinach when cooked. However, purslane may also have some medicinal value as it has been utilised for many years as a cure for headaches, coughs, intestinal, liver and stomach pain, for relief of burns, and as a diuretic and muscle relaxant. Aboriginal people in Australia have traditionally used purslane for various medicinal purposes [2]. It has been suggested that purslane is an important part of the Mediterranean diet, especially in Crete and, thus it has been the topic of many investigations which have revealed various health benefits. Purslane is an excellent source of minerals [3], vitamin C, vitamin E and carotenoids [4-6] and a rich source of α-linolenic acid, a major omega-3 fatty acid [7-8].

A number of authors have promoted the positive attributes of purslane [7-9] but they have given only a small mention to any negative features. A few authors have measured high levels of oxalates in either the whole plants or the leaves of purslane ranging from 2.55 to 12.94 g total oxalate kg^{-1} fresh weight (FW) [3, 4, 10-12]. This range of oxalate values in the edible portions of purslane should be compared with the highest values reported for Thai vegetables, such as bamboo shoots with 1.63 g kg^{-1} FW [13], spinach grown in Italy with 5.43 g kg^{-1} FW [14], taro leaves grown in New Zealand with 5.89 g kg^{-1} FW [15] and oca tubers grown in New Zealand with 1.62 g kg^{-1} FW [16]. Oxalate is found in many plant families [17] and the oxalate content of a wide range of vegetables, fruits, nuts and wild edible plants have been determined in a number of comprehensive studies [13, 14, 18]. The possible adverse effect of consuming purslane on human health has already been discussed [19]. As oxalate has no metabolic use in the body, once it has been absorbed it will be transported to the kidneys to be excreted in the urine as a waste product. The amount of oxalate excreted in the urine is an important risk factor in the development of calcium oxalate crystals, the most common component of kidney stones.

The adverse effect of ruminants consuming large amounts of raw purslane has been demonstrated by Obied *et al.* [20]. Goats fed a diet containing only purslane showed significant liver and kidney damage and died within 15 to 45 days of commencing the diet.

Oxalate can be found as soluble and insoluble forms in plants. Soluble salts are formed when oxalate binds with potassium, sodium and magnesium (magnesium oxalate is less soluble than the potassium and sodium salts) while insoluble salts are produced when the oxalate binds with calcium and iron. Finally, oxalate can also be found as free oxalic acid [18]. Cooking can reduce the soluble oxalate content of many common vegetables, but not the insoluble fraction, if the cooking water containing some of the leached soluble oxalate is discarded [13, 21].

Purslane is commonly eaten raw as a garnish on foods or mixed into a salad because it has a characteristic tangy taste. It is also cooked lightly similarly to spinach. This process is likely to result in losses of soluble oxalates into the cooking water. Some recipes describe the use of pickled purslane. Pickled purslane is a less common but interesting garnish. Soaking the plant tissue in vinegar (essentially a dilute acetic acid solution) may well lead to a reduction of soluble oxalates in the tissue as they may be leached into the vinegar, which is usually discarded.

The positive nutritional characteristics of purslane are underexploited and underutilised in Western diets but its high oxalate content may limit its wider use particularly for people who have a tendency to form kidney stones. Even Gonnella *et al.* [9] who promoted its positive features, suggested that the consumption of purslane everyday is inadvisable. This paradoxical situation motivated this present study. Moreover, information about the oxalate content of various parts of the plant is scarce. Therefore, this study aimed to determine the oxalate content of different parts of the plant and the effects of cooking and pickling on the edible portions.

Materials and Methods

Sample materials: Golden purslane (*Portulaca oleracea* L.) plants (Koanga Gardens, Maungaturoto, N.Z.) were grown in a Wakanui silt loam soil at the Horticulture Research Area, Lincoln University, Canterbury, New Zealand (43°38'S, 172°27'E) 19 m above sea level. The seeds were sown in potting mixture in a greenhouse on the 13th September 2005 and the seedlings were planted out on the 4th November 2005 (late spring in New Zealand). They were planted in four 5 m length rows. The plants were irrigated on demand throughout the growing season. Leaves, stems and buds were harvested on the 11th April 2006 (early autumn in New Zealand) and dried at 105°C and ground to a powder using a Sunbeam Multigrinder (model no. EMO400 Sunbeam Corporation Ltd., New South Wales, Australia). Stem diameters were measured on representative samples of purslane stems using a digital micrometer (Mitutoyo, Digimatic CD-8C, Mitutoyo, Sakado 1-chome, Takatsu-ku, Kawasaki, Kanagawa, Japan). Weights of representative sample of leaves were weighed using a Mettler Toledo analytical balance Model No. AB 204 (Mettler Toledo, GmbH., Langacher, Greifensee, Switzerland). Whole plants for the pickling were harvested on the 25th April 2006.

Cooking and processing treatments: Leaves, stems and buds were boiled (500 g fresh material was added to 700 ml boiling tap water) for 5 minutes and allowed to drain for 2 minutes. The cooked samples were then dried at 105°C and ground to a powder using a Sunbeam Multigrinder. Whole fresh purslane plants were pickled by adding 500 g of whole plants to 500 ml white vinegar (4% acetic acid, Pam's White Vinegar, (Pam's Products Ltd., Mt Roskill, Auckland)). Ten g Gregg's Whole Pickling Spice (Gregg's Ltd., East Tamaki, Auckland) was added and the bottle was sealed and stored at 4°C. Samples were taken for analysis after two weeks. The pickled samples were then dried at 105°C and ground to a powder using a Sunbeam Multigrinder.

Chemical analysis: Dry matter (DM) contents of the fresh, cooked and pickled purslane were determined, in duplicate, by drying in an oven at 105°C for 24 h [22]. Soluble and total oxalate contents of each sample of raw, cooked or pickled purslane were extracted and measured as described in detail by Savage *et al.* [21]. Triplicate 5 g samples of chopped purslane leaves, stems or buds were chopped into small pieces (5 mm × 5 mm) placed in a 100 ml beaker, 50 ml nanopure water added and incubated in a water bath at 80°C for 15 minutes to extract soluble oxalates. Total oxalates were extracted using 50 ml 0.2 M HCl at 80°C for 15 minutes. 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 3500 rpm for 15 min. The supernatant was filtered through a 0.45 mm cellulose nitrate filter. The chromatographic separation was carried out using a 300 mm × 7.8 mm ion exclusion column (Alltech Associates Inc., Deerfield, Illinois, USA) attached to a cation H+ guard column (Bio-Rad, Richmond, California, USA). The analytical column was held at 25°C. The equipment consisted of 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 Cromatopac C-R3A integrator (Shimadzu, Corporation, Kyoto, Japan). The mobile phase used was an aqueous solution of 25 mM sulphuric acid. Samples of 20 µl 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 [23]. Each sample was analysed in triplicate and all data are presented as oxalate g kg^{-1} wet matter (WM) as this is how this food is normally consumed. A recovery study was carried out where 5 mg of oxalic acid was added to 100 mg of dried leaves, and 99.8±0.22% of added oxalate was recovered in subsequent analysis.

Statistical analysis: The data were analysed using General Linear Model (GLM) in Minitab ver. 14.0 (Minitab Ltd., Coventry, UK).

Results

The total soluble and insoluble content of the raw leaves, stems and buds are shown in Table 1 compared with the values for the same plant parts after boiling in tap water for 5 minutes. The leaves contain significantly ($p<0.001$) more total and insoluble oxalates than the stems and buds. The soluble oxalate in the raw leaves was 27.5% of the total oxalate while buds and the stems contain respectively 71.1 and 78.6% of soluble oxalate. Cooking reduced significantly ($p<0.001$) the total oxalate content of the leaves but the ratio of soluble to insoluble oxalates remained similar to that observed in the raw tissues. Cooking purslane resulted in a reduction in soluble oxalate concentration in every part of the plant (Table 1), however, different percentage losses occurred in different plant parts. The highest loss was observed in the buds with a 49.5% reduction of soluble oxalate content, while the leaves lost 33.5% and stems 18% respectively.

Pickling in white vinegar resulted in a 66.7% reduction ($p<0.001$) of soluble oxalate on a wet matter basis (Table 2). Large losses of dry matter content were also observed.

The purslane plants grown in this experiment contained a wide range of leaf sizes and different diameters of stem (Table 3). The same ratio of soluble to insoluble oxalate was observed for all leaf sizes (mean 33.2% soluble oxalates). The small leaves contained the lowest concentration of total, soluble and insoluble oxalates

Table 1. Mean (± SE) of dry matter and oxalate content (g kg^{-1} FW) and percentage of soluble and insoluble forms in different parts of purslane.

Treatment	DM	Total	Soluble	[1]Insoluble	Soluble	Insoluble
	%		(g kg^{-1}FW)		%	%
Raw						
Leaves	9.56 ± 0.21	23.45 ± 0.45	6.45 ± 0.09	17.00 ± 0.38	27.5	72.5
Stems	5.84 ± 0.20	5.58 ± 0.18	4.39 ± 0.16	1.19 ± 0.14	78.6	21.4
Buds	8.69 ± 0.14	9.09 ± 0.12	6.46 ± 0.07	2.63 ± 0.14	71.1	28.9
Cooked						
Leaves	9.20 ± 0.22	16.13 ± 0.51	4.29 ± 0.15	11.84 ± 0.39	26.6	73.4
Stems	5.98 ± 0.25	5.17 ± 0.10	3.60 ± 0.06	1.57 ± 0.09	69.6	30.4
Buds	6.51 ± 0.31	5.21 ± 0.04	3.28 ± 0.05	1.93 ± 0.06	63.0	37.0
Source of variation			Probability			
Treatment		***	***	***		
Location		***	***	***		

*** $p<0.001$, [1]Insoluble oxalate = total oxalate – soluble oxalate [23].

Table 2. Mean (± SE) oxalate content (g kg^{-1}FW) in the whole raw and pickled purslane.

	DM	Total	Soluble	Insoluble	Soluble	Insoluble
	%		(g kg^{-1} FW)		%	%
Whole raw plant	8.65	12.88 ± 0.36	4.75 ± 0.34	8.13 ± 0.42	36.9	63.1
Whole pickled plant	5.73	10.88 ± 0.32	1.58 ± 0.10	9.30 ± 0.33	14.5	85.5
Source of variation			Probability			
Treatment		**	***	*		

*** $p<0.001$, [1]Insoluble oxalate = total oxalate – soluble oxalate [23].

Table 3. Mean (± SE) weight (mg) of leaves, diameter (mm) of stems and oxalate content (g kg^{-1} FW) and percentage of soluble and insoluble forms in different size cooked leaves and stems.

Leaf or stem size	Weight of the leaves	Diameter of the stems	Total	Soluble	Insoluble	Soluble	Insoluble
	mg	mm		(g kg^{-1} FW)		%	%
Small leaves	34 ± 6.0	-	11.27 ± 0.16	4.00 ± 0.02	7.27 ± 0.17	35.5	64.5
Medium leaves	122 ± 29	-	14.76 ± 0.14	4.72 ± 0.06	10.04 ± 0.19	32.0	68.0
Large leaves	276 ± 39	-	15.83 ± 0.22	5.07 ± 0.18	10.76 ± 0.15	32.1	67.9
Small stems	-	11.75 ± 0.69	6.23 ± 0.07	3.75 ± 0.02	2.48 ± 0.10	60.2	39.8
Medium stems	-	7.94 ± 0.47	5.63 ± 0.15	3.88 ± 0.01	1.75 ± 0.14	68.9	31.1
Large stems	-	4.35 ± 0.37	4.88 ± 0.10	3.51 ± 0.10	1.37 ± 0.01	71.9	28.1

rising significantly ($p<0.001$) for the medium and larger sized leaves. The total oxalate content of the stems decreased ($p<0.01$) with an increase in stem diameter while, in contrast, the medium-sized stems contained the highest soluble oxalate contents when compared to the smaller and largest-size stems. Overall, the stems contained 67.0% soluble oxalates in contrast to the leaves which contained a mean of 33.2% soluble oxalates.

Discussion

The highest concentration of oxalate, 23.45 g kg^{-1} FW, could be found in the raw leaves (Table 1). This value is 2.4 times higher than previously reported values for purslane [11]. The high levels of oxalate measured in this study may result from different growing conditions, and the improved methods of extraction and analysis used in this study. It is interesting to note that the mean oxalate content of the three different sizes of leaves harvested from the same plants two weeks later was 14.0 g kg^{-1} FW, much lower that the mean value for raw leaves harvested on the 11th April 2006. These leaves were harvested in a different manner as compared to the material harvested in the first part of the experiment and perhaps shows the variability of oxalate content of the leaf material. The buds and leaves contained very similar amounts of soluble oxalate (respectively 6.46 and 6.45 g kg^{-1} FW). These results are, however, comparable to earlier reported values [4, 12].

Oxalate is almost ubiquitous in plants and oxalate formation is said to be closely linked to the glycolate cycle and also to photosynthesis [18]. Therefore, the accumulation in leaves of purslane can be explained by photosynthetic activity. However, no generalisations can be made about oxalate accumulation in plant tissues since different trends can be observed in different plants [25]. In the present study an accumulation of oxalate in the leaves of soil-grown plants appears to occur as the leaves enlarge with age (Table 3). In contrast, older leaves of plants grown hydroponically contained lower levels of oxalate than younger leaves [8].

Studies on other vegetables have shown that boiling an oxalate-rich plant allows them to lose soluble oxalate into the cooking water [21]. A recent study reported losses of soluble oxalate between 38 and 87% from various vegetables such as spinach, Swiss chard and Brussels sprouts [24]. In this study the purslane leaves lost only 33.5% soluble oxalate on cooking. This difference is likely to have occurred because Chai and Liebman [24] boiled their foods for 12 minutes while purslane in this study was only boiled for 5 minutes. Boiling purslane for 25 minutes and draining the water resulted in a significant decrease of both soluble and insoluble oxalate levels ($p<0.001$), however, this treatment also resulted in an undesirable loss of total solids and valuable nutrients [11]. Therefore, it seems unlikely that boiling purslane for such a long time will allow it to retain its health benefits. However, it was reported that a reduction in oxalates occurred without excessive losses of minerals when purslane was boiled in a 3% salt solution [11].

Two studies have shown that boiling and steaming are the two cooking methods that can reduce soluble oxalates in yam tubers [26, 27]. These results suggest that steaming might be a positive option for purslane.

It has been reported that the formation of oxalate and calcium oxalate salts is genetically controlled [25] and a considerable variation in oxalate content between different cultivars has been shown for various vegetables [28, 29]. A wide range of purslane cultivars exists and it is possible that low oxalate cultivars may already exist. However, a selected low oxalate cultivar may not retain its tangy taste and may be less popular. It has been suggested that as there is a negative correlation between malate and oxalate in rhubarb tissue and because they together constitute 80% of the total organic acid content, it may be possible to breed a low-oxalate strain without losing the acidic taste which is a main feature of rhubarb [18]. Consequently, a study of both the taste and the oxalate content of several cultivars of purslane could bring to light a low oxalate variety with an acceptable taste.

In a PhD thesis, cited by Gonnella *et al.*[9], it was reported that the buds of purslane grown hydroponically contained lower oxalic acid concentrations [30]. It has also been reported that the oxalate concentration in purslane leaves could be lowered from 6.23 to 3.80 g kg^{-1} FW when grown with a NO_3^-/NH_4^+ ratio of 25:75 [8]. The same pattern was also observed in the stems. Nitrate has to be reduced by the plant before it can be utilised and this leads to the formation of oxalic acid and other organic acids which accumulate in the plant [17].

This study has shown that pickling purslane allows a significant decrease ($p<0.001$) in the concentration of soluble oxalate of the plant tissue as soluble oxalates are leached into the vinegar. Similar losses of soluble oxalate were observed when taro leaves were soaked in cold water [31]. There was a reduction in total dry matter content from 8.7 to 5.7% in the whole pickled plant. During pickling there was a reduction in soluble oxalate content but there was also a significant loss of other acid soluble materials from the leaf tissues.

Earlier studies have reported the reduction of soluble oxalates in spinach [32] and taro [15] from 49 to 73% when cooked with a milky product. Consuming purslane with a calcium rich food such as milk or sour cream would allow a reduction in the absorption of soluble oxalate since calcium binds with soluble oxalate forming insoluble oxalates which will not be absorbed in the gastrointestinal tract. Therefore, the oxalate bioavailability from purslane would be significantly reduced if it was cooked with a milky product.

Noonan and Savage [18] placed purslane in Group 1, the group containing the highest levels of oxalate, because reported literature values of total oxalate ranged from 9.1 to 16.8 g kg^{-1} FW. The values for the raw leaves in this experiment were much higher than these previously reported ranges. It was concluded that foods in group 1 should be consumed in moderation and cooked to reduce their overall oxalate content [18]. The results from this experiment confirm these recommendations.

Conclusions

Overall, the results of this experiment confirm that cooking and pickling purslane reduce the soluble oxalate content of the processed tissue. The loss of soluble oxalate with cooking was up to 33.5% in the leaves and 18% in the stems, the two edible parts of purslane. Pickling in vinegar leads to an even greater loss (66.7%) of soluble oxalate. Purslane is usually used as a garnish and not consumed everyday. The two treatments, boiling for a short period and pickling could allow an increase in purslane consumption in a healthy diet since the risk of kidney stone formation resulting from a large intake of soluble oxalates and decreasing mineral bioavailability appears to be reduced by these treatments. Moreover, the consumption of such properly boiled or pickled purslane would supply a valuable source of omega-3 fatty acids and other nutrients not only in the diets of western societies but also in countries where malnutrition is widespread and where purslane grows wild.

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References

[1]Coquillat, M. 1951. Bulletin Mensuel de la Société Linnéenne de Lyon **21**:165.

[2]Isaacs, J. (ed.) 1987. Bush Food. Weldons, Sydney, pp. 114.

[3]Bianco, V.V., Santamaria, P. and Elia, A. 1998. Nutritional value and nitrate content in edible wild species used in southern Italy. Acta Hort. **467**:71-87.

[4]Guil, J.L., Rodríguez-García, I. and Torija, E. 1997. Nutritional and toxic factors in selected wild edible plants. Plant Foods Human Nutr. **51**:99-107.

[5]Liu, L., Howe, P., Zhou, Y, Xu, Z., Hocart, C. and Zhang, R. 2000. Fatty acids and β-carotene in Australian purslane (*Portulaca oleracea*) varieties. J. Chromat. A **893**:207-213.

[6]Simopoulos, A. P. 2004. Omega-3 fatty acids and antioxidants in edible wild plants. Biol. Res. **37**:263-277.

[7]Simopoulos, A.P., Norman, H.A., Gillapsy, J.E. and Duke, J.A. 1992. Common purslane: A source of omega-3 fatty acids and antioxidants. J. Am. Coll. Nutr. **11**:374-382.

[8]Palaniswamy, U.R., Bible, B.B. and McAvoy, R.J. 2004.Oxalic acid concentrations in purslane (*Portulaca oleraceae* L.) is altered by the stage of harvest and the nitrate to ammonium ratios in hydroponics. Sci. Hort. **102**:267-275.

[9]Gonnella, M., Charfeddine, M., Conversa, G. and Santamaria, P. 2005. Portulaca: From weed to function as a foodstuff? Colture-Protette **34**:49-55.

[10]Gontzea, I. and Sutzescu, P. 1968. Natural Antinutritive Substances in Foodstuffs and Forages. S. Karger, Basel.

[11]Tabekhia, M.M., Toma, R.B. and El-Mahdy, A.R. 1978. Effects of Egyptian cooking methods on total, free oxalates and mineral content of two leafy, green vegetables (Jew's mellow and purslane). Nutr. Rep. Intern. **18**:611-617.

[12]Prakash, D. and Pal. M. 1991. Nutritional and antinutritional composition of vegetables and grain amaranth leaves. J. Sci. Food Agric. **57**:73-83.

[13]Judpresong, K., Chaoenkiatkul, S., Sungpuag, P., Vasanachitt, K. and Nakjamanong, Y. 2006. Total and soluble oxalate contents in Thai vegetables, cereal grains and legume seeds and their changes after cooking. J. Food Comp. Anal. **19**:340-347.

[14]Santamaria, P., Elia, A., Serio, F. and Todaro, E. 1999. A survey of nitrate and oxalate content in fresh vegetables. J. Sci. Food Agric. **79**:1882-1888.

[15]Oscarsson, K.V. and Savage, G.P. 2007. Composition and availability of soluble and insoluble oxalates in raw and cooked taro (*Colocasia esculenta* var. Schott) leaves. Food Chem. **101**:559-562.
[16]Savage, G.P., Deo, B., Mason, S. and Vanhanen, L. 2006. The effect of storage on the oxalate content of New Zealand grown oca. Intern. J. Food Sci. Nutr. (submitted).
[17]Libert, B. and Franceschi, V.R. 1987.Oxalate in crop plants. J. Agric. Food Chem. **35**:926-938.
[18]Noonan, S.C. and Savage, G.P. 1999. Oxalate content of foods and its effect on humans. Asia Pacific J. Clin. Nutr. **8**:64-74.
[19]Mathams, R.H. and Sutherland, A.K. 1952. The oxalate content of some Queensland pasture plants. Queensland J. Agric. Sci. **9**:317-334.
[20]Obied, W.A., Mohamoud, E.N. and Mohamed, O.S.A. 2003. *Portulaca oleracea* (purslane): Nutritive composition and clinical-pathological effects on Nubian goats. Small Rum. Res. **48**:31-36.
[21]Savage, G.P., Vanhanen, L., Mason, S.M. and Ross, A.B. 2000. Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. J. Food Comp. Anal. **13**:201-206.
[22]AOAC 2002. Official Methods of Analysis of AOAC International. 17th edn, AOAC International, Gathersberg, MD, USA.
[23]Holloway, W., Argall, M., Jealous, W., Lee, J. and Bradbury, J. 1989.Organic acids and calcium oxalate in tropical root crops. J. Agric. Food Chem. **37**:337-340.
[24]Chai, W. and Liebman, M. 2005. Effect of different cooking methods on vegetable oxalate content. J. Agric. Food Chem. **53**:3027-3030.
[25]Franceschi, V.R. and Nakata, P.A. 2005. Calcium oxalate in plant: Formation and function. Ann. Rev. Plant Biol. **56**:41-71.
[26]Albihn, P. and Savage, G.P. 2000. Effect of cooking on the soluble oxalate content of three cultivars of oca. Proc. Nutr. Soc. New Zealand **25**:66-70.
[27]Bhandari, M.R. and Kawabata, J. 2006.Cooking effects on oxalate, phytate, trypsin and α-amylase inhibitors of wild yam tubers of Nepal. J. Food Comp. Anal. **19**:524-530.
[28]Libert, B. and Creed, C. 1985. Oxalate content of seventy eight rhubarb cultivars and its relation to some other characters. J. Hort. Sci. **60**:257-261.
[29]Massey, L.K., Palmer, R.G. and Horner, H.T. 2001. Oxalate content of soybean seeds (*Glycine max:* Leguminosae), soyfoods and other edible legumes. J. Agric. Food Chem. **49**:4262-4266.
[30]Charfeddine, M. 2004. Miglioramento del Valore Nutrizionale di Ortaggi da Foglia del Bacino Mediterraneo. PhD dissertation, Università di Bari, Italy.
[31]Savage, G.P. and Dubois, M. 2006.The effect of soaking and cooking on the oxalate content of taro leaves. Intern. J. Food Sci. Nutr. **57**:376-381.
[32]Brogren, M. and Savage, G.P. 2003. Bioavailability of soluble oxalate from spinach eaten with and without milk products. Asia Pacific J. Clin. Nutr. **12**:219-224.