A Comparison of Two Extraction Methods for Food Oxalate Assessment

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Abstract

Hyperoxaluria is a primary risk factor for the formation of calcium oxalate-containing kidney stones. Increased dietary oxalate intake and/or intestinal absorption may provide the critical quantity of additional oxalate that triggers the formation of kidney stones. The accurate determination of food oxalate is highly dependent on oxalate extraction, the first step in oxalate analysis. Potential problems include the possibility of elevated oxalate due to in vitro conversion from various oxalate precursors such as ascorbate and failure to dissolve all pre-existing calcium oxalate crystals. The primary objective was to compare the efficiency of the hot and cold extraction methods in extracting oxalate from 50 dry herb and 10 fresh fruit samples. Regardless of the method of extraction, leaves of Atriplex halimus and kiwifruit exhibited the highest concentrations of both total and soluble oxalate among the herbs and the fruits, respectively. The hot extraction method appeared to extract more total oxalate compared to the cold extraction method while there was no significant difference between the methods in efficiency of extracting soluble oxalate. The overall data suggested that the use of the hot acid method will yield a more accurate assessment of the total oxalate content of foods.

Keywords: Kidney stones, Oxalate extraction, Soluble oxalate, Total oxalate, Herbs, Fruits

1. Introduction

Oxalate is the conjugate base of oxalic acid which can bind to metal ions such as $Ca2^{+}$ and $Mg2^{+}$ to form precipitates in the body. Consumption of high oxalate-containing foods may result in hyperoxaluria and subsequent formation of insoluble calcium oxalate (CaOx) crystals, a primary component of kidney stones. Although urinary oxalate originates from two sources, endogenous synthesis from various precursors and exogenous intake from oxalate-containing foods, increased dietary oxalate intake and/or intestinal absorption may provide the critical quantity of additional oxalate that triggers the formation of CaOx kidney stones (Robertson et al. 1978; Robertson & Peacock, 1980).

Dietary oxalate is mainly derived from foods of plant origin. Efficiency of oxalate absorption depends on the amount present and form (soluble or insoluble), as well as other constituents of the diet such as calcium, magnesium and various fibers (Liebman & Al-Wahsh, 2011). Determination of oxalate content of foods is of special interest for kidney stone patients for whom decreasing urinary oxalate excretion by avoiding consumption of oxalate-rich foods may help prevent stone recurrence (Massey, 2007). However, data on the oxalate content of foods are incomplete and sometimes inaccurate. The accurate determination of food oxalate is highly dependent on oxalate extraction, the first step in oxalate analysis. Oxalate exists in plants in a crystalline form either as calcium oxalate or as a soluble anion (Holmes & Kennedy, 2000). Soluble oxalates, which consist of oxalic acid and soluble salts, can be released when foods are extracted with water; insoluble oxalates, presumed to be mainly calcium oxalate, freely dissolve in acid (Liebman & Al-Wahsh, 2011). Traditionally, dilute acid is used in extracting total oxalate including both the soluble and insoluble fractions.

Problems associated with oxalate extraction from food include the possibility of elevated oxalate due to in vitro conversion from various oxalate precursors such as ascorbate and failure to dissolve all pre-existing CaOx crystals (Hönow & Hesse, 2002). Oxalate extraction with hot acid has been used to ensure complete dissolution of CaOx. However, this method may result in in vitro oxalate generation with subsequent overestimation of oxalate content (Zarembski & Hodgkinson, 1962a). On the other hand, although cold acid extraction has been suggested to minimize in vitro oxalate synthesis, CaOx crystals may not completely solubilize which in turn may lead to underestimation of oxalate content (Hönow & Hesse, 2002). The primary objective of the present study was to compare the efficiency of the hot and cold extraction methods in extracting oxalate from dry and fresh food samples.

2. Materials and Methods

2.1 Samples

After short interviews with local herbalists to identify the most popular commercially available herbs, representative samples were purchased from local herbalist shops in Amman, Jordan. Fifty herbs were taxonomically identified at Hashemite University laboratories (Zarqa, Jordan) and then transported to the nutrition research laboratory at the University of Wyoming (Laramie, Wyoming). The herbs were ground into a fine powder using a coffee mill prior to soluble and total oxalate analyses.

The fruits used in this study were apples, strawberries, blueberries, grapes, kiwifruits, peaches, pears, oranges, bananas and cantaloupes. All fruits were purchased from local supermarkets in Laramie, Wyoming. Individual fruits were cut into small pieces and homogenized using a tissue homogenizer before oxalate extraction.

2.2 Hot extraction

0.5 g of finely ground herb or 4 g of homogenized fruit sample were weighed into 250 ml volumetric flasks and 50 ml of 2 N HCl (for total oxalate extraction) or 50 ml of distilled deionized water (for soluble oxalate extraction) were added. The flasks were placed in a shaking water bath at 80 ℃ for 30 min. The extracts were further diluted with 50 ml of distilled deionized water and then transferred into 15 ml centrifuge tubes and centrifuged at 4200 rpm for 10 min. The supernatants were filtered through Whatman #1 filter paper and kept frozen until the time of oxalate analysis. Each sample was extracted in duplicate.

2.3 Cold extraction

Total and soluble oxalates were extracted from the samples using the method of Ohkawa (Ohkawa, 1985) with some modifications. 0.15 g of the herb or 1.0 g of the fruit was weighed into a 15-ml centrifuge tube and 5 ml of 2 N HCl (for total oxalate extraction) or 5 ml of distilled deionized water (for soluble oxalate extraction) were added to the sample. The tube was tightly capped and vortexed for 5 min and then centrifuged at 4200 rpm for 10 min. The supernatant was transferred to a 25-ml volumetric flask and the remaining oxalate in the pellet was extracted two additional times. The final volume of the collected supernatant from the 3 successive extractions was diluted to 25 ml with distilled deionized water. The extracts were kept frozen until the time of oxalate analysis. Each sample was extracted in duplicate.

2.4 Quantification of total and soluble oxalate

The extracts were analyzed in duplicate for oxalate by using a commercially available enzymatic kit (Trinity Biotech, Berkeley Heights, New Jersey), which is based on measuring the amount of hydrogen peroxide liberated from the oxidation of oxalate by oxalate oxidase. Oxalate concentrations are expressed in mg/100 g of dry herbal sample weight and mg/100 g of fresh fruit weight (± standard deviation of two separate extractions).

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2.5 Statistical Analysis

The paired t test was used to test the null hypothesis that the average amount of extracted oxalate was the same between the two methods. To satisfy the assumption of normality, oxalate values were analyzed in transformed scale (loge(x)). In addition, simple linear regression was employed to describe the relation between the two extraction methods. Regression was conducted on the square root of the oxalate values to satisfy the assumption of normality. Statistical significance was declared at $\alpha = 0.05$ level. Statistical computations were made by using the Statistical Analysis System (SAS institute, version 9.2, Cary, North Carolina USA).

3. Results

Oxalate concentrations corresponding to the two extraction methods (hot and cold) for 50 herbs and 10 fruits are shown in Tables 1 and 2, respectively. Mean total oxalate concentrations using the hot extraction method were significantly higher than the values obtained from the cold extraction method (t_{49}=4.27, $p<0.01$ for herbs; t_9= 2.10, $p< 0.05$ for fruits). The mean difference (± standard error) between the two extraction methods (hot - cold) in total oxalate for the herb and fruit samples were 119.4 ± 32.5 and 1.6 ± 0.8 mg /100 g, respectively. However, with respect to extracting the soluble oxalate from both herb and fruit samples, there was no significant difference in mean oxalate concentrations between the two extraction methods. Regardless of the method of extraction, leaves of Atriplex halimus and kiwifruit exhibited the highest concentrations of both total and soluble oxalate among the herbs and the fruits, respectively.

Linear regression analysis of the total oxalate concentration values in herbs revealed a significant linear correlation ($r^2 = 0.97$, $p<0.0001$) between the two extraction methods (Figure 1). Back transformation indicated that for every 1 mg total oxalate/100 g increase using the cold extraction method, there was on average a 1.2 mg total oxalate/100 g increase using the hot extraction method. A similar correlation between the two methods occurred for the fruit samples ($r^2 = 0.98$, $p< 0.0001$), with an average 1.2 times more total oxalate obtained using the hot extraction method (Figure 2). Linear regression analysis of soluble oxalate in herbs also indicated a significant ($r^2 = 0.87$, $p<0.0001$) linear correlation between the two methods. In fruits, however, after excluding kiwifruit, which was considered to be an outlier because of its markedly higher oxalate concentration, the correlation between the two extraction methods was not significant ($r^2 = 0.25$, $p= 0.17$).

4. Discussion

The oxalate content of foods is of interest because consumption of a high oxalate diet may result in hyperoxaluria thereby increasing risk of kidney stones. Most very high oxalate-containing plants (more than 5% oxalate by dry weight) belong to the three families Amaranthaceae, Chenopodiaceae, and Polygonaceae (Siener et al. 2006). It is possible that a similar biosynthetic pathway and functional role for oxalate exists within one family or group of families that account for a specific range of oxalate levels (Libert & Franceschi, 1987). Among fifty herbs that were presently analyzed, Atriplex halimus contained the highest amount of both total and soluble oxalate, which exceeded 5% of dry weight when extracted by the hot extraction method. Atriplex halimus belongs to the Chenopodiaceae family; oxalate levels within this family vary from 1% to over 30% of the plant dry weight (Libert & Franceschi, 1987). Other important species of plants in the Chenopodiaceae family include beetroot, mangold, spinach, and quinoa. Eight tested herbs, Achillea millefolium, Artemisia abrotanum, Artemisia herba-alba, Calendula officinalis, Carthamus tinctorius, Chrysanthemum vulgare, Cichorium intybus and Matricaria chamomilla belong to the Asteraceae family, the largest family of flowering plants. In the present study, their total oxalate levels ranged from over 200 to 868 mg oxalate/100 g. The lowest oxalate levels were found in Trigonella foenum-graecum which belongs to the Fabaceae (Leguminosa) family and Citrullus colocynthis from the Cucurbitaceae family. One study reported that the total oxalate level of another plant, Trichosanthes cucumerina, of the Cucurbitaceae family, was 2.4–2.6 mg/100 g fresh weight (Adebooye & Oloyede, 2007). Because both Citrullus colocynthis and Trichosanthes cucumerina are fruits, it appears that fruit tissues from the Cucurbitaceae family of plants are relatively low in oxalate.

The distribution of oxalate within plants is uneven. Generally, oxalate contents are highest in the leaves, followed by the seeds; levels tend to be lowest in the stem (Noonan & Savage, 2002). Leaves are typically the part of the plant used to make herbs. In this study, most herbs that were high in oxalate (>1000 mg total oxalate/100 g) were made from dry leaf tissue including Atriplex halimus which had the highest total oxalate level among all the herbs tested.

In addition, high levels of oxalates are commonly observed in tropical plants like taro and sesame seeds (Savage & Mårtensson, 2010; Ishi & Takiyama, 1994). According to the present results, species of Atriplex halimus, Laurus nobilis and Sarothamnus scoparius were rich in total oxalate; these species grow in tropical or low latitude areas.

In contrast to the dietary intake of most plant products, the herbs analyzed in the present study are always prepared raw and consumed in the form of an infusion or tea. Soluble oxalates would be expected to reach out and dissolve in the boiling water. Thus, with the use of traditional herbs in this manner, it may be important to consider oxalate levels, particularly in individuals predisposed to CaOx stone formation.

Previous work suggested that most fruits contain only small quantities of oxalate (Zarembski & Hodgkinson, 1962b), while some such as kiwifruit and star fruit (Carambola) were reported to be moderately high (Wang et al., 2006; Rassam & Laing, 2005). Kiwi (Actinida chinesis) was reported to contain a range of 18-45 mg total oxalate/100 g fresh weight in different genotypes (Rassam & Laing, 2005). The present study yielded 37.4 mg total oxalate and 6.6 mg soluble oxalate per 100 g in kiwifruits when using the hot extraction method, while the cold extraction method yielded 30.6 mg total oxalate and 6.5 mg soluble oxalate per 100 g.

Oranges, bananas, peaches and pears have been previously reported to contain within the range of 2-10 mg total oxalate/100 g fresh weight (Hönow & Hesse, 2002) which are consistent with the presently reported values. Apples and and grapes were previously reported to be low oxalate fruits with total oxalate levels between 0-2 mg/100 g (Holmes & Kennedy, 2000; Hönow & Hesse, 2002). In the present study, the two extraction methods yielded a range of 1.3-2.6 mg total oxalate/100 g for apple and 1.5-2.8 mg total oxalate/100 g for grape.

Earlier studies are inconsistent with regard to the reported oxalate level of strawberries with total oxalate levels ranging from 2.9 mg/100g (Hönow & Hesse, 2002) to 23.4 mg/100g (Ogawa, Takahashi & Kitagawa, 1984). In the present study, strawberries were reported to contain 6.0 mg and 2.5 mg of total and soluble oxalate/100g, respectively. The variation in oxalate values in different sources of plants can be affected by factors such as soil quality, climate or different state of fruit ripeness (Libert & Franceschi, 1987). In addition, discrepancies could also be due to differences in preparation of the samples and analytical techniques.

Controversy remains with respect to the ideal temperature for oxalate extraction. Room temperature could cause erroneously low values due to an incomplete extraction while high temperature could lead to high values due to the possible in vitro generation of oxalate from various food constituents such as ascorbate and other precursors. Therefore, although hot acid and water methods ensure a complete extraction of oxalate, there is a possibility that certain compounds contained in food could be converted to oxalate.

Ascorbate can be converted to oxalate non-enzymatically. Therefore, the presence of ascorbate is one of the major factors that can affect the measurement of oxalate. Chalmers, Cowley & McWhinney (1985) reported that, in urine samples, ascorbate is most stable at about pH 4.5-5.0, while there was a considerable conversion of ascorbate to oxalate at pH 7.0. In addition to pH, heating temperature and time may also be important factors.

In the present study, comparison of the two extraction methods indicated that the hot acid extraction yielded significantly more total oxalate than cold acid extraction for both herb and fruit samples. There are two possible reasons for the elevation: a greater efficiency of hot acid in extracting oxalate and oxalate generation from precursors upon heating. Because ascorbate has been reported to be stable at pH < 5 and because the dried herbs and most of the analyzed fruits were low in ascorbate, it is unlikely that ascorbate conversion to oxalate would account for the elevated oxalate values. In addition, the fruit samples highest in ascorbate (orange, strawberries, and kiwifruit) were not characterized by a relatively greater elevation in both total and soluble oxalate levels when comparing the hot and cold extraction methods, which should have occurred if there had been significant in vitro conversion from ascorbate.

The possibility that oxalate was generated from other precursors when using the hot acid and water extraction method should also be acknowledged. However, the consistently observed increase in total oxalate with the hot acid method (i.e., an average of 1.2 times more oxalate for both herb and fruit samples) suggested that the discrepancy between the two methods is most likely due to a higher efficiency of oxalate extraction with heating. This assertion is further supported by the finding of greater total oxalate levels with the use of hot compared to cold acid in conjunction with no difference in soluble oxalate levels between the hot and cold water extractions, which would be predicted if there was a more efficient dissolution of insoluble oxalate crystals, such as calcium oxalate, with hot acid.

In conclusion, the overall data suggested that the use of the hot acid method will yield a more accurate assessment of the total oxalate content of foods while either method can be used for the assessment of soluble oxalate.

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Table 1. Mean (± standard error) of total and soluble oxalate concentrations in 50 herbs (mg/100 g dry weight) using two extraction methods (means of n = 2)

Herb	Family	Parts analyzed	Total oxalate		Soluble oxalate	
			Hot extraction	Cold extraction	Hot extraction	Cold extraction
Achillea millefolium	Asteraceae	Flowers and stems	533 ± 13	466 ± 4	129 ± 8	101 ± 8
Alchemilla vulgaris	Rosaceae	Leaves	1104 ± 1	1050 ± 13	100 ± 3	72 ± 0
Althaea rosea	Malvaceae	Leaves and flowers	1798 ± 20	1653 ± 60	112 ± 6	114 ± 7
Angelica officinalis	Apiaceae	Aerial parts	535 ± 12	485 ± 18	140 ± 10	102 ± 10
Arctostaphylos uva-ursi	Ericaceae	Leaves	2269 ± 11	2162 ± 6	60 ± 2	55 ± 5
Artemisia abrotanum	Asteraceae	Aerial parts	530 ± 18	488 ± 30	309 ± 32	18 ± 2
Artemisia herba-alba	Asteraceae	Aerial parts	868 ± 15	700 ± 40	107 ± 4	127 ± 4
Atriplex halimus	Chenopodiacea	Leaves	5311 ± 50	4452 ± 177	2293± 48	2298 ± 219
Calendula officinalis	Asteraceae	Flowers	502 ± 4	408 ± 9	131 ± 1	144 ± 1
Capparis spinosa	Capparaceae	Leaves	190 ± 16	209 ± 8	91 ± 5	97 ± 2
Capsella bursa pastoris	Brassicaceae	Aerial parts	353 ± 3	291 ± 2	131 ± 1	124 ± 1
Carthamus tinctorius	Asteraceae	Flowers	392 ± 1	370 ± 29	63 ± 1	112 ± 3
Cassia senna	Caesalpiniaceae	Leaves	1898 ± 29	1727 ± 9	120 ± 9	123 ± 1
Cetraria islandica	Parmeliaceae	Leaves	584 ± 15	470 ± 45	83 ± 1	215 ± 23
Chrysanthemum vulgare	Asteraceae	Flowers	562 ± 1	495 ± 9	81 ± 5	81 ± 8
Cichorium intybus	Asteraceae	Leaves	298 ± 7	236 ± 11	109 ± 1	73 ± 4
Citrullus colocynthis	Cucurbitaceae	Fruits and seeds	48 ± 1	37 ± 1	32 ± 1	34 ± 1
Crataegus oxyacantha	Rosaceae	Leaves and fruits	1843 ± 77	1939 ± 151	114 ± 9	267 ± 26
Cuminum cyminum	Apiaceae	Seeds	714 ± 19	639 ± 21	140 ± 4	120 ± 11
Cuscuta epithymum Mur	Cuscutaceae	Aerial parts	273 ± 25	240 ± 6	130 ± 12	136 ± 6
Erythraea centaurium	Gentianaceae	Aerial parts	193 ± 14	111 ± 4	78 ± 1	5 ± 1
Foeniculum vulgare	Apiaceae	Seeds	1086 ± 63	935 ± 24	194 ± 5	263 ± 4
Galium aparine	Rubiaceae	Aerial parts	1031 ± 25	825 ± 9	461 ± 9	322 ± 11
Humulus lupulus	Cannabaceae	Leaves	2292 ± 47	2568 ± 20	110 ± 2	256 ± 10
Hypericum perforatum	Clusiaceae	Flowers	187 ± 4	188 ± 6	82 ± 6	62 ± 3
Hyssopus officinalis	Lamiaceae	Aerial parts	201 ± 20	279 ± 2	104 ± 5	144 ± 4
Juniperus communis	Cupressaceae	Aerial parts	4493 ± 74	3592 ± 191	63 ± 5	195 ± 2
Laurus nobilis	Lauraceae	Leaves	1972 ± 15	1710 ± 11	168 ± 1	154 ± 6
Lavandula officinalis	Lamiaceae	Flowers	1078 ± 2	971 ± 5	310 ± 0	241 ± 19
Matricaria chamomilla	Asteraceae	Aerial parts	202 ± 4	152 ± 2	85 ± 1	76 ± 1
Melilotus officinalis	Fabaceae	Aerial parts	514 ± 29	795 ± 10	107 ± 2	93 ± 0
Melissa officinalis	Lamiaceae	Leaves	692 ± 27	676 ± 11	132 ± 7	101 ± 9
Myrtus communis	Myrtaceae	Leaves	1574 ± 13	1440 ± 35	176 ± 11	136 ± 4
Origanum vulgare	Lamiaceae	Leaves	442 ± 2	307 ± 28	137 ± 5	126 ± 12
Ortie urticadiocia	Urticaceae	Leaves	545 ± 19	864 ± 5	176 ± 1	33 ± 2
Paronychia argentea	Caryophyllaceae	Aerial parts	2664 ± 122	1858 ± 49	367 ± 16	278 ± 20
Peganum harmala	Zygophyllaceae	Seeds	1921 ± 39	1547 ± 7	1452 ± 12	1510 ± 83
Pimpinella anisum	Apiaceae	Seeds	1011 ± 7	920 ± 16	273 ± 11	505 ± 36
Plantago lanceolata	Plantaginaceae	Aerial parts	367 ± 20	446 ± 17	100 ± 0	117 ± 6
Primevere officinalis	Primulaceae	Leaves	2254 ± 54	2135 ± 18	209 ± 4	188 ± 16
Rosa canina	Rosaceae	Flowers	1100 ± 33	820 ± 23	115 ± 6	99 ± 5
Rosmarinus officinalis	Lamiaceae	Aerial part	403 ± 11	291 ± 4	101 ± 4	82 ± 4
Ruta graveolens	Rutaceae	Leaves	1908 ± 1	1661 ± 45	78 ± 3	127 ± 6
Salvia triloba	Lamiaceae	Leaves	798 ± 16	694 ± 8	163 ± 8	150 ± 6
Sarothamnus scoparius	Fabaceae	Aerial parts	1627 ± 16	1351 ± 55	72 ± 6	57 ± 5
Teucrium polium	Lamiaceae	Aerial part	1132 ± 10	1068 ± 6	93 ± 2	183 ± 18
Thymus serpyllum	Lamiaceae	Leaves	394 ± 27	327 ± 14	162 ± 11	147 ± 13
Tilia playtphyllos	Tiliaceae	Leaves	1783 ± 21	1512 ± 14	510 ± 46	440 ± 37
Trigonella foenum-graecum	Fabaceae	Seeds	42 ± 2	36 ± 3	18 ± 2	27 ± 1
Viscum album	Viscaceae	Aerial part	355 ± 11	302 ± 16	45 ± 4	54 ± 4

Table 2. Mean (± standard error) of the total and soluble oxalate contents in 10 fruits (mg/100 g fresh weight) using two extraction methods (means of n = 2)

Fruit	Brand	Total oxalate		Soluble oxalate	
		Hot extraction	Cold extraction	Hot extraction	Cold extraction
Strawberry	Dole Strawberry Fraises	6.0 ± 1.8	6.1 ± 0.0	2.5 ± 0.1	2.5 ± 0.1
Pears	Asia Pear	3.3 ± 0.4	3.5 ± 0.4	2.3 ± 1.0	3.3 ± 0.6
Banana	Del Monte	6.1 ± 1.5	2.8 ± 0.1	2.5 ± 0.3	1.4 ± 0.6
Apple	Red Delicious	2.6 ± 0.3	1.3 ± 0.2	2.2 ± 0.2	1.1 ± 0.0
Grape	PLU Raisins Rouge Sans Pepius	1.5 ± 0.1	2.8 ± 0.1	1.1 ± 0.4	1.7 ± 0.5
Kiwifruit	Zespri green	37.4 ± 2.5	30.6 ± 0.3	6.6 ± 0.2	6.5 ± 1.3
Peach	Tree Ripe Chile	1.8 ± 0.5	2.0 ± 0.4	0.3 ± 0.3	0.1 ± 0.1
Blueberry	Gourmet Trading Company Argentina	5.4 ± 0.6	4.4 ± 0.3	3.7 ± 0.5	1.4 ± 0.1
Orange	Sunkist Satsuma Mandarins	10.2 ± 0.2	6.0 ± 0.6	2.9 ± 0.3	3.2 ± 0.0
Cantaloupe	Unknown	5.4 ± 1.0	3.8 ± 0.1	3.5 ± 0.2	2.3 ± 0.0

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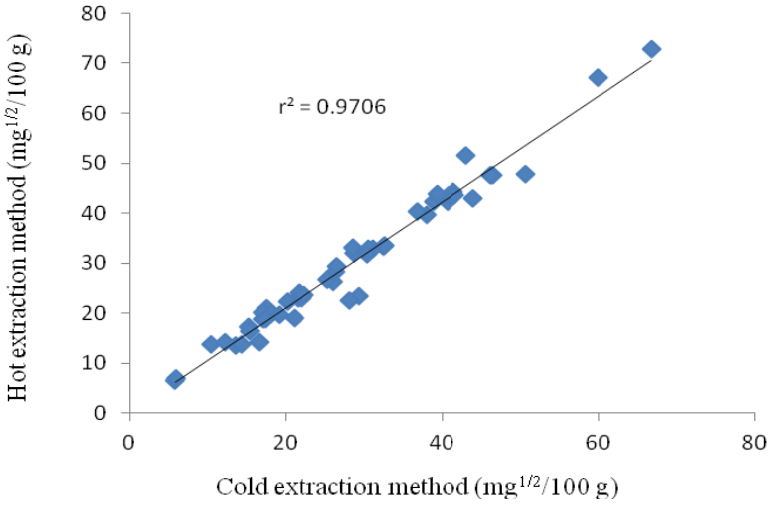

Figure 1. Regression analysis of the total oxalate values of herbs by using hot extraction against cold extraction after square root transformation ($\sqrt{x}$)

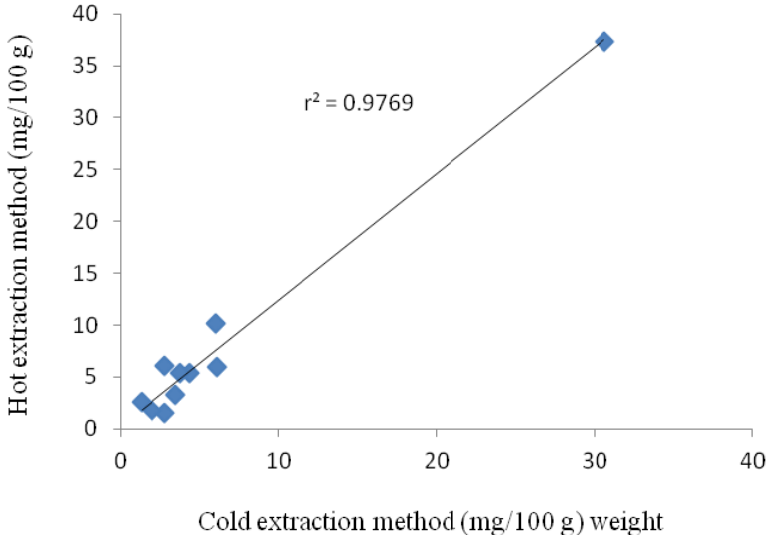

Figure 2. Regression analysis of the total oxalate values of fruits by using hot extraction against cold extraction