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RESEARCH NOTE

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Analysis of Oxalic Acid of Various Vegetables Consumed in Korea

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Abstract Many vegetables contain oxalate at various levels depending on their type and family. Oxalate is known to reduce mineral bioavailability from foods. The following study was conducted to determine soluble and total oxalate contents in 32 plant samples commonly consumed in Korea using high-performance liquid chromatography (HPLC). Chard, amaranth, and spinach contained total oxalate of >1,000 mg/100 g. Approximately 45% of the oxalate in amaranth is insoluble, whereas 74.46 and 92.45% of the oxalates in chard and spinach, respectively, was soluble oxalates, which may be removed by blanching. Eggplant, carrot, leak, ginger root, spinach, burdock, and sweet pepper contained more than 90% soluble oxalate in total oxalate content. However, all oxalates detected in lettuce and celery were insoluble. Oxalate was not detected in shepherd's purse, bellflower root, garlic, radish root, broccoli, cabbage, onion, lotus root, adlay, cucumber, kale, and pumpkin. These observations provide useful information needed for selection of vegetables.

Keywords: oxalic acid, vegetable, analysis, method validation

Introduction

Vegetables are commonly consumed as sources of essential vitamins and minerals, dietary fiber, and complex carbohydrates. However, oxalic acid interferes with absorption of the minerals available in vegetables (1, 2). Oxalic acid is known to reduce calcium availability, which can lead to kidney stones (3). It can also leak into blood and tissue fluid resulting in corrosive action in buccal and gastrointestinal track epithelia and abnormal function of the kidney (4).

Many leafy vegetables contain oxalic acid and its concentrations vary depending on the family and type of vegetables. Many studies have reported oxalate contents in vegetables such as spinach (5), taro (6), sweet potato (7), nuts, and legumes (8). Vegetables belonging to families of *Chenopodiaceae*, *Amaranthaceae*, and *Polygonaceae* have generally been considered high oxalate-containing vegetables. Oxalates are most highly concentrated in the leaves of vegetables as compared to other parts such as seeds or stems (9).

Oxalate content is categorized into water-soluble, insoluble, and total oxalate (both water-soluble and insoluble) categories. Oxalate forms water-soluble salts with Na^+, K^+, and NH_4^+, it also binds with Ca^{++}, Fe^{++}, and Mg^{++} rendering these minerals unavailable. Numerous studies have shown that the amount of oxalate in vegetables is reduced through the cooking process and the reduction rate varies depending on cooking time and temperatures (10). Results suggest blanching vegetables as the preferable method to reduce oxalate concentration (7).

Methods such as permanganate (11) and colormetric (12) to determine oxalate in food samples have been reported. However, since these methods have been somewhat inefficient, complicated, and inaccurate, several new methods have been developed to measure oxalate in foods. Methods using gas chromatography, HPLC and capillary electrophoresis (10, 13, 14) are now widely used. The objective of this study was to determine oxalate content in 32 vegetable samples consumed in Korea using HPLC and to note any differences of the oxalate content in the vegetable samples. Also, the analytical method validation parameters such as recovery, repeatability, and reproducibility were provided to ensure the method's validity.

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Materials and Methods

Sample preparation Table 1 lists 32 raw vegetables purchased at local markets in Cheongju, Korea in 2004 and tested for their oxalate contents. After peels or stems were removed, edible portions of the vegetables were washed with distilled water and dried with absorbent papers. Each sample was homogenized with a blender. After homogenization, the samples were analyzed for total and soluble oxalate content and moisture content. Samples were stored at 4°C prior to use in experiments.

Moisture content The moisture contents of the samples were determined by measuring weight differences between samples (1-2 g) before and after 24 hr of drying in an oven (vacuum oven VOS-300SD; Eyela, Tokyo, Japan) at 105±5°C.

Total and soluble oxalic acid Samples were homogenized using a grinder (Super Mill HM-180; Hwaseng, Korea). Forty mL of 2 N HCl were added to the homogenized samples (5 g) to extract total oxalic acid. The samples were ground to fine particles using a homogenizer (Polytron® Ultra Turrax T25 basic; IKA

Table 1. English name, scientific name, and family of analyzed vegetables

Family	English name	Scientific name
Amaranthaceae	Amaranth	*Amaranthus mangostanus*
Campanulaceae	Bellflower root	*Platycodon grandiflorum*
Chenopodiaceae	Chard	*Beta vulgaris* var. *cicla* L.
	Spinach	*Spinacia oleracea*
Compositae	Lettuce	*Lactuca sativa*
	Crown daisy	*Chrysanthemum coronarium* var. *spatiosum*
	Burdock	*Arctium lappa*
	Chicory, leaves	*Cichorium intybus*
Cruciferae	Shepherd's purse	*Capsella bursa-pastoris*
	Cabbage	*Brassica oleracea* var. *capitata*
	Kale	*Brassica oleracea* L. var. *acephala* DC.
	Radish root	*Raphanus sativus*
	Broccoli	*Brassica oleracea* var. *italica plenck*
	Adlay	*Raphanus sativus*
Cucurbitaceae	Cucumber	*Cucumis sativus*
	Pumpkin	*Cucurbita* spp.
Labiatae	Perilla leaf	*Perilla frutescens* var. *japonica*
Leguminosae	Soybean sprout	*Glycine max Merrill*
Liliaceae	Garlic	*Allium scorodorpasum* var. *viviparum Regel*
	Leek	*Allium tuberosum*
	Onion	*Allium cepa*
	Spring onion	*Allium fistulosum*
Nymphaeaceae	Lotus root	*Nelumbo nucifera*
Pteridaceae	Bracken	*Pteridium aquilinum* var. *latiusculum*
Solanaceae	Eggplant	*Solanum melongena*
	Green pepper	*Capsicum annuum* L.
	Sweet pepper	*Capsicum annuum* var. *angulosum*
Umbelliferae	Water dropwort	*Oenanthe javanica*
	Carrot	*Daucus carota* var. *sativa*
	Celery	*Apium graveolens* var. *dulce*
	Parsley	*Petroselinum crispum (Mill.)*
Zingiberaceae	Ginger root	*Zingiber officinale*

labortechnik, Kuala lumpur, Malaysia) for 1 min, washed in 20 mL of 2 N HCl, and shaken in water bath at 80°C for 15 min. After shaking, samples were cooled and filtrated with filter paper (No. 2; Whatman, Maidstone, England). The filtrates were placed in 100 mL volumetric flasks and brought up to 100 mL with 2 N HCl. Then, the samples were diluted with deionized water (90 mL). The diluted samples were filtered through 0.45 μm regenerated cellulose microfilters (Sartorius AG, Goettingen, Germany) and injected to an HPLC system. For extraction of soluble oxalic acid, water was used as a substitute for 2 N HCl in the above procedure. All samples were analyzed in duplicate.

HPLC conditions An HPLC system consisted of Biorad Aminex ion exclusion column HPX-87H (300×7.8 mm) attached to an Aminex Cation-H guard column, using an isocratic elution at 0.6 mL/min with 0.008 N sulphuric acid as a mobile phase. UV detector (M720; Young-Lin, Korea) at 215 nm was used.

Standard calibration The standard curve of oxalic acid was analysed in the range 16.8-1,313.1 μg/mL. Standard solution was prepared by filling up a 100 mL volumetric

Table 2. Three parameters for the precision and accuracy of assay of freeze-dried spinach sample

Oxalates	Parameter[1)]	Precision		Accuracy
		Repeatability	Reproducibility	Recovery (%)
Total oxalate	Mean	15,825.4	16,092.3	107.3
	SD	171.17	132.46	2.44
	CV, %	1.08	0.82	0.82
Soluble oxalate	Mean	15,637.9	15,120.0	102.8
	SD	25.39	580.38	2.23
	CV, %	0.16	3.84	2.17

[1)]Mean (n=5, mg/100 g); SD, standard deviation; CV, coefficient of variation.

flask containing 1.0 mL of oxalic acid (Sigma-Aldrich, St. Louis, MO, USA) with water.

Recovery study The recovery of oxalic acid from vegetable samples were determined by adding oxalic acid standard solution (9 mL) to freeze-dried spinach sample (1 g). Oxalate in spinach was extracted using the same method as described above.

Results and Discussion

Prior to data collection, the analytical methods were validated for use on freeze-dried spinach samples by determining the accuracy and precision. Table 2 shows precision and accuracy. Precision is made up of 2 components: repeatability (intra-day assay) and reproducibility (inter-day assay). The accuracy (recovery) was evaluated by analyzing a spiked sample (15). Recoveries of total and soluble oxalates in the freeze-dried spinach were 107.3 and 102.8%, respectively (Table 2). The linear relationship between the concentrations of oxalate and the peak areas of standard curve for oxalate values was R^2=0.9995 under the HPLC condition (data not shown). The repeatability and reproducibility for total and soluble oxalate contents were less than 5%. The chromatograms of standard and the dried spinach sample are shown in Fig. 1. Retention time for total and soluble oxalates in the vegetable samples was about 6.5 min.

Mean values of oxalate contents in the 32 vegetable samples expressed on a fresh weight (FW) basis are listed in Table 3. Oxalate contents for same vegetable samples have been reported in other studies (10, 13, 16). Higher levels of oxalate content in broccoli and carrots, and lower levels of that in spinach were reported by Savage *et al.* (10). Differences in oxalate content in the same samples between studies may be due in part to different analytical methods, location, and handling skills. Judprasong *et al.* (13) found 29±7, 23±13, and 55±6 mg of total oxalate and 24±6, <3, and 45±1 mg of soluble oxalate in carrot, kale, and eggplant, respectively. As with our study, the ratio of soluble oxalate to total oxalate contents of kale was not calculated since soluble oxalate was not detected. It was also reported that 82 and 83% of total oxalate in eggplant and carrot, respectively, were soluble oxalate (13). The results of this study showed a ratio of 98.8 and 99.1% soluble oxalate to total oxalate content in eggplant and carrot, respectively.

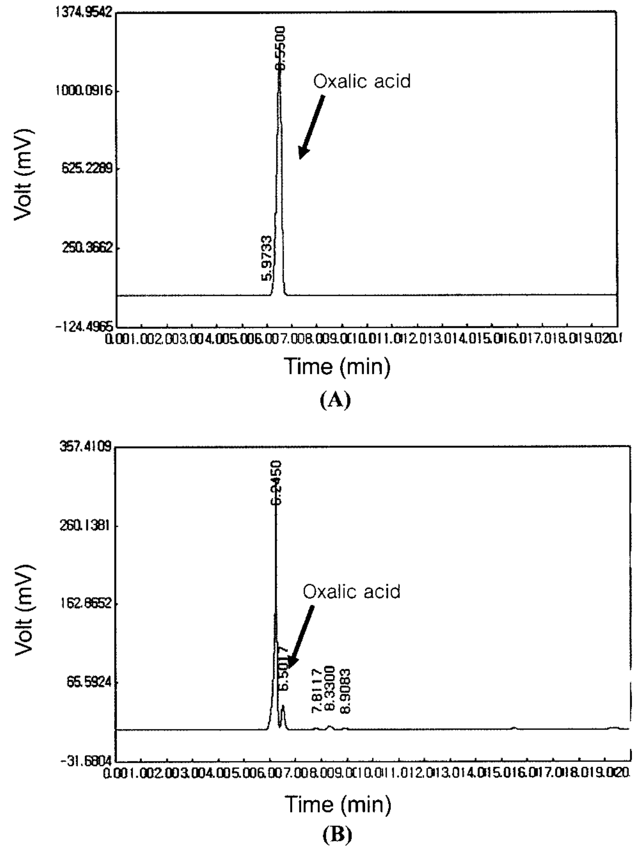

Fig. 1. Reverse-phase LC chromatograms of oxalic acid using UV detection (215 nm), flow rate of 0.6 mL, injection volume of 20 uL, and mobile phase of 0.008 N sulphuric acid. (A), Standard; (B), extract of the spinach sample.

Significantly high levels of total and soluble oxalate contents were detected in chard, amaranth, and spinach among the tested vegetable samples. Chard, amaranth, and spinach contained 1,458.0, 1,510.8, and 1,272.2 mg of total oxalate/100 g FW of samples, respectively, and 1,082.7, 835.1, and 1,176.1 mg of soluble oxalate/100 g FW of samples, respectively. Spinach has been considered as high-oxalate containing vegetable (12, 17). Amaranth had the highest value of total oxalate (1,510.8 mg/100 g FW), but the ratio of soluble oxalate to total oxalate contents was only 55.3%. The highest concentration (675 mg/100 g FW) of insoluble oxalic acid was found in amaranth among the test vegetables. The ratios of soluble oxalate to total oxalate contents in chard and spinach were 74.3 and 92.5%, respectively, and significantly higher than that in amaranth.

Oxalates was not found in shepherd's purse, bellflower root, garlic, radish, broccoli, cabbage, onion, lotus root, adlay, cucumber, kale, and pumpkin samples. Lettuce and celery contained 40.0 and 23.2 mg of total oxalate/100 g FW, respectively, but did not contain soluble oxalate (or contained it at an undetectable level). With the exceptions of perilla leaf, amaranth, crown daisy, soybean sprout, and

Table 3. Oxalate, moisture contents, and percentage soluble oxalate in various vegetables consumed in Korea[1)]

Food items	Moisture (%)	Oxalate (mg/100g)			% Soluble oxalate
		Total	Soluble	Insoluble	
Amaranth	90.2	1,510.8	835.1	975.7	55.3
Bracken	90.6	35.9	30.3	4.6	86.9
Broccoli	89.7	Tr	Tr	Tr	NC
Burdock	81.2	64.8	62.7	2.1	96.7
Cabbage	94.7	Tr	Tr	Tr	NC
Carrot	90.6	16.4	16.2	0.2	99.1
Celery	92.2	23.2	Tr	23.2	0
Chard	92.5	1,458.1	1,082.7	375.4	74.3
Chicory, leaves	94.4	47.6	42.3	5.3	88.9
Crown daisy	94.0	96.0	58.8	37.2	61.3
Cucumber	96.7	Tr	Tr	Tr	NC
Bellflower root	88.1	Tr	Tr	Tr	NC
Eggplant	93.1	54.4	53.7	0.7	98.8
Garlic	63.9	Tr	Tr	Tr	NC
Ginger root	92.8	241.1	236.7	4.4	98.2
Green pepper	89.9	31.0	27.5	3.5	88.6
Kale	92.6	Tr	Tr	Tr	NC
Leek	93.9	48.6	45.1	3.5	92.8
Lettuce	94.4	40.0	Tr	40.0	0
Lotus root	80.2	Tr	Tr	Tr	NC
Onion	93.2	Tr	Tr	Tr	NC
Parsley	86.2	270.7	72.0	198.8	26.6
Perilla leaf	85.9	440.0	96.6	353.4	21.5
Pumpkin	94.9	Tr	Tr	Tr	NC
Radish root	95.3	Tr	Tr	Tr	NC
Shepherd's purse	89.1	Tr	Tr	Tr	NC
Soybean sprout	89.9	26.5	12.7	13.8	47.8
Spinach	92.3	1,272.2	1,176.1	96.0	92.5
Sweet pepper	94.6	11.4	10.6	0.8	92.7
Water dropwort	92.9	93.0	64.7	28.3	69.5
Spring onion	93.4	33.3	28.2	5.1	84.6
Adlay	94.7	Tr	Tr	Tr	NC

[1)]Mean of duplicate measurements; Insoluble oxalate = total oxalate – soluble oxalate; Tr, trace amount; NC, not calculated.

parsley, ratios of soluble oxalate to total oxalate contents in all samples, in which oxalate was found, ranged from 84 to 99%. However, only 21-61% of total oxalate in perilla leaf, amaranth, crown daisy, soybean sprout, and parsley was composed of soluble oxalate.

Oxalate has been known to have deteriorative effects on nutrient absorption and kidney function, but soluble oxalate in foods was determined to be removed by boiling (4, 18). Since oxalate has been considered to inhibit absorption of iron, an excessive intake of vegetables containing large amounts of oxalate may reduce bioavailability of essential minerals (19), and may cause acute renal failure (20). However, oxalate concentration in foods could be reduced depending on cooking methods. These results suggest that vegetarians should carefully monitor their intake of high oxalate-containing vegetables, and consume other foods that assist in absorption of minerals.

In this study, oxalates in an assortment of vegetable samples were determined through HPLC. Due to the disadvantages of oxalate in the human organism, oxalate in foods should be removed before consumption. However, in addition to total oxalate content, the amount of insoluble oxalate in foods should also be considered since soluble oxalate is easily removed by boiling or blanching process of foods. This study provides valuable information regarding vegetable selection.

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References

1. Massey LK, Roman-Smith H, Sutton RA. Effect of dietary oxalate and calcium on urinary oxalate and risk of formation of calcium oxalate kidney stones. J. Am. Diet Assoc. 93: 901-906 (1993)
2. McConn MM, Nakata PA. Oxalate reduces calcium availability in the pads of the prickly pear cactus through formation of calcium oxalate crystals. J. Agr. Food Chem. 52: 1371-1374 (2004)
3. Ross AB, Savage GP, Martin RJ, Vanhanen L. Oxalates in Oca (New Zealand yam)(*Oxalis tuberose* Mol.). J. Agr. Food Chem. 47: 5019-5022 (1999)
4. Noonan SC, Savage GP. Oxalate content of foods and its effect on humans. Asia Pac. J. Clin. Nutr. 8: 64-74 (1999)
5. Grazyna J. Content of nitrates, nitrites, and oxalates in New Zealand spinach. Food Chem. 89: 235-242 (2005).
6. Holloway WD, Argall ME, Jealous WT, Lee JA, Bradbury JH. Organic acids and calcium oxalate in tropical root crops. J. Agr. Food Chem. 37: 337-341 (1989)
7. Mosha TC, Gaga HE, Pace RD, Laswai HS, Mtebe K. Effect of blanching on the content of antinutritional factors in selected vegetables. Plant Food Hum. Nutr. 47: 361-367 (1995)
8. Liebman M, Chai WW. Oxalate content of legumes, nuts, and grain-based flours. J. Food Compos. Anal. 18: 723-729 (2005)
9. Libert B, Franceschi VR. Oxalate in crop plants. J. Agr. Food Chem. 35: 926-938 (1987)
10. Savage GP, Vanhanen L, Mason SM, Ross AB. Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. J. Food Compos. Anal. 13: 201-206 (2000)
11. Baker CJL. The determination of oxalates in fresh plant material. Analyst 77: 340-344 (1952)
12. Zarembski PM, Hodgkinson A. The oxalic acid content of English diets. Brit. J. Nutr. 16: 627-634 (1962)
13. Judprasong K, Charoenkiatkul S, Sungpuang P, Vasanachit K, Nakjamanong Y. Total and soluble oxalate contents in Thai vegetables, cereal grains, and legume seeds and their changes after cooking. J. Food Compos. Anal. 19: 340-347 (2006)
14. Hönow R, Bongartz D, Hesse A. An improved HPLC-enzyme-reactor method for the determination of oxalic acid in complex matrices. Clin. Chim. Acta 261: 131-139 (1997)
15. Lee SM, Lee J. Tocopherol and tocotrienol contents of vegetable oils, margarines, butters, and peanut butters consumed in the Korean diet. Food Sci. Biotechnol. 15: 183-188 (2006)
16. U.S. Department of Agriculture, Agricultural Research Service.

USDA composition of foods, vegetables, and vegetable products. p.11. In: Agriculture Handbook. No. 11. Human Nutrition Information Service, Washington, DC, USA (1984)
17. Hönow R, Hesse A. Comparison of extraction methods for the determination of soluble and total oxalates in foods by HPLC-enzyme-reactor. Food Chem. 78: 511-521 (2002)
18. Albihn PB, Savage GP. The bioavailability of oxalate from Oca (Oxalis tuberosa). J. Urology 166: 420-422 (2001)
19. Chawla S, Saxena A, Seshadri S. *In-vitro* availability of iron in various green leafy vegetables. J. Sci. Food Agr. 46: 125-127 (1988)
20. Chen CL, Fang HC, Chou KJ, Wang JS, Chung HM. Acute oxalate nephropathy after ingestion of star fruit. Am. J. Kidney Dis. 37: 418-422 (2001)