Note

Determination of Vitamin U in Food Plants

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Vitamin U levels were determined on 26 kinds of food plants well known to be healthy vegetables in Asian or Western countries. Spinach showed the highest level of 45.2 mg/100 g DW followed by Pak-choi (34.3 mg/100 g DW) > kale (23.4 mg/100 g DW) > Sumssukbujaengi (19.8 mg/100 g DW) > leaf mustard (19.6 mg/100 g DW) > bud of aralia (19.3 mg/100 g DW) > broccoli (18.9 mg/kg DW) > asparagus (18.7 mg/100 g DW). Among Korean wild medicinal plants, Sumssukbujaengi showed the highest value followed by Sanmanul (a kind of wild garlic) with the level of 14.4 mg/100 g DW. For Chinese and Western cabbages, the level of vitamin U differed according to the parts of the plant such as core, middle and outside leaves. In both cabbages, level in the middle parts of leaves including midribs was the highest. As stated, the level of vitamin U was dependent on the part of the plant sample. Leaf parts of turnip and white radish showed higher value of 8.5 and 12.5 mg/100 g DW, respectively, than those of roots such as white radish in which levels were in the order of middle (11.3 mg/100 g DW), top (8.5 mg/100 g DW) and bottom (8.5 mg/100 g DW) portions in the white radish. In the analysis of amino acids, we did not find either a distinctive relationship between methionine and vitamin U synthesis or a significant connection between various free amino acids and vitamin U level in food plants.

Keywords: vitamin U (S-methylmethionine), food plants, methionine, amino acids

Recently, much attention has been focused on health and the extension of life worldwide. There is increasing evidence that increased consumption of fruit and vegetables leads to a decreased risk of cancer and other chronic diseases. The healthy effect of fruit and vegetables on the prevention of chronic disease and cancer has been investigated focusing on the functional constituents in food plants (Williams *et al.*, 1996). Functional foods are defined not as a remedy for disease at the stage of disease development but as an aid in the prevention of a particular disease at the premonitory stage (Okubo *et al.*, 1996). To reach this purpose of functional foods, it is necessary to establish a database of origins of food materials which contain certain bioactive compounds, and their function in maintaining the health of human beings.

As a natural amino acid, vitamin U is chemically S-methylmethionine and a vitamin-like substance. It belongs to the group of natural physiologically active compounds (Maw, 1981). Vitamin U extracted from *Brassica* vegetables has been known to be a good source of an anti-ulcer factor (Leung & Leung, 1989). Its deficiency may cause gastric ulcers. Other valuable pharmacological properties of vitamin U are their anti-inflammatory, analgesic, hypolipidemic (Bukin & Anisimov, 1973) and radio-protective effects (Gessler *et al.*, 1991).

Vitamin U has been isolated from many varieties of *Brassica* vegetables (Larina *et al.*, 1991), asparagus (Challenger & Hayward, 1954), green tea (Kiribuchi & Yamanishi, 1963), and cabbages (McRorie *et al.*, 1954). The metabolism of vitamin U is associated with sulfur-containing compounds since detachment of a methyl group converts this vitamin into methionine and its

enzymatic hydrolysis produces dimethyl sulfide (Gessler *et al.*, 1996). Vitamin U is a major metabolite of methionine and also can be synthesized from methionine and S-adenosylmethionine. It plays a role in reserving a form of methionine (Giovannelli *et al.*, 1980) and its synthesis could decrease the concentration of free methionine and its active derivative, S-adenosylmethionine (Baum *et al.*, 1983).

Even though many scientists have reported on bioactive components of some food plants, the physiological activities and nature of vitamin U have not yet been clearly identified. Therefore, more research in relation to its physiochemical characteristics and application are required. Most of the research on vitamin U has been carried out on Western vegetables, and not on Asian food plants such as Chinese cabbage, white radish and so on. Moreover, these results were mainly in the field of horticultural science, not in the components of the food itself.

The objective of this study was to determine the vitamin U level in 26 kinds of food plants which are known to have bioactive compounds using LC (liquid chromatography)-amino acid analyzer. Amino acids were also investigated to determine the relationship between vitamin U level and amino acids in several food plants.

Materials and Methods

Plant materials Twenty six kinds of food plants that are widely consumed as healthy vegetables in Asian or Western countries were selected for this experiment. All samples had highly marketable quality. After obtaining various food plants from wholesale markets and farms, samples were trimmed and divided into several groups for analysis. Outside, middle, and core parts of Chinese and Western cabbages, and leaf and root

parts of turnip and white radish were prepared. All parts of other plants were mixed analysis.

Preparation of extracts for vitamin U and amino acid analyses Samples were cut into small pieces mixed thoroughly and then freeze dried. One-2 g of each freeze-dried sample was combined with 50 ml of 80% ethanol and 100 μ l of 10 μ M/l norvaline as an internal standard. For extraction of vitamin U and amino acids, treated samples were stored overnight at room temperature with occasional gentle shaking. The samples were filtered (Advantec Toyo No. 2 $\times$ 2, Tokyo) using a vacuum pump and measured up to 100 ml volume with 80% ethanol. From these ethanol extracts, 10 ml aliquots were taken and evaporated to dryness using a rotary evaporator at 35°C with reduced pressure. After drying, 10 ml of 0.2 N sodium citrate buffer solution (pH 2.2) was added to each dried sample and mixed well using a sonicator. These buffer solutions contained vitamin U, amino acids, and pigments. To remove the pigments, 5 ml of each solution was passed through a syringe attached to a Sep-pak C18 cartridge (Waters, MA) with a 0.45 μ m pore size filter (13A, GL Science, Tokyo) and 2 ml of purified solution was collected for sample analysis after discarding the initial 3 ml. All samples were analyzed in triplicate.

Instrumentation For analysis of vitamin U and amino acids, a Shimadzu liquid chromatograph for amino acid analysis (ALC-1000, Shimadzu Corp., Kyoto) was used, consisting of a Shim-Pack Amino-Na (6.0 mm $\times$ 10 cm) column and a fluorescence detector (ex. 340 nm, em. 450 nm). Amino acid standard solutions (Type H, Wako Pure Chemical Industries, Ltd., Osaka)

and DL-methionine-S-methylsulfonium chloride (Sigma, St. Louis, Mo) were used for standards (Fig. 1). Norvaline (Wako Pure Chemical Industries, Ltd., Osaka) was used for an internal standard. The concentration of all standards was 100 n mol/ml.

Statistical analysis The data are summarized as simple mean values and standard deviation of 3 replications.

Results and Discussion

The level of vitamin U Vitamin U levels of 22 kinds of food plants were determined (Table 1). Spinach showed the highest level of 45.2 mg/100 g followed by Pak-choi (34.3 mg/100 g DW), kale (23.4 mg/100 g DW), Sumssukbuaengi (a wild Korean leafy vegetables, 19.8 mg/100 g DW), and leaf mustard (19.6 mg/100 g DW). Spinach is one of the most popular vegetables in both Asian and Western countries. This vegetable showed an approximately 1.3–2.3 times higher vitamin U level than that of the other three and leaf mustard which have been recommended as healthy food plants containing bioactive components (Jeong *et al.*, 1999; Kim *et al.*, 2000; Choi *et al.*, 2001). Asparagus and broccoli which are popular healthy vegetables in Western countries, showed moderately high vitamin U level, 18.7 mg/100 g DW and 18.9 mg/100 g DW, respectively. Among Korean wild vegetables, Sumssukbuaengi had the highest value followed by Sanmanul (a kind of wild garlic) in which the level was 14.4 mg/100 g DW. These wild vegetables have been used in folk remedies for years. Recently, Sanmanul has been reported to have antioxidant and anticarcinogenic activities (Lim *et al.*, 1996). Other food plants such as the bud of aralia (Ma *et al.*, 1995), burdock and crown daisy (Jeong *et al.*, 1999), which are also known for their functionality both in folk remedies and modern science were studied and found to have moderately higher levels of vitamin U. However, vitamin U was not detected in ginger, seaweed, red chili or several Korean wild medicinal vegetables (data not shown).

Unlike other food plant resources, Chinese cabbages, Western cabbage, turnip and white radish are big sized vegetables. It can be assumed that the level of nutritional composition would be dependent on the parts of those vegetables used. In Chinese and Western cabbages, there were different levels of vitamin U in the parts of the core, the middle and the outside leaves (Table 2). In both cabbage samples, the middle parts of leaves contained the highest level of vitamin U. However, other research on Western cabbages reported that core parts (a mixture of the leaf and mid-rib parts) showed the highest vitamin U level (Takigawa & Ishii, 1998). This difference seems to be caused by the portion sampled from the cabbages. The midribs contained 2–4 times less vitamin U than the leaf parts (data not shown). As shown in Table 2, levels of this vitamin were dependent on the part of the plant sampled. Leaf parts of turnip and white radish showed higher values of 8.5 and 12.5 mg/100 g DW, respectively, than those of roots in which the levels were in the order of middle (11.3 mg/100 g DW)>top (8.5 mg/100 g DW)>bottom (8.5 mg/100 g DW) portions in the white radish (Table 2). This result indicates that the leaf part of root vegetables is as nutritious as the more common edible parts.

The level of amino acids Fifteen kinds of amino acids were investigated using an amino acid analyzer for various food plants. In this study, asparagus and Sanmanul showed higher levels in most of the amino acids (data not shown). As a vitamin U

Fig. 1. The chromatographic profile of standards for vitamin U and amino acids.

Table 1. Levels of vitamin U and methionine in various food plant resources.

Common name	Scientific name	Vitamin U level (mg/100 g dry wt.)	Methionine ($\mu\text{mol}/100\text{ g dry weight}$)
Asparagus	<i>Asparagus officinalis</i> L.	18.7 $\pm$ 1.0	146.6 $\pm$ 2.3
Broccoli	<i>Brassica oleracea</i> var. <i>italica</i> Plenck	18.9 $\pm$ 1.1	37.7 $\pm$ 8.9
Bud of aralia	<i>Aralia elata</i> Seem.	19.3 $\pm$ 1.0	14.3 $\pm$ 2.3
Burdock	<i>Arctium lappa</i> L.	11.0 $\pm$ 1.0	ND
Celery	<i>Apium graveolens</i> L. var. <i>dulce</i>	8.3 $\pm$ 0.9	11.0 $\pm$ 2.1
Chamchi*	<i>Aster scaber</i> THUNB	4.0 $\pm$ 0.4	ND
Crown daisy	<i>Chrysanthemum coronarium</i> L.	11.1 $\pm$ 0.9	1.7 $\pm$ 0.2
Garlic	<i>Allium sativum</i> f. <i>pekinense</i> Makino	2.8 $\pm$ 0.2	46.4 $\pm$ 4.5
Green onion, large	<i>Allii fistulosum</i>	2.6 $\pm$ 0.6	12.9 $\pm$ 1.4
Green tea	<i>Theasinensis</i> var. <i>Bohea</i>	0.1 $\pm$ 0.0	ND
Kale	<i>Brassica oleracea</i> L. var. <i>acephala</i> DC.	23.4 $\pm$ 1.6	44.7 $\pm$ 3.2
Komchi*	<i>Ligularia fischeri</i> TURCZ	4.7 $\pm$ 0.5	2.0 $\pm$ 0.1
Laver, fresh	<i>Porphyra tenera</i>	2.2 $\pm$ 0.3	25.8 $\pm$ 1.4
Leaf mustard	<i>Brassica juncea</i> L.	19.6 $\pm$ 1.4	20.0 $\pm$ 1.2
Nurukchi*	<i>Pleurospermum kamtschaticum</i> HOFFM	0.8 $\pm$ 0.2	3.3 $\pm$ 0.1
Onion	<i>Allium cepa</i> L.	2.7 $\pm$ 0.2	55.2 $\pm$ 2.1
Pak-choi	<i>Brassica campestris</i> L. var. <i>chinensis</i>	34.3 $\pm$ 2.1	25.0 $\pm$ 2.9
Sanmanul*	<i>Allium victorialis</i> var. <i>platyphyllum</i> Makino	14.4 $\pm$ 0.7	161.9 $\pm$ 14.2
Shepherd's purse	<i>Capsella bursa-pastoris</i> Medicus	3.4 $\pm$ 0.3	12.9 $\pm$ 1.5
Spinach	<i>Spinacia oleracea</i> L.	45.2 $\pm$ 1.3	91.0 $\pm$ 7.1
Sumssukbuaengi*	<i>Aster glehni</i>	19.8 $\pm$ 0.7	21.9 $\pm$ 1.2
Wasabi	<i>Wasabia koreana</i>	4.7 $\pm$ 0.3	ND

*Korean wild food plants.

ND: not detected.

Each value is the mean of 3 replicates $\pm$ SD.**Table 2.** Levels of vitamin U and methionine in different parts of food plant resources.

Common name	Scientific name	Part of food plants	Vitamin U level (mg/100 g dry wt.)	Methionine ($\mu\text{mol}/100\text{ g dry weight}$)
Chinese cabbage	<i>Brassica campestris</i> L. var. <i>perkinensis</i>	Core	26.7 $\pm$ 1.3	139.6 $\pm$ 11.2
		Middle	39.3 $\pm$ 1.2	167.6 $\pm$ 9.8
		Outside	27.8 $\pm$ 1.4	254.2 $\pm$ 15.2
Turnip	<i>Brassica campestris</i> L. var. <i>rapifera</i>	Leaf	8.5 $\pm$ 0.5	170.7 $\pm$ 3.6
		Root	7.3 $\pm$ 0.9	153.9 $\pm$ 7.3
Cabbage	<i>Brassica oleracea</i> L. var. <i>capitata</i> L.	Core	30.6 $\pm$ 1.7	51.3 $\pm$ 2.1
		Middle	46.4 $\pm$ 1.7	14.6 $\pm$ 0.3
		Outside	26.1 $\pm$ 1.2	20.8 $\pm$ 2.3
White radish	<i>Raphanus sativus</i> var. <i>acanthiformis</i> Makino	Leaf	12.5 $\pm$ 0.8	24.6 $\pm$ 2.1
		Root (top)	8.5 $\pm$ 0.4	12.8 $\pm$ 0.5
		Root (middle)	11.2 $\pm$ 0.7	26.1 $\pm$ 1.9
		Root (bottom)	8.5 $\pm$ 0.3	19.9 $\pm$ 1.3

Each value is the mean of 3 replicates $\pm$ SD.

precursor, methionine was either not detected or showed low levels in vegetables except asparagus (146.6 $\mu\text{mol}/100\text{ g DW}$), Sanmanul (161.9 $\mu\text{mol}/100\text{ g DW}$), and spinach (91.0 $\mu\text{mol}/100\text{ g DW}$), which showed high vitamin U values (Table 1). However, there was no clear relationship between methionine and vitamin U levels in this experiment. From this result, it is not conclusive that various free amino acids relate to levels of vitamin U in food plants.

Different parts of food plants showed different types of amino acid (data not shown). This does not mean that the levels of vitamin U were affected by other free amino acids. Furthermore, comparison of different parts of the plants, methionine levels showed no discernable relationship with vitamin U content (Table 2). In the case of Chinese cabbage, it has been reported that a relationship was found between methionine level and vitamin U synthesis in the young seedling stage (Kim & Ishii, unpublished data). However, the result in this experiment did not clarify the involvement of methionine in vitamin U synthesis. It seems to be due to the fast turnover rate of the methionine cycle in mature food plants.

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