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Vitamin U in Medicinal Food Plants

Gun-Hee Kim

Department of Food and Nutrition, DukSung Women's University, Seoul, Korea
E-mail: ghkim@duksung.ac.kr

ABSTRACT

Vitamin U levels in 26 kinds of food plants are well known to healthy vegetables in Asian or Western countries were determined. Spinach showed the highest level of 452.04 mg/kg and in order Pack-choi (343.18 mg/kg) > Kale (234.18 mg/kg) > Sumssukbujaengi (197.66 mg/kg) > leaf mustard (196.21 mg/kg) > aralia bud (192.50 mg/kg) > broccoli (189.03 mg/kg) > Asparagus (187.35 mg/kg). Among Korean wild medicinal plants, Sumssukbujaengi showed the highest value followed by Sanmanul (a kind of wild garlic) level of 143.46 mg/kg. For Chinese cabbages and cabbages, vitamin U showed different levels according to the parts of plant such as core, middle, outward leaves. In both samples, middle parts of leaves including midribs contained the highest level of vitamin U. The level of vitamin U was dependent on the part of the plant sample and cultivars. Leaf parts of turnip and white radish showed higher value of 84.82 mg/kg and 124.62 mg/kg than those of roots which were in order of middle (112.39 mg/kg), top (84.84 mg/kg) and bottom (84.61 mg/kg) portions in the white radish. In the analysis of amino acids, we didn't find either distinctive relationship between methionine and vitamin U synthesis or significant connection various free amino acids and vitamin U level in food plants.

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

INTRODUCTION

Fruit and vegetable consumption has been increasing with concerning health and life prolongs. The healthy effect of fruit and vegetable on the prevention chronic disease and cancer has been investigated with focusing on the functional constituents in food plants (Williams et al., 1996). Recently, functional foods are defined not as the remedy for disease at the stage of disease development but as the prevention of particular disease at the premonitory stage (Okubo, 1996). To reach modern concept of functional foods, developing origins of food resources, which have bioactive compounds as well as its function for the human health benefit are required.

As a natural amino acid, S-methylmethionine (SMM), namely vitamin U is vitamin-like substance with natural physiological active compounds (Maw, 1981). Other valuable pharmacological properties of Vitamin U are known for anti-inflammatory, analgesic, hypolidemic (Bukin and Anisimov, 1973) and radio-protective effects (Gessler et al., 1991). Extractions of *Brassica* vegetables are known to be good sources of anti-ulcer factor (Leung and Leung, 1989). Its deficiency may cause gastric ulcers.

Vitamin U has been isolated from many varieties of *Brassica* vegetables (Larina et al., 1991), asparagus (Challenger and Hayward, 1954), green tea (Kiribuchi and Yamanishi, 1963), and cabbages (McRorie et al., 1954). The metabolism of vitamin U is associated with sulfur-containing compounds since detachment of a methyl converts vitamin U into methionine and then enzymatic hydrolysis of vitamin U 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. As a major metabolite of methionine, Vitamin U can be synthesized from methionine and S-adenosylmethionine (Green and Davis, 1960). It plays a role in reserving form of methionine (Giovanelli et al., 1980) and its sythesis could decrease the concentration of free methionine and its active derivative, S-adenosylmethionine (Baum et al., 1983).

There are many reports on bioactive components in several food plants. However, physiological activities and properties of Vitamin U are not clearly identified. Furthermore, many studies on Vitamin U have been conducted on Western vegetables and studied in the field of horticultural science not on Asian plants such Chinese cabbage, white radish. Therefore, much attention on vitamin U related to physio-chemical components, specific function, and application are suggested.

The objective of this study was to determine the vitamin U level in 26 kinds of food plants which are well known to bioactive compounds by using LC (liquid chromatography)-amino acid analyzer. Amino acids were also investigated to determine 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 worth condition. After obtaining various food plants from wholesale market and farm, samples were trimmed and divided into several groups for analysis. Outer, middle, and core parts group of Chinese cabbages and cabbages and leaf, and root parts for turnip and white radish were prepared. Other plants are mixed with all parts for analysis.

Preparation of analysis sample solutions for vitamin U and amino acids

Samples were cut into small pieces and mixed thoroughly and then freeze dried. Fig. 1 shows that sample preparation diagrams for extraction and isolation of vitamin U. About 1～2 g of each freeze-dried sample was combined with 50 mL of 80% ethanol and 100 μL of 10 μmole 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. Extracted samples were filtered (Advantec Toyo No.2×2) using a vacuum pump and bulked up to 100 mL volume with 80% ethanol. From these ethanol fractions, 10 mL aliquots were taken and condensed using a rotary evaporator at 35°C with reduced pressure. After condensing, 10 mL of 0.2 N-sodium citrate buffer solution (pH 2.2) was added into each dried sample and then ultrasonic treatment was applied for 2～3 minutes. These buffer solutions contained vitamin U, amino acids, and pigments. To remove pigments, 5 mL of each solution was passed through a syringe attached to a Sep-pak C18 cartridge (Waters, Ma, USA) with a 0.45 μm pore size filter (13A, GL Science, Tokyo, Japan) and collected 2 mL of purified solution for sample analysis after discarding initial 3 mL. All samples were replicated three times.

Instrumentation

For analysis of vitamin U and amino acids, a Shimadzu liquid chromatograph using amino acid analyzer (ALC-1000, Shimadzu Corp., Kyoto, Japan) was used, consisting of Shim-Pack Amino-Na (6.0 mm×10 cm) column and fluorescence detector (ex. 340 nm, em. 450 nm). Amino acid standard solutions (Type H, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and DL-methionine-s-methylsulfonium chloride (Sigma, St. Louis, Mo, USA) were used for standards (Fig. 2). Norvaline (Wako Pure Chemical) was used for internal standard. The concentration of all standards was 100 nmol/mL.

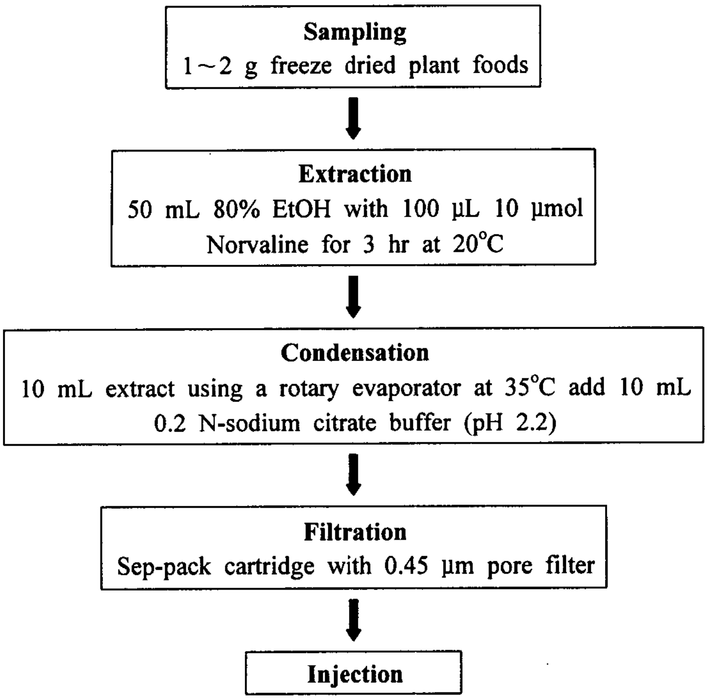

Fig. 1. Flow diagram for sample preparation by using LC with amino acid analyzer

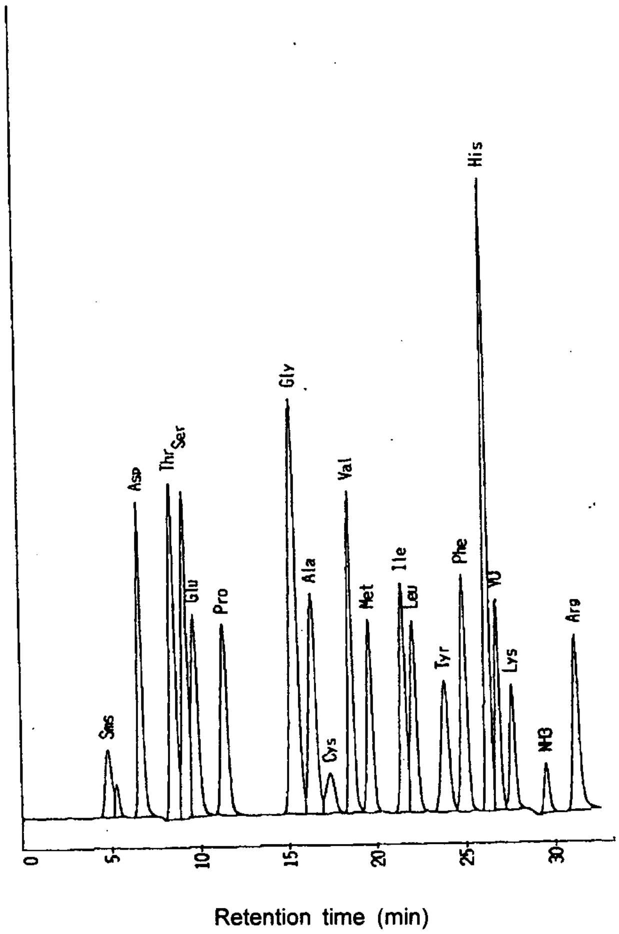

Fig. 2. The chromatogram profile of standards for vitamin U and amino acids.

Statistical analysis

The data are summarized as simple mean values and standard deviation for 3 replications. No statistical test of this data was done.

RESULTS AND DISCUSSION

The level of vitamin U

Vitamin U levels of 25 kinds of food plants were determined (Table 1). Spinach showed the highest level of 452.04 mg/kg followed 343.18 mg/kg of Pak-choi, 234.18 mg/kg of kale, 197.66 mg/kg of Sumssukbujaengi, and 196.21 mg/kg of leaf mustard. Spinach is one of the most popular vegetables in both Asia and Western countries. This vegetable showed approximately 1.3~2.3 times higher vitamin U level than that of Pak-choi, kale, Sumssukbujaengi and leaf mustard which have been recommended as healthy plant foods containing bioactive components (Jeong et al., 1999; Kim et al., 2000; Choi et al., 2001). Asparagus and broccolis which are well known to popular healthy vegetables in Western countries, showed moderately high vitamin U level, 187.35 mg/kg and 189.03 mg/kg. Among Korean wild vegetables, Sumssukbujaengi had the highest value followed by Sanmanul (a kind of wild garlic), level of 143.46 mg/kg. These wild vegetables have been used in a folk remedy for years. Recently, those functional properties of antioxidant and anticarcinogen activities were found (Lim et al., 1996). Other food plants such as bud of aralia (Ma et al., 1995), burdock and crown daisy (Jeong et al., 1999), which are also known for their functionality both in a folk remedy and modern sciences were investigated and showed moderately higher levels of vitamin U. However, vitamin U was not detected in ginger, seaweed, red chili and

Table 1. Vitamin U level in various food plant resources

Common name	Scientific name	Vitamin U level (mg/kg^{-1} dry wt.)
Asparagus	*Asparagus officinalis* L.	187.35±10.21
Broccoli	*Brassica oleracea* var. *italica* Plenck	189.03±11.44
Bud of aralia	*Aralia elata* Seem.	192.50±9.89
Burdock	*Arctium lappa* L.	109.59±10.44
Celery	*Apium graveolens* L. var. *dulce*	82.59±8.98
Chamchi*	*Aster scaber* Thunb	39.67±3.79
Crown daisy	*Chrysanthemum coronarium* L.	111.04±9.44
Garlic	*Allium sativum* f. *pekinense* Makino	28.03±1.86
Green onion, large	*Allii fistulosum*	26.05±6.24
Green tea	*Theasinensis* var. *Bohea*	1.26±0.19
Kale	*Brassica oleracea* L. var. *acephala* DC.	234.18±16.23
Komchi*	*Ligularia fischeri* Turcz	46.60±4.89
Laver, fresh	*Porphyra tenera*	22.27±2.65
Leaf mustard	*Brassica juncea* L.	196.21±14.23
Nurukchi*	*Pleurospermum kamtschaticum* Hoffm	8.23±2.41
Onion	*Allium cepa* L.	27.19±2.11
Pak-choi	*Brassica campestris* L. var. *chinensis*	343.18±21.23
Sanmanul*	*Allium victorialis* var. *platyphyllum* Makino	143.46±6.54
Shepherd's purse	*Capsella bursa-pastoris* Medicus	34.18±3.25
Spinach	*Spinaoia oleracea* L.	452.04±12.51
Sumssukbujaengi*	*Aster glehni*	197.66±6.59
Wasabi	*Wasabia koreana*	46.57±3.24

*Korean wild food plants.

several Korean wild medicinal vegetables (data not shown).

Unlike other food plant resources, Chinese cabbages, cabbage, turnip and white radish are big sized samples. This indicated that the level of nutritional composition would be dependent on the parts of the sample. For example, Chinese cabbages and cabbages, vitamin U showed different levels in the parts of core, middle and outward leaves. In both samples, middle parts of leaves contained the highest level of vitamin U (Table 2). However, other research on cabbages reported that core parts showed the highest vitamin U level due to only leaves used for sample not midribs included (Takigawa and Ishii, 1998). In this study, leaves and midribs were mixed together for samples. Midribs contained 2～4 times less vitamin U than only leaves parts (data not shown). Levels of vitamin U were influenced by the part of the plant sampled and cultivars. Leaf parts of turnip and white radish showed higher values of 84.82 mg/kg and 124.62 mg/kg than those of roots which were in order middle (112.39 mg/kg) > top (84.84 mg/kg) > bottom (84.61 mg/kg) portions in the white radish (Table 2). This result indicates that leaf part of root vegetables can be considered as edible food plants.

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 amino acids. The most prevalent amino acids were glycine with value of 9,295 μmol/kg, 29,034 μmol/kg of serine in asparagus, 18,855 μmol/kg of isoleucine, phenylalanine of 16,472 μmol/kg, and 7,829 μmol/kg of tyrosine in Samanul, 94,509 μmol/kg of alanine in fresh laver, and 54,485 μmol/kg of arginine in Burdock (Table 3). As a vitamin U precursor, methionine was not either detected or showed low levels excluded asparagus (1,465 μmol/kg), Sanmaul (1,618 μmol/kg), and spinach (910 μmol/kg). These food plants showed high vitamin U value. However, there was no clear relationship between methionine and vitamin U synthesis 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 had different type of amino acids. In cabbages, core parts were determined high value of alanine, glutamic acid, and isoleucine, whereas middle parts showed arginine, glycine, histidine, lycine, proline, serine, threonine, tyrosine, and valine, and outward parts contained aspartic acid, methionine, and phenylalanine (Table 4). This trend also occurred in Chinese cabbages, turnip and white radish. It does not mean

Table 2. Vitamin U level in different parts of food plant resources

Common name	Scientific name	Part of food plants	Vitamin U level (mg/kg dry wt.)
Chinese cabbage	*Brassica campestris* L. var. *perkinensis*	Core	266.52±13.21
		Middle	393.10±12.19
		Outward	277.77±14.13
Turnip	*Brassica campestris* L. var. *rapifera*	Leaf	84.82±4.78
		Root	72.82±8.99
Cabbage	*Brassica oleracea* L. var *capitata* L.	Core	305.89±17.11
		Middle	464.40±16.98
		Outward	261.11±12.43
White radish	*Raphanus sativus* var. *acanthiformis* Makino	Leaf	124.62±7.68
		Root (top)	84.84±3.86
		Root (middle)	112.39±7.23
		Root (bottom)	84.61±2.96

Table 3. Amino acids level in various food plant resources

Food plants	Amino acids (μmol/kg dry weight)													
	Alanine	Arginine	Aspartic acid	Glutamic acid	Glycine	Histidine	Isoleucine	Lysine	Methionine	Phenylalanine	Proline	Serine	Threonine	Tyrosine	Valine
Asparagus	33062.64	14669.55	7800.80	23229.01	9295.96	6132.78	9198.02	5503.09	1465.74	11665.24	8583.85	29034.84	57729.19	1439.68	22674.97
Broccoli	10391.17	19017.14	9888.16	15396.42	810.51	3120.28	10297.83	2148.06	377.01	3308.64	30030.89	18301.27	35720.48	1855.66	19119.23
Bud of aralia	1989.24	9194.40	7855.53	2903.33	534.96	3821.76	7894.65	1465.97	142.45	5513.58	2754.08	15052.07	78319.30	3700.69	8209.37
Burdock	6094.26	54485.65	1822.36	2679.28	300.84	4677.38	6771.30	3232.32	ND	3130.59	38170.75	1875.52	43594.34	1361.51	6765.48
Celery	3222.14	179.28	1511.54	7438.15	181.36	850.83	2193.63	605.53	110.29	455.65	1125.77	5515.71	59106.25	1274.40	4981.60
Chamchi	2741.97	1591.66	4113.76	12076.66	125.61	478.88	1723.64	665.55	ND	3117.59	1764.63	3715.61	7271.35	604.63	2705.18
Crown daisy	9694.20	4840.14	4546.28	12016.33	492.67	1861.48	5187.07	683.36	17.22	3778.26	24119.55	14010.29	39399.10	344.50	9465.05
Garlic	4288.94	16596.56	620.04	3615.52	895.93	1515.50	2938.51	1074.38	463.68	3727.19	7128.87	6419.10	23458.13	7061.01	6854.31
Green onion, large	20900.52	1088.73	798.58	5067.10	152.13	620.95	711.54	279.06	128.64	1993.98	885.09	8869.69	25472.83	1064.98	2647.05
Green tea	1288.84	390.75	2528.16	3952.80	75.89	274.92	441.15	41.79	ND	544.35	504.74	ND	13560.79	429.35	623.20
Kale	10367.89	6939.40	5809.14	18566.11	595.59	2065.13	8523.02	4559.01	446.51	6574.60	5311.44	16754.53	45778.09	1331.25	15893.94
Komchi	7351.39	4891.94	6546.97	21182.32	302.01	1049.57	4468.42	595.64	20.40	2420.92	13741.45	13798.31	12797.14	2492.78	6863.54
Laver, raw	94509.05	ND*	2638.20	12732.83	1512.26	344.62	1089.71	1417.83	257.92	5342.26	1489.90	1274.43	10636.60	3414.34	2098.67
Leaf mustard	6538.14	5847.65	9257.71	16403.33	432.53	1711.97	4691.54	3840.61	199.56	7267.77	4012.16	8694.19	35102.84	786.23	10168.14
Nurukchi	2773.13	725.24	3774.81	2368.91	92.59	481.74	1130.12	242.95	32.59	1020.06	1099.45	2848.61	5073.97	119.63	1959.15
Onion	3355.69	19614.21	3907.73	8899.25	718.79	1839.38	1616.03	1972.07	552.10	2028.72	3049.08	7298.07	22458.14	2557.85	3196.54
Pak-choi	26717.83	10083.732	9990.25	20337.40	2376.93	2365.44	4944.39	3688.78	250.00	3861.12	3462.96	13061.76	74205.97	1435.38	11105.05
Sanmanul	24996.83	5033.93	5529.67	10028.00	2225.61	6287.48	18855.09	5090.64	1618.83	16472.06	12645.74	15557.22	77017.35	7829.54	27810.90
Shepherd's purse	7663.96	3001.50	1855.16	5328.93	593.39	670.45	1896.01	544.55	128.88	1323.61	6147.56	4575.33	18699.65	625.77	3603.57
Spinach	10657.67	29326.95	14254.37	11956.34	645.98	4986.84	9790.71	4282.43	910.10	7194.19	7460.10	14373.79	46118.17	5771.90	14428.38
Sumssukbujaengi	11643.05	2844.93	10871.23	9356.81	649.54	2261.10	6784.67	1402.07	218.70	6078.31	5770.97	12998.70	59768.05	2171.16	11783.44
Wasabi	5765.00	31497.95	7342.95	13727.95	2028.97	2137.84	1755.22	1162.27	ND	1464.20	20861.59	3542.27	23329.43	1315.11	4175.22

*ND: not detected.

Table 4. Amino acids level in different parts of food plant resources

Food plants	Amino acids (μmol/kg dry weight)													
	Alanine	Arginine	Aspartic acid	Glutamic acid	Glycine	Histidine	Isoleucine	Lysine	Methionine	Phenylalanine	Proline	Serine	Threonine	Tyrosine	Valine
Cabbage (core part)	69034.31	10548.43	7825.29	39859.60	1769.60	7809.02	9749.02	2150.00	1395.49	21501.56	15166.47	27552.94	80017.45	4417.84	14514.11
Cabbage (middle part)	60584.31	12895.49	12002.74	30736.66	2104.51	9117.45	8656.86	2327.05	1676.07	29213.52	15230.39	29037.05	153034.90	6532.94	15844.90
Cabbage (outward part)	28145.96	10013.26	28164.42	30011.34	1242.11	5448.07	4536.53	892.69	2542.30	53343.26	13666.73	14024.80	148075.57	697.88	15270.76
Chinese cabbage (core part)	124757.50	1092.11	4461.92	38082.69	6376.92	3508.84	4784.61	417.30	1706.73	33197.50	16423.65	15913.84	202528.46	7159.61	8230.96
Chinese cabbage (middle part)	107210.57	9892.30	13754.03	48497.30	4316.53	2736.15	8018.84	949.23	1539.03	37632.69	13266.15	15668.84	173694.42	5097.69	13140.96
Chinese cabbage (outward part)	14038.67	11548.11	3866.41	7714.15	1001.32	549.62	2095.66	254.52	512.45	14128.67	3715.28	4597.73	67844.52	433.39	5182.45
Turnip (leaf)	15233.50	4639.74	1467.80	2044.26	719.19	797.21	1888.39	1783.12	146.32	1806.66	1927.97	5674.51	20801.22	801.68	4748.81
Turnip (root)	10883.35	518.39	4830.51	2193.93	3599.28	767.50	1133.28	534.41	208.15	594.62	5970.61	5220.10	29391.91	493.08	2458.15
White radish (leaf)	12363.18	4408.72	2490.38	4035.90	1619.01	1350.31	2873.07	1580.98	246.14	1929.54	4253.25	8517.38	29945.12	1126.92	8655.26
White radish (root-top)	8687.07	2581.61	3490.24	3444.78	1431.95	1212.09	4070.43	1046.48	127.75	1438.29	4342.04	5219.51	34878.58	530.43	6904.19
White radish (root-middle)	31634.52	3148.60	4960.34	5815.92	5333.78	1536.07	4876.81	1435.32	261.24	1816.36	2139.10	8328.60	40690.10	604.77	8908.15
White radish (root-bottom)	18107.96	3455.67	5637.39	8980.26	2621.87	1431.09	4315.05	1304.94	199.11	1450.62	1395.41	10126.09	32892.24	525.46	8031.87

levels of vitamin U affected by other free amino acids. Furthermore, comparing different parts of food plants, methionine levels was not showed any discernable relationship with vitamin U content. Since there was a report on relationship with methionine and vitamin U synthesis in young seedlings of Chinese cabbages (Kim GH and Ishii G unpublished), this finding is not able to determine the involvement of methionine for vitamin U synthesis. It seems to be due to fast turnover rate of the methionine cycle. When comparing amino acids, core parts of Chinese cabbages had extremely high values in alanine of 124,757 μmol/kg and threonine of 202,528 μmol/kg.

CONCLUSION

Spinach showed the highest vitamin U level of 452.04 mg/kg among the selected food plant resources, which are known to healthy vegetables. Pak-choi, kale, Sumssukbujaengi, leaf mustard, asparagus and broccoli showed high vitamin U level ranged from 343.18 mg/kg to 187.35 mg/kg. Among Korean wild vegetables, Sumssukbujaengi showed the highest value followed by Sanmanul, which is a kind of wild garlic, level of 143.46 mg/kg. Vitamin U level was influenced by the part of the plant sampled and cultivars. Leaf parts of turnip and white radish showed higher levels of 84.82 mg/kg and 124.62 mg/kg than those of roots.

In amino acids, asparagus, and Sanmanul showed high levels in most amino acids. As a vitamin U precursor, methionine was neither detected nor contained low level except asparagus (1,465 μmol/kg), Sanmaul (1,618 μmol/kg), and spinach (910 μmol/kg). These food plants showed high vitamin U level. However, there was no discernable relationship between methionine and vitamin U synthesis in this study. From this result, it is not conclusive whether various free amino acids were related to vitamin U level in food plants. Different parts of food plants seem to be based on amino acids. This trend also showed in Chinese cabbages, turnip and white radish. It didn't appear vitamin U level effect by other free amino acids. Furthermore, the level of methionine didn't show any distinctive relationship with vitamin U content in comparing different parts of food plants. It could be due to quick turnover rate of the methionine cycle in the mature food plants. Among levels of amino acids, core parts of Chinese cabbages contained extremely high values of alanine, 124,757 μmol/kg and threonine, 202,528 μmol/kg.

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