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Change in Levels of Vitamin U and Amino Acids in Korean Chinese Cabbages Under Various Drying Methods

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ABSTRACT – S-methylmethionine, vitamin U levels were affected by cultivars, parts of Korean Chinese cabbages, and drying methods. Among drying conditions, freeze drying method appeared the best condition to maintain vitamin U content compared to oven and air drying methods. In the case of Korean Chinese cabbages, outward leaves have high levels of chlorophyll and fiber. From this study, the outward parts contained high levels of vitamin U in two cultivars. Leaf parts were 1.1-21.2 times higher in vitamin U levels than midribs in both cultivars. This difference was shown most distinctively in freeze dried outward parts of Winter Pride cultivar. Like vitamin U, free amino acids also showed much higher levels in leaves. Levels of amino acids showed irregularly changing patterns at different parts and cultivars of Korean Chinese cabbages with various drying methods. Alanine and threonine appeared relatively abundant amino acids in most parts of samples. Since no distinctive trends were observed in this result, it seems no relationship exists between amino acids and vitamin U levels. Levels of methionine in different parts and cultivars of Korean Chinese cabbages dried with various methods did not show clear relationship with the level of vitamin U. Moreover, methionine was not detected in freeze dried outward leaf parts which were the highest parts of vitamin U levels in Winter Pride and 55 days cultivars. There were similar levels of methionine between oven and freeze drying. Samples prepared by air drying showed significantly lower levels than those by oven and freeze drying. Methionine as a precursor of vitamin U, may not play a role in an increase of vitamin U during drying of Korean Chinese cabbages

Key words: Korean Chinese cabbages (*Brassica campestris* L. P*erkinensis)*, S-methylmethionine, vitamin U, amino acids, drying methods

Introduction

Recently, much attention has been focused on health and life extension world-wide. There is increasing evidence that increased consumption of fruit and vegetables leads to a decreased risk of cancer and other chronic diseases[1]. Hence, much research was conducted on functionality of food materials itself mainly on food plants. In modern concepts of functional foods, it should be used for prevention of a particular disease at the premonitory stage, not for remedy of the disease at the stage of development[2]. To meet this purpose, it is necessary to establish data base of origins of food materials which contain certain bioactive compounds, and its function towards the health of human beings.

Vitamin U which is chemically S-methylmethionine(SMM) is a vitamin-like substance and a natural amino acid. It belongs to the group of natural physiologically active compounds[3] and has been known as an anti-ulcer factor extracted from *Brassica* vegetables[4]. Its deficiency is thought to be a possible cause of gastric ulcers. Other valuable pharmacological properties of vitamin U have been known to have anti-inflammatory, analgesic, hypolipidemic[5] and radio-protective effects[6].

Since vitamin U was isolated from cabbages[7], it has been identified in food plants such as asparagus[8], green tea(9) and various *Brassica* vegetables[10]. Vitamin U metabolism is known to be closely associated with sulfur-containing compounds because the detachment of a methyl group converts vitamin U into methionine, and enzymatic hydrolysis of vitamin U produces dimethyl sulfide[11]. Vitamin U is a major metabolite of methionine and also can be synthesized from methionine and S-adenosylmethionine[12]. Vitamin U plays a role as a reserve form of methionine[13]. Vitamin U synthesis decreases the concentration of free methionine and its

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active derivative, S-adenosylmethionine[14].

Even though many scientists have reported on bioactive components of some food plants, vitamin U is not yet clearly identified for its physiological activities and nature. Therefore, more research is needed on vitamin U concerning physico-chemical components, characteristics and application. Most of the research on vitamin U has been carried out on Western vegetables not on Asian food plants such as Chinese cabbage, white radish and so on. Moreover, these results showed mainly in the field of horticultural science, not in view of food materials.

The aim of this study is to determine the level of vitamin U in Korean Chinese cabbages dried with various conditions using liquid chromatography with amino acid analyzer for providing basic database for active research on functionality of vitamin U. Also, amino acids were investigated to determine their relationship with vitamin U levels in various drying conditions of Korean Chinese cabbages.

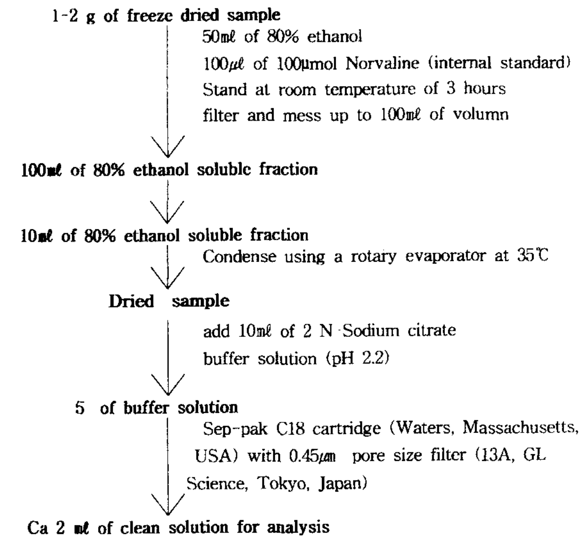

Fig. 1. Procedure of sample preparation for vitamin U and amino acids.

Materials and Methods

Plant materials

Two different cultivars, Winter Pride and Hungnong 55 days (Hungnong Seeds, Korea), were sampled for this experiment. Sampling occurred at fully developed stages of growth was prepared for determination of vitamin U and amino acids.

Korean Chinese cabbages were obtained from wholesale market. After purchasing, Chinese cabbages were stored at 4°C until required for use in the experiment. These samples were divided into outward (green/partly green leaves), middle (completely yellow leaves) and core parts (small, yellow leaves, less than 18 g/leaf). The experiments were conducted on leaf and mid-rib sections separately. The sample size was about 10g for each section for analysis of vitamin U and amino acids.

Preparation of analysis sample solutions for vitamin U and amino acids

The samples of all prepared Korean Chinese cabbages were cut into small pieces and mixed thoroughly, and then freeze dried. Fig. 1 shows sample preparation for extraction and isolation of vitamin U. About 1-2 g of each freeze dried samples was combined with 50 mL of 80% ethanol and 100 μL of 10 μmol Norvaline as an internal standard. For extracting vitamin U and amino acids, treated samples were stored overnight at room temperature with occasional gentle shaking. Extracted samples were filtered (Advantec Tokyo 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 ultrasonic treatment was applied for 2-3 minutes. These buffer solutions included 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, Massachusetts, 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 washing initial 3 mL. Three replication sample solutions were provided for each treatment.

Instrumentation

For analysis of vitamin U and amino acids, a Shimadzu liquid chromatography using amino acid analyzer (ALC-1000, Shimadzu Corp., Kyoto, Japan) was used, consisting of Shim-PackAmino-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) were used for standards (Fig. 2). Norvaline (Wako Pure Chemical) was used as an internal standard. The concentration of all standards was 100 nmol/mL.

Statistical analysis

Each value of results was the mean of three replications. The level of vitamin U was expressed in mean with standard deviation.

Results and Discussion

Levels of vitamin U in different parts under various drying conditions

The results showed significantly different levels of vitamin U from different parts of Korean Chinese cabbages and drying methods as shown in Table 1. There are significant differences in vitamin U levels with drying conditions and parts of Korean Chinese cabbages. Among drying conditions, freeze drying method appeared the best condition to maintain vitamin U content compared to oven and air drying methods. It has been reported that core parts contained the highest levels of vitamin U in fresh cabbages[15]. In the case of Korean Chinese cabbages, outward leaves have high levels of chlorophyll and fiber. From this study, the outward parts showed high levels of vitamin U in two cultivars. Leaf parts were 1.1-21.2 times higher vitamin U levels than those in midribs in both cultivars. This difference was shown most distinctively in freeze dried outward parts of Winter Pride cultivar. From the these results, vitamin U levels can be affected by cultivars and parts of Korean Chinese cabbages. These results agree with Larina *et al.*[10] that cultivar-related features, anatomical structure of plant and physiological state affect vitamin concentration in the vegetables.

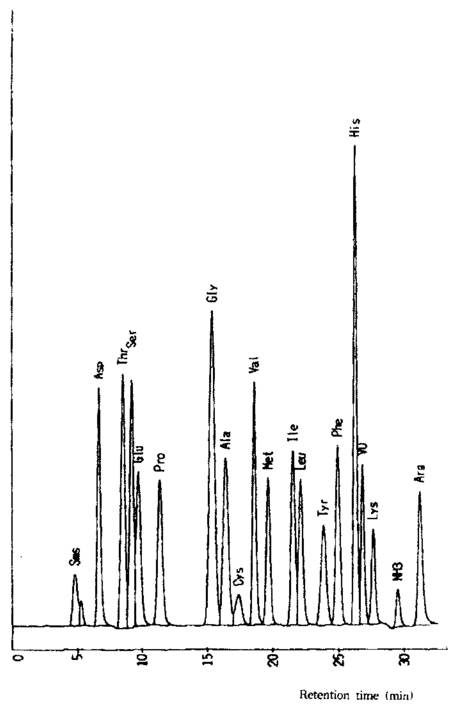

Fig. 2. The pattern of standard solution for vitamin U and amino acids.

Amino acids compostion of Korean Chinese cabbages during storage

Methionine has been known a precursor of vitamin U synthesis(8). Levels of major amino acids in core, middle and outward parts in two cultivars of Korean Chinese cabbages are shown in Table 2-1, 2-2, 3-1, 3-2, 4-1, 4-2. Like Vitamin U, free amino acids also showed much higher levels in leaves. Levels of amino acids showed irregularly changing patterns at different parts and cultivars of Korean Chinese cabbages with various drying methods. However, alanine and threonine appeared rel-

Table 1. Levels of vitamin U in different parts of Korea Chinese cabbages dried with different methods Each vale is the mean of 3 replicates

cultivar	part	Drying condition (mg/100g)		
		Oven-drying	Freeze-drying	Air-drying
winter pride	core leaf	41.08	153.86	22.47
	core midrib	20.05	95.91	20.83
	middle leaf	40.79	112.65	14.57
	middle midrib	12.85	28.91	11.66
	outward leaf	71.37	154.86	35.67
	outward midrib	6.85	7.29	4.59
55 Days	core leaf	49.12	137.04	18.91
	core midrib	14.76	85.88	11.55
	middle leaf	56.34	176.42	16.67
	middle midrib	16.00	51.46	6.50
	outward leaf	85.26	244.09	48.34
	outward midrib	6.96	24.61	5.81

Each vale is the mean of 3 replicates

Table 2-1. Levels of various amino acids in core parts of Winter Pride Korean Chinese cabbage cultivar dried with different methods

Amino acid	Part of Korean Chinese cabbage(μmol/100 g)					
	core leaf			core midrib		
	Oven drying	Freeze drying	Air drying	Oven drying	Freeze drying	Air drying
L-Aspartic acid	1059.65	812.68	360.94	519.80	269.36	547.10
Threonine	3438.38	13079.16	2448.53	4297.85	12111.53	2446.26
L-Serine	1780.87	4200.31	816.04	1655.30	3012.26	1177.56
Glutamic acid	4254.17	9315.27	805.28	3958.40	3533.02	666.97
L-Proline	752.76	2669.43	-	741.84	1773.16	-
Glycine	477.50	868.20	171.02	528.44	782.39	-
L-Alanine	4323.8	14643.5	1529.41	4438.95	9626.19	-
Cystain	236.73	371.74	14.78	191.58	-	-
Valine	2088.15	4685.76	1045.36	1741.37	4167.69	1289.06
Isoleucine	2226.41	2892.28	646.75	1133.39	2382.83	1243.71
Leucine	2140.49	1243.44	164.85	731.86	398.34	268.30
Tyrosine	492.86	447.63	58.54	2411.60	129.08	66.45
Phenylalanine	1106.55	1552.92	-	517.33	596.28	-
GABA*	151.30	147.67	93.21	203.68	92.20	294.34
L-Histidine	396.51	1486.57	457.95	297.72	1339.99	428.15
Lysine	254.77	489.43	46.79	147.41	551.46	102.38
L-Arginine	1454.56	2278.06	414.28	564.71	774.20	159.99

*r-amino-n-butyric acid

Table 2-2. Levels of various amino acids in core parts of 55 days Korean Chinese cabbage cultivar dried with different methods

Amino acid	Part of Korean Chinese cabbage(μmol/100 g)					
	core leaf			core midrib		
	Oven drying	Freeze drying	Air drying	Oven drying	Freeze drying	Air drying
L-Aspartic acid	845.34	1404.60	420.40	350.65	389.59	164.89
Threonine	4112.82	-	2753.63	2848.68	10068.45	1984.82
L-Serine	2546.11	6921.31	894.97	1583.03	4554.68	1158.30
Glutamic acid	5021.41	10855.37	884.12	2593.65	3908.61	312.97
L-Proline	822.89	2747.24	-	535.24	1443.30	2775.43
Glycine	640.09	1010.14	153.92	698.79	2927.05	272.87
L-Alanine	5225.33	15393.02	1418.85	3306.62	10916.86	911.80
Cystain	-	188.63	-	166.33	-	14.79
Valine	2263.63	3590.31	496.60	1193.15	1688.51	788.87
Isoleucine	2052.35	1670.34	219.64	833.62	1422.20	473.27
Leucine	1561.06	686.25	69.84	484.60	254.23	113.94
Tyrosine	326.14	233.10	30.78	152.68	99.66	51.07
Phenylalanine	1023.73	1224.89	-	395.42	-	186.67
GABA*	146.31	65.71	88.40	94.27	39.54	84.31
L-Histidine	477.00	962.68	442.63	201.79	648.95	345.73
Lysine	268.37	10.25	40.84	110.30	329.09	48.73
L-Arginine	1593.86	2861.93	505.30	296.58	372.52	118.68

*r-amino-n-butyric acid

atively abundant amino acids in most parts of samples. Vitamin U is known as a natural amino acid [11,13], and it could be possible to infer that level of vitamin U influences other amino acids in composition or degradation.

Table 3-1. Levels of various amino acids in middle parts of Winter Pride Korean Chinese cabbage cultivar dried with different methods

Amino acid	Part of Korean Chinese cabbage (μmol/100 g)					
	Middle leaf			Middle midrib		
	Oven drying	Freeze drying	Air drying	Oven drying	Freeze drying	Air drying
L-Aspartic acid	1038.45	2354.13	483.38	262.99	1033.85	112.33
Threonine	3413.63	11852	2413.97	2970.47	10919.29	2186.81
L-Serine	1486.11	3662.20	326.84	6980.16	1906.48	550.16
Glutamic acid	3733.72	13081.56	976.88	2260.91	5843.81	437.18
L-Proline	39.21	2063.94	-	489.74	1587.40	-
Glycine	79.14	659.87	81.82	256.57	439.37	-
L-Alanine	414.21	13170.17	916.30	3000.05	5478.10	-
Cystain	4319.01	164.09	-	64.80	-	-
Valine	2044.59	4343.64	928.29	959.34	1514.87	519.21
Isoleucine	1960.15	2219.41	197.05	292.31	555.76	148.85
Leucine	2141.05	2142.68	100.35	352.25	485.81	96.45
Tyrosine	525.51	528.82	47.30	86.48	140.17	45.45
Phenylalanine	1122.83	853.40	98.32	180.34	231.03	154.34
GABA*	209.39	89.28	73.41	115.16	74.09	89.86
L-Histidine	296.98	801.99	379.35	112.45	315.08	228.38
Lysine	234.98	497.07	42.32	115.29	172.46	52.60
L-Arginine	1411.51	2739.96	819.87	414.69	928.81	463.53

*γ-amino-n-butyric acid

Table 3-2. Levels of various amino acids in middle parts of 55 days Korean Chinese cabbage cultivar dried with different methods

Amino acid	Part of Korean Chinese cabbage(μmol/100 g)					
	Middle leaf			Middle midrib		
	Oven drying	Freeze drying	Air drying	Oven drying	Freeze drying	Air drying
L-Aspartic acid	995.38	2366.59	432.66	386.35	672.65	19.88
Threonine	4550.27	17704.27	2089.92	4680.46	11873.52	-
L-Serine	2667.89	8659.82	857.04	1551.82	2993.11	116.61
Glutamic acid	5097.72	15732.07	1164.34	3224.94	5047.44	69.16
L-Proline	738.42	2578.61	-	680.89	1533.93	2409.10
Glycine	511.59	1003.10	106.51	451.11	870.59	23.52
L-Alanine	5459.31	18849.97	825.72	4877.91	10594.81	100.26
Cystain	-	-	-	-	-	-
Valine	2259.17	4451.45	857.46	1073.42	1085.04	243.89
Isoleucine	1808.23	2350.62	291.56	518.71	351.59	47.73
Leucine	1805.09	1570.35	107.52	498.64	255.54	25.5
Tyrosine	254.57	323.85	38.54	158.83	86.23	12.29
Phenylalanine	1178.00	43.62	115.01	371.97	141.44	57.29
GABA*	161.58	41.33	70.31	94.16	29.89	40.01
L-Histidine	407.14	1055.21	405.76	170.20	338.04	95.27
Lysine	262.93	627.38	41.81	142.90	307.02	21.52
L-Arginine	1709.61	4674.86	909.50	405.47	996.32	107.55

*γ-amino-n-butyric acid

Since no distinctive trends were observed in this result, it seems no relationship exists between amino acids and vitamin U levels.

Table 5 shows levels of methionine in different parts

Table 4-1. Levels of various amino acids in outward parts of Winter Pride Korean Chinese cabbage cultivar dried with different methods

Amino acid	Part of Korean Chinese cabbage(μmol/100 g)					
	Outward leaf			Outward midrib		
	Oven drying	Freeze drying	Air drying	Oven drying	Freeze drying	Air drying
L-Aspartic acid	1178.08	4562.17	537.45	81.49	337.93	5.06
Threonine	3688.25	15229.36	3762.17	-	625.63	299.45
L-Serine	1715.14	4220.10	707.29	322.83	469.18	429.54
Glutamic acid	4092.40	12400.77	1221.37	1104.02	1451.46	-
L-Proline	619.81	2324.81	4943.90	395.31	583.17	-
Glycine	320.33	522.24	100.19	117.27	72.36	-
L-Alanine	4311.65	12086.44	588.87	1854.14	648.16	-
Cystain	-	-	-	30.30	7.72	1.64
Valine	2110.10	4569.70	1420.66	529.02	317.96	189.67
Isoleucine	1866.49	1759.41	306.58	124.81	50.83	25.43
Leucine	1818.35	1076.03	135.80	208.02	39.27	19.14
Tyrosine	424.29	306.98	84.99	45.22	-	8.46
Phenylalanine	1197.73	0.384	248.53	114.28	-	41.59
GABA*	208.77	97.81	80.85	117.94	19.84	50.47
L-Histidine	265.55	442.10	266.23	21.10	33.17	31.86
Lysine	263.28	323.83	64.12	64.56	33.73	19.14
L-Arginine	1999.89	3533.32	1835.93	151.22	1236.58	33.83

*v-amino-n-butyric acid

Table 4-2. Levels of various amino acids in outward parts of 55 days Korean Chinese cabbage cultivar dried with different methods

Amino acid	Part of Korean Chinese cabbage(μmol/100 g)					
	Outward leaf			Outward midrib		
	Oven drying	Freeze drying	Air drying	Oven drying	Freeze drying	Air drying
L-Aspartic acid	1211.76	4006.74	481.57	68.77	48.49	12.38
Threonine	6402.59	15916.74	3438.64	-	5575.33	-
L-Serine	3152.02	6933.23	1209.18	392.82	1079.50	312.16
Glutamic acid	6068.70	10948.63	1317.00	695.99	1802.11	111.03
L-Proline	938.89	1660.51	3974.18	349.23	221.36	3024.29
Glycine	332.45	486.52	123.90	121.92	164.10	42.15
L-Alanine	6843.66	13279.98	538.80	2213.07	958.04	145.43
Cystain	-	-	-	-	-	-
Valine	3240.70	5211.45	1271.35	560.47	592.18	362.42
Isoleucine	1807.79	2170.60	346.15	130.21	55.21	-
Leucine	1151.50	515.40	143.58	183.38	24.18	43.16
Tyrosine	311.80	229.88	79.54	49.66	-	-
Phenylalanine	1600.48	1204.45	459.27	159.00	52.23	-
GABA*	179.46	56.47	107.91	83.71	8.78	99.44
L-Histidine	598.68	662.05	385.68	36.47	69.04	59.48
Lysine	351.61	417.70	85.08	63.95	65.22	36.39
L-Arginine	2932.40	10538.15	2787.21	149.30	404.81	84.57

*v-amino-n-butyric acid

and cultivars of Korean Chinese cabbages dried with various methods. These results do not a support clear relationship of methionine and vitamin U. Futhermore, methionine was not detected in parts of outward leaves

Table 5. Levels of methionine in different parts of Korean Chinese cabbages

cultivar	part	Drying condition (mg/100 g)		
		Oven-drying	Freeze-drying	Air-drying
winter pride	core leaf	64.57	78.61	11.56
	core midrib	48.62	49.40	13.57
	middle leaf	68.47	62.24	3.81
	middle midrib	32.29	23.84	3.04
	outward leaf	47.98	-*	-
	outward midrib	24.95	2.12	-
55 Days	core leaf	63.99	63.81	4.94
	core midrib	50.83	32.94	11.60
	middle leaf	66.25	74.67	9.23
	middle midrib	38.48	17.25	-
	outward leaf	63.20	-	12.26
	outward midrib	21.43	-	-

*not detected
Each value is the mean of 3 replicates

which were the highest parts of vitamin U in Winter Pride and 55 days cultivars. Mid ribs of each part appeared relatively higher levels of methionine compared to vitamin U levels in the same parts. Moreover, there were similar levels of methionine between oven and freeze drying. Samples prepared by air drying showed significantly lower levels than those by over and freeze drying. Methionine as a precursor of vitamin U, may not play a role in an increase of vitamin U during drying of Korean Chinese cabbages

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국문요약

S-methylmethionine(vitamin U)은 천연 아미노산의 일종으로 *Brassica* vegetables 중 양배추에 풍부하게 함유된 항위궤양성 인자로 알려져 있다. 본 연구에서는 우리나라에서 많이 소비되고 있는 배추를 대상으로, 건조방법을 달리하여 품종 및 부위별로 vitamin U 함량 변화를 조사하였다. 건조방법에 따른 함량변화는 동결건조 처리군이 vitamin U 최대 보존 효과를 보여주었다. 부위별로는 전체적으로 잎 부분 (leaf parts)이 줄기 (mid-rib)보다 최소 1.1배, 최고 21.2배 높았으며 그중에서 배추 잎 바깥쪽(outward parts)에서 가장 높은 vitamin U 함량을 나타내었다. 주요 아미노산의 함량은 vitamin U와 같이 잎 부분이 전반적으로 높았으며, 특히, alanine과 threonine이 가장 풍부한 아미노산으로 조사되었다. Vitamin U의 전구물질인 methionine의 함량은 건조방법에 따라 큰 차이를 보여주었으나, vitamin U 함량에 미치는 영향은 뚜렷한 상관관계를 보이지 않았다.

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