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Changes in Vitamin U, Amino acid and Sugar Levels in Chinese Cabbages during Storage

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배추 저장동안 비타민 U, 아미노산, 유리당 함량 변화

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Abstract

Vitamin U (S-methylmethionine) levels of Chinese cabbages at 4℃ were investigated to establish its physiological characteristics and also amino acids and sugars levels to find out their relationship with vitamin U were determined. The levels of vitamin U showed different from parts of Chinese cabbages. The highest value was shown in outward leaf in Wineter Pride (12.70 mg/100 g fresh wt.) and core leaf in 55 Days cultivars (18.60 mg/100 g fresh wt.). Leaf parts were 1.7-9.0 times higher in vitamin U levels than those in midribs in both cultivars. Levels of vitamin U in stored Chinese cabbages increased with storage time. Moreover, two cultivars used for this experiment showed different pattern during storage. In Winter Pride, vitamin U levels sharply increased in leaf and midrib of core part during storage. This value reached about 2.5 times for leaf and 4 times for midrib compared to the levels of initial storage time in core part. In 55 Days cultivars, outward leaf showed an increased level of vitamin U of 1.8 times compared to that of 1 month storage time. Methionine known as a precursor of vitamin U synthesis did not showed clear relationship with vitamin U levels. Methionine was either not detected or at negligibly low levels in Chinese cabbages during storage. Methionine may not play a role in an increase of vitamin U during storage of Chinese cabbages at 4℃. No clear relationship of free amino acids and soluble sugars for vitamin U accumulation during storage of Chinese cabbages was shown in this study.

Key words : Chinese cabbage, *Brassica campestris* L. *Perkinensis*, vitamin U, S-methylmethionine, amino acids

Introduction

S-methylmethionine (SMM), which is also called vitamin U, is a natural amino acid that has been identified in the free state in higher plants (1). It belongs to the group of natural physiologically active compounds (2), and has been known as an anti-ulcer factor extracted from cabbages and other green vegetables (3). Its deficiency is thought to be a possible cause of gastric ulcers. Vitamin U is also known to have other valuable pharmacological properties such as being anti-inflammatory, analgesic, hypolipidemic (4) and radio protective (5).

Since vitamin U was isolated from cabbages (6), it has been identified in plant foods such as asparagus (7), green tea (8) and various *Brassica* vegetables (9). Vitamin U metabolism is known to be closely associated with sulfur-containing compounds because the detachment of a methyl converts vitamin U into methionine, and enzymatic hydrolysis of vitamin U produces dimethyl sulfide (10). Vitamin U is a major metabolite of methionine and also can be synthesized from methionine and S-adenosylmethionine (11). Vitamin U plays a role as a reserve form of methionine (12). Vitamin U synthesis decreases the concentration of free methionine and its active derivative, S-adenosylmethionine (13).

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Even though many scientists have reported on bioactive components of some plant foods, vitamin U is not yet clearly identified for its physiological activities and nature. Therefore, more research is needed on vitamin U concerning physio-chemical components, characteristics and application. It has been reported that a good source of vitamin U is *Brassica* vegetables. Most of the research has been carried out on European style cabbages in *Brassica* vegetables, not on Chinese cabbages (*Brassica campestris* L. *Perkinensis*) which is one of the most popular vegetables in China, Japan and Korea.

The aim of this study is to determine the level of vitamin U in 2 cultivars of Chinese cabbage at fully grown stages using liquid chromatograph and amino acid analyzer. Also, amino acids and sugars were investigated to determine their correlation with vitamin U levels in Chinese cabbages.

Materials and methods

Plant materials

For this experiment, two different cultivars were sampled, Winter Pride and 55 Days (Hungnong Seeds, Korea). Sampling occurred at fully developed stages of growth and were prepared for determination of vitamin U, amino acid and sugar analysis.

Whole Chinese cabbages were harvested in November, 2000 at National Agricultural Research Center for Hokkaido Region, Sapporo, Japan. After harvesting, Chinese cabbages were stored at 4℃ 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 10 g for each section for analysis of vitamin U, amino acids and sugar contents.

Preparation of analysis sample solutions for vitamin U and amino acids

The samples of fresh Chinese cabbages were cut into small pieces and mixed thoroughly. Uniformly mixed samples were 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 Toyo No.2x2) 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℃ with reduced pressure. After condensing, 10 ml of 0.2N-sodium citrate buffer solution (pH 2.2) was added to 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 C_{18} 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 wasting initial 3mL.

Preparation of analysis sample solutions for soluble sugar levels

Chinese cabbages were divided and cut into small pieces and were combined with 50 mL of 80% ethanol and bulked up to 100 mL volume. A 10 mL aliquot of solution from each sample was condensed by rotary evaporator at 35℃ and bulked up to 10 mL with distilled water. After passing through a Sep-pak C_{18} cartige with a 0.45 μm pore size of filter, about 2 mL of sugar solution was applied for HPLC anlysis.

Instrumentation

For analysis of vitamin U and amino acids, a Shimadzu liquid chromatograph using a amino acid analyzer (ALC-1000, Shimadzu Corp., Kyoto, Japan) was used, consisting of Shim-Pack Amino-Na (6.0mm x 10cm) column and fluorescence detector (ex. 340nm, em. 450nm). This Na type column have no capability to separate glutamic acid and glutamine, and asparagine and threonine. Amino acid standard solutions (Type H, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and DL-methionine-s-methylsulfonium chloride (Sigma) were used for standards. Norvaline (Wako Pure Chemical) was used as an internal standard. The concentration of all standards was 100 nmol/mL.

Soluble sugar contents was measured using a HPLC (Shimadzu 10A model system) equipped with YMC-Pack Polyamine II(250x4.6mm) column (YMC, Inc., Wilmington, USA), coupled with a RI detector(RID-10A, Shimadzu). Elution solvent was H_2O(25)/acetonitrile(75) without gradient. The flow rate was 1 mL/min. Temperatures were 25℃ for column and 40℃ for detector.

Statistical analysis

Version of 6.12 of the Statistical Analysis System (SAS Institute, 1998) was used for analysis. Means were compared using Duncan's multiple range test at P<0.05 level of significance

Results and discussion

Vitamin U levels in different parts and cultivars of Chinese cabbages during storage

The results showed significantly different levels of vitamin U from different parts of Chinese cabbages as shown in Table 1. The highest value appeared in outward leaf in Winter Pride and core leaf in 55 Days cultivars. It has been reported that core parts contained highest levels of vitamin U in cabbages (14). In the case of 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 1.7-9.0 times higher vitamin U levels than those in midribs in both cultivars. This deference was shown most distinctively in the outward parts of 55 Days cultivars. There are also significant differences in water content between core and other parts, vitamin U levels in outward parts had high levels of 12.70 mg/100 g in Winter Pride and 10.88 mg/100 g fresh weight in 55 Days. From the these results, vitamin U levels can be affected by cultivars and parts of Chinese cabbages. These results agree with Larina et al.(9) that cultivar-related features, anatomical structure of plant and physiological state affect vitamin concentration in the vegetables.

Vitamin U levels during storage at 4℃ are shown in Fig. 1. Two cultivars showed different patterns during storage. In Winter Pride, vitamin U levels sharply increased in leaf and midrib of core part during storage. This value reached about 2.5 times for leaf and 4 times for midrib compared to levels of initial storage time in core part. In 55 Days, outward leaf showed an increase level of vitamin U of 1.8 times compared to that of 1 month storage time. In general, levels of vitamin U increased with storage time. Russian scientists (9) reported that the level of the vitamin U in *Brassica* vegetables did not change during storage which is opposite to the results from our research results. Since vitamin U level is quite a cultivar dependent component, this difference may come from the different cultivars used, which are Russian and Asian cultivars.

Amino acids compostion of Chinese cabbages during storage

Methionine has been known as a precursor of vitamin U synthesis (15). Table 2 shows the ratio of methionine and vitamin U during storage of Chinese cabbages. These results

Table 1. Vitamin U content in different parts of Chinese cabbages

Cultivar	Part	Water content (%)	Vitamin U (mg/100 g fresh wt.)
Winter Pride	Core leaf	88.12 ± 1.08[1)b2)]	9.95 ± 0.09[b]
	Core midrib	87.82 ± 0.69[b]	5.99 ± 0.90[c]
	Middle leaf	90.38 ± 1.99[a]	9.39 ± 0.77[b]
	Middle midrib	91.63 ± 0.32[a]	3.49 ± 0.77[d]
	Outward leaf	91.41 ± 2.02[a]	12.70 ± 1.04[a]
	Outward midrib	92.45 ± 1.93[a]	2.65 ± 0.44[d]
55 Days	Core leaf	88.88 ± 1.04[b]	18.60 ± 0.80[a]
	Core midrib	89.17 ± 0.92[b]	7.55 ± 0.62[d]
	Middle leaf	91.66 ± 0.83[a]	14.58 ± 0.62[b]
	Middle midrib	92.69 ± 0.90[a]	3.39 ± 0.94[e]
	Outward leaf	91.81 ± 1.46[a]	10.88 ± 1.85[c]
	Outward midrib	93.21 ± 1.85[a]	1.21 ± 0.24[f]

[1)]Values are means±standard deviation from three replications.
[2)]Means within a column with different superscript letters are significantly different ($p \leq 0.05$).

Table 2. The ratio of vitamin U and methionine produced during storage of Chinese cabbage at 4℃

Cultivar	Part	Storage time (months)			
		1	2	3	4
Winter Pride	Core leaf	2.9	4.9	6.5	5.6
	Core midrib	5.3	4.6	4.6	0.3
	Middle leaf	2.7	3.5	7.3	0.5
	Middle midrib	3.3	4.6	8.1	1.5
	Outer leaf	ND*	0.9	0.9	ND
	Outer midrib	ND	ND	ND	ND
55 Days	Core leaf	3.1	3.2	6.6	1.4
	Core midrib	ND	4.6	6.1	5.0
	Middle leaf	ND	3.9	4.1	2.6
	Middle midrib	ND	6.5	6.8	9.9
	Outer leaf	ND	ND	0.6	0.2
	Outer midrib	ND	ND	ND	ND

Each value is the percentage of ratio of methionine to vitamin U which are basis on μ mol/100 g fresh weight.
*ND : not detected.

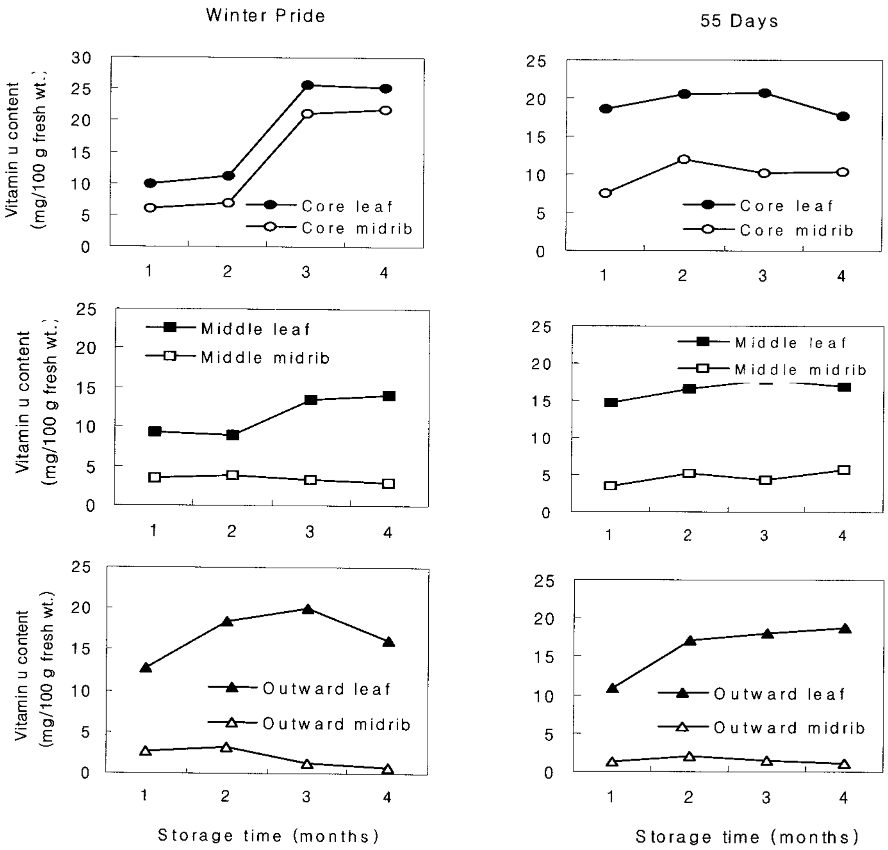

Fig.1. Changes in vitamin U levels during storage of Chinese cabbages stored at 4℃.

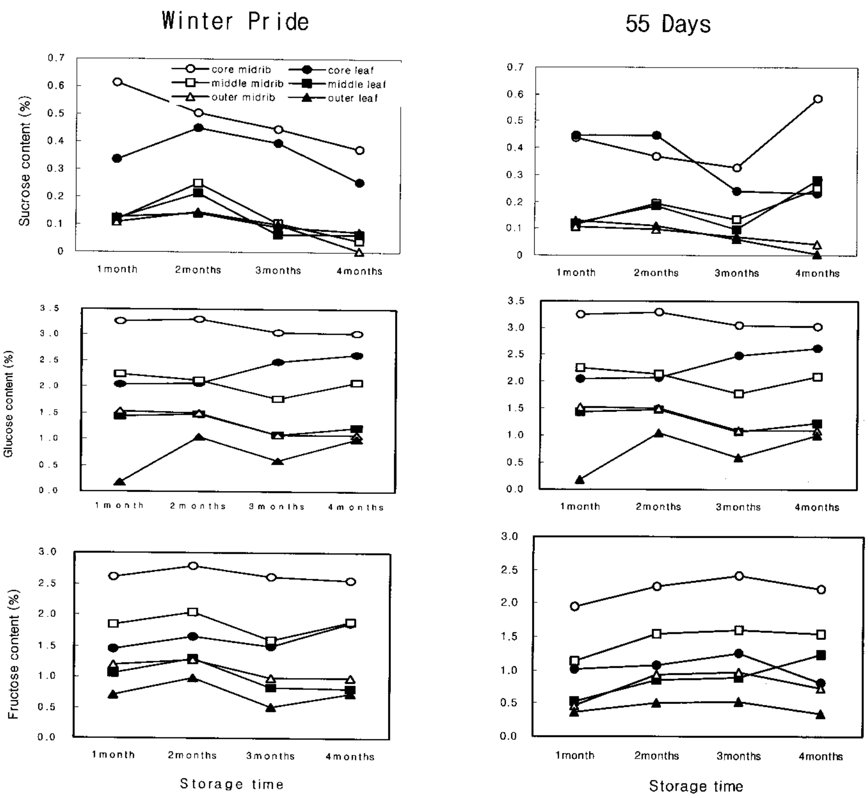

Fig. 2. Changes in soluble sugar levels during storage of Chinese cabbages at 4℃.

does not a support clear relationship of methionine and vitamin U. However, the ratio increased up to 3 months storage then after decreased in both cultivars. Methionine levels was either not detected or at negligably low levels in Chinese cabbages during storage. It may be due to quick turnover rate of the methionine cycle in full grown Chinese cabbages. Distinct relation of vitamin U and methionine has been reported in young seedling stage of Chinese cabbage. It seems that methionine can be used as a precursor of vitamin U during maturation of Chinese cabbages. However, methionine may not play a role in an increase of vitamin U during storage of Chinese cabbages at 4℃.

Major amino acids in different parts and cultivars of Chinese cabbages are shown in Table 3-1,2,3. Like Vitamin U, free amino acids also showed much higher levels in leaves. Amino acids examined showed irregularly changing patterns at different parts and cultivars of Chinese cabbages during storage. Most amino acids changed levels significantly after 1 month storage. Vitamin U is known as a natural amino acid (10), and it could be possible to infer that level of vitamin U influences other amino acids in composition or degradation. Since no distinctive trends were observed in this result, it seems no relationship exists between amino acids and vitamin U levels during storage.

Table 3-1. Changes of amino acids during storage of Chinese cabbages at 4℃

Amino acid	Part of Chinese cabbage μmol/100 g fresh wt.							
	Core leaves				Core midribs			
	1 mon	2 mon	3 mon	4 mon	1 mon	2 mon	3 mon	4 mon
Winter Pride cultivar								
L-Alanine	1509.09	407.01	2045.93	1523.44	1173.01	1381.09	1176.68	965.50
L-Arginine	249.92	250.86	358.64	384.85	112.79	83.60	63.41	104.30
L-Aspartic acid	262.32	220.21	261.84	224.52	124.26	97.49	60.40	64.66
L-Glutamic acid	982.89	1068.17	1390.50	1165.54	457.08	478.12	365.63	404.94
Glycine	69.66	68.13	120.36	101.77	96.29	112.49	116.63	85.86
L-Histidine	79.25	104.82	222.55	179.16	90.13	112.38	157.23	148.79
L-Proline	1310.54	2195.39	791.95	297.01	994.58	1665.91	423.00	179.97
L-Serine	382.52	438.73	680.84	521.95	230.88	290.64	366.17	355.81
L-Threonine	1336.88	983.03	1662.63	1467.15	1542.77	1932.36	1545.62	1194.56
55 days cultivar								
L-Alanine	2085.12	162.73	1424.74	1773.57	1491.90	1281.82	777.27	935.45
L-Arginine	408.39	539.79	469.87	428.49	79.47	95.88	41.83	49.21
L-Aspartic acid	308.56	266.24	233.16	186.93	102.41	103.61	72.24	69.33
L-Glutamic acid	1577.74	1437.80	1439.43	1182.29	544.69	472.30	345.64	422.71
Glycine	90.36	71.59	105.73	113.07	133.04	146.83	197.54	294.49
L-Histidine	108.62	141.02	126.01	97.94	68.84	95.85	64.52	66.50
L-Proline	1895.51	2153.65	638.23	384.70	827.35	1170.73	317.70	133.46
L-Serine	849.75	753.27	956.54	781.56	379.65	477.94	453.63	449.25
L-Threonine	2296.59	1582.24	994.17	1230.03	2751.54	2614.67	1202.95	888.75

Table 3-2. Changes of amino acids during storage of Chinese cabbages at 4℃

Amino acid	Part of Chinese cabbage μmol/100 g fresh wt.							
	Middle leaves				Middle midribs			
	1 mon	2 mon	3 mon	4 mon	1 mon	2 mon	3 mon	4 mon
Winter Pride cultivar								
L-Alanine	857.31	627.51	1261.66	1309.68	329.83	354.62	184.50	306.16
L-Arginine	176.74	208.48	375.80	871.21	59.68	68.92	88.92	88.00
L-Aspartic acid	312.72	310.93	479.67	436.72	90.90	95.33	97.11	120.61
L-Glutamic acid	770.24	1233.08	1407.74	1455.49	398.78	491.70	449.59	454.98
Glycine	42.68	44.42	55.52	60.08	32.01	25.94	24.76	29.05
L-Histidine	45.14	62.53	73.14	76.57	19.56	25.08	19.29	22.57
L-Proline	841.20	1084.78	414.68	241.38	311.90	336.02	240.89	126.41
L-Serine	256.27	319.99	374.21	362.69	112.72	130.46	117.80	138.56
L-Threonine	1200.02	964.26	1512.31	1269.45	842.48	702.99	961.95	839.12
55 days cultivar								
L-Alanine	1054.98	1169.09	617.14	1448.52	256.44	454.62	293.72	957.27
L-Arginine	347.30	482.83	234.99	471.81	41.46	94.76	73.59	119.70
L-Aspartic acid	281.38	265.56	189.28	340.41	92.07	87.91	116.05	101.37
L-Glutamic acid	1078.63	1142.66	690.70	1285.54	401.39	452.51	448.68	545.39
Glycine	48.50	48.49	29.79	75.30	41.47	49.10	41.24	84.03
L-Histidine	59.84	87.50	39.51	85.70	15.86	30.38	23.41	39.73
L-Proline	609.04	926.37	331.20	221.33	245.19	355.20	196.19	141.88
L-Serine	548.91	571.56	353.31	732.01	146.22	229.01	197.52	308.99
L-Threonine	1921.12	2123.30	1064.75	1458.62	1102.40	1795.65	1050.06	1077.93

Table 3-3. Changes of amino acids during storage of Chinese cabbages at 4℃

Amino acid	Part of Chinese cabbage μmol/100 g fresh wt.							
	Outward leaves				Outward midribs			
	1 mon	2 mon	3 mon	4 mon	1 mon	2 mon	3 mon	4 mon
Winter Pride cultivar								
L-Alanine	423.60	640.56	1287.47	1177.60	85.31	68.48	88.25	29.51
L-Arginine	242.28	363.74	558.58	361.46	18.78	20.90	17.77	9.45
L-Aspartic acid	243.44	299.56	469.95	562.34	95.58	81.18	40.87	33.72
L-Glutamic acid	519.98	864.73	1102.69	1057.16	303.62	270.36	160.78	114.31
Glycine	21.57	25.97	48.43	42.34	9.19	4.64	8.51	3.27
L-Histidine	35.14	48.87	71.21	40.29	7.17	9.38	4.70	2.20
L-Proline	513.51	841.69	427.39	211.81	131.81	100.46	41.87	31.46
L-Serine	190.51	304.96	367.67	511.77	59.17	62.03	61.72	31.22
L-Threonine	886.79	1114.44	1722.88	860.47	210.70	215.09	183.03	43.66
55 days cultivar								
L-Alanine	355.39	600.96	712.77	1024.33	31.44	36.61	36.21	39.27
L-Arginine	377.61	420.80	630.36	815.05	8.07	24.45	20.31	19.37
L-Aspartic acid	259.64	338.58	424.09	393.39	59.65	61.81	54.77	41.21
L-Glutamic acid	540.06	753.56	872.69	864.08	193.45	195.69	130.75	107.39
Glycine	15.84	19.17	28.13	34.01	3.42	2.89	4.02	7.69
L-Histidine	34.84	68.64	56.27	58.48	4.21	8.40	6.01	4.09
L-Proline	362.34	463.56	342.01	151.21	50.13	80.80	24.51	7.99
L-Serine	276.80	326.22	391.39	503.63	44.64	17.69	52.10	48.46
L-Threonine	851.82	215.55	1578.97	1459.32	117.43	182.64	166.27	248.29

Soluble sugar levels during storage of Chinese cabbages

Soluble sugars during storage of Chinese cabbages at 4℃ are shown in Fig. 2. These sugar levels were higher levels in midribs compared to leaf parts. Core parts in both cultivars showed higher levels of sucrose, glucose and fructose compared to middle and outward samples. Among soluble sugars, glucose levels were higher than sucrose and fructose in all samples used. Comparing to vitamin U levels, no correlation was observed between sugars and vitamin U during storage. This may mean that soluble sugars do not affect vitamin U accumulation during storage.

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요 약

본 연구에서는 배추를 품종별, 부위별, 저장기간 동안의 vitamin U, amino acids 및 유리당 함량을 분석하고 변화 경향을 조사하여 보았다. Vitamin U의 함량은 배추의 품종과 부위에 따라 차이를 나타내는 것으로 조사되었다. 동풍배추(Winter Pride)의 겉잎(outward leaf)에서 12.70 mg/100 g, 55일배추(55 Days)의 속잎(core leaf)에서 18.60 mg/100 g으로 vitamin U의 함량이 가장 높은 것으로 나타나 품종별 함량차이가 있음을 확인할 수 있었으며, 부위별 함량을 측정하여 본 결과 두 품종 모두 배추의 leaf 부위가 midrib 부위에 비하여 1.7-9.0배 이상 높은 것으로 조사되었다. 4℃ 저장 동안의 vitamin U 함량은 두 품종 모두 다소 증가하는 것으로 나타났다. 저장 동안의 vitamin U의 전구체인 methionine 함량을 조사하여 본 결과 아주 낮거나 존재하지 않는 것으로 조사되었는데 이는 배추가 성숙하는 동안 methionine이 vitamin U 전구물질로 사용된 것으로 추정되어진다. 그러나, 4℃ 저장 동안의 vitamin U과 methionine의 함량에 대한 뚜렷한 상관관계는 보이지 않았으며 유리 amino acid와 유리당 함량과의 관계에 대한 더 많은 연구가 수행되어야 할 것으로 사료되어진다.

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