Stability of Anthocyanins in Various Vegetables and Fruits

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Anthocyanins were isolated from 19 kinds of vegetables and fruits. Individual anthocyanins were analyzed by HPLC using a photodiode array detector and were examined for the heating and ultraviolet irradiation stabilities of these anthocyanins. This report deals with the relationship between anthocyanin composition and heating or ultraviolet irradiation stabilities of anthocyanins. Stable anthocyanins showed many anthocyanin peaks and a high ratio of acylated anthocyanin peaks. This result shows that the anthocyanin composition and the rate of acylation affect the stability of anthocyanins.

Keywords: various vegetable and fruit anthocyanins, stability, HPLC analysis

Anthocyanins obtained from plants have been used as a coloring material for 'umezuke,' wine, jam and juice, and recently anthocyanins are known to show anti-oxidant activities (Igarashi *et al.*, 1989, 1993; Ishii, 1995).

Several theories have been proposed to explain the stabilization mechanism of anthocyanins such as self-association (Asen *et al.*, 1977), copigmentation (Robinson & Robinson, 1931) and intramolecular copigmentation (Saito *et al.*, 1972; Goto *et al.*, 1982). Anthocyanins are unstable and easily degraded by heat and light, etc. (Ishii *et al.*, 1990). Therefore, the anthocyanin colors practically change to yellow or fade away during food processing. Studies of various effects on anthocyanin stability in foods, such as heating, light irradiation, pH, metal ions, sugars and organic acids, have been reported (Tsukui, 1988 1989; Tsukui *et al.*, 1989).

In this study, anthocyanins were extracted from 19 kinds of vegetables and fruits, and we examined the relationship between anthocyanin composition, i.e., the number of anthocyanin peaks, and anthocyanin stability to heating and ultraviolet light (UV) irradiation.

Materials and Methods

Anthocyanin samples Red cabbage (*Brassia oleracea* L.), perilla 'Katame shiso' (*Perilla* forma *discolor* Makino), chicory 'Chicoria rossa' (*Cichorium endivia* L.), eggplant 'Naga nasu' (*Solanum melongena* L.), purple onion (*Allium cepa* L.), red radish (*Raphanus sativus* L.), red turnip (*Brassica campestris* L.), black soybean (*Glycine max* Merr.), water chestnut (*Trapa japonica* Flerov), purple yam (*Dioscorea alata* L.), strawberry 'Nyohou' (*Fragaria* × *ananassa*), red raspberry (*Rubus idaeus* L.), prune (*Prunus domestica* L.), grape 'Kaizi' (*Vitis vinifera* L.), 'Kyohou' (*Vitis vinifera* L. × *V. labrusca* L.), apple 'Sun tsugaru' (*Malus pumila* Mill.), and sweet potato 'Tanegashima murasaki (TM)' (*Ipomoea batatas* Lam.) were purchased from the market. Sweet potato varieties 'Yamagawa murasaki (YM)' and 'Kankei 55 (K55)'

(*Ipomoea batatas* Lam.) were donated by the Sweet Potato Breeding Laboratory of the National Agriculture Research Center.

Red cabbage, eggplant, purple onion, red turnip, and black soybeans were produced in Abiko city of Chiba Prefecture. Perilla was produced in Odawara city of Kanagawa Prefecture. Red radish was produced in Miura city of Kanagawa Prefecture. Water chestnut was produced in the Aso area of Kumamoto Prefecture. Strawberry was produced in the Tochigi area of Tochigi Prefecture. Grape 'Kaizi' and 'Kyohou' were produced in Koufu and Katsunuma cities of Yamanashi Prefecture. Apple was produced in the Tsugaru area of Aomori Prefecture. Sweet potato 'TM' was produced in the Aira area of Kagoshima Prefecture. Sweet potato 'YM' and 'K55' were obtained in the fields of the Tsukuba Sweet Potato Breeding Laboratory. Chicory was imported from Italy. Red raspberry and prune were imported from the USA. Purple yam (powdered) was imported from Philippines.

Preparation of anthocyanins Samples were pulverized with a blender, extracted with 3% trifluoroacetic acid (TFA) solution overnight at 4°C and filtered. The extract was passed through an Amberlite XAD-7 column (30 mm i.d. × 450 mm). The column was washed with 1 l of H₂O and the absorbed anthocyanins were eluted with 0.3% TFA-methanol. The pigment eluate was concentrated and then dissolved in 0.1% TFA-methanol, filtered and dried. The residue was dissolved in a small amount of 0.1% TFA-methanol, and anthocyanin was precipitated by the addition of diethyl ether. The precipitate was collected by centrifugation and dried under vacuum at 40°C.

The color of 0.2 mg of the anthocyanin dissolved in 1 ml of pH 3.16 MacIlvain's buffer was measured by a ZE-2000 colorimeter (Nihon Densyoku, Tokyo) and expressed as on L.a.b. value of the Hunter Solid Diagrams.

HPLC analysis HPLC analysis was carried out with a Waters MillenniumTM 2010J HPLC system, and the absor-

bance in the range from 220 nm to 700 nm was measured with a WatersTM996 photodiode array detector. Separation was accomplished on a TSK gel ODS-80TM (4.6 mm i.d.×25 cm, Tosoh, Tokyo) using an isocratic solution of CH₃CN and 0.1% TFA (1:4, v/v) at a flow rate of 1.0 ml/min. The anthocyanin composition was determined from the HPLC peak area recorded at 525 nm. Acylated anthocyanin peaks were identified based on absorption at two λ max in 310–335 nm (Hayashi, 1991).

Heat stability of anthocyanins Anthocyanins dissolved in pH 3.16 MacIlvain's buffer and also individual anthocyanin solutions (0.2 mg/ml) were heated at 80°C for 0.0, 0.5, 1.0, 3.0, 5.0 and 18.0 h. After the heat treatment, each anthocyanin solution was cooled in an ice-water bath. The color stabilities of anthocyanins were determined by comparing the pre- and post-heating absorbances at 525 nm.

UV irradiation stability of anthocyanins Anthocyanins dissolved in pH 3.16 MacIlvain's buffer and individual anthocyanin solutions (0.2 mg/ml) were irradiated with ultraviolet light at 20°C for 0.0, 0.5, 1.0, 3.0, 5.0 and 18.0 h. The sample solutions were irradiated at a rate of 880 μ W/cm² using two 6 W germicidal lamps (254 nm). The color stability of each anthocyanin was determined by comparing the pre- and post-irradiation absorbances at 525 nm.

Results

Yields and colorimeter readings of anthocyanins from vegetables and fruits The yields of anthocyanins and the colorimeter readings (Hunter solid diagrams) of anthocyanin solutions are shown in Table 1.

The greatest yield (1.30%) was obtained from grape among 19 kinds of vegetables and fruits. The yields of purple yam, red cabbage, red turnip and strawberry anthocyanins were less than 0.1% but the yields of anthocyanins from other vegetables and fruits were in the range of 0.15–0.68%.

According to the Hunter solid diagrams, chicory, purple onion, strawberry, raspberry and apple anthocyanins were red

with *b* values of 4.37–13.53 and *a* values of 21.79–31.29, and red radish and red turnip anthocyanins were yellow-red with *b* values of 12.20–15.06 and *a* values of 39.44–41.53. Red cabbage, perilla, black soybean, water chestnut, sweet potato, 'YM', 'K55', 'TM', purple yam, prune, grape 'Kaizi' and 'Kyohou' anthocyanins were red-purple with *b* values of –6.64–11.66 and *a* values of 13.95–58.56. Eggplant anthocyanin was purple with a *b* value of –9.96 and an *a* value of 29.31.

Composition of vegetable and fruit anthocyanins The compositions of anthocyanins expressed as the number of anthocyanin peaks by HPLC analysis are shown in Table 2.

Table 2. HPLC analysis of anthocyanin contents of 19 vegetable and fruit anthocyanins.

Samples	Main peaks (%) ^{a)}				Numbers	
	P-1	P-2	P-3	P-4	Peaks ^{b)}	Acylated ^{c)}
Red cabbage	38.05	20.93	—	—	12	4
Perilla	49.92	16.72	—	—	10	3
Chicory	49.00	27.00	17.65	—	6	—
Eggplant	93.54	—	—	—	4	1
Purple onion	41.51	10.34	10.24	—	10	4
Red radish	39.07	33.20	10.78	—	8	3
Red turnip	63.16	12.10	—	—	5	4
Black soybean	71.40	14.81	—	—	6	1
Water chestnut	84.62	—	—	—	5	—
Yamaga murasaki	29.97	15.68	14.60	14.51	9	8
Kankei 55	26.94	16.45	12.14	10.02	11	6
Tanegashima murasaki	24.67	23.56	—	—	13	6
Purple yam	37.96	25.75	11.00	10.78	9	4
Strawberry	90.96	—	—	—	3	—
Raspberry	62.05	21.22	—	—	6	—
Kyohou	49.69	21.15	10.88	—	7	2
Kaizi	74.63	12.93	10.17	—	3	—
Prune	44.24	40.22	—	—	6	—
Apple	84.48	—	—	—	3	—

^{a)}Anthocyanins contain over 10%, ^{b)}anthocyanin peak numbers of contents over 1%, ^{c)}number of acylated anthocyanin peaks.

Table 1. Anthocyanin yields and *L*, *a*, *b*, values of 19 vegetables and fruits.

Samples	Yield (%)	Hunter's chromatic value		
		<i>L</i>	<i>a</i>	<i>b</i>
Red cabbage	0.07	65.51	33.24	–6.64
Perilla	0.42	68.97	31.47	–5.41
Chicory	0.22	71.23	39.29	5.01
Eggplant (skin)	0.49	82.02	29.31	–9.96
Purple onion	0.15	79.46	33.29	2.10
Red radish (skin)	0.43	76.95	41.53	12.20
Red turnip	0.08	77.51	39.44	15.06
Black soybean	0.45	70.74	23.82	11.66
Water chestnut	0.12	91.12	13.95	0.94
Sweet potato Yamagawa murasaki	0.68	66.67	31.02	–8.26
Sweet potato Kankei 55	0.38	60.32	35.53	–7.05
Sweet potato Tanegashima murasaki	0.18	71.11	25.17	–5.69
Purple yam	0.05	58.74	58.56	3.19
Strawberry	0.09	90.11	13.52	13.53
Raspberry	0.28	75.53	21.79	4.37
Prune (skin)	0.36	71.45	15.35	–0.77
Grape Kyohou (skin)	1.30	71.54	35.63	11.21
Grape Kaizi (skin)	0.15	77.28	19.42	9.68
Apple (skin)	0.22	86.88	22.60	4.90

Table 3. Effect of heating on the stability of 19 vegetable and fruit anthocyanins.

Anthocyanins	Relative absorbance (525 nm)					
	0.0	0.5	1.0	3.0	5.0	18.0 (h)
Red cabbage	100	79	77	74	73	60
Perilla	100	76	74	69	64	44
Chicory	100	87	80	72	65	31
Eggplant	100	72	66	56	48	22
Purple onion	100	89	84	74	68	38
Red radish	100	81	81	76	71	54
Red turnip	100	80	80	74	69	46
Black soybean	100	63	60	51	44	19
Water chestnut	100	87	85	75	65	40
Yamagawa murasaki	100	89	89	86	84	71
Kankei 55	100	95	94	92	89	75
Tanegashima murasaki	100	80	79	78	76	63
Purple yam	100	97	97	94	92	77
Strawberry	100	86	86	76	69	24
Raspberry	100	93	86	76	67	30
Kyohou	100	75	70	65	58	32
Kaizi	100	58	57	49	42	17
Prune	100	80	78	70	65	33
Apple	100	89	80	60	47	12

Table 4. Effect of UV irradiation on the stability of 19 vegetable and fruit anthocyanins.

Anthocyanins	Relative absorbance (525 nm)							
	0.0	0.5	1.0	2.0	3.0	4.0	5.0	18.0 (h)
Red cabbage	100	99	97	91	83	74	74	43
Perilla	100	99	97	89	86	78	78	48
Chicory	100	88	86	71	64	59	56	25
Eggplant	100	93	80	64	60	42	36	18
Purple onion	100	92	87	79	74	71	64	47
Red radish	100	107	109	110	107	105	102	69
Red turnip	100	105	109	111	109	107	104	59
Black soybean	100	94	90	85	76	67	58	26
Water chestnut	100	98	98	95	93	92	92	60
Yamagawa murasaki	100	100	99	98	96	92	93	74
Kankei 55	100	100	99	99	99	97	95	80
Tanegashima murasaki	100	97	96	94	92	90	87	68
Purple yam	100	98	96	94	92	90	89	65
Strawberry	100	102	65	78	62	50	41	6
Raspberry	100	94	88	76	66	56	48	8
Kyohou	100	98	95	89	83	76	68	44
Kaizi	100	106	101	85	69	60	55	22
Prune	100	94	88	78	69	63	58	18
Apple	100	100	97	87	77	66	58	10

HPLC analysis revealed that red cabbage, perilla, purple yam, red radish, purple onion and sweet potato anthocyanins were separated into 8–13 pigment peaks. Other vegetable and fruit anthocyanins had less than 7 anthocyanin peaks.

Heat stability of vegetable and fruit anthocyanins

Anthocyanins stable to heating at 80°C for 18 h were purple yam anthocyanins (the percentage residual being 77%), sweet potato 'K55' (75%), 'YM' (71%), 'TM' (63%) and red cabbage (60%) (Table 3). By HPLC analysis, these anthocyanins had many peaks including acylated anthocyanin peaks. However, anthocyanins composed of a small number of anthocyanin peaks were unstable as shown for eggplant, chicory, strawberry, raspberry and apple (Table 3).

UV irradiation stability of vegetable and fruit anthocyanins

The results of UV irradiation for 18 h are shown in Table 4. The anthocyanins stable to UV irradiation were sweet potato 'K 55' (the residual color ratio being 80%), 'YM' (74%), red radish (69%) and 'TM' (68%). These anthocyanins had a high ratio of acylated anthocyanin peaks. However,

anthocyanins with low acylated ratios such as grape, strawberry, raspberry and apple were unstable. Red radish and red turnip were extremely stable and even increased slightly in absorbance at 525 nm in the first 5 h.

Relationship between residual color ratio and anthocyanin peaks The relationships between color stability of anthocyanins and anthocyanin composition are shown in Fig. 1.

Upon heating, a positive correlation was observed between the residual color ratios and the number of anthocyanin peaks ($r=0.79$, $p<0.01$). A positive correlation also existed between the residual color ratios and the number of acylated anthocyanin peaks ($r=0.85$, $p<0.01$).

Concerning the effects of UV irradiation, a positive correlation was observed between the residual color ratios and the number of acylated anthocyanin peaks ($r=0.83$, $p<0.01$).

Discussion

Generally, anthocyanins become stable due to transesterification of the flavylium cation structure in acidic aqueous solution.

Anthocyanins are very unstable in weakly acidic or neutral aqueous solution, and these colors rapidly disappear under such conditions. Anthocyanin discoloring is generally recognized to be caused by transforming of the anhydro base formation which is easily hydrated into the colorless pseudo-base formation.

Keeping the colors is based on preventing the hydration of anthocyanins. Color stabilization mechanisms of anthocyanin have already been proposed such as metal ion complexation (Hayashi *et al.*, 1958), complex formation of anthocyanins with flavonoid copigments (co-pigmentation) (Robinson & Robinson, 1931), self-association (Asen *et al.*, 1977; Hoshino *et al.*, 1982) and intramolecular sandwich-type stacking of the anthocyanin nucleus and acyl groups (intramolecular copigmentation) (Saito *et al.*, 1972; Goto *et al.*, 1982; Goto & Kondo, 1988).

Tsukui (1988, 1989) and Tsukui *et al.* (1989) reported that various factors affect anthocyanin stability in foods, such as heating, light irradiation, pH, metal ions, sugars and organic acids.

Fig. 1. Relationships between anthocyanin stabilities and anthocyanin composition.

In this study, we extracted and purified anthocyanins from 19 kinds of vegetables and fruits and examined the relationship between anthocyanin composition and the stability of anthocyanins.

A positive correlation was observed between the color stability on heating and the number of anthocyanin peaks and also acylated anthocyanins. Anthocyanins composed of many anthocyanins and acylated anthocyanins, such as purple yam, sweet potato and red cabbage, were stable. This would indicate that anthocyanin stability is caused by self-association.

The stability to UV irradiation was different from the stability to heating. A positive correlation was observed between residual color ratios and the number of acylated anthocyanin peaks. Anthocyanins of sweet potato were the most stable, which were acylated with many organic acids such as caffeic acid, ferulic acid and *p*-hydroxybenzoic acid (Shimizu & Nakamura, 1994; Miyazaki *et al.*, 1991). The number of acylated anthocyanins was more influential on the UV irradiation stability of anthocyanins than the number of anthocyanins. From this result, it was presumed that the UV irradiation stability of anthocyanin was derived from intramolecular sandwich-type stacking with acyl groups. However, red radish and red turnip were extremely stable. In this case, anthocyanin stability cannot be elucidated by only self-association or intramolecular copigmentation. These results are interesting and worthy of further study.

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