Contents and Cooking Loss of Three Quinic Acid Derivatives from Garland (*Chrysanthemum coronarium* L.)

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Three quinic acid derivatives, i.e., chlorogenic acid (CA), 3,5-dicaffeoylquinic acid (SP-1), and 3,5-dicaffeoyl-4-succinylquinic acid (SP-2), determined in five garland (*Chrysanthemum coronarium* L.) cultivars were analyzed using HPLC. Young leaves contained greater amounts of these compounds than other parts, and mean dry weight content was 5.4 mg/g for CA, 22.9 mg/g for SP-1, and 20.8 mg/g for SP-2. No significant difference in CA, SP-1, or SP-2 content was seen in cultivars except for the rosette type. To clarify the extent of cooking loss, amounts of the three compounds were compared before and after boiling of garland. Approximately 44−62% of the quinic acid derivatives was retained after 5 min of boiling compared to 14−37% after 30 min.

Keywords: *Quinic acid; Chrysanthemum coronarium; stability; cooking loss*

INTRODUCTION

Quinic acid derivatives such as chlorogenic acid are widely distributed in the plant kingdom. Such compounds were found recently as antioxidants in the *Compositae* family, burdock (Maruta et al., 1995) and garland (Tsushida et al., 1994). Three quinic acid derivatives with antioxidant properties, i.e., chlorogenic acid (CA), 3,5-dicaffeoylquinic acid (SP-1), and 3,5-dicaffeoyl-4-succinylquinic acid (SP-2) (Figure 1), were identified from garland (Chuda et al., 1996). CA and SP-1 are well-known components of potato and sweet potato, but an SP-2 other than garland has not been reported.

Garland originated in the Mediterranean region, spreading from there to Europe, Africa, and Asia (Larkcom, 1991). It became very popular in Japan, where more than 40 are varieties registered. This vegetable is rich in mineral and vitamins; potassium, for example, is present at 610 mg/100 g and carotene at 3400 μg/100 g in edible portions (Resources Council, Science and Technology Agency, 1982).

The intake of antioxidants appears to have potential long-term benefits to human health (Miller, 1995). Fresh raw garland is not eaten in Japan and is usually heated before consumption, suggesting that quinic acid derivatives would decompose or leach during cooking. Quinic acid derivatives of potato and sweet potato have been studied extensively (Hayase and Kato, 1984; Thompson, 1981; Walter et al., 1979). CA in home-cooked and commercially processed potato products, for example, have been measured. The CA content in baked potato is 0% and in boiled potato is about 40% (Dao and Friedman, 1992). However, no attempt has been made to quantify these compounds in garland.

In the present study, to shed more light on garland as a foodstuff, the quinic acid derivatives from three edible parts (i.e., young and mature leaves and stems) of major garland cultivars and their heat stability in cooking were determined.

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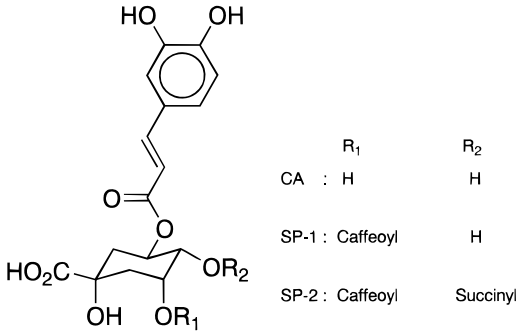

Figure 1. Quinic acid derivatives in garland.

MATERIALS AND METHODS

Reagents. CA was obtained from Wako (Osaka, Japan), and high-performance liquid chromatography (HPLC)-grade acetonitrile and formic acid (98−100%) were purchased from Nacalai Tesque (Kyoto, Japan), which were filtered and degassed before use. Analytical-grade methanol was obtained from Nacalai Tesque. The preparation of SP-1 and SP-2 were described previously (Chuda et al., 1996).

Apparatus. HPLC analysis was conducted using a JUSCO PU-980 (Tokyo, Japan) equipped with a JUSCO 875-UV detector and a Shimadzu C-R6A integrator (Tokyo, Japan). The column (250 × 6 mm i.d., COSMOSIL 5C_{18}-MS, Nacalai Tesque) was placed in a JUSCO CO-965 theremostated control system, operated at 40 °C throughout analysis.

Plants. Five garland cultivars were obtained from three locations in Japan, namely, cv. Okiku-3 and cv. Kiwame-chuba from Tochigi Prefecture, cv. Satoyutaka from Iwate Prefecture, and cv. Satoakira and cv. Natsunosei from Ibaraki Prefecture. All plants were harvested June 26−30, 1997, when about 30 cm high. Cultivar Natsunosei is a rosette type with medium-sized leaves, while the other cultivars are of the up-right type, also with medium-sized leaves. Thirty plants were divided into three parts, the stem 5 cm from the root, mature leaves branching from the stem and less than 5 cm from the root, and young leaves branching from the stem less than 5 cm from the top.

Sample Preparation and HPLC Analysis. Samples were lyophilized for 48 h and pulverized in a Waring blender. Approximately 15 mg of the homogenate was directly weighed into a sample tube (2 mL, Assist Trading Co., Ltd., Tokyo, Japan) and mixed with 70% methanol (1.5 mL), then let stand

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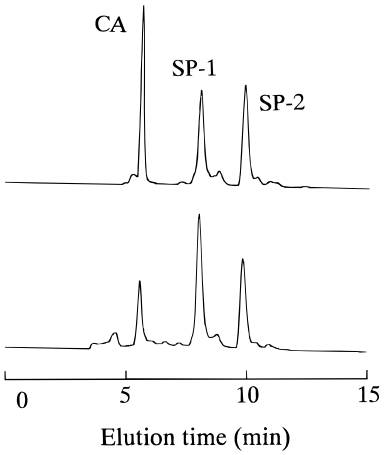

Figure 2. Chromatograms of 50 μg/mL standard solution (top) and 70% methanolic extract of young cv. Kiwame-chuba leaves (bottom). The eluate was monitored at 280 nm.

for 24 h at room temperature with occasional vigorous shaking, and centrifuged at 10 000 rpm for 1 min, as described by Hayase and Kato (1984). The supernatant was decanted and residue re-extracted as described above. Combined extracts were filtered through a 0.45-μm filter (Cosmo Nice Filter-W; Nacalai Tesque) and diluted to 10 mL with 70% methanol in water before injection of a 20 μL aliquot. The following solvents and elution profiles were used: solvent A, 10% (v/v) acetonitorile in water containing 0.2% (v/v) formic acid; solvent B, 100% acetonitorile containing 0.2% formic acid; elution profiles: 0−15 min 87% solvent A (isocratic); 15−25 min 87−0% solvent A (linear gradient); flow rate, 1.0 mL/min. Separation was achieved on a COSMOSIL $5C_{18}$-MS (250 × 6 mm i.d.) (Nacalai Tesque) column, and the eluate was monitored with a UV detector at a wavelength of 280 nm. CA, SP-1, and SP-2 were identified by comparison with the retention times of authentic standards (Figure 2). Three components on the chromatogram were isolated by HPLC and identified by nuclear magnetic resonance (NMR).

Heat Stability Evaluation. Garland is commonly used in pot-cooked winter meals in Japan, so we determined the recovery of the quinic acid derivatives after boiling. An aerial part of cultivar Kiwame-chuba (ca. 15 g fresh wt) was heated separately in a beaker with 300 mL of boiling water. After boiling for 0, 1, 5, 10, and 30 min, heated samples were immediately frozen, kept at −20 °C for 2 h, then lyophilized for 48 h and pulverized in a Waring blender. The broth was also measured after concentration. Further samples were prepared, and HPLC analysis was conducted as described above.

In additional experiments, each solution of CA, SP-1, and SP-2 was prepared at a concentration of 50 μg/mL in water and pipetted into 10-mL sample tubes. Solutions were kept at 100 °C for 30 min. After heat treatment, the solutions were cooled to room temperature before HPLC quantification. All of the above experiments were replicated three times each.

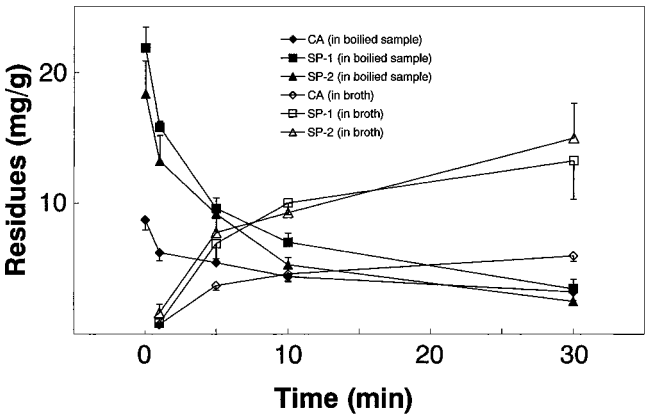

Figure 3. Retention of quinic acid derivatives in garland during boiling in water and of broth. Initial dry weight CA content was 8.7 ± 0.8 mg/g, that of SP-1 was 21.8 ± 1.6 mg/g, and that of SP-2 was 18.3 ± 2.5 mg/g. After 30 min, the remaining dry weight CA content was 3.2 ± 0.7 mg/g, that of SP-1 was 3.4 ± 0.7 mg/g, and that of SP-2 was 2.5 ± 0.6 mg/g.

RESULTS

HPLC Quantification or Analysis. Several mobile phases and column conditions were examined. Mobile phases exceeding 30% acetonitrile in water containing 0.2% formic acid satisfactorily separated three antioxidants peaks from impurities. The COSMOSIL $5C_{18}$-MS column, of several column tested, gave the best separation of the quinic acid derivatives. The profile of the separation of the standards was shown in Figure 2. Retention time was 5.3 min for CA, 7.8 min for SP-1, and 9.9 min for SP-2.

Content of Quinic Acid Derivatives in Edible Parts of Garland. The contents of CA, SP-1 and SP-2 in three edible parts of garland are presented in Table 1. Young leaves contained the greatest amount of SP-1 and SP-2, but the CA content was more in the mature leaves. The relative quantities of the respective quinic acid derivatives differed, i.e., cv. Kiwame-chuba (K) > cv. Satoyutaka (SY) = cv. Okiku-3 (O) > cv. Natsunosei (N) = cv. Satoakira (SA) for CA; (K) > (SY) = (SA) = (O) > (N) for SP-1; and (K) = (SY) > (SA) ≫ (O) = (N) for SP-2. Furthermore, the order of total amounts of three antioxidants was as follows: (K) > (SY) > (SA) = (O) > (N). The Natsunosei, rosette type, contained the smallest amount of the three compounds in garland.

Heat Stability. The effect of heating time on the quinic acid derivatives in garland is shown in Figure 3. The amount of CA, SP-1, and SP-2 in garland decreased with time and the amount in broth increased. The amounts of CA, SP-1, and SP-2 observed finally after 30 min as compared to the fresh sample were approximately 37% for CA equaling 3.2 ± 0.7 mg/g dry wt (n = 3); 16% for SP-1 equaling 3.4 ± 0.7 mg/g dry wt;

Table 1. Concentration (mg/g, Dry Weight) of Quinic Acid Derivatives in Different Parts of Garland Cultivars[a]

plant part		cv. Okiku-3			cv. Kiwame-chuba			cv. Satoyutaka			cv. Natsunosei			cv. Satoakira			mean		
		CA	SP-1	SP-2	CA	SP-1	SP-2	CA	SP-1	SP-2	CA	SP-1	SP-2	CA	SP-1	SP-2	CA	SP-1	SP-2
leaves																			
young	mean	5.2	19.8	13.6	8.5	39.2	27.7	5.8	22.7	28.2	3.8	11.5	10.2	3.6	21.0	24.3	5.4	22.9	20.8
	SD	0.4	0.4	1.6	0.6	7.3	3.1	0.4	1.3	2.8	0.1	0.1	0.8	0.1	1.4	0.5	1.9	9.8	7.9
mature	mean	5.5	13.8	7.6	8.7	20.2	10.6	7.5	13.4	18.4	5.3	13.2	9.0	3.2	13.0	8.7	6.0	14.7	10.9
	SD	0.6	0.9	0.7	1.6	3.1	1.6	1.1	2.9	1.2	0.5	1.0	1.2	0.3	1.6	2.4	2.1	3.4	4.2
stem	mean	2.1	3.0	1.4	2.9	7.3	1.9	2.4	3.6	2.3	1.2	3.0	1.5	1.4	3.1	1.4	2.0	4.0	1.7
	SD	0.3	0.4	0.1	0.1	1.1	0.5	0.5	0.7	0.2	0.2	0.4	0.2	0.1	0.2	0.1	0.7	1.8	0.4

[a] Data were derived from three replications for all samples. CA, chlorogenic acid; SP-1, 3,5-dicaffeoylquinic acid; SP-2, 3,5-dicaffeoyl-4-succinylquinic acid.

14% for SP-2 equaling 2.5 ± 0.6 mg/g dry wt. Broth amounts after 30 min of boiling were about 69% for CA, 60% for SP-1, and 83% for SP-2. In the case of standard solutions, the recovery after heating at 100 °C for 30 min was 99.3 ± 1.7% (n = 3) for CA, 96.4 ± 4.5% for SP-1, and 96.4 ± 2.4% for SP-2.

DISCUSSION

Natural antioxidants have drawn the attention of the food industry and customers, because synthetic antioxidants have been found to be carcinogenic and harmful to the lungs and liver (Colbert and Decker, 1991; Ito et al., 1986). Putting the matter of taste aside, it is noteworthy, from the viewpoint of health and industrial use, that garland leaf contains several "natural" antioxidants.

Young leaves contained the most quinic acid derivatives, followed by mature leaves and stem. This agreed with the sequence for ascorbic acid and carotenoids (Nakamura et al., 1997), which are major, widely studied antioxidants. These compounds protect plants against oxidative damage by inhibiting or quenching free radical and reactive oxygen species (Larson, 1988; Friedman, 1997). Other compounds possessing these functions, such as catechins, quercetin, and luteolin, accumulate in many plant species. The tea plant (*Camellia sinensis*) contains a considerable amount of catechins in its leaves. The order of catechins content in parts of the fresh tea shoot (Lin et al., 1996) agrees well with that of antioxidants in garland. It is suggested that the high content of antioxidants in young leaves rather than other parts is an essential component of protection against oxidation because the young parts of the plant are actively growing.

Several reports suggest that antioxidants in foods such as potato (*Solanum tuberosum*) and sweet potato (*Ipomoea batatas*) may protect the human body from free radicals, which may cause disease and aging (Cultar, 1992; Player, 1982). CA constitutes about 90% of all phenolic compounds in potato tubers (Mondy and Gosselin, 1988; Malmberg and Theander, 1985). CA may produce an undesirable appearance, e.g., bluish-gray discoloration in boiled or steamed potatoes, following exposure to air (Swain, 1962). Garland does not discolor after cooking since chlorophyll degradation products probably mask discoloration. Further, quinic acid derivatives have not been reported to lower the quality of garland used in cooking recipes.

Judging from the more than 95% recovery of standard solution after heating at 100 °C for 30 min and the recovery of compounds in broth, the antioxidants decrease found for the plant material (Figure 3) indicates that the compounds did not decompose but were leached out during boiling in water. Since boiling time is generally several minutes, it can be assumed that garland retains significant amounts of compounds in conventional cooking.

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