

## Changes in Polyphenolic Content and Radical-Scavenging Activity of Sweetpotato (*Ipomoea batatas* L.) during Storage at Optimal and Low Temperatures

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Polyphenolic content and radical-scavenging activities (RSA) of four sweetpotato (*Ipomoea batatas* L.) cultivars were characterized after storage at optimal (15 °C) or low temperature (5 °C) for 0, 13, 26, and 37 days. The polyphenolic content increased during storage in three cultivars but not in 'Murasakimasari'. The change in 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity (DPPH-RSA) correlated very well with polyphenolic content. The increases in polyphenolics and the RSA in 'Benimasari' were significantly greater during storage at 5 °C than at 15 °C. The main polyphenolic components in all cultivars were chlorogenic acid (ChA) and 3,5-di-*O*-caffeoylquinic acid (3,5-diCQA). ChA level increased more at 5 °C than at 15 °C, whereas that of 3,5-diCQA was greater at 15 °C. Caffeoylquinic acids and RSA in 'Murasakimasari', which contains a large amount of anthocyanin in flesh tissue, were extremely high at the beginning of storage and remained nearly constant or decreased over time. A non-caffeoylquinic acid component that increased during storage, especially in 'J-Red' at 15 °C, was purified by successive chromatographic steps. The isolate was identified as caffeoyl sucrose [CSu, 6-*O*-caffeoyl-( $\beta$ -D-fructofuranosyl-(2 $\rightarrow$ 1))- $\alpha$ -D-glucopyranoside] by fast atom bombardment–mass spectroscopy (FAB-MS), infrared spectroscopy (IR), and nuclear magnetic resonance spectroscopy (NMR). These results suggest that storage under cultivar-dependent, controlled temperature is one approach for increasing desirable physiologic function associated with RSA of polyphenolic compounds in sweetpotato roots.

**KEYWORDS:** Sweetpotato (*Ipomoea batatas* L.); polyphenolic; caffeoylquinic acid; DPPH radical-scavenging activity (DPPH-RSA); caffeoyl sucrose; storage

### INTRODUCTION

Polyphenolics from various plant sources are receiving increasing attention due to the desirable physiological functions associated with their antioxidant activities. They prevent oxidative stress that increases risk for many diseases (1, 2). Sweetpotato (*Ipomoea batatas* L.) roots contain polyphenolics, which are mainly caffeoylquinic acid derivatives, namely, caffeic acid (CA), chlorogenic acid (ChA), 3,4-di-*O*-caffeoylquinic acid (3,4-diCQA), 3,5-di-*O*-caffeoylquinic acid (3,5-diCQA), and 4,5-di-*O*-caffeoylquinic acid (4,5-diCQA) (3). Caffeoylquinic acids have been shown to exhibit radical-scavenging activity (RSA), antimutagenicity, anticancer activity, antidiabetic activity, antibacterial activity, anti-inflammatory activity, and anti-HIV activity and to inhibit melanin production in vitro or in

vivo (3–10). Polyphenolics also play an important role in vivo defense systems against insects and other pathogens. The caffeoylquinic acids in sweetpotato roots have antifungal and antiviral activities (11–15). The concentration of these polyphenolics has been reported to increase as a function of stress, that is, wounding, infection, and drought (16–18).

Sweetpotatoes are a tropical root crop, susceptible to physiological damage during low-temperature storage. The optimal storage temperature for sweetpotatoes is 13–16 °C, and the optimal relative humidity is 80–85% (19). When exposed to lower temperatures for a prolonged period, sweetpotato roots undergo irreversible deterioration and are more easily infected by nonpathogenic fungi (16).  $\alpha$ - and  $\beta$ -amylases have been reported to increase during the early stage of storage at optimal temperature in a cultivar-dependent manner (20–22). Some biochemical changes observed in roots during storage depend on temperature. Sucrose and total soluble sugar content was reported to be enhanced in roots held at 7 °C compared to those held at 15.6 °C (23). A decrease in starch content at 4 °C was

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observed to be greater than in control roots stored at 13–15 °C (24). Carotenoids were reported to increase slightly during short-term storage at 7, 15.6, or 26.6 °C (25). However, little information is available on changes in the concentration of hydrophilic antioxidants in sweetpotato roots during storage, except for the report that ChA increased and ascorbic acid decreased during storage at 7.5 °C (26).

In the present study, we investigated changes in polyphenolic content and RSA in four cultivars during storage at optimal and low temperatures. Changes in the concentration of individual caffeic acid derivatives, including a component produced during storage, were also analyzed.

## MATERIALS AND METHODS

**Chemicals.** ChA was purchased from Sigma Chemical (St. Louis, MO) and Trolox from Aldrich Chemical Co. (Milwaukee, WI). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and other chemicals used were of the highest grade available from Wako Pure Chemicals Industries Ltd. (Osaka, Japan). Dicafeoylquinic acids (3,4-diCQA, 4,5-diCQA, and 3,5-diCQA) were prepared as previously reported (27).

**Sample Preparation.** The four cultivars, 'Benimasari' and 'Koganesengan' with yellow-colored flesh, 'J-Red' with orange-colored flesh, and 'Murasakimasari' with purple-colored flesh, were grown in an experimental plot at the National Agricultural Research Center for the Kyushu Okinawa Region. Plants were transplanted on April 21 and roots harvested on August 5, 2003. Harvested sweetpotato roots were initially held at ambient temperature in a storage facility. The next day (first full day of storage), the storage temperature was reduced to 15 °C and the relative humidity maintained at >80.0%. Half the roots of each cultivar were then moved to storage at 5 °C and >80.0% relative humidity to begin the storage treatment. All sweetpotato roots stored at 15 °C and 'Murasakimasari' and 'Benimasari' roots held at 5 °C were found to be healthy during storage, whereas chilling injury (discoloration of skin and internal tissue, followed by softening in tissue) was evident in 'Koganesengan' after 37 days and in 'J-Red' after 49 days of storage at 5 °C. Five healthy roots of each cultivar were taken from the initial sample (day 0) and after 13, 26, and 37 days of storage with the following exceptions. Four healthy roots of 'J-Red' were sampled after 26 days of storage at 15 °C and after 37 days at 5 °C for 'Koganesengan'. Whole roots were freeze-dried and held at -35 °C until analysis.

**Total Polyphenolic Content.** Total polyphenolics were measured according to a modified Folin–Ciocalteu method (27). Freeze-dried samples (50 mg) were vigorously mixed in 2.5 mL of 80% ethanol and were heated in a boiling water bath for 5 min. The ethanol was evaporated and the extract was redissolved in the same volume of distilled water. The extract (25  $\mu$ L) was added to 125  $\mu$ L of phenol reagent and reacted for 4 min. The reaction was stopped by the addition of 125  $\mu$ L of sodium carbonate, followed by measurement at 600 nm. Total polyphenolics are expressed as milligrams of ChA equivalents per gram of dry weight (DW).

**DPPH-RSA.** RSA was assayed as described (28) with the following modifications. The extract prepared for measurement of total polyphenolics was diluted with 80% ethanol to various concentrations. Diluted samples (75  $\mu$ L) were transferred to a 96-well microplate, and 150  $\mu$ L of 2-morpholinoethanesulfonic acid (MES) buffer (100 mM MES in 50% ethanol, pH 6.0) and 75  $\mu$ L of DPPH solution (200  $\mu$ M DPPH in 50% ethanol) were added. After reaction for 2 min, absorbance at 520 nm was measured by a densitometer using a microplate program (CS-9300PC; Shimadzu Co., Kyoto, Japan). Reaction solutions without DPPH were used as color controls, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as a standard.  $A_{520}$  values were plotted against the diluted sample, and a linear decrease in  $A_{520}$  of the DPPH radical was recorded. DPPH-RSA was expressed as micromoles of Trolox equivalents per gram of DW.

**HPLC Analysis.** A portion of the total polyphenolic extract was filtered through a 0.45  $\mu$ m membrane, and the caffeoylquinic acids were determined quantitatively by high-performance liquid chromatography (HPLC) as described (27). The HPLC system consisted of two pumps

(model LC-10AT; Shimadzu, Kyoto, Japan), an autoinjector (model SIL-10AXL; Shimadzu), a column oven (model CTO-10AC; Shimadzu), and a photodiode array detector (model SPD-M10AVP UV-vis; Shimadzu). A reversed phase column was used (150  $\times$  4.6 mm i.d., YMC-Pack ODS-AM AM-302; YMC, Kyoto, Japan). The mobile phase consisted of water containing 0.2% formic acid (A) and methanol (B). The elution profile was as follows: 2% B from 0 to 15 min, a linear gradient of 2–45% B from 15 to 50 min, and 45% B from 50 to 65 min at a flow rate of 1 mL/min. The column oven was set at 40 °C. The caffeoylquinic acids were identified by the retention time and the UV-vis spectra of standards. Quantification of caffeoylquinic acids was carried out according to an external standard method using calibration curves based on detection at 326 nm. Caffeoylquinic acids are quantified as micromoles per gram of DW.

**Structural Identification of Caffeoyl Sucrose (CSu).** One hundred grams of freeze-dried sweetpotato roots of 'J-Red' stored at 15 °C for 5 months were extracted with 80% methanol (2  $\times$  2 L) at room temperature. The extract was dried by rotary evaporation and redissolved in 250 mL of distilled water. The extract was partitioned using an equal volume of *n*-hexane. The water layer was chromatographed on MCI gel CHP20P (400 mm  $\times$  30 mm i.d., Mitsubishi Chemical Ind., Tokyo, Japan) and eluted with distilled water, followed by 10, 20, and 30% methanol in succession. The 30% methanol eluate was dried by rotary evaporation and dissolved in 20% methanol. The extract was chromatographed on an octadecylsilyl (ODS) column (400 mm  $\times$  30 mm i.d., Fuji Silisia Ltd., Kasugai, Japan) and eluted with 20% methanol. The major peak was isolated (54 mg) and subjected to the instrumental analyses. The isolate was identified as caffeoyl sucrose [CSu, 6-*O*-caffeoyl-( $\beta$ -D-fructofuranosyl-(2 $\rightarrow$ 1))- $\alpha$ -D-glucopyranoside] by fast atom bombardment–mass spectroscopy (FAB-MS), infrared spectroscopy (IR), and  $^1$ H and  $^{13}$ C nuclear magnetic resonance spectroscopy (NMR). The IR spectrum was recorded on a JEOL JIR-6500W spectrometer (Tokyo, Japan) using a microspectroscopy FT-IR method. The one- and two-dimensional NMR spectra were recorded on a JEOL A-500 (Tokyo, Japan)  $^1$ H (500 MHz) and  $^{13}$ C (125 MHz) spectrometer using standard pulse sequences with TMS as the internal standard. Chemical shifts are reported in  $\delta$ , and coupling constants (*J*) are given in hertz. FAB-MS was obtained using a JEOL DX-303HF spectrometer (Tokyo, Japan).

**Statistical Analysis.** Data are the average of five roots per cultivar (four after 26 days of storage at 15 °C for 'J-Red' and after 37 days at 5 °C for 'Koganesengan') stored at 5 or 15 °C for each storage period at each temperature. All analyses for each root were performed in triplicate. Statistical calculations were performed using SPSS 10.0J for Windows (SPSS Inc.). The statistical significance of differences found in total polyphenolic content and DPPH-RSA during storage at 15 or 5 °C was analyzed using two-way repeated measures analysis of variance. Statistical differences among storage periods at a given temperature per cultivar were evaluated by Tukey's multiple-range test at *P* = 0.05. Differences among temperatures within a storage period for a cultivar were evaluated by the single degree of freedom *F* test at *P* = 0.05. The Pearson correlation coefficient (*R*) was used to show the correlation between total polyphenolics content and DPPH-RSA.

## RESULTS AND DISCUSSION

**Change in Polyphenolic Content.** Total polyphenolics during storage were determined according to the Folin–Ciocalteu method to obtain a global view of changes in the sweetpotato roots. Changes in total polyphenolics in the four types of sweetpotato roots during storage at 15 or 5 °C are shown in **Table 1**. The polyphenolic content in 'Murasakimasari' was 31.2 mg of ChA/g of DW, much higher than that in 'Benimasari' (3.4 mg/g of DW), 'Koganesengan' (2.3 mg/g of DW), or 'J-Red' (4.2 mg/g of DW) in the initial sample prior to storage. Storage time had a significant influence on all cultivars tested, whereas temperature did not in the case of 'Koganesengan' and 'Murasakimasari'. Significant interactions were observed between storage time and temperature for 'Benimasari' and 'J-Red' by the two-way repeated measures analysis of variance.

**Table 1.** Mean Polyphenolic Content (Milligrams of Chlorogenic Acid per Gram of Dry Weight) during Storage at 15 and 5 °C

cultivar		0 days <sup>a</sup>	13 days	26 days	37 days
Benimasari	15 °C	3.4 a <sup>b</sup>	3.8 ab	5.6 c	5.1 bc
	5 °C	3.4 a	5.4 b A <sup>c</sup>	8.6 c A	12.5 d A
Koganesengan	15 °C	2.3 a	3.6 b	4.0 b	4.7 b
	5 °C	2.3 a	3.2 ab	4.3 bc	4.7 c
J-Red	15 °C	4.2 a	7.5 b A	8.7 b	9.0 b
	5 °C	4.2 a	6.6 b	9.0 d	7.6 c
Murasakimasari	15 °C	31.2 bc	27.5 ab	35.4 c	23.7 a
	5 °C	31.2 bc	30.5 ab	37.3 c	25.4 a

<sup>a</sup> The values in the 0 days column represent the mean of the prestorage sample. The data were filled in both 15 and 5 °C rows to indicate the significant differences at each temperature for the same cultivar. <sup>b</sup> Small letters indicate significant differences ( $P < 0.05$ ) at a given temperature for the same cultivar. <sup>c</sup> Mean polyphenolic content followed by an "A" at one temperature is significantly greater than that at the other storage temperature ( $F$  test with single degree of freedom at  $P < 0.05$ ).

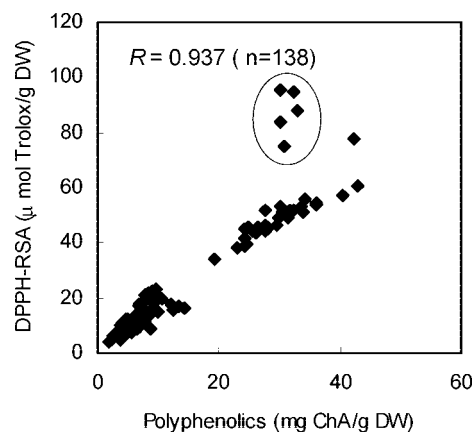
Polyphenolics increased during storage in all cultivars except 'Murasakimasari'. The largest increase was observed in 'Benimasari' after 13 days of storage at 5 °C, which was significantly greater than that at 15 °C. At 37 days of storage at 5 °C, the polyphenolic content in 'Benimasari' increased 3.7-fold relative to the initial sample. In 'Murasakimasari', the content increased from day 13 to day 26 and decreased thereafter. Lieberman et al. (26) previously reported that ChA increased in sweetpotato roots during storage at 7.5 °C relative to storage at 15 °C. Our results for 'Benimasari' were consistent with those of Lieberman et al. (26), but with a cultivar-dependent pattern. Exposure to nonfreezing temperatures has been shown to stimulate an increase in unique phenolics in various fruits and vegetables (29–31). Lattanzio et al. (32) reported that there is a low, critical temperature below which an increase in phenylpropanoid metabolism including phenylalanine ammonia-lyase (PAL) is stimulated during the storage of plant tissues and that this temperature varies from commodity to commodity. It has been suggested that the temperature sensitivity at 5 °C of sweetpotato roots differs as a function of genotype.

**RSA.** RSA was analyzed because polyphenolics are known as radical scavengers. Changes in DPPH-RSA during storage at 15 or 5 °C are shown in **Table 2**. Activity in 'Murasakimasari' was 87.3  $\mu\text{mol}$  of Trolox/g of DW, much higher than that in 'Benimasari' (6.9  $\mu\text{mol}$ /g of DW), 'Koganesengan' (5.0  $\mu\text{mol}$ /g of DW), or 'J-Red' (11.1  $\mu\text{mol}$ /g of DW) in the initial sample before storage. Storage time had a significant influence on all cultivars tested, whereas temperature had no significant effect on 'Koganesengan' or 'Murasakimasari'. Significant interactions between storage time and temperature were observed for 'Benimasari' and 'J-Red' by the two-way repeated measures analysis of variance. Activity was found to increase during storage in all cultivars except 'Murasakimasari'. The increase in 'Benimasari' stored at 5 °C was greater than that at 15 °C, and after 37 days of storage, its activity increased up to 2.4-fold. Activity at 15 °C in 'J-Red' was greater than that at 5 °C after 13 days of storage. In 'Murasakimasari', a decrease in activity was observed during storage. After 37 days of storage, its activity decreased to <50% of the initial prestorage sample. DPPH-RSA correlated very well with total polyphenolic content ( $R = 0.937$ ,  $P < 0.01$ ,  $n = 138$ ; **Figure 1**). This result indicates that the major radical-scavenging components in sweetpotato roots during storage are polyphenolics. DPPH-RSA in 'Murasakimasari' before storage was greater than after storage and

**Table 2.** Mean DPPH-RSA (Micromoles of Trolox per Gram of Dry Weight) during Storage at 15 and 5 °C

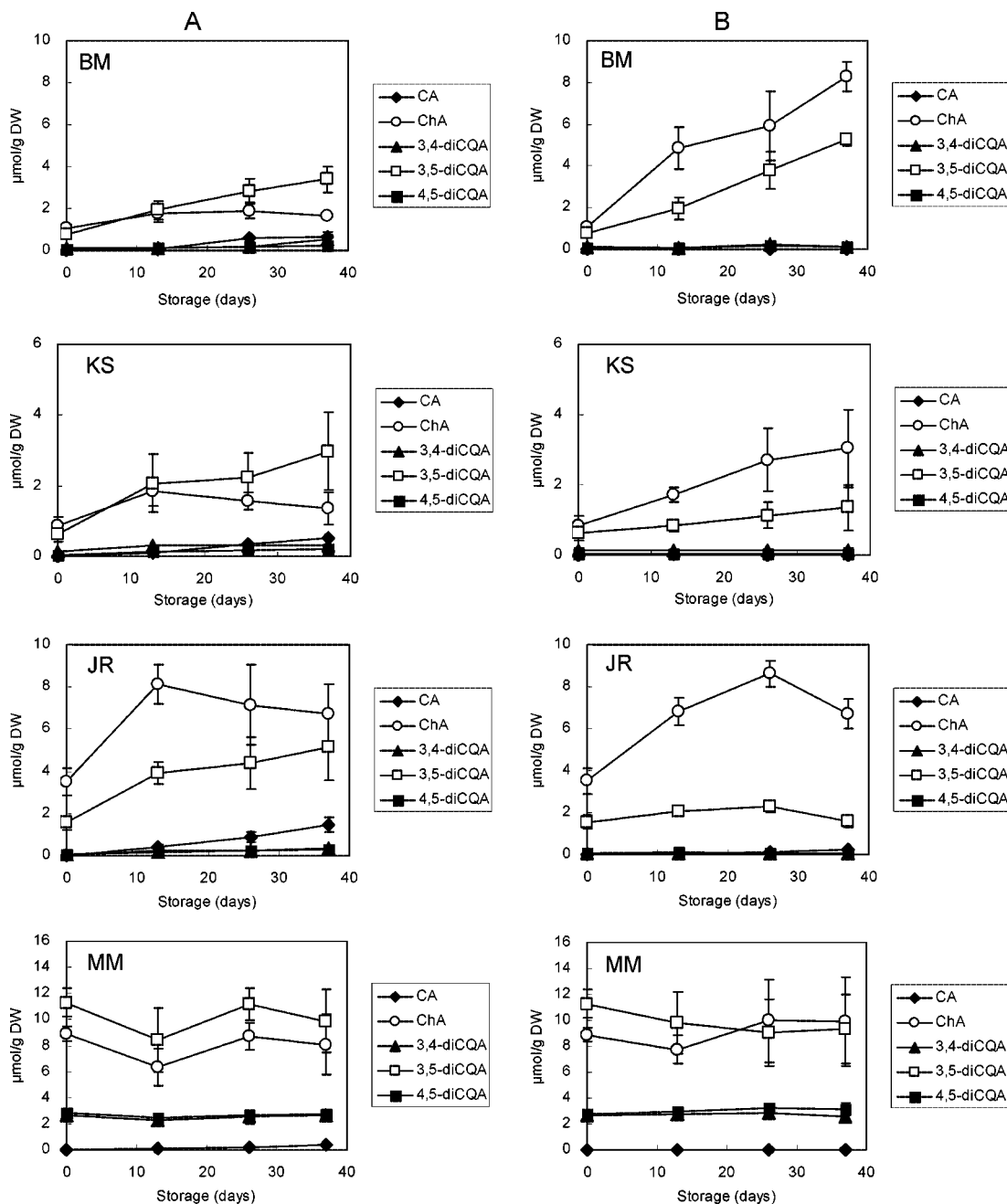
cultivar		0 days <sup>a</sup>	13 days	26 days	37 days
Benimasari	15 °C	6.9 a <sup>b</sup>	9.0 a	12.2 b	8.9 a
	5 °C	6.9 a	10.4 a	15.3 b	16.3 b A
Koganesengan	15 °C	5.0 a	8.7 b	9.5 b	8.8 b
	5 °C	5.0 a	6.9 b	7.7 b	6.6 ab
J-Red	15 °C	11.1 a	18.7 b A <sup>c</sup>	22.7 c A	18.0 c A
	5 °C	11.1 a	10.9 a	18.7 a	11.3 b
Murasakimasari	15 °C	87.3 c	48.5 ab	54.5 b	41.9 a
	5 °C	87.3 c	49.8 ab	58.4 b	41.1 a

<sup>a</sup> The values in the 0 days column represent the mean of the prestorage sample. The data were filled in both 15 and 5 °C rows to indicate the significant differences at each temperature for the same cultivar. <sup>b</sup> Small letters indicate significant differences ( $P < 0.05$ ) at a given temperature for the same cultivar. <sup>c</sup> Mean DPPH-RSA followed by an "A" at one temperature is significantly greater than that at the other storage temperature ( $F$  test with single degree of freedom at  $P < 0.05$ ).

**Figure 1.** Correlation between polyphenolic content and DPPH-RSA. The values of prestorage sample of 'Murasakimasari' are circled.

did not correlate with polyphenolic content (**Figure 1**). This result suggests that components other than polyphenolics are responsible for the additional RSA in 'Murasakimasari' before storage. Low-temperature storage may induce active-oxygen species, radicals, or superoxide formation as a consequence of an imbalance in oxidative and reductive processes (32). 'Benimasari' and 'Murasakimasari', which have higher RSA due to inducible or preformed polyphenolics during storage, showed excellent stability to low-temperature storage. This suggests that the cold resistance of sweetpotato cultivars may be, at least in part, mediated by the RSA of polyphenolics.

**Change in Caffeoylquinic Acids.** Individual caffeoylquinic acids, CA, ChA, 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA, were analyzed by HPLC during storage to assess changes. The main caffeoylquinic acids in all cultivars at the beginning of storage were ChA and 3,5-diCQA, which increased extensively during storage except in 'Murasakimasari' (**Figure 2**). The pattern of increase in ChA and 3,5-diCQA differed as a function of storage temperature. The increase in ChA stored at 5 °C was significantly greater than that at 15 °C in 'Benimasari' and 'Koganesengan' after 13 and 26 days of storage, respectively, whereas 3,5-diCQA was greater at 15 °C than at 5 °C in 'Koganesengan' and 'J-Red' after 13 days of storage. Kojima et al. (33) reported that ChA was converted enzymatically to 3,5-diCQA in a one-step reaction. Lower enzyme activity could explain the lower amounts of 3,5-diCQA at 5 °C. In 'J-Red', DPPH-RSA was significantly greater at 15 °C than at 5 °C after 13 days of storage



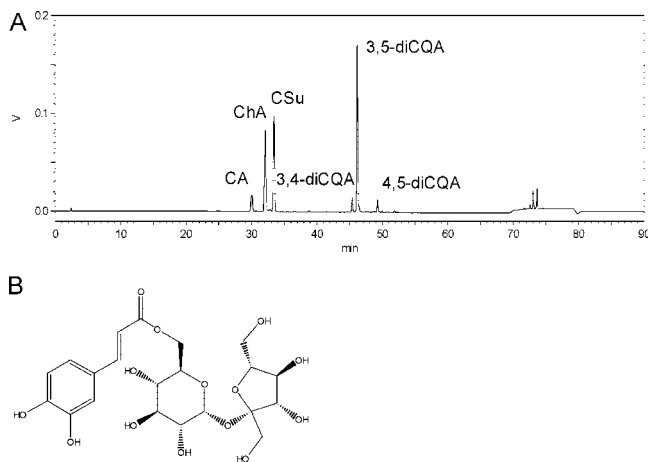
**Figure 2.** Changes in caffeoylquinic acids during storage 15 °C (A) or 5 °C (B). Data are represented as means  $\pm$  SD of five different roots. BM, Benimasari; KS, Koganesengan; JR, J-Red; MM, Murasakimasari.

and thereafter (Table 2), although a significant difference in total polyphenolics between 15 and 5 °C was observed only after 13 days of storage (Table 1). DPPH-RSA correlates with the number of caffeoyl groups bound to quinic acid as dicaffeoylquinic acids had about 2 times higher activity than a monocaffeoylquinic acid, ChA (6). It is possible that the higher content of 3,5-diCQA resulted in stronger RSA at 15 than at 5 °C after 13 days of storage and thereafter in 'J-Red'. In 'Murasakimasari', caffeoylquinic acid content except for CA was higher than in other cultivars, with level and composition remaining nearly constant.

**Identification and Change in Caffeoyl Sucrose (CSu) during Storage.** A component other than the five aforementioned caffeoylquinic acids was observed during storage (Figure 3A) and was purified by successive chromatography steps.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data are shown in Table 3. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals were assignable by 1D  $^1\text{H}$  and  $^{13}\text{C}$  NMR data

and 2D HH-COSY, TOCOSY, HMQC, and HMBC spectra data. The IR spectrum showed  $\nu_{\text{max}}$  (neat) at 3317 (OH), 1689 (C=O), and negative FAB mass spectrum gave  $[\text{M} - \text{H}]^-$  at  $m/z$  503, indicating that the compound was composed of one molecule each of caffeic acid, glucose, and fructose (sucrose). This was confirmed by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra. The acylation shifts to lower field of H-6 protons of glucose [4.27 (1H, dd,  $J = 5.5, 12.2$  Hz, g-6), 4.50 (1H, dd,  $J = 1.8, 12.2$  Hz, g-6)] indicated that the caffeic acid was attached to the 6-OH of glucose, which was supported by HMBC (8 Hz), HMQC, and TOCOSY analyses. This structure was identified as gluco-6-*O*-caffeoyl sucrose [CSu; 6-*O*-caffeoyl-( $\beta$ -D-fructofuranosyl-(2 $\rightarrow$ 1))- $\alpha$ -D-glucopyranoside] (Figure 3B).

CSu was not detected in 'Benimasari', 'Koganesengan', or 'J-Red', whereas 0.013  $\mu\text{mol/g}$  of DW was found in the prestorage sample of 'Murasakimasari' (Figure 4). Generally speaking, CSu content significantly increased during storage,



**Figure 3.** HPLC chromatogram of polyphenolics in 'J-Red' sweetpotato roots stored for 37 days at 15 °C (A) and the structure of CSu (B).

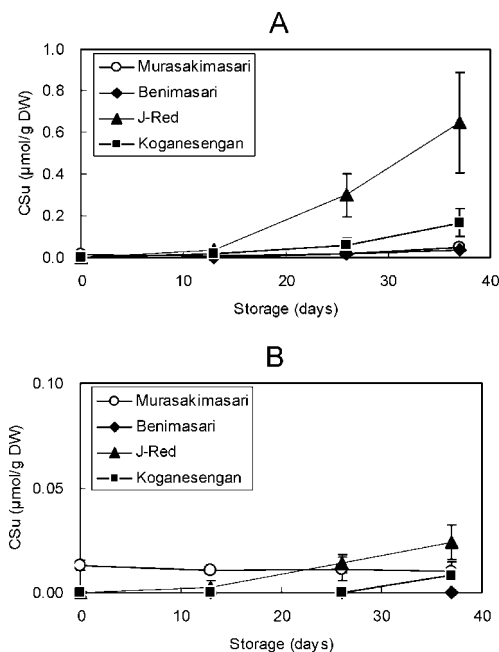
**Table 3.** <sup>1</sup>H and <sup>13</sup>C NMR Spectral Data of CSu

<sup>1</sup> H NMR (CD <sub>3</sub> OD + D <sub>2</sub> O)				<sup>13</sup> C NMR (CD <sub>3</sub> OD + D <sub>2</sub> O)			
position	δ		J, Hz	position	δ		
g-4 <sup>a</sup>	3.36	1H	t 9.1	f-1	64.2	CH <sub>2</sub>	
g-2	3.46	1H	dd 3.7, 9.1	f-6	64.4	CH <sub>2</sub>	
f-1 <sup>b</sup>	3.60, 3.64	each 1H	d 12.2	g-6	65.0	CH <sub>2</sub>	
g-3	3.74	1H	t 9.1	g-4	71.1	CH	
f-5	3.81	1H	m	g-5	72.2	CH	
f-6	3.78	1H	m	g-2	73.3	CH	
f-6	3.85	1H	dd 6.1, 11.0	g-3	74.7	CH	
f-4	4.03	1H	t 8.5	f-4	76.2	CH	
f-3	4.09	1H	d 8.5	f-3	79.5	CH	
g-5	4.10	1H	m	f-5	84.0	CH	
g-6	4.27	1H	dd 5.5, 12.2	g-1	93.5	CH	
g-6	4.50	1H	dd 1.8, 12.2	f-2	105.4	C	
g-1	5.41	1H	d 3.7	caff-8	115.1	CH	
caff-8 <sup>c</sup>	6.34	1H	d 15.9	caff-2	115.4	CH	
caff-5	6.77	1H	d 7.8	caff-5	116.6	CH	
caff-6	6.97	1H	dd 1.8, 7.8	caff-6	123.1	CH	
caff-2	7.03	1H	d 1.8	caff-1	129.5	C	
caff-7	7.58	1H	d 15.9	caff-3	146.9	C	
				caff-7	147.3	CH	
				caff-4	149.7	C	
				caff-9	169.3	C	

<sup>a</sup> Glucopyranoside moiety. <sup>b</sup> Fructofuranosyl moiety. <sup>c</sup> Caffeoyl moiety.

and the magnitude of the increase was greater at 15 °C than at 5 °C (Figure 4). This result is consistent with more CSu being found in sweetpotato subjected to long-term storage than in fresh sweetpotato (34). The change was the greatest in 'J-Red' stored at 15 °C and after 37 days of storage, when the content increased to 0.646 μmol/g of DW (Figure 4). In 'Benimasari', CSu was not detected even after 37 days of storage at 5 °C. The DPPH-RSA of CSu was almost the same as that of CA (data not shown). It has been suggested that CSu contributes DPPH-RSA as a function of increasing storage time in 'J-Red' at 15 °C. The CA might react with the sucrose, which is generated during storage, at the optimal storage temperature of 15 °C.

To summarize, RSA in sweetpotato roots increases during storage, likely due to the increase in polyphenolics at both optimal and low temperatures in a cultivar-dependent manner. The increase in polyphenolics in 'Benimasari' was greater at 5 °C than at 15 °C. The change of individual caffeic acid derivatives was dependent on temperature. ChA increased more at 5 °C, whereas 3,5-diCQA and CSu increased more at 15 °C. It is worth nothing that 'Murasakimasari', which contains anthocyanin pigments in the root, exhibited a different pattern of change in polyphenolics during storage. Caffeoylquinic acids



**Figure 4.** Changes in CSu during storage at 15 °C (A) and 5 °C (B). Data are represented as means ± SD of five different roots.

and RSA were extremely high at the beginning of the storage period and remained nearly constant or decreased (Figure 2; Table 2). Because this cultivar has a naturally greater polyphenolic content in the absence of storage, increased synthesis of polyphenolics to enhance RSA may be unnecessary to ensure resistance to cold stress or pathogens.

A variety of plant polyphenolics are receiving greater scientific attention because of their perceived beneficial physiological functions. Postharvest storage at controlled temperature with appropriate duration is one possible approach for increasing the functional value of sweetpotato roots.

#### ABBREVIATIONS USED

CA, caffeic acid; ChA, chlorogenic acid; 3,4-diCQA, 3,4-di-*O*-caffeoylquinic acid; 3,5-diCQA, 3,5-di-*O*-caffeoylquinic acid; 4,5-diCQA, 4,5-di-*O*-caffeoylquinic acid; CSu, caffeoyl sucrose; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ODS, octadecylsilyl; IR, infrared spectroscopy; FAB-MS, fast atom bombardment–mass spectroscopy; NMR, nuclear magnetic resonance spectroscopy.

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