

# Influence of Jam Processing on the Radical Scavenging Activity and Phenolic Content in Berries

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Six selected phenolic aglycons (caffeic and ellagic acids, kaempferol, quercetin, myricetin, and morin) in nine types of berries, and their changes as influenced by jam processing, have been evaluated using optimized HPLC with diode-array detection. The berry samples, fresh and after jam processing, were analyzed, and the total amounts of selected phenolics as aglycons were identified and determined by acid hydrolysis. Their contents in fresh and jam samples did not indicate appreciable changes; therefore, the influence of jam processing on these selected phenolics in berries was suggested to be small, and was mostly present in berries as several conjugated forms that were glycosylated, esterified, etc., in the samples. The total phenolic content of each sample was also determined by the Folin-Ciocalteu method. The three samples of each berry, namely fresh, jam, and acid hydrolysate of the berry, had similar total phenolic contents. On the other hand, the scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was measured, and acid hydrolysates showed stronger activity than that of the fresh and jam-processed samples for all of the berry types.

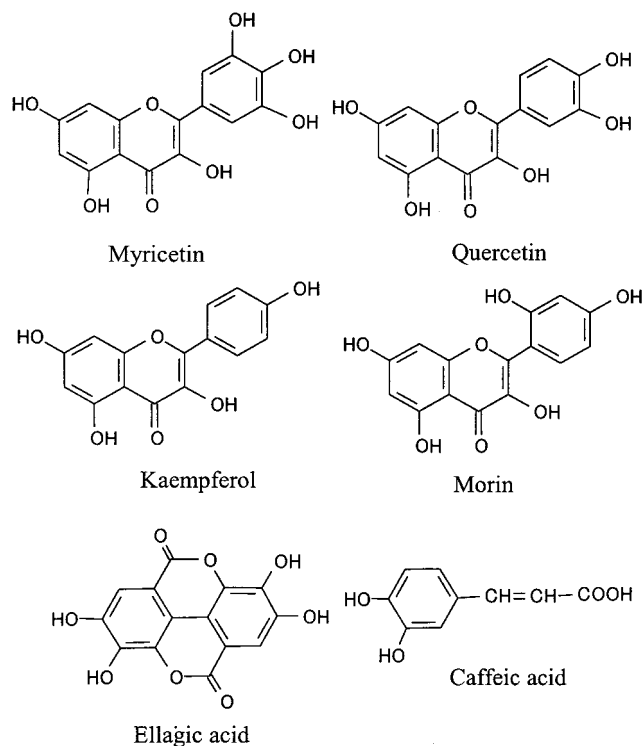
**Keywords:** berry; phenolic; HPLC; jam; radical scavenger

## INTRODUCTION

Phenolics such as flavonoids and phenolic acids are important secondary plant metabolites and are widely distributed in many fruits, vegetables, teas, and beverages (Haslam, 1977, 1981; King and Young, 1999; Merken and Beecher, 2000; Okuda et al., 1995; Tanaka, 1999). Most of the phenolics in plants occur as glycosides or esters (Daniel et al., 1989; Henning, 1981; Herrmann, 1989; Rommel and Wrolsted, 1993; Wilson and Hagerman, 1990). Among them, typical low-molecular-weight phenolics in foods (namely caffeic and ellagic acids, quercetin, kaempferol, morin, and myricetin, Figure 1), have been reported to exert potential health-promoting effects as antioxidants, and as antitumor and anticarcinogenic agents (Hatano, 1995; Okuda et al., 1984, 1992; Rapisarda and Tomaino, 1999). Additionally, ellagic acid and morin are included in the List of Existing Food Additives as natural antioxidants in Japan [Notification No. 120 (April 16, 1996), Ministry of Health and Welfare, Japan].

Berries such as strawberry, blueberry, and raspberry are traditionally favorite desserts all over the world. They are also used as processed food materials for juice, jam, dried fruit, ice cream, etc., and therefore are quite prevalent in our lives. Berries contain many phenolic substances, and attention is being paid to health-promoting foods that have phenolic bioactivities (Häkkinen et al., 1998, 1999; Versari, 1998).

The analysis of phenolics (flavonols, phenolic acids, etc.) in berries using HPLC has already been discussed in several reports (Amakura et al., 2000; Häkkinen et al., 1998, 1999; Rommel and Wrolstad, 1993; Shahrzad



**Figure 1.** Structures of selected phenolics.

et al., 1996). However, reports describing changes of the actual aglycon amounts between fresh and processed berries has been rarely found. As mentioned above, the berries, including their phenolics, have often been proposed as health-promoting materials. Therefore, in view of the importance of these substances to health and to food chemistry, the determination of changes in selected phenolic contents upon processing of berries is required and is deemed important.

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In this paper, we studied the fluctuations of six selected phenolics and the total phenolic amounts in fresh berries and in jam as processed berries. Furthermore, the scavenging activity on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was measured, and the relationship between the phenolic contents and the radical scavenging effect was estimated and discussed.

## MATERIALS AND METHODS

**Samples, Chemicals, and Reagents.** The nine edible types of berries used for this study were imported and donated by the Kobe quarantine station in Japan. All fresh berries were frozen and stored for 2–3 months at  $-20^{\circ}\text{C}$  until processed and analyzed. Ellagic acid dihydrate, quercetin dihydrate, and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Kaempferol, morin, and caffeic acid were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Myricetin was obtained from Aldrich Chemical (Milwaukee, WI). The standards were dissolved in methanol or ethanol. Methanol and acetonitrile were of HPLC grade. The Sep-Pak Plus tC18 cartridge (900 mg) used for refinement was from Waters (Milford, MA).

**Apparatus.** The HPLC analysis was performed on a Shimadzu class LC–VP HPLC system with class LC–VP software, a pump (LC-10Advp), an autosampler (SIL-10AD), and a diode-array detector (SPD-M10Avp) (Shimadzu, Kyoto, Japan). An L-column ODS ( $5\ \mu\text{m}$ ,  $250 \times 4.6\ \text{mm}$  i.d., Chemicals Inspection and Institute, Japan) was used for the analysis.

**Analytical Conditions.** The HPLC conditions were modified from the method of Häkkinen (1998): flow-rate, 1.0 mL/min; volume injected, 10  $\mu\text{L}$ ; temperature,  $40^{\circ}\text{C}$ ; detection, 360 nm. The mobile phase used two solvents. Solvent A was 5 mmol/L potassium dihydrogenphosphate solution (pH 2.5) and solvent B was acetonitrile. The elution profile was 0–5 min, 0–10% B in A (linear gradient); 5–20 min, 10–40% B in A (linear gradient); 20–35 min, 40% B in A (isocratic); followed by 5 min of equilibrating with 100% A. The UV spectrum was recorded between 200 and 400 nm. The retention time and UV spectrum of the peak compared to the standard were used to identify the compound. An ambiguous peak in agreement with the standard on HPLC chromatogram was distinguished by photodiode array detection. Quantitative determination was carried out using calibration graphs obtained from standard solutions diluted with methanol or ethanol in the concentration range of 0.1–100  $\mu\text{g}/\text{mL}$ . Results were expressed as mg of each phenolic per 100 g of fresh berries.

**Sample Preparations.** All of the raw materials used were the same type of fresh berries homogenized.

**Jam Processing Procedure.** The mixture (total amount, 20 g), consisting of the raw material (10 g) with added granulated sugar (10 g), was mildly heated to concentrate to ca. 16 g (80% of total amount) with slow stirring for 10–15 min. This mixture was then allowed to stand to cool to room temperature, and the prepared substance was used as the jam sample (55–65 °Brix, 10 g equivalent of each fresh berry).

**Preparation of Test Solution.** The raw material (10 g) (or the jam sample, 10 g equivalent of fresh berry) was homogenized in 80% methanol (50 mL), and then the homogenate was filtered in vacuo. The filtrate was evaporated to ca. 10 mL, and then 0.1 mol/L HCl solution (100  $\mu\text{L}$ ) was added. The extract was directly loaded onto the Sep-Pak Plus tC18 cartridge (previously conditioned with 10 mL of methanol), followed by 10 mL of distilled water, and washed with 10 mL of distilled water. The cartridge was eluted with 10 mL of methanol. The eluate was collected in a flask and then evaporated to dryness under reduced pressure below  $40^{\circ}\text{C}$  using a rotary evaporator. Every extract sample was dissolved in 10 mL of methanol then filtered through a 0.5- $\mu\text{m}$  filter to give the test solution.

**Preparation of Acid Hydrolysate.** A test solution (5 mL) in 1 mol/L HCl (5 mL) was heated in a boiling-water bath for 2 h, and the reaction mixture was evaporated to ca. 5 mL. The

concentrate was directly loaded onto the Sep-Pak Plus tC18 cartridge, washed with 10 mL of distilled water, and then eluted with 10 mL methanol. The eluate was evaporated to dryness and dissolved in 5 mL of methanol.

**Determination of Total Phenolics.** The total phenolic contents in the samples were determined with Folin-Ciocalteu reagent according to the method of Julkunen-Tiitto (1985) using gallic acid as a standard. Results were expressed as mg of gallic acid equivalent per 100 g of fresh berries.

**DPPH Radical Scavenging Assay.** The free-radical scavenging capacity of the samples was tested as bleaching of the stable DPPH. A solution (4 mL) of a test sample in MeOH was added to a solution (1 mL) of DPPH in MeOH (final concentration of DPPH,  $2.0 \times 10^{-4}\ \text{mol/L}$ ). After the solution was mixed for 10 s, it was left to stand for 30 min, and the absorbance of the resulting solution at 520 nm was measured. All experiments were carried out in triplicate and repeated at least three times. The scavenging activity on the DPPH radical was expressed as  $\text{EC}_{50}$ , the concentration of the test sample required to give a 50% reduction in the absorbance from that of  $2.0 \times 10^{-4}\ \text{mol/L}$  DPPH in MeOH (Hatano, 1997). The potency is graphically displayed as  $1/\text{EC}_{50}$ .

## RESULTS AND DISCUSSION

The contents of the selected phenolics in fresh berries, jam, and acid hydrolysates of the berries, which were determined by RP–HPLC, are given in Table 1. All of the results were obtained in the three tests. Figure 2 illustrates the chromatograms of standards and strawberry (fresh, jam, and acid hydrolysate), at 360 nm, as examples.

**Selected Phenolics in Fresh Berries.** Most fresh berry samples contained quercetin and kaempferol as shown in Table 1. In contrast, morin was not detected in the tested berries. Ellagic acid was detected in strawberry, blackberry, blueberry, and raspberry samples. Myricetin was detected in black current, cowberry, bayberry, and red current samples, but was not detected in berries that contained ellagic acid.

On the other hand, samples hydrolyzed by acid showed the selected phenolics in amounts of about 5–100 times those of fresh samples (Table 1). In particular, bayberry was found to have a high increment of aglycon content (0.15–15.93 mg/100 g), but strawberry (1.02–23.81 mg/100 g), black current (0.77–22.40 mg/100 g), raspberry (0.92–20.01 mg/100 g), and blackberry (7.11–49.30 mg/100 g) also showed high aglycon contents, quantitatively. Caffeic acid was not detected in any of the fresh berry samples, but it was detected in the blueberries hydrolyzed by acid (0.89 mg/100 g). For each aglycon, ellagic acid was detected at a high content in strawberry (1.01–19.75 mg/100 g), raspberry (0.82–17.91 mg/100 g), and blackberry (6.87–42.44 mg/100 g), and quercetin was detected in cranberry (0.78–14.02 mg/100 g), blueberry (0.07–7.08 mg/100 g), black current (0.17–9.09 mg/100 g), bayberry (0.04–8.77 mg/100 g), and blackberry (0.22–6.21 mg/100 g). Myricetin was detected in bayberry (0.10–5.79 mg/100 g) and black current (0.57–11.12 mg/100 g). The total selected phenolic contents were comparatively high in strawberry (23.81 mg/100 g), black current (22.40 mg/100 g), blackberry (49.30 mg/100 g), and raspberry (20.01 mg/100 g).

**Effects of Jam Processing on Selected Phenolics Contents.** The amounts of the selected flavonols and phenolic acids in the berry jams were determined (Table 1). The production rate from conjugated forms to aglycons by jam processing was calculated as shown in Table 2. Consequently, the rates of selected phenolics pro-

**Table 1. Effects of Jam Processing and Acid Hydrolysis on Contents of Phenolics in Fresh Berries<sup>a</sup>**

	contents (mg/100 g of fresh berry weight, means $\pm$ SD, $n = 3$ )						TSP <sup>b</sup>	TP <sup>c</sup>
	caffeic acid	ellagic acid	myricetin	quercetin	kaempferol			
bayberry								
fresh	nd	nd	0.10 $\pm$ 0.01	0.04 $\pm$ 0.01	0.01 $\pm$ 0.01	0.15	36.87 $\pm$ 1.35	
after acid hydrolysis	nd	nd	5.79 $\pm$ 0.04	8.77 $\pm$ 0.37	1.37 $\pm$ 0.04	15.93	36.58 $\pm$ 0.84	
after jam processing	nd	nd	0.22 $\pm$ 0.03	0.11 $\pm$ 0.01	0.01 $\pm$ 0.01	0.34	31.49 $\pm$ 1.62	
after acid hydrolysis of jam	nd	nd	4.18 $\pm$ 0.48	7.91 $\pm$ 0.19	1.29 $\pm$ 0.04	13.38	28.27 $\pm$ 0.98	
black current								
fresh	nd	nd	0.57 $\pm$ 0.01	0.17 $\pm$ 0.01	0.03 $\pm$ 0.01	0.77	61.90 $\pm$ 0.66	
after acid hydrolysis	nd	nd	11.12 $\pm$ 0.18	9.09 $\pm$ 0.22	2.19 $\pm$ 0.01	22.40	66.48 $\pm$ 1.85	
after jam processing	nd	nd	0.73 $\pm$ 0.05	0.48 $\pm$ 0.17	0.06 $\pm$ 0.03	1.27	60.47 $\pm$ 1.09	
after acid hydrolysis of jam	nd	nd	8.83 $\pm$ 0.95	7.06 $\pm$ 1.10	1.57 $\pm$ 0.15	16.46	58.37 $\pm$ 1.89	
blackberry								
fresh	nd	6.87 $\pm$ 0.16	nd	0.22 $\pm$ 0.01	0.02 $\pm$ 0.01	7.11	76.23 $\pm$ 1.76	
after acid hydrolysis	nd	42.44 $\pm$ 0.42	nd	6.21 $\pm$ 0.58	0.65 $\pm$ 0.08	49.30	79.23 $\pm$ 1.26	
after jam processing	nd	15.78 $\pm$ 0.85	nd	1.65 $\pm$ 0.13	0.05 $\pm$ 0.03	17.48	76.91 $\pm$ 1.29	
after acid hydrolysis of jam	nd	43.87 $\pm$ 0.94	nd	5.90 $\pm$ 0.83	0.62 $\pm$ 0.08	50.39	68.91 $\pm$ 1.02	
blueberry								
fresh	nd	0.78 $\pm$ 0.14	nd	0.07 $\pm$ 0.01	nd	0.85	33.50 $\pm$ 0.19	
after acid hydrolysis	0.89 $\pm$ 0.05	0.85 $\pm$ 0.08	nd	7.08 $\pm$ 0.33	1.72 $\pm$ 0.04	10.54	37.04 $\pm$ 0.33	
after jam processing	nd	0.70 $\pm$ 0.02	nd	0.45 $\pm$ 0.07	0.04 $\pm$ 0.02	1.19	30.24 $\pm$ 1.32	
after acid hydrolysis of jam	0.50 $\pm$ 0.06	0.75 $\pm$ 0.10	nd	6.04 $\pm$ 0.80	1.52 $\pm$ 0.08	8.81	29.56 $\pm$ 1.02	
cowberry								
fresh	nd	nd	1.17 $\pm$ 0.03	0.12 $\pm$ 0.00	0.02 $\pm$ 0.01	1.31	78.43 $\pm$ 1.26	
after acid hydrolysis	nd	nd	3.89 $\pm$ 0.43	2.46 $\pm$ 0.08	0.50 $\pm$ 0.25	6.85	82.12 $\pm$ 1.54	
after jam processing	nd	nd	1.80 $\pm$ 0.06	0.93 $\pm$ 0.05	0.11 $\pm$ 0.05	2.84	85.35 $\pm$ 1.79	
after acid hydrolysis of jam	nd	nd	4.27 $\pm$ 0.27	2.90 $\pm$ 0.41	0.53 $\pm$ 0.03	7.70	72.54 $\pm$ 2.01	
cranberry								
fresh	nd	nd	nd	0.78 $\pm$ 0.05	0.04 $\pm$ 0.02	0.82	81.55 $\pm$ 0.65	
after acid hydrolysis	nd	nd	nd	14.02 $\pm$ 0.19	0.64 $\pm$ 0.33	14.66	85.70 $\pm$ 1.19	
after jam processing	nd	nd	nd	2.19 $\pm$ 0.09	0.04 $\pm$ 0.02	2.23	84.48 $\pm$ 1.19	
after acid hydrolysis of jam	nd	nd	nd	10.58 $\pm$ 0.38	0.51 $\pm$ 0.02	11.09	80.18 $\pm$ 1.21	
raspberry								
fresh	nd	0.82 $\pm$ 0.10	nd	0.10 $\pm$ 0.01	nd	0.92	32.28 $\pm$ 1.22	
after acid hydrolysis	nd	17.91 $\pm$ 0.32	nd	1.71 $\pm$ 0.08	0.38 $\pm$ 0.02	20.01	33.80 $\pm$ 0.39	
after jam processing	nd	1.23 $\pm$ 0.03	nd	0.06 $\pm$ 0.02	nd	1.29	31.02 $\pm$ 1.04	
after acid hydrolysis of jam	nd	15.09 $\pm$ 0.80	nd	2.10 $\pm$ 0.07	0.36 $\pm$ 0.07	17.55	29.16 $\pm$ 1.05	
red current								
fresh	nd	nd	0.07 $\pm$ 0.02	0.34 $\pm$ 0.02	0.03 $\pm$ 0.01	0.44	74.48 $\pm$ 0.82	
after acid hydrolysis	nd	nd	0.37 $\pm$ 0.03	2.06 $\pm$ 0.02	0.36 $\pm$ 0.03	2.79	71.71 $\pm$ 0.77	
after jam processing	nd	nd	0.07 $\pm$ 0.01	0.40 $\pm$ 0.01	0.30 $\pm$ 0.02	0.77	72.17 $\pm$ 1.95	
after acid hydrolysis of jam	nd	nd	0.41 $\pm$ 0.03	2.11 $\pm$ 0.07	0.36 $\pm$ 0.05	2.88	69.31 $\pm$ 1.82	
strawberry								
fresh	nd	1.01 $\pm$ 0.19	nd	0.01 $\pm$ 0.01	nd	1.02	36.50 $\pm$ 0.66	
after acid hydrolysis	nd	19.75 $\pm$ 0.23	nd	3.42 $\pm$ 0.17	0.64 $\pm$ 0.32	23.81	37.61 $\pm$ 1.76	
after jam processing	nd	1.38 $\pm$ 0.18	nd	0.61 $\pm$ 0.06	0.01 $\pm$ 0.01	2.00	32.50 $\pm$ 1.74	
after acid hydrolysis of jam	nd	16.30 $\pm$ 0.76	nd	3.69 $\pm$ 0.18	0.70 $\pm$ 0.06	20.69	30.34 $\pm$ 1.82	

<sup>a</sup> nd, not detected (<0.01 for myricetin, quercetin and kaempferol; < 0.02 for ellagic acid; < 0.03 for caffeic acid). Morin was not detected in any samples. <sup>b</sup> TSP, total selected phenolics are the sum of the individual selected phenolics content. <sup>c</sup> TP, total phenolics are determined using the Folin-Ciocalteu method (gallic acid equivalents).

duced were 3–51% of the total amount of selected aglycons (jam/AH column in Table 2); most of them showed below 20%. Dissolution of the conjugated forms to the selected aglycons by jam processing was considered, but for the most part, they retained their conjugated forms.

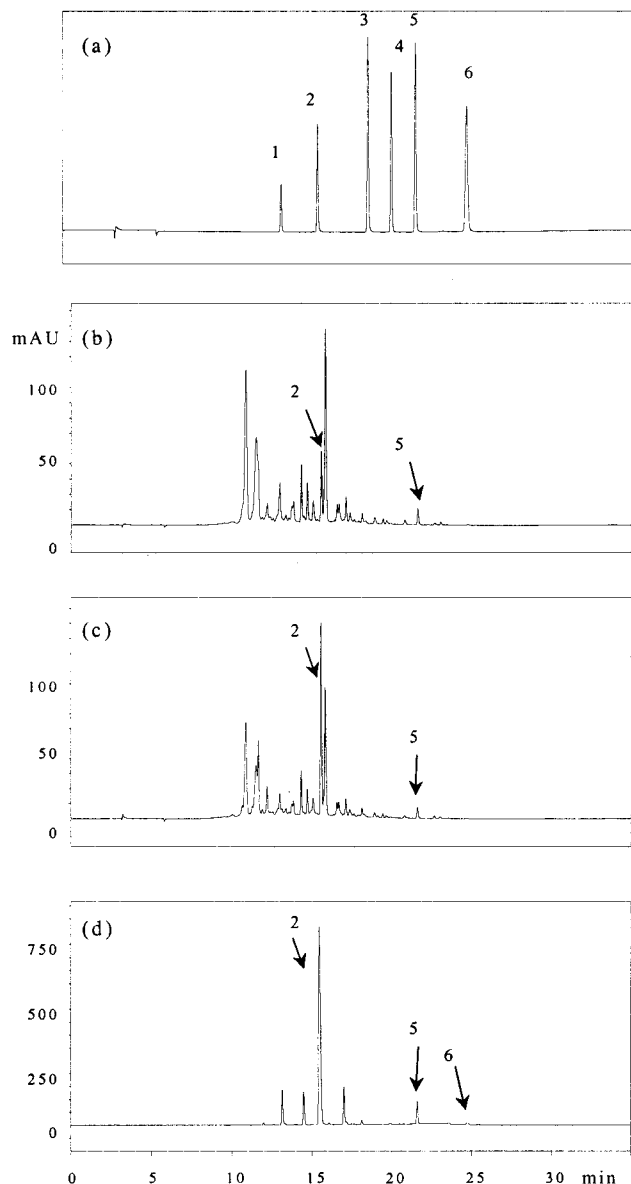
Next, these samples of jam were treated with acid solution (Table 1). The production rate from the conjugated forms to the selected aglycons by acid hydrolysis of jam were calculated as the value of acid hydrolysates based on 100 percent (Table 2). This result was 73–112% of the total selected aglycon amounts (jam-AH/AH column in Table 2). Therefore, the conjugated forms were considered to remain in the jam but it might have been partially changed to polymerization degrees of sugar, etc.

**Total Phenolics.** The amounts of total phenolics in each sample are given in Table 1. The levels of total phenolics were determined by the Folin-Ciocalteu method based on gallic acid. The contents of total phenolics were about 32–82 mg/100 g of fresh berry weight; thus, it

was also apparent in this experiment that the berries included many phenolic substances. In the samples from the same berry, namely fresh, jam, and acid hydrolysate, the total phenolics remained nearly the same. This indicated that the total phenolic contents were not appreciably changed by jam processing and acid hydrolysis.

Furthermore, the quantitative proportions of the total selected phenolics per total phenolics were calculated as shown in Table 2 (TSP/TP). Strawberry, blackberry, and raspberry showed 59–63% of total phenolics consisted of the selected phenolics. To the contrary, cranberry, cowberry, and red current phenolics were only 4–17% TSP, which suggested that these berries might contain mainly phenolic substances other than the selected ones.

**DPPH Radical Scavenging Effects.** The selected phenolics used in this study are known as typical natural antioxidants (Fujimoto, 1996). DPPH is a free radical compound and has been widely used to test the free radical scavenging ability of various samples (Ha-



**Figure 2.** Reversed-phase HPLC chromatograms of strawberry: (a) standard solution (each 50  $\mu\text{g/mL}$ , injected 10  $\mu\text{L}$ ); (b) fresh; (c) after jam processing; (d) after acid hydrolysis. Peak: 1, caffeic acid; 2, ellagic acid; 3, myricetin; 4, morin; 5, quercetin; 6, kaempferol.

tano et al., 1989, 1995, 1997; Mitsuda, 1966; Yoshida et al., 1989). In this experiment, the prepared samples have been measured by a DPPH test (Table 3). Among the fresh berries, blackberry showed the strongest DPPH radical scavenging activity with an  $\text{EC}_{50}$  value of 2.06 mg/mL; black current was second strongest with an  $\text{EC}_{50}$  value of 2.45 mg/mL, and cowberry had an  $\text{EC}_{50}$  value of 2.64 mg/mL. On the other hand, in their acid hydrolysates, the tested samples except for cranberry and red current, showed stronger activities of 0.56–0.59 mg/mL than did the fresh samples. The relationships between DPPH radical scavenging activity and total selected phenolics and total phenolic contents in fresh berries after jam processing and acid hydrolysis are depicted in Figure 3. The varying capacities of the nine berries for DPPH radical scavenging activity appeared to be associated with the contents of the total selected phenolics. Previously, Hatano et al. (1989, 1997) reported that the radical-scavenging activity of polyphenols depends largely on the number and position (*ortho*-

**Table 2.** Percentages of Selected Phenolics in Berry Jams and Acid Hydrolysates of Jams, and of Total Selected Phenolics to Total Phenolics

	total selected phenolics (%)		TSP <sup>d</sup> /TP <sup>e</sup> (%)
	jam/AH <sup>a</sup>	jam-AH <sup>b</sup> /AH <sup>c</sup>	
bayberry	3	84	44
black current	6	73	34
blackberry	41	102	62
blueberry	12	84	29
cowberry	51	112	8
cranberry	16	76	17
raspberry	7	88	59
red current	33	103	4
strawberry	8	87	63

<sup>a</sup> AH in this case is the value obtained by subtracting the content of fresh berries from that of acid hydrolysates. <sup>b</sup> jam-AH, content of acid hydrolysate of jam. <sup>c</sup> AH in this case, content of acid hydrolysate. <sup>d</sup> TSP, total selected phenolics of acid hydrolysate. <sup>e</sup> TP, total phenolics of acid hydrolysate.

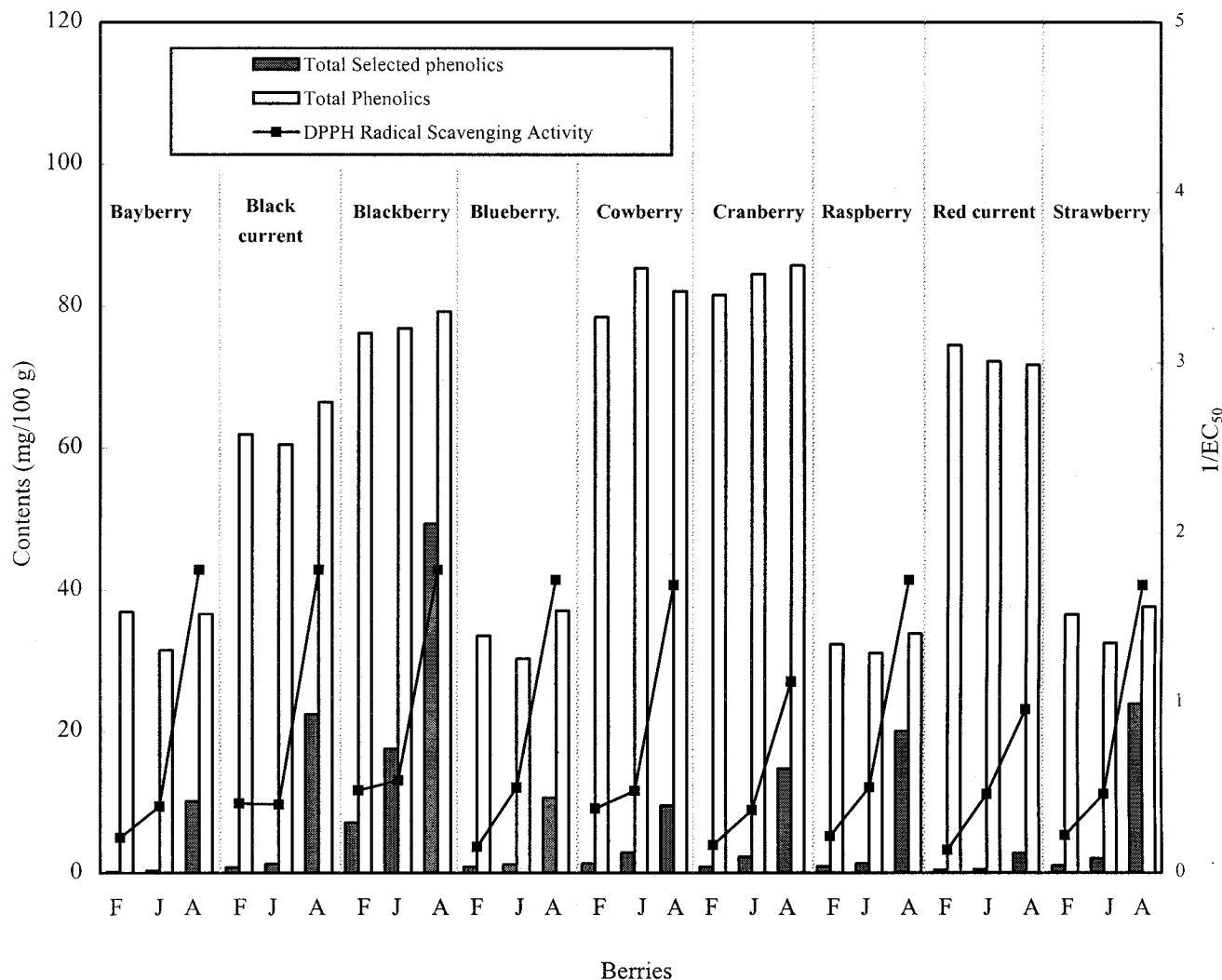
**Table 3.** Scavenging Effects of Fresh Berries after Jam Processing and Acid Hydrolysis on DPPH Radical, and Correlation Coefficients of DPPH activity and Total Selected Phenolics or Total Phenolic Content in Berries

		$\text{EC}_{50}$ (mg/mL) <sup>a</sup>	correlation coefficient ( <i>R</i> ) <sup>b</sup>	
			TSP <sup>c</sup> - DPPH	TP <sup>d</sup> - DPPH
bayberry	fresh	4.86		
	after jam processing after acid hydrolysis	2.56 0.56	0.9918	0.1297
black current	fresh	2.45		
	after jam processing after acid hydrolysis	2.48 0.56	0.9995	0.9495
blackberry	fresh	2.06		
	after jam processing after acid hydrolysis	1.84 0.56	0.9611	0.9685
blueberry	fresh	6.53		
	after jam processing after acid hydrolysis	2.00 0.58	0.9677	0.5736
cowberry	fresh	2.64		
	after jam processing after acid hydrolysis	2.08 0.59	0.9884	0.0116
cranberry	fresh	6.11		
	after jam processing after acid hydrolysis	2.72 0.89	0.9879	0.7231
raspberry	fresh	4.63		
	after jam processing after acid hydrolysis	2.00 0.58	0.9742	0.6359
red current	fresh	7.38		
	after jam processing after acid hydrolysis	2.16 1.04	0.8609	-0.7809
strawberry	fresh	4.52		
	after jam processing after acid hydrolysis	2.16 0.59	0.9867	0.2986

<sup>a</sup> Concentration required for a 50% reduction in absorbance of the DPPH radical at 520 nm in MeOH. <sup>b</sup> Relationship between the amount of phenolic contents in fresh, jam, and acid hydrolysate (mg/100 g of fresh berry) and the reciprocal number of the  $\text{EC}_{50}$ . <sup>c</sup> TSP, Total selected phenolics. <sup>d</sup> TP, Total phenolics.

phenol) of the hydroxyl groups they possess. The so-called aglycons that included the selected phenolics used in this study were mostly induced from the conjugated form. That is to say, they produced many hydroxyl groups after deglycosylation, demethylation, demethoxylation, etc., by acid hydrolysis. It was discussed that the decomposition from the conjugated forms to the aglycon by acid hydrolysis produced many free hydroxyl groups and *ortho*-phenolic groups; therefore, strong activity was observed in the DPPH test.

In three samples, namely fresh, after jam processing, and after acid hydrolysis samples originated from the



**Figure 3.** Relationship between DPPH radical scavenging activity and contents of total selected phenolics and total phenolics in fresh berries, after jam processing, and after acid hydrolysis. The scavenging activity on the DPPH radical was expressed as  $1/EC_{50}$ , where  $EC_{50}$  is the concentration in mg/mL of the test sample required to give a 50% reduction in the absorbance from that of  $2.0 \times 10^{-4}$  mol/L DPPH in MeOH. Total phenolics were determined by the Folin-Ciocalteu colorimetric assay (results expressed as mg/100 g of gallic acid equivalents). Total selected phenolics were the sum of individual selected phenolic contents determined by RP-HPLC. F, fresh; J, jam; A, acid hydrolysate.

same berry, a direct correlation between the total selected phenolics or total phenolic contents and DPPH activity was demonstrated by linear regression analysis (TSP- and TP-DPPH), as reported in Table 3. In fact, high correlation levels ( $R = 0.8609-0.9995$ ) were indicated in the total selected phenolic contents and the DPPH activity (TSP-DPPH) of the berries. These results suggested the participation of the selected phenolics in the DPPH activity. On the other hand, the correlation in total phenolic contents and DPPH activity (TP-DPPH) showed good correlation ( $R = 0.9495$  and  $0.9685$ ) in black current and blackberry. Blueberry, cranberry, and raspberry showed relatively good correlation ( $R = 0.5736$ ,  $0.7231$ , and  $0.6359$ ); hence, these results were considered to show that the influence on the DPPH activity was also correlated with phenolics other than the selected phenolics. Conversely, bayberry, cowberry, red current, and strawberry showed a minor role ( $R = -0.7809-0.2986$ ). This result indicated that their contribution to DPPH activity was mostly due to the selected phenolics among the total phenolics.

**Discussion.** It has become apparent that flavonoids and phenolic acids widely distributed as conjugated forms in berries, for the most part, retained their

conjugated forms during jam processing. Fluctuation in the selected phenolic contents due to jam processing was slight; hence, it was suggested that they might be present as the conjugated forms in jam.

Most biological and pharmacological activities, such as antioxidant activity etc., have been demonstrated by aglycons rather than by the conjugated forms themselves, although phenolics are usually present in dietary plants in conjugated forms. When we eat them in foods, it is thought that the conjugated forms are mostly converted to aglycons, which showed a stronger radical scavenging activity through deconjugation by enzymes, bacteria, etc., in digestive organs, and are then absorbed in the body (Ameer et al., 1996; King et al., 1998). In concepts of food chemistry, when we orally ingest functional compounds in foods, it is useful to consider dissolution (water solubility), etc., whereby they exist as the conjugated forms (mainly glycosides, etc.). Water solubility is an important factor for bioavailability and affects the biological activities especially with orally ingestion. Thus, the intake of fresh berries containing many phenolics as conjugated forms is significant. Jam containing them as conjugated forms can also be expected to have similar effects.

## ACKNOWLEDGMENT

The authors thank the Kobe quarantine station for providing the samples.

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Received for review July 10, 2000. Revised manuscript received September 25, 2000. Accepted September 25, 2000.

JF000849Z