

# Phenolic Composition of Strawberry Genotypes at Different Maturation Stages

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The effects of maturation (green, pink, and ripe) on phenolic composition of strawberry cultivars Camarosa, Dorit, Chandler, and Osmanli and their hybrids were investigated using a high-pressure liquid chromatography (HPLC) method. *p*-Hydroxybenzoic acid, *p*-coumaric acid, ellagic acid, cyanidin-3-glucoside, pelargonidin-3-glucoside, kaempferol, quercetin, and myricetin were individually quantified for each stage. The highest amounts of anthocyanins were obtained from ripe fruits whereas ellagic acid was found as the main phenolic in the green fruits. Phenolic concentrations were found statistically different in green and ripe fruits. One hybrid was found to have higher phenolic contents than the other genotypes. The *p*-hydroxybenzoic and *p*-coumaric acid levels changed during maturation, but no differences in contents of flavonoids in green and ripe fruit were detected.

KEYWORDS: Strawberry; phenolic composition; maturation stages; anthocyanins; flavonoids; phenolic acids; ellagic acid; HPLC

## INTRODUCTION

Phenolics, the components responsible for antioxidant capacity in fruits and vegetables, constitute one of the most numerous and widely distributed groups of substances in the plant kingdom, with more than 8000 phenolic structures currently known. Natural phenolics can range from simple molecules, such as phenolic acids, to highly polymerized compounds, such as tannins. Flavonoids refer to a specific class of plant phenolics. Anthocyanins are the main flavonoids found in ripe grapes, red wine, berries, and berry-based dietary supplements. Anthocyanins are responsible for the red, violet, and blue colors observed in fruits and berries. Flavonoids are absorbed and found in human plasma in the glycosylated form, as are anthocyanins. Flavonoids have been reported to inhibit, and sometimes induce, a large variety of mammalian enzyme systems. Some of the flavonoids have been shown to have chemopreventive effects against various cancers in numerous animal models. Thus, some of the beneficial effects of the consumption of fruits and vegetables in reducing cancer risk may be attributed, in part, to the flavonoid content of fruits and vegetables (1, 2).

Strawberries (*Fragaria*  $\times$  *ananassa* Duch., Rosaceae) have a unique, highly desirable taste and flavor and are one of the most popular edible spring and summer fruits. Of the many factors that can affect the taste quality of a product, ripeness, maturity, cultivar, irrigation, and fertilization are especially important (3). The beneficial effects have been well-known for

	5		y
hybrid no.	parents	hybrid no.	parents
2	Osmanli × Douglas	11	$499/1 \times Chandler$
4	499/1 × Oso Grande	12	$499/1 \times Oso Grande$
5	499/1 × Oso Grande	13	$499/1 \times Chandler$
6	$489/1 \times Oso Grande$	14	$489/1 \times Oso Grande$
8	499/1 × Oso Grande	15	Osmanli × Aiko
9	504/7 × 216	17	504/7 × 216
10	504/7 $ imes$ Early Glow		

Table 1. Hybrids and Their Parents of Experimental Strawberry Fruits

many years. Strawberry is a source of phenolics such as flavonoids, phenolic acids, and anthocyanidins. The most commonly occurring anthocyanins in strawberry are based on cyanidin and pelargonidin. The main pigment in cultivated strawberries has been identified as pelargonidin-3-glucoside, and the presence of cyanidin-3-glucoside is also well-documented (4). Ellagic acid occurs in particularly high concentrations in strawberries and raspberries, being about three times higher than the other fruits and nuts (5). Häkkinen et al. (6) indicated that ellagic acid is the main phenolic compound in berries of the family Rosaceae, genus Fragaria (strawberry), forming 51% of the phenolic compounds analyzed. In contrast, other researchers have indicated that ellagic acid is qualitatively important but generally it is not the main phenolic compound in strawberries (7, 8). Flavonoid composition of fruits and their variations has been reported in a few literatures. Quercetin was reported to be present in most fruits whereas kaempferol and myricetin were rarely found. Flavonoid contents within strawberries were reported in some studies, and all results relate to ripe fruits.

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Table 2. HPLC Quantitative Data of p-Hydroxybenzoic Acid, p-Coumaric Acid, and Ellagic Acid in Strawberry Genotypes<sup>a</sup>

<i>p</i> -OH-benzoic acid (mg/100 g frozen fruit)			<i>p</i> -coumaric acid (mg/100 g frozen fruit)			ellagic acid (mg/100 g frozen fruit)			
sample	G <sup>a</sup>	Р	R	G	Р	R	G	Р	R
hybrid 1	$0.36 \pm 0.02^{b}$	$0.11 \pm 0.01$	$0.44 \pm 0.02$			$1.74 \pm 0.03$	$0.54 \pm 0.03$	$0.17 \pm 0.01$	$0.31 \pm 0.01$
hybrid 2	$0.82\pm0.01$	$0.43 \pm 0.02$	$0.63 \pm 0.02$	$1.23 \pm 0.10$	$1.12 \pm 0.04$	$2.32 \pm 0.02$	$2.20 \pm 0.07$	$0.57 \pm 0.01$	$0.69 \pm 0.01$
hybrid 4	$0.32\pm0.01$	$0.17 \pm 0.02$	$0.13 \pm 0.02$	$0.47 \pm 0.02$	$0.48 \pm 0.03$	$0.88\pm0.02$	$0.45 \pm 0.03$	$0.58 \pm 0.01$	$0.31 \pm 0.01$
hybrid 5	$0.47\pm0.02$	$1.21 \pm 0.02$	$0.47 \pm 0.02$		$0.76 \pm 0.03$	$3.28 \pm 0.01$	$1.10 \pm 0.03$	$0.82 \pm 0.01$	$0.96 \pm 0.05$
hybrid 6	$0.17 \pm 0.01$	$0.27\pm0.02$	$0.35\pm0.02$	$0.47\pm0.02$	$0.54\pm0.03$	$5.83\pm0.02$	$1.24\pm0.01$	$2.08\pm0.05$	$1.20\pm0.02$
hybrid 7	$0.55\pm0.02$	$0.65 \pm 0.02$	$0.27 \pm 0.01$	$0.48 \pm 0.02$	$1.00 \pm 0.01$	$3.27 \pm 0.01$	$0.59 \pm 0.01$	$0.67 \pm 0.01$	$0.57 \pm 0.02$
hybrid 8	$0.21 \pm 0.01$	$0.28 \pm 0.02$	$0.16 \pm 0.02$		$1.62 \pm 0.02$	$3.57 \pm 0.02$	$0.76 \pm 0.02$	$0.76 \pm 0.01$	$0.59 \pm 0.01$
hybrid 9	$0.23\pm0.02$	$0.16 \pm 0.02$	$0.23 \pm 0.02$	$0.36 \pm 0.01$	$2.16 \pm 0.02$	$3.84 \pm 0.02$	$1.39 \pm 0.05$	$0.64 \pm 0.01$	$1.19 \pm 0.11$
hybrid 10	$0.12 \pm 0.02$	$0.17 \pm 0.02$	$0.29 \pm 0.01$	$0.45 \pm 0.02$	$0.94 \pm 0.04$	$4.10 \pm 0.02$	$0.66 \pm 0.01$	$1.69 \pm 0.01$	$0.84\pm0.01$
hybrid 11	$0.16 \pm 0.02$	$0.33 \pm 0.02$	$0.36 \pm 0.03$	$0.53 \pm 0.02$	$2.04 \pm 0.03$	$2.32 \pm 0.02$	$2.10 \pm 0.07$	$1.06 \pm 0.08$	$0.91 \pm 0.01$
hybrid 12	$0.19 \pm 0.01$	$0.22 \pm 0.01$	$0.27 \pm 0.03$			$2.44 \pm 0.03$			$0.23 \pm 0.03$
hybrid 13	$0.16 \pm 0.02$	$0.21 \pm 0.01$	$0.26 \pm 0.02$	$0.67 \pm 0.01$	$2.62 \pm 0.02$	$5.62 \pm 0.02$	$1.40 \pm 0.02$	$1.39 \pm 0.02$	$0.68 \pm 0.01$
hybrid 14	$0.36 \pm 0.02$	$0.19 \pm 0.01$	$0.38 \pm 0.02$	$0.62 \pm 0.02$	$1.61 \pm 0.00$	$1.46 \pm 0.02$	$1.36 \pm 0.03$	$0.67 \pm 0.04$	$0.76 \pm 0.02$
hybrid 16	$0.38 \pm 0.01$	$0.23 \pm 0.01$	$0.11 \pm 0.01$			$3.11 \pm 0.03$	$1.22 \pm 0.03$	$0.74 \pm 0.02$	$0.37 \pm 0.01$
hybrid 17	$0.31 \pm 0.02$	$0.24 \pm 0.02$	$0.12 \pm 0.01$	$0.44 \pm 0.03$	$2.90 \pm 0.01$	$1.44 \pm 0.02$	$0.57 \pm 0.02$	$0.84 \pm 0.02$	$0.47\pm0.02$
cv. Camarosa		$0.13 \pm 0.01$	$0.15 \pm 0.02$		$0.96 \pm 0.01$	$2.07 \pm 0.04$	$0.71 \pm 0.01$	$0.13 \pm 0.03$	$0.36\pm0.02$
cv. Dorit	$0.17 \pm 0.01$	$0.15 \pm 0.01$	$0.59\pm0.01$		$0.47\pm0.03$	$0.90 \pm 0.01$	$0.66\pm0.02$	$0.32\pm0.01$	$0.11\pm0.01$
cv. Chandler	$0.12 \pm 0.01$	$0.23\pm0.02$	$0.26\pm0.02$		$0.32\pm0.03$	$1.38\pm0.02$	$1.22 \pm 0.03$	$1.07 \pm 0.10$	$0.42\pm0.01$
cv. Osmanli	$0.17\pm0.01$	$0.19\pm0.02$	$0.17\pm0.02$	$0.64\pm0.01$	$0.47\pm0.02$	$0.42\pm0.01$	$2.09\pm0.05$	$0.84\pm0.02$	$0.52\pm0.01$

<sup>a</sup> G, green stage; P, pink stage; R, ripe stage. <sup>b</sup> Values (mg/100 g frozen fruit) are expressed as means  $\pm$  SD (SD for n = 9 of three extraction replicates) (p < 0.05).

While quercetin (0.86 mg/100 g frozen weight) and kaempferol (1.2 mg/100 g frozen weight) were present in ripe strawberry, myricetin was not detected (6, 9, 10). Some benzoic and hydroxycinnamic acids, especially *p*-OH-benzoic and *p*-coumaric acid, were also detected in strawberry genotypes (10, 11).

Eating quality is becoming more important in determining the value of new varieties. The color, taste, shape, and size are essential characteristics for import and export of strawberries. The fruit of Osmanli is very rich in volatile aromatic substances but rather small in size (2-3 g), pink-colored, rather soft, and therefore not resistant to transportation. Its flowers are male sterile, and yield per plant is very low. Therefore, Osmanli cannot be considered as a commercial strawberry cultivar in modern strawberry growing. For transferring pleasant aroma from Osmanli to the other commercial cultivars, a classical breeding program was initiated at the University of Cukurova in 1984. This breeding program has the major objective to develop new strawberry varieties especially well-adapted and able to provide a better productive and qualitative standard. In this study, we aimed at comparing the phenolic profiles of Osmanli, some commercial cultivars such as Camarosa, Chandler, and Dorit, and some cultivar candidates of strawberries at different maturation stages (green, pink, and ripe) by a highpressure liquid chromatography (HPLC)-DAD method. There is no comparative study between the phenolic contents and the different maturation stages of strawberry genotypes. The compounds of interest were kaempferol, quercetin, myricetin, ellagic acid, pelargonidin-3-glucoside, cyanidin-3-glucoside, *p*-coumaric acid, and *p*-OH-benzoic acid.

#### MATERIALS AND METHODS

**Plant Material and Reagents.** Strawberries (*Fragaria* × *ananassa* Duch., Rosaceae) were grown in the experimental field of the University of Cukurova, Faculty of Agriculture, Department of Horticulture in Adana province of the Mediterranean Region in Turkey. Four varieties (Osmanli, Chandler, Camarosa, and Dorit) and 15 hybrids were used as plant materials (**Table 1**). The plants were planted in the beginning of September 2001 and grown in walk-in plastic tunnels. The fruits of the genotypes were harvested at green, pink, and ripe maturation stages, immediately treated with liquid nitrogen, and stored at -80 °C until

 Table 3. HPLC Quantitative Data of Anthocyanins in Strawberry Genotypes<sup>a</sup>

	,	3-glucoside frozen fruit)	pelargonidin-3-glucoside (mg/100 g frozen fruit)		
sample	P <sup>a</sup>	R	Р	R	
hybrid 1		$0.94\pm0.02^b$	$0.76 \pm 0.01$	$12.09\pm0.04$	
hybrid 2		$0.94 \pm 0.03$	$1.68 \pm 0.02$	$44.10 \pm 0.13$	
hybrid 4		$0.63\pm0.03$	$1.30 \pm 0.01$	$6.74\pm0.04$	
hybrid 5		$1.15 \pm 0.08$	$2.42 \pm 0.03$	$13.85\pm0.04$	
hybrid 6		$0.94 \pm 0.02$	$1.12 \pm 0.02$	$21.13\pm0.02$	
hybrid 7		$0.99 \pm 0.01$	$0.79 \pm 0.01$	$14.83\pm0.02$	
hybrid 8		$1.21 \pm 0.02$	$2.78 \pm 0.03$	$9.94\pm0.05$	
hybrid 9		$0.94 \pm 0.02$	$5.08 \pm 0.05$	$7.66\pm0.04$	
hybrid 10		$0.47 \pm 0.01$	$2.94 \pm 0.06$	$15.19 \pm 0.02$	
hybrid 11		$0.53 \pm 0.01$	$6.67 \pm 0.03$	$6.86\pm0.01$	
hybrid 12		$0.71 \pm 0.01$		$8.64\pm0.02$	
hybrid 13	$1.07 \pm 0.06$	$1.75 \pm 0.02$	$6.60 \pm 0.02$	$13.61 \pm 0.01$	
hybrid 14		$0.45 \pm 0.01$	$1.61 \pm 0.02$	$5.33\pm0.04$	
hybrid 16		$0.47 \pm 0.01$	$1.12 \pm 0.04$	$5.84\pm0.02$	
hybrid 17		$0.48\pm0.01$	$2.84 \pm 0.02$	$13.77 \pm 0.03$	
cv. Camarosa		$0.72 \pm 0.01$	$1.43 \pm 0.04$	$11.72 \pm 0.12$	
cv. Dorit		$0.51\pm0.00$	$3.09\pm0.02$	$12.29\pm0.02$	
cv. Chandler cv. Osmanli		$0.95\pm0.01$	$3.09\pm0.035$	$16.24 \pm 0.08$	

<sup>*a*</sup> P, pink stage; R, ripe stage. <sup>*b*</sup> Values (mg/100 g frozen fruit) are expressed as means  $\pm$  SD (SD for n = 9 of three extraction replicates) (p < 0.05).

extraction. Ultrapure water (18.2 M $\Omega$ cm) was prepared by using a Millipore system (Millipore Corp., Bedford, MA). The chromatography standards, all reagents, and solvents were purchased from Sigma Chemical Co. (St. Louis, MO).

**Extraction and Hydrolysis Procedure of Phenolics.** Approximately 500 g of frozen samples was used as each replicate, and three replicates were used separately. Frozen samples were powdered with liquid nitrogen in a mortar. Three grams of this powder, 10 mL of acetone/ water (1:4, v/v) mixture, and 0.1 mL of trifluoroacetic acid were added into the flask and refluxed for 1 h (*12*). After it was cooled, the mixture was filtered and made up to 10 mL with distilled water. These samples were directly used for HPLC analyses.

**HPLC.** The liquid chromatographic apparatus (Hewlett-Packard HP-1100) consisted of an in-line degasser, pump, and controller coupled to a photodiode array detector equipped with an automatic injector (20  $\mu$ L injection volume) interfaced to a PC running ChemStation chro-

 
 Table 4. HPLC Quantitative Data of Flavonoids at Green, Pink, and Ripe Stages of Strawberry Genotypes

	kaempferol	quercetin	myricetin
	(mg/100 g	(mg/100 g	(mg/100 g
sample			frozen fruit)
	Gree	en	
hybrid 2	$1.12 \pm 0.05^{a}$	2.40 ± 0.02	$2.93 \pm 0.10$
hybrid 7	$1.98 \pm 0.10$		
hybrid 11			$0.40 \pm 0.02$
hybrid 14	$0.54 \pm 0.02$	$0.85 \pm 0.01$	$1.13 \pm 0.10$
cv. Dorit			$0.41 \pm 0.02$
	Pin	k	
hybrid 2		$0.52 \pm 0.02$	$0.67 \pm 0.03$
hybrid 7	$2.20\pm0.02$		
cv. Camarosa			$0.38 \pm 0.02$
	Rip	P	
hybrid 5	i tip	$0.50 \pm 0.04$	
hybrid 6			$3.60 \pm 0.01$
hybrid 10			$0.65 \pm 0.03$
hybrid 11			$0.47 \pm 0.01$
hybrid 13			$0.33 \pm 0.02$
hybrid 14		$0.94 \pm 0.25$	
cv. Camarosa			$0.69 \pm 0.01$
cv. Dorit			$0.31 \pm 0.01$
cv. Chandler			$0.36 \pm 0.03$

<sup>a</sup> Values (mg/100 g frozen fruit) are expressed as means  $\pm$  SD (SD for n = 9 of three extraction replicates) (p < 0.05).

matography manager software (Hewlett-Packard). Separations were performed on a 150 mm  $\times$  4.6 mm i.d., 5  $\mu$ m, reverse-phase Nucleosil C18 analytical column (Supelco, PA) operating at room temperature with a flow rate of 1 mL/min. Detection was carried out with a sensitivity of 0.1 a.u.f.s. between the wavelengths of 200 and 600 nm. Elution was effected using a nonlinear gradient of the solvent mixture 2.5% HCOOH in water (solvent A) and 2.5% HCOOH in acetonitrile (solvent B). The composition of B was increased from 5 to 13% in 15 min, increased to 15% in 5 min and to 30% in a further 5 min and held for 3 min, increased to 45% in 4 min and held for 3 min, increased to 90% in 5 min and held for 5 min, and then returned to initial conditions in 5 min. Components were identified by comparison of their retention times to those of authentic standards under analysis conditions and UV spectra with our in-house PDA library. A 10 min equilibrium time was allowed between injections.

**Quantitative Analyses.** All of the samples were directly injected to the reverse phase chromatography column after filtration. *p*-Hydroxybenzoic acid, *p*-coumaric acid, ellagic acid, cyanidin-3-glucoside, pelargonidin-3-glucoside, kaempferol, quercetin, and myrice-tin were dissolved in methanol at a concentration of 1 mg/mL, and five dilute solutions from these stock solutions were used to prepare calibration curves of each standard. Recoveries were measured by extracting the recovered amounts of pure substances added to frozen strawberries before the experiment. Three replicates from each sample were used for HPLC analyses. All samples and standards were injected three times, and mean values were used.

Statistical Analyses. All statistical analyses were carried out using SPSS 10.0.1 (SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) was performed by ANOVA procedures. Significant differences between means were determined by Tukey's pairwise comparison test at a level of p < 0.05.

#### **RESULTS AND DISCUSSION**

The qualitative–quantitative analyses of the strawberry genotypes carried out using HPLC coupled with PDA detection are presented in **Tables 2–4**. Phenolic compounds were calculated at 260, 320, 360, and 520 nm as benzoates, hydroxycinnamates, flavonoids, and anthocyanidins, respectively.

The components *p*-hydroxybenzoic acid, *p*-coumaric acid, ellagic acid, cyanidin-3-glucoside, pelargonidin-3-glucoside, quercetin, kaempferol, and myricetin were identified by comparison to the retention times and UV spectra of authentic standards, while quantitative data were calculated from their calibration curves. Retention time, maximum wavelength, concentration range, recovery, calibration curve, and calibration coefficient of these standards are given in **Table 5**.

Accordingly, ellagic acid in the green stage, pelargonidin-3glucoside and p-coumaric acid in the pink stage, and pelargonidin-3-glucoside and p-coumaric acid in the ripe stage were identified as the major compounds. The content of ellagic acid decreased with maturity except in the hybrid 12 where a slight increase was observed. However, the amount of the ellagic acid was significantly decreased in the ripe stage of experimental genotypes. The concentration of ellagic acid was found to be between 0.45 (hybrid 4) and 2.20 mg/100 g (hybrid 2) in the green stage between 0.12 (cv. Camarosa) and 2.08 mg/100 g (hybrid 6) in the pink stage, and it changed from 0.22 (hybrid 12) to 1.19 mg/100 g (hybrid 6) in ripe stage (Table 2). Previously, ellagic acid had been reported generally as the main compound in green strawberry fruit (12). Williner (5) reported that ellagic acid concentration decreased in Camarosa (from 12.9 to 4.47 mg/100 g) and Chandler (from 17.8 to 4.13 mg/100 g) varieties during the ripening progress. The amount of ellagic acid in the ripe fruit of some strawberry genotypes was found in various levels such as 8.1 (13), 35.5-46.5 (10), and 40.3 mg/100 g (14).

Anthocyanins were not detected in all green stages. The contents of both cyanidin-3-glucoside and pelargonidin-3-glucoside were significantly increased during ripening in all genotypes. The highest content of cyanidin-3-glucoside was obtained from hybrid 13, whereas pelargonidin-3-glucoside was detected in hybrid 2. In addition, the lowest concentrations of pelargonidin-3-glucoside and cyanidin-3-glucoside were detected in hybrid 14. The amount of pelargonidin-3-glucoside was about 20 times more than cyanidin-3-glucoside in strawberry genotypes (**Table 3**). Garcia-Viguera et al. (*15*) worked with both frozen and fresh strawberry samples and reported the amount

 Table 5.
 Concentration Ranges, Retention Times, Calibration Equations, and Coefficients and Maximum Wavelengths of Reference Compounds

 Used for Calibration of the HPLC Analysis<sup>a</sup>

components	retention time (min)	maximum wavelength $(\lambda, nm)$	recovery (%)	concentration range (mg/mL)	calibration equation
p-OH-benzoic acid	6.7	260	88	0.000-0.005	y = 111703x - 24.997
<i>p</i> -coumaric acid	13.2	320	90	0.001-0.059	y = 76831x - 77.9
, cyanidin-3-glucoside	13.9	520	92	0.001-0.015	$y = 34\ 100x - 13.17$
pelargonidin-3-glucoside	15.8	520	95	0.001-0.200	$y = 24 \ 172x - 23.977$
ellagic acid	20.7	260	90	0.000-0.009	$y = 158\ 486x - 39.399$
myricetin	21.5	360	70	0.001-0.012	y = 13824x - 0.8704
quercetin	24.3	360	78	0.001-0.011	y = 24777x - 7.0033
kaempferol	28.3	360	72	0.002-0.009	$y = 22\ 859x + 0.0821$

<sup>a</sup> All calibration coefficients > 0.99.

of cyanidin-3-glucoside and pelargonidin-3-glucoside as 6.64-7.39 and 65.20-72.61 mg/100 g, respectively. Wang et al. (13) reported the major anthocyanins in strawberry as pelargonidin-3-glucoside (32.4-70.8 mg/100 g) and cyanidin-3-glucoside (1.6-6.67 mg/100 g). This experiment was carried out in open field, whereas in our experiment the cultivation was done under walk-in plastic tunnels. The fruit color is affected by many ecological factors such as light and temperature. Wang et al. used a different fertilization program and fertilizer types, such that they applied NH<sub>4</sub>NO<sub>3</sub> whereas we applied NH<sub>4</sub>SO<sub>4</sub> as the nitrogen source. In addition, the climatic conditions of the two experimental areas were different since our experiment was carried out in the Mediterranean coastal region of Turkey and the other was in Maryland, U.S.A. The above reasons may have caused the differences of anthocyanin concentration of strawberries between the two experiments.

The content of *p*-coumaric acid significantly increased during the ripening stages. p-Coumaric acid ranged from 0.35 (hybrid 9) to 1.22 mg/100 g (hybrid 2) in the green stage and from 0.32 (cv. Dorit) to 2.90 mg/100 g (hybrid 17) in the pink stage, and it was between 0.41 (cv. Osmanli) and 5.82 mg/100 g (hybrid 6) in the ripe stage (**Table 3**). The amount of *p*-coumaric acid significantly increased during maturity in experimental genotypes. Häkkinen and Törrönen (11) found the amount of p-coumaric acid in Senga Sengana as 3.4 mg/100 g. A study performed by Häkkinen and Törrönen (10) showed the importance of growing region in the amount of *p*-coumaric acid in strawberry fruit. The authors studied the Senga Sengana variety and found it to be 0.7 mg/100 g in Poland and 1.8 mg/100 g in Finland. There was no significant difference in the concentration of *p*-hydroxybenzoic acid between different maturation stages. p-Hydroxybenzoic acid was also detected by Häkkinen and Törrönen (11) in Senga Sengana variety (0.4 mg/100 g).

The amounts of flavonoids in strawberry samples are shown in Table 4. The concentrations of flavonoids such as kaempferol, quercetin, and myricetin were not significantly changed during maturity. Amounts of kaempferol, quercetin, and myricetin were less than the other phenolic compounds such as phenolic acids and anthocyanidins. Kaempferol was not detected in any genotypes at the ripe stage. When the previous results were concluded, quercetin and kaempferol were detected in strawberry samples but myricetin was rarely found. The concentration of quercetin has been reported as 0.4-0.7 mg/100 g and kaempferol content as 0.7-0.9 mg/100 g in the fresh ripe berries (6, 9, 11). Lugasi and Hovari (16) reported that quercetin was present at a concentration of 10-53 mg/kg whereas myricetin was found at a concentration of 994 mg/kg. In addition, kaempferol was not detected in strawberry samples (16). A high variation of flavonoid contents of strawberry cultivars was found in the literature cited. The reasons for these variations on the amounts of flavonoids within the strawberries may be due to different extraction/hydrolysis and analytical methods used. In another study, the effect of storage on the flavonoid content was investigated and the amount of quercetin was reported to increase while kaempferol and myricetin amounts decreased during storage at -20 °C (9).

The concentration of phenolic compounds within the fruits is important for their beneficial effects and quality. Antioxidant activities of fruits are well-known and reported in the literature. Some phenolic compounds such as phenolic acids, flavonoids, and anthocyanins are known natural antioxidants. Therefore, breeding for high phenolic content of fruits is important. The data presented in this study demonstrate that the amount of phenolic compounds significantly changes during maturation. In addition, varietal differences among the experimental genotypes are observed. Our results and those published previously compare well. The levels of phenolic compounds of strawberry fruits are affected from varietal variation, growing site, and degree of ripeness. Further research on strawberry is needed to evaluate the effect of storage and processing on the phenolic composition of these genotypes.

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## LITERATURE CITED

- Prior, R. L.; Cao, G. Flavonoids: Diet and health relationships. *Nutr. Clin. Care* 2000, *3*, 279–288.
- (2) Shahidi, F.; Naczk, M. Food Phenolics: Sources, Chemistry, Effects, Applications; Technomic Publishers: Lancester, PA, 1995; pp 75–108.
- (3) Hancock, J. F. Strawberries. Crop Production Science in Horticulture; University Press: Cambridge, 1999; p 237.
- (4) Lopes-Da-Silva, F.; Pascual, T.; Rivas, G.; Santos-Buelga, C. Identification of anthocyanin pigments in strawberry (cv. Camarosa) by LC using DAD and ESI-MS detection. *Eur. Food Res. Technol.* 2003, 8, 217–226.
- (5) Williner, M. R.; Pirovani, M. E.; Guemes, D. R. Ellagic acid content in strawberries of different cultivars and ripening stages. *J. Sci. Food Agric.* 2003, *83*, 842–845.
- (6) Häkkinen, S. H.; Kärenlampi, S. O.; Heinonen, I. M.; Mykkänen, H. M.; Ruuskanen, J.; Törrönen, A. R. Screening of selected flavonoids and phenolic acids in 19 berries. *Food Res. Int.* **1999**, *32*, 345–353.
- (7) Cordenunsi, B. R.; Nascimento, J. R. O.; Genovese, M. I.; Lajolo, F. M. Influence of cultivation quality parameters and chemical composition of strawberry fruits grown in Brazil. *J. Agric. Food Chem.* 2002, *50*, 2581–2586.
- (8) Holcroft, D. M.; Kader, A. A. Carbon-dioxide induced changes in color and anthocyanin synthesis of stored strawberry fruit. *HortScience* **1999**, *34*, 1244–1248.
- (9) Häkkinen, S. H.; Kärenlampi, S. O.; Mykkänen, H. M.; Törrönen, A. R. Influence of domestic processing and storage on flavonol contents in berries. J. Agric. Food Chem. 2000, 48, 2960–2965.
- (10) Häkkinen, S. H.; Törrönen, A. R. Content of flavonols and selected phenolic acids in strawberries and *Vaccinium* species: Influence of cultivar, cultivation site and technique. *Food Res. Int.* **2000**, *33*, 517–524.
- (11) Häkkinen, S. H.; Kärenlampi, S. O.; Heinonen, I. M.; Mykkänen, H. M.; Törrönen, A. R. HPLC method for screening of flavonoids and phenolic acids in berries. *J. Sci. Food Agric.* **1998**, 77, 543– 551.
- (12) Maas, J. L.; Wang, S. Y.; Galletta, G. J. Evaluation of strawberry cultivars for ellagic acid content. *HortScience* **1991**, *26*, 66– 68.
- (13) Wang, S. Y.; Zheng, W.; Galletta, G. J. Cultural system affects fruit quality and antioxidant capacity in strawberries. *J. Agric. Food Chem.* 2002, *50*, 6534–6542.
- (14) Häkkinen, S. H.; Kärenlampi, S. O.; Mykkänen, H. M.; Heinonen, I. M.; Törrönen, A. R. Ellagic acid content in berries: Influence of domestic processing and storage. *Eur. Food Res Technol.* 2000, 212, 75–80.
- (15) Garcia-Viguera, C.; Zafrilla, P.; Tomás-Barberán, F. A. The use of acetone as an extraction solvent for anthocyanins from strawberry fruit. *Phytochem. Anal.* **1998**, *9*, 274–277.
- (16) Lugasi, A.; Hovari, J. Flavonoid aglycones in foods and plant origin II. Fresh and dry fruits. *Acta Aliment.* 2002, *31*, 63–71.

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