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Polyphenol Composition and Antioxidant Activity in Strawberry Purees; Impact of Achene Level and Storage

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In this study the impact of achenes on polyphenolic compounds, ascorbic acids, and antioxidant activities in strawberry purees at production and after storage at 6 and 22 °C for 8 and 16 weeks was investigated. Strawberry purees were made from flesh, berry, and achene-enriched homogenate and contained 0, 1.2, and 2.9% achenes, respectively. At production, strawberry purees made from flesh contained more anthocyanins, *p*-coumaroyl glycosides, and ascorbic acids, whereas increasing achene levels caused increasing levels of ellagic acid derivatives, proanthocyanidins, flavonols, total phenolics (TP), and antioxidant activities. In addition, the anthocyanins, TP, and ferric reducing ability power (FRAP) in purees with more achenes were better retained during storage. Ascorbic acids and anthocyanins declined rapidly during storage, whereas other polyphenols and antioxidant activities were more stable; that is, the contributions from anthocyanins and ascorbic acids to TP and antioxidant activities decreased. The findings that achenes contributed significantly to polyphenol content and stability of strawberry purees may be interesting in a nutritional and, thus, commercial, perspective.

KEYWORDS: Strawberry; *Fragaria* × *ananassa;* puree; storage; achenes; antioxidant activity; ORAC; FRAP; HPLC; MS; anthocyanins; phenolic compounds; ellagic acid; ellagitannins; ascorbic acid

INTRODUCTION

Epidemiological studies have shown that a diet high in fruits and vegetables is associated with lower risk for heart diseases and cancer (*1*−*3*). Strawberries (*Fragaria* × *ananassa*) are consumed in high quantities, both fresh and processed, and can thus be a valuable source of compounds with potential health benefits, for example, phenolic compounds with antioxidative and antiproliferative activities (*4*−*6*). The phenolic compounds detected in strawberries are anthocyanins, responsible for the red color in strawberry flesh, flavonols, flavanols, and derivatives of hydroxycinnamic acid and ellagic acid (**Figure 1**) (*5*, *7*−*9*). The ellagic acid derivatives, comprising ellagic acid, ellagic acid glycosides, and ellagitannins, represent an important group of phenolic compounds in strawberries, and together with raspberries and blackberries, strawberries are a major dietary source of ellagic acid-containing compounds (*10*). Strawberry extracts and their individual components are shown to demonstrate anticancer activity, anti-inflammatory effect, and prevention of heart disease by decreased oxidation of low-density lipoprotein and platelet aggregation (reviewed in ref *11*). Especially for ellagic acid and quercetin there is a huge body of literature, but also ellagitannins are shown to have anticarcinogenic properties, probably due to their hydrolysis product, ellagic acid (*12*, *13*).

During fruit processing cell structures are disrupted and the fruits become more prone to enzymatic and nonenzymatic oxidation (*14*, *15*). Thus, the general opinion used to be that fresh, unprocessed fruits and berries contain more of the components responsible for health benefits than products such as jams, purees, and juices. The influence of processing and storage on different components in berries varies. Whereas ascorbic acids (AA and DHAA) are very susceptible to degradation when subjected to physical stress, other phenolic compounds and antioxidant activities in berry products are reported to be unchanged or even to increase during processing or storage (*15*−*20*).

Localization of components within plant materials becomes important when berries are processed and separated into various fractions, as in the production of juices and seedless purees. Strawberry fruits consist of seeds, called achenes, which are the actual fruits of the berries, and flesh, which is modified receptacle tissue. Previous studies have shown that the phenolic composition in strawberry achenes is different from that of flesh (*5*) and that the achenes possess high antioxidant activity and are high in phenolic compounds, especially ellagic acid deriva-

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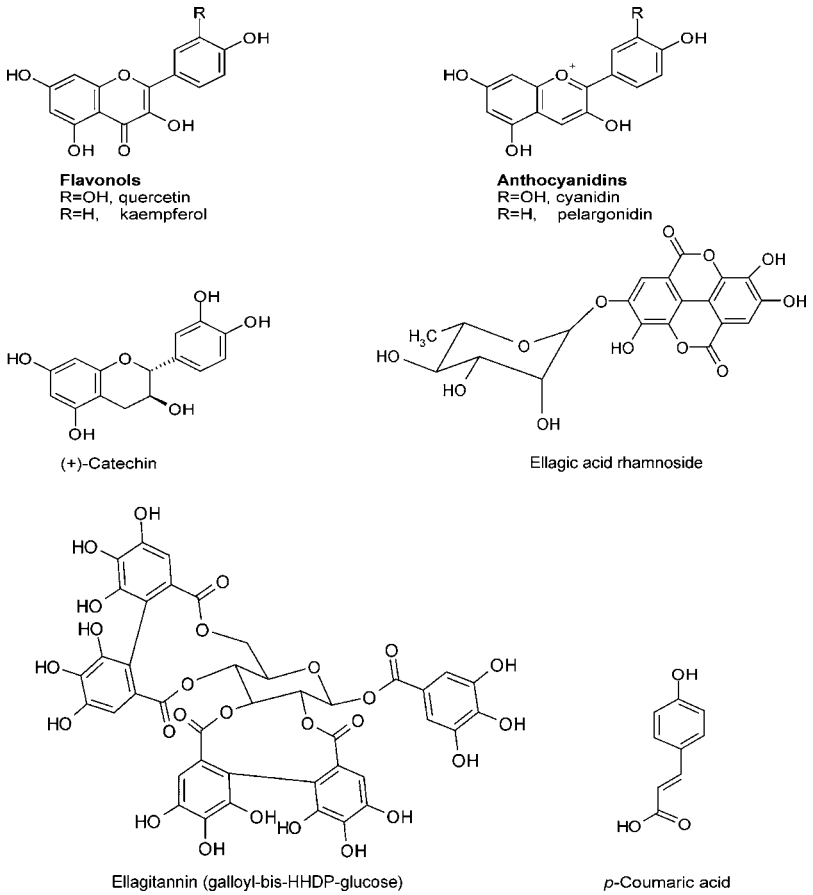

Figure 1. Structures of phenolic compounds.

tives (*5*, *21*, *22*). However, knowledge of the impact of achene level on phenolic compounds, ascorbic acids, and antioxidant activities in strawberry products, especially during storage, is missing.

In the present study phenolic compounds, ascorbic acids, and antioxidant activities in whole strawberry fruits and strawberry purees with different achene levels were analyzed. The impact of achene levels in purees at production and after storage at 6 and 22 °C was evaluated.

MATERIALS AND METHODS

Chemicals. Gallic acid, (+)-catechin, ellagic acid, *p*-coumaric acid, dehydro-L-(+)-ascorbic acid (DHAA) dimer, fluorescein sodium salt, tris[2-carboxyethyl]phosphine (TCEP), and Folin−Ciocalteu's phenol reagent were purchased from Sigma Chemical Co. (St. Louis, MO). Quercetin-3-glucoside was obtained from Carl Roth GmbH (Karlsruhe, Germany). 2,2′-Azobis(2-amidinopropane)dihydrochloride (AAPH), sodium benzoate, potassium sorbate, and metaphosphoric acid were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Chlorogenic acid, 2,4,6-tripyridyl-*s*-triazine, and 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) were obtained from Fluka Chemie GmbH (Buchs, Switzerland). Pelargonidin-3-glucoside and cyanidin-3-glucoside were obtained from Polyphenols AS (Sandnes, Norway). $FeCl_3 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, L-(+)-ascorbic acid (AA), citric acid, sodium acetate, sodium carbonate, sodium dihydrogen phosphate dihydrate ($Na_2H_2PO_4 \cdot 2H_2O$), *n*-dodecyltrimethylammonium chloride, and disodium EDTA (Na_2-EDTA) were obtained from Merck KGAa (Darmstadt, Germany). All solvents were of HPLC grade (Merck KGAa), and water was of Milli-Q quality (Millipore Corp., Cork, Ireland).

Materials. Strawberries (*Fragaria* × *ananassa*, cv. Senga Sengana) grown in western Norway were harvested at commercial ripeness in July 2004. The berries were single-frozen and stored at −20 °C for 7 months until processing.

Partly thawed strawberries (overnight at −1 °C, followed by 2 h at room temperature) were homogenized in a food processor (CombiMax 700, Braun GmbH, Kronberg, Germany). Berry homogenate was separated into a flesh and an achene-rich fraction by centrifugation of berry homogenate (0.75 kg each in four tubes, 2000*g*, 15 min, 10 °C; Heraus Multifuge 4 KR, Kendro Laboratory Products GmbH, Hanau, Germany). The weight proportion of the achene fraction was recorded. Achene-enriched raw material was made by adding the achene fraction to berry homogenate (1:2, w/w).

Dry weights of the raw materials (berry, flesh, and achene-enriched homogenate) were roughly determined on a dry weight balance (Sartorius Thermo control, Sartorius GmbH, Göttingen, Germany), and water was added to adjust dry matter to the same level in all purees (8%). Berry raw material and water were heated to 75−80 °C in a boiling water bath. The temperature was held for 3 min, and then the preservatives (0.3 g of sodium benzoate and 0.4 g of potassium sorbate per kilogram), dissolved in hot water, were added. The purees were cooled in ice−water before transfer to plastic reagent tubes (50 mL).

The whole process, from thawing the berries to making purees and the succeeding storage, was repeated three times (**Figure 2**). The purees were stored in the dark at 6 and 22 °C for 16 weeks. The purees were analyzed at production and after 8 and 16 weeks of storage. Prior to extraction, the samples were kept at −80 °C.

Extraction of Phenolic Compounds. Purees (5 g) or liquid nitrogen-milled strawberries (5 g) were extracted with acetone (10 mL) by sonication for 10 min. After centrifugation (1500*g* in 10 min at 4 °C; Heraus Multifuge 4 KR), the supernatant was collected and the insoluble plant material re-extracted twice with 70% acetone (10 mL). Acetone

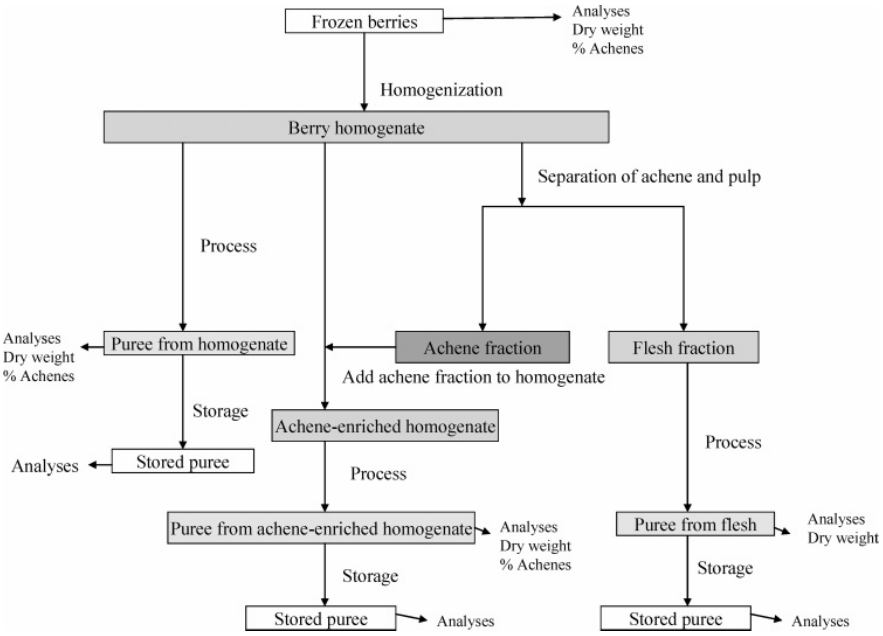

Figure 2. Flowchart of the process from frozen berries to stored purees. The whole process was performed three times.

was removed from pooled extracts by a nitrogen flow at 37 °C (Pierce, Reacti-Therm III, Heating/Stirring Module, Rockford, IL). The volume of the extract was made up to 15 mL by water. The purees were extracted in duplicate, and the fresh strawberries were extracted in triplicate. The extracts were stored at −80 °C until analysis.

Dry Weight, Achene Content, Soluble Solids, and pH. Dry weights of the berries and the purees at production were determined by using the vacuum drying method (*23*). Proportions of achenes in the purees at production were determined by separating the achenes from the flesh in a strainer. The collected achenes were rinsed several times by running cold water and left to dry at room temperature. The weight of starting material and achenes after drying was recorded. Soluble solids (percent) was determined at 20 °C in a refractometer (Mettler Toledo RE40 refractometer). pH was measured by a pH-meter (Thermo Orion, Beverly, MA).

Ascorbic Acid. AA and DHAA were determined in the purees directly after production and storage. The method was an HPLC method previously described (*24*), with some modifications. Sample (5 g) was mixed with 4.5% metaphosphoric acid (25 mL) by shaking. The mixture was filtered through a folded filter (Schleicher & Schuell GmbH, Dassel, Germany), followed by filtering through a Millex HA 0.45 μm filter (Millipore Corp.). For measurement of AA, supernatant (100 μL) was diluted with sodium phosphate buffer, pH 6.5 (400 μL). For measurement of total ascorbic acid, DHAA was reduced to AA by mixing TCEP (800 mM Trizma base containing 5 mM TCEP, pH 10) (50 μL) with supernatant (100 μL). After 20 min of reaction in the dark at room temperature, sodium phosphate buffer, pH 6.5 (350 μL), was added. The samples were analyzed using an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany). Chromatographic separation was performed on a monolithic HPLC column, Chromolith Performance RP-18e (100 mm × 4.6 mm i.d., Merck KGaA) fitted with a Chromolith RP-18e guard cartridge (5 mm × 4.6 mm i.d., Merck KGaA). The mobile phase consisted of 2.5 mM $Na_2H_2PO_4 \cdot 2H_2O$, 2.5 mM *n*-dodecyltrimethylammonium chloride, 1.25 mM Na_2-EDTA, and 2% acetonitrile, adjusted to pH 4.7 with 0.27 M citric acid. Column temperature was 25 °C, and the solvent flow rate was 1 mL/min. A 15 μL sample was injected. AA was detected at 264 nm. Concentration of AA was quantified by external standard. The concentration of DHAA was calculated by subtracting the AA concentration from the total ascorbic concentration determined after reduction of the DHAA present. The contents of ascorbic acids were expressed as milligrams of AA and milligrams of DHAA per 100 g of fresh weight (fw). The samples were analyzed in duplicates.

Total Phenolics (TP). TP was determined according to the Folin−Ciocalteu procedure (*25*). Appropriately diluted extract (0.2 mL) was mixed with Folin−Ciocalteu's phenol reagent (1:10 v/v, diluted with water) (1.0 mL) and incubated for 2 min, before sodium carbonate (7.5% w/v) (0.8 mL) was added. The mixture was incubated for 60 min at room temperature before absorption was measured at 765 nm (Agilent 8453 spectrophotometer, Agilent Technologies). Total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per 100 g of fresh weight (mg of GAE/100 g of fw). All extracts were analyzed in duplicate.

Ferric Reducing Activity Power (FRAP) Assay. The FRAP assay was carried out as described by Benzie and Strain (*26*) with some modifications as previously reported (*5*, *27*). Briefly, freshly prepared FRAP reagent (2.4 mL) was mixed with appropriately diluted extract (80 μL). The mixture was allowed to stand for 60 min at room temperature before absorption was measured at 593 nm (Agilent 8453 spectrophotometer, Agilent Technologies). Aqueous solutions of Fe-(II) ($FeSO_4 \cdot 6H_2O$) in the concentration range of 125−1000 μmol/L were used for calibration of the FRAP assay. FRAP values were expressed as millimoles of Fe(II) per 100 g of sample (mmol of Fe/100 g of fw). All extracts were diluted and analyzed in duplicate.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC assay was performed using a fluorescence plate reader (*28*). The measurements were carried out on a FLUOstar OPTIMA plate reader (BMG Labtech GmbH, Offenburg, Germany) using the 485 nm excitation and the 520 nm emission filters. Fluorescein (120 μL; 70 nM final concentration) was the fluorescent substrate and AAPH (60 μL; 24 mM final concentration) the source for peroxyl radicals. The reaction was conducted in 75 mM potassium phosphate buffer, pH 7.4, at 37 °C with Trolox (2 μM) as control standard and phosphate buffer as blank. The extracts were automatically diluted to four concentrations in duplicates by Biomek 2000 Workstation (Beckman Coulter, Fullerton, CA), and 20 μL of samples, blanks, and Trolox were added to a black 96-well microplate (96F, Nunc, Roskilde, Denmark). The fluorescein fluorescence was recorded every 3 min after the addition of AAPH until the fluorescence was <5% of the initial reading. Final results were calculated using the differences of areas under the fluorescein decay curves between the blank and a sample and were expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight (μmol of TE/g of fw).

High-Performance Liquid Chromatography (HPLC) Analyses of Phenolic Compounds. The analyses were carried out on an Agilent 1100 deries HPLC system (Agilent Technologies) equipped with an

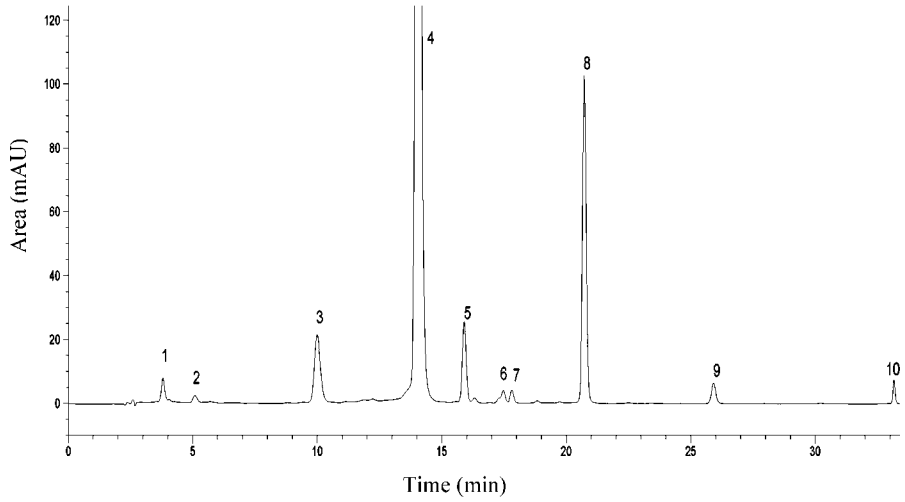

Figure 3. HPLC chromatogram (at 500 nm) of anthocyanins in strawberries (cv. Senga Sengana). Peak numbers refer to **Table 1**.

autosampler cooled to 6 °C, a diode array detector (DAD), and an MSD XCT ion trap mass spectrometer (MS) fitted with an electrospray ionization (ESI) interface. Chromatographic separation was performed on a Betasil C18-column (250 mm × 2.1 mm i.d., 5 μm particles) equipped with a 5 μm C18 guard column (4.0 mm × 2.1 mm i.d.), both from Thermo Hypersil-Keystone (Bellefonte, PA). The separation of polyphenols, other than anthocyanins, was as described by Aaby et al. (*7*), with mobile phases consisting of (A) acetic acid/water (2:98, v/v) and (B) acetic acid/acetonitrile/water (2:50:48, v/v/v). For separation of anthocyanins the mobile phases were (A) phosphoric acid/acetic acid/water (1:10:89, v/v/v) and (B) acetonitrile. When the sample was directed to the MS, mobile phase A was acetic acid/water (5:95, v/v). The gradient elution was as follows: 0−2 min, 2% B; 2−25 min, 2−20% B; 25−30 min, 20−40% B; 30−34 min, 40−2% B. Column temperature was held at 40 °C, and injection volume was 5 μL. In both separation systems the solvent flow rate was 0.25 mL/min and the column was allowed to equilibrate for 6 min between injections. The extracts were filtered through a Millex HA 0.45 μm filter (Millipore Corp.) before injections. The HPLC eluate was introduced directly to the ESI interface and analyzed by MS as earlier described (*7*).

The anthocyanins were quantified by external standard of pelargonidin-3-glucoside (at 500 nm). The other phenolic compounds were classified on the basis of their characteristic UV−vis spectra (*7*) and quantified by external standards. Hydroxycinnamic acid derivatives were quantified as chlorogenic acid (at 320 nm), (+)-catechin and proanthocyanidins as (+)-catechin (at 280 nm), flavonols as quercetin-3-glucoside (at 260 nm), ellagic acid and ellagic acid glycosides as ellagic acid (at 260 nm) and ellagitannins as gallic acid (at 260 nm). The concentration of the polyphenolic compounds was expressed as milligrams per 100 g of fw.

HPLC-DAD-MS analyses of phenolic compounds, other than anthocyanins, were performed on all samples (not on extraction parallels). HPLC-DAD analyses of anthocyanins were carried out on all extracts, whereas only an anthocyanin fraction isolated by solid-phase extraction (*29*) was analyzed with MS detection.

Contributions of Anthocyanins and Ascorbic Acids to TP and Antioxidant Activities. TP, FRAP, and ORAC values of standards of pelargonidin-3-glucoside, cyanidin-3-glucoside, AA, and DHAA were determined and expressed as milligrams of GAE, millimoles of Fe, and micromoles of TE per milligram of standard, respectively. The contribution of the anthocyanins and ascorbic acids to TP, FRAP, and ORAC values in strawberries and strawberry purees was calculated by multiplying the amount of anthocyanins or ascorbic acids in the samples with the TP, FRAP, or ORAC values of the standards. The values for pelargonidin-3-glucoside were used for the calculation of all pelargonidin glycosides.

Statistical Analysis. The results reported for strawberries were based on triplicate extractions ($n = 3$). The results reported for the purees were based on analyses of extraction parallels of purees from three productions ($n = 2 \times 3$), except for phenolic compounds other than anthocyanins, where only one sample from each production was analyzed by HPLC ($n = 1 \times 3$). Analysis of variance (ANOVA) was performed to evaluate significant differences between purees (Minitab Release 14.2, Minitab Inc., State College, PA). Significant differences ($p \leq 0.05$) between average responses were evaluated by using Tukey's multiple-comparisons test. ANOVA was performed to determine effects of the experimental factors. The production number (1, 2, and 3) and all interactions involving the production number were considered to be random effects, whereas the remaining effects, achene level (flesh, berry, and achene-enriched homogenate), time (0, 8, and 16 weeks), and temperature (6 and 22 °C), were fixed. Production number was nested within achene level. A general linear model was used to determine effects of all experimental factors during storage of the purees. In addition, one-way ANOVA was performed to determine the effect of achene level in the purees at production. Differences between variables were regarded as significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

Preliminary extraction experiments of phenolic compounds from strawberry puree using 80% methanol in water (v/v) or 70% acetone in water (v/v) showed that acetone extracts contained more phenolic compounds than methanol extracts (results not presented). Aqueous acetone was therefore chosen for extraction in the present study.

Fresh Strawberries. A chromatogram of the colored compounds in strawberries, the anthocyanins, is shown in **Figure 3**. Identification of the anthocyanins (**Table 1**) was based on chromatographic retention, UV−vis and MS spectra, and comparison with previous descriptions of anthocyanins in strawberries (*5*, *7*−*9*, *30*, *31*). Characterization of anthocyanins in strawberries used in the current experiment was also described in Aaby et al. (*7*). However, the HPLC conditions in that study were primarily for analyses of polyphenols other than anthocyanins, and thus not all of the anthocyanins found with the present HPLC methodology were detected. The most abundant anthocyanin in the strawberries was pelargonidin-3-glucoside, contributing 76.1% of the total anthocyanin content (**Table 2**). The second most abundant anthocyanin was pelargonidin-3-malonylglucoside (12.5%), followed by cyanidin-3-glucoside (3.6%) and pelargonidin-3-rutinoside (3.1%). Total content of anthocyanins in the strawberries (52.6 mg/100 g of fw) as determined by HPLC-DAD was comparable to previous determination with the pH differential method (*5*).

Table 1. Characterization of Anthocyanins in Strawberries (Cv. Senga Sengana) Using HPLC with Diode Array and MS^n Detection in Positive Mode

no.	t_R[a] (min)	λ_{max}[b] (nm)	$[M^+]$ (*m*/*z*)	MS^2 (*m*/*z*)[c]	MS^3 (*m*/*z*)	tentative identification	ref[d]
1	3.7	495, 434sh, 286, 240	595	433 ([M – 162]$^+$)	271 ([M – 162 – 162]$^+$)	pelargonidin-diglucoside	*9*, *30*
2	5.0	515, 432, 297, 239	465	285 ([M – 180]$^+$)	267 ([M – 180 – 18]$^+$)	unknown	
3	9.8	516, 279	449	287 ([M – 162]$^+$)		cyanidin-3-glucoside	*5*, *7*–*9*, *30*
4	13.9	502, 429sh, 330, 277	433	271 ([M – 162]$^+$)		pelargonidin-3-glucoside	*5*, *7*–*9*, *30*
5	15.8	503, 430sh, 33, 277	579	433 ([M – 162]$^+$), 271 ([M – 162 – 146]$^+$)		pelargonidin-3-rutinoside	*5*, *7*–*9*, *30*
6	17.3	489, 359, 262sh, 239	501	339 ([M – 162]$^+$)	295 ([M – 162 – 44]$^+$)	5-carboxypyranopelargonidin-3-glucoside	*7*, *30*, *31*
7	17.7	518, 281, 239	422	331 ([M – 91]$^+$)	313 ([M – 91 – 18]$^+$)	unknown	*30*
8	20.6	503, 429sh, 331, 277	519	433 ([M – 86]$^+$) 271 ([M – 86 – 162]$^+$)		pelargonidin-3-malonylglucoside	*5*, *7*, *8*, *30*
9	25.9	505, 431sh, 332, 272	475	271 ([M – 204]$^+$)		pelargonidin-3-acetylglucoside	*30*
10	33.1	506, 432sh, 333, 277	503	271 ([M – 232]$^+$)		pelargonidin-3-malonylrhamnoside or -3-malonylarabinoside	*7*, *30*

[a] Retention times using the mobile phases for LC-DAD detection. [b] sh, shoulder. [c] Suggested structural units of masses in brackets; 162, hexose; 180, hexose, without elimination of water; 18, water; 146, deoxyhexose (rhamnose or arabinose); 44, CO_2; 86, malonyl; 204, acetylhexose; 232, malonyldeoxyhexose (malonylrhamnose or malonylarabinose). [d] Described in literature cited.

Table 2. Concentrations (Milligrams per 100 g of Fresh Weight) of the Major Anthocyanins in Strawberries and Strawberry Purees[a–c]

sample		cyanidin-3-glucoside	pelargonidin-3-glucoside	pelargonidin-3-rutinoside	5-carboxypyrano-pelargonidin-3-glucoside	pelargonidin-3-malonyl-glucoside	pelargonidin-3-acetyl-glucoside	total[d]
berries		1.90	40.00	1.63	0.24	6.59	0.39	52.56
puree made from flesh	at production	1.34 def	32.28 h	1.29 g	0.35 g	4.95 g	0.36 g	41.42 h
	6 °C, 8 weeks	1.17 cde	23.55 f	1.01 ef	0.33 fg	3.50 e	0.27 ef	30.43 f
	6 °C, 16 weeks	0.77 b	16.00 d	0.74 d	0.32 f	2.33 d	0.20 d	20.77 e
	22 °C, 8 weeks	0.17 a	4.13 ab	0.28 b	0.21 b	0.44 bc	0.03 ab	5.36 bc
	22 °C, 16 weeks	0.00 a	1.55 a	0.18 a	0.20 b	0.13 a	0.00 a	2.13 a
puree made from berry homogenate	at production	1.58 f	31.94 h	1.29 g	0.20 b	4.82 g	0.35 g	40.80 h
	6 °C, 8 weeks	1.23 cdef	23.91 f	1.01 ef	0.24 c	3.50 e	0.27 ef	30.56 f
	6 °C, 16 weeks	0.94 bc	17.78 de	0.82 d	0.29 e	2.58 d	0.20 d	22.84 e
	22 °C, 8 weeks	0.23 a	5.26 bc	0.34 bc	0.28 de	0.55 c	0.07 bc	6.79 cd
	22 °C, 16 weeks	0.08 a	2.07 a	0.13 a	0.28 de	0.17 ab	0.00 a	2.74 a
puree made from achene-enriched fraction	at production	1.50 ef	27.74 g	1.06 f	0.17 a	4.23 f	0.29 f	35.57 g
	6 °C, 8 weeks	1.30 cdef	23.27 f	0.95 e	0.21 b	3.41 e	0.25 e	29.82 f
	6 °C, 16 weeks	1.00 bcd	17.94 e	0.76 d	0.24 c	2.54 d	0.19 d	22.90 e
	22 °C, 8 weeks	0.34 a	6.83 c	0.37 c	0.25 cd	0.67 c	0.08 c	8.60 d
	22 °C, 16 weeks	0.15 a	3.17 a	0.18 a	0.26 d	0.23 ab	0.00 a	3.98 ab
significance								
at production[e]	achene level (AL)	$p = 0.535$	$p = 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.003$	$p = 0.002$	$p = 0.002$
during storage[f]	AL	$p = 0.145$	$p = 0.113$	$p = 0.003$	$p < 0.001$	$p = 0.054$	$p = 0.059$	$p = 0.124$
	time	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.019$	$p < 0.001$	$p < 0.001$	$p < 0.001$
	temp	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.018$	$p < 0.001$	$p < 0.001$	$p < 0.001$
	AL × time	$p = 0.395$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
	AL × temp	$p = 0.939$	$p = 0.032$	$p = 0.010$	$p < 0.001$	$p = 0.310$	$p = 0.017$	$p = 0.044$

[a] The anthocyanins were quantified by external standard pelargonidin-3-glucoside (at 500 nm). [b] The results are from analyses of two extracts of each of the three purees from each treatment ($n = 2 \times 3$). [c] Values in a column with different letters are different ($p < 0.05$) based on Tukey's comparison test. [d] Concentration of minor anthocyanins are included in the total value. [e] Significance of achene level on anthocyanin concentrations in purees at production determined by one-way ANOVA. [f] Significance of all experimental factors on anthocyanin concentrations in newly made and stored purees determined by a general linear model ANOVA.

Identification of phenolic compounds, other than anthocyanins, is described in Aaby et al. (*7*). As in previous studies (*5*, *8*), ellagitannins were, together with the anthocyanins, the major class of phenolic compounds in strawberries (**Table 3**). The concentrations of ellagitannins (25.2 mg/100 g of fw), ellagic acid glycosides (2.7 mg/100 g of fw), and ellagic acid (1.4 mg/100 mg of fw) were in the same order of magnitude as previously reported for strawberries (*8*, *19*, *32*). Variation in concentration of phenolic compounds in strawberries can be due to factors such as cultivar, growing conditions, maturity, and storage (*21*, *32*–*34*). Due to the lack of authentic standards for all analytes, the compounds are quantified as equivalents of other compounds, preferably compounds with similar structure and size. The quantification method has to be taken into account when results from different studies are compared. In addition the extraction procedure, for example, extraction solvent, can affects the result. The concentrations of (+)-catechin (2.7 mg/100 g of fw) and proanthocyanidins (6.1 mg/100 g of fw) were within the ranges reported for strawberries (*5*, *8*). The *p*-coumaroyl glycosides were present at the same levels as previously reported in strawberry flesh, about 8 mg/100 g of fw (*5*). Total flavonol content (1.3 mg/100 g of fw) was lower than previously reported in flesh (*5*), but similar to values reported for whole strawberries (*8*, *18*, *32*).

Total ascorbic acid in strawberries (40.4 mg/100 g of fw) (**Table 4**) was in the same order of magnitude as previously determined with analogous methodology (*33*, *34*). TP and FRAP

Table 3. Concentrations (Milligrams per 100 g of Fresh Weight) of Phenolic Compounds in Strawberries and Strawberry Purees[a,b]

sample		ellagitannins[c]	ellagic acid glycosides[d]	ellagic acid[d]	catechin[e]	proanthocyanidins[e]	*p*-coumaroyl glycosides[f]	flavonols[g]
berries		25.2	2.7	1.4	2.7	6.1	8.5	1.3
puree made from flesh	at production	11.4 a	1.8 cd	0.8 a	2.9 g	6.0 bcd	8.4 g	0.9 a
	6 °C, 8 weeks	11.8 ab	1.5 bc	1.3 ab	2.7 efg	6.8 cde	8.4 g	1.2 ab
	6 °C, 16 weeks	12.0 abc	1.3 ab	1.5 abc	1.9 dce	5.8 bc	8.5 g	1.2 abc
	22 °C, 8 weeks	11.6 a	1.0 a	1.5 abc	1.2 abc	4.4 a	8.1 fg	1.0 a
	22 °C, 16 weeks	10.8 a	1.0 a	1.7 abc	0.8 ab	4.0 a	7.7 f	0.9 a
puree made from berry homogenate	at production	18.5 de	2.3 e	1.6 abc	3.0 g	6.7 dce	8.1 fg	1.2 ab
	6 °C, 8 weeks	16.1 cd	2.1 de	2.3 bc	2.3 efg	4.9 ab	7.7 f	1.4 bcd
	6 °C, 16 weeks	17.5 de	1.9 d	2.0 abc	1.8 dce	4.6 a	7.8 fg	1.5 bcd
	22 °C, 8 weeks	15.9 bcd	1.5 bc	2.1 abc	1.2 abc	5.0 ab	7.5 ef	1.1 ab
	22 °C, 16 weeks	14.8 abcd	1.4 ab	2.8 cd	0.8 ab	5.0 ab	6.9 de	1.1 ab
puree made from achene-enriched fraction	at production	29.3 h	3.3 f	2.9 cd	2.8 g	7.5 e	6.4 cd	1.4 bcd
	6 °C, 8 weeks	26.3 gh	3.2 f	3.9 de	2.0 def	7.2 e	6.2 c	1.7 d
	6 °C, 16 weeks	25.1 fgh	2.9 f	4.7 e	1.4 bcd	6.9 de	6.0 bc	1.6 cd
	22 °C, 8 weeks	23.4 fg	2.3 e	5.0 e	0.9 ab	6.9 cde	5.4 b	1.4 bcd
	22 °C, 16 weeks	21.2 ef	2.0 de	6.9 f	0.8 a	6.6 cde	4.6 a	1.4 bcd
significance								
at production[h]	achene level (AL)	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.251$	$p = 0.137$	$p < 0.001$	$p = 0.040$
during storage[i]	AL	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.032$	$p = 0.001$	$p < 0.001$	$p < 0.001$
	time	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.011$
	temp	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.002$	$p < 0.001$	$p < 0.001$
	AL × time	$p < 0.001$	$p = 0.231$	$p < 0.001$	$p = 0.314$	$p = 0.007$	$p < 0.001$	$p = 0.785$
	AL × temp	$p = <0.001$	$p < 0.001$	$p = 0.003$	$p = 0.160$	$p < 0.001$	$p = 0.001$	$p = 0.847$

[a] The results are from analyses of the three purees from each treatment ($n = 3$). [b] Values in a column with different letters are different ($p < 0.05$) based on Tukey's comparison test. [c] Quantified as gallic acid (at 260 nm). [d] Quantified as ellagic acid (at 260 nm). [e] Quantified as (+)-catechin (at 280 nm). [f] Quantified as chlorogenic acid (at 320 nm). [g] Quantified as quercetin-3-glucoside (at 260 nm). [h] Significance of achene level on phenolic compounds in purees at production determined by one-way ANOVA. [i] Significance of all experimental factors on phenolic compounds in newly made and stored purees determined by a general linear model ANOVA.

Table 4. Ascorbic Acid (AA and DHAA), TP, FRAP, and ORAC Values in Strawberries and Strawberry Purees[a,b]

sample		AA (mg/100 g of fw)	DHAA (mg/100 g of fw)	TP (mg of GAE/100 g of fw)	FRAP (mmol of Fe/100 g of fw)	ORAC (μmol of TE/g of fw)
berries		34.7	5.7	223	3.63	23.5
puree made from flesh	at production	21.6 d	11.6 d	157 ef	2.27 de	18.4 bcde
	6 °C, 8 weeks	2.3 a	2.8 c	145 cde	1.95 bcd	18.6 bcde
	6 °C, 16 weeks	na[c]	na	136 bcd	1.89 bc	17.4 abcd
	22 °C, 8 weeks	0.0 a	2.0 bc	118 ab	1.77 ab	16.0 ab
	22 °C, 16 weeks	na	na	106 a	1.54 a	14.8 a
puree made from berry homogenate	at production	17.6 c	12.7 de	164 ef	2.54 ef	19.0 cde
	6 °C, 8 weeks	1.9 a	0.2 ab	156 de	2.27 de	18.9 cde
	6 °C, 16 weeks	na	na	162 ef	2.21 cde	17.0 abc
	22 °C, 8 weeks	0.0 a	1.5 abc	131 bc	2.07 bcd	17.2 abc
	22 °C, 16 weeks	na	na	127 bc	1.90 bc	15.8 ab
puree made from achene-enriched fraction	at production	6.7 b	14.3 e	197 h	3.41 i	21.3 e
	6 °C, 8 weeks	2.2 a	0.9 abc	186 gh	3.12 hi	20.3 de
	6 °C, 16 weeks	na	na	185 gh	3.19 hi	19.2 cde
	22 °C, 8 weeks	0.0 a	0.0 a	166 fg	2.90 gh	17.8 bc
	22 °C, 16 weeks	na	na	160 ef	2.78 fg	17.1 abc
significance						
at production[d]	achene level (AL)	$p < 0.001$	$p = 0.060$	$p = 0.001$	$p < 0.001$	$p = 0.069$
during storage[e]	AL	$p < 0.001$	$p = 0.613$	$p < 0.001$	$p < 0.001$	$p = 0.001$
	time	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
	temp	$p = 0.024$	$p = 0.782$	$p < 0.001$	$p < 0.001$	$p < 0.001$
	AL × time	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.024$	$p = 0.560$
	AL × temp	$p = 0.985$	$p = 0.106$	$p = 0.252$	$p = 0.780$	$p = 0.500$

[a] The results are from analyses of two extracts of each of the three purees from each treatment ($n = 2 \times 3$). [b] Values in a column with different letters are different ($p < 0.05$) based on Tukey's comparison test. [c] Not analyzed. [d] Significance of achene level on ascorbic acids, TP, and antioxidant activities in purees at production determined by one-way ANOVA. [e] Significance of all experimental factors on ascorbic acids, TP, and antioxidant activities in newly made and stored purees determined by a general linear model ANOVA.

values were comparable with previous findings, as well (*4*, *5*, *35*). The ORAC value was higher than reported before (*5*, *32*), which was most likely due to different fluorescence probes (fluorescein vs phycoeryhtrin) used in the assays (*28*, *36*).

Impact of Processing on Constituents in Strawberries. The impact of processing was evaluated by comparing fresh strawberries with newly made puree from berry homogenate. When the effect of processing was calculated, dry weights of the fresh strawberries and the purees (**Table 5**), and of

Table 5. Percentage of Achenes, Dry Weight, and Soluble Solids and pH in Strawberries and Purees at Production[a]

	% achenes	% dry weight	% soluble solids	pH
berries	1.0 ± 0.1	9.3 ± 0.0	8.0 ± 0.2	3.80 ± 0.01
puree made from flesh	0.0 ± 0.0	8.5 ± 0.3	8.1 ± 0.3	3.75 ± 0.01
puree made from berry homogenate	1.2 ± 0.1	8.6 ± 0.1	7.5 ± 0.0	3.82 ± 0.05
puree made from achene-enriched raction	2.9 ± 0.3	8.5 ± 0.2	6.0 ± 0.1	3.78 ± 0.01

[a] The mean values and standard deviations are determined in triplicate for strawberries ($n = 3$) and in duplicate samples in purees at production after the triplicate productions ($n = 2 \times 3$).

preservatives added to the purees (0.07 g/100 g), were taken into account; that is, the results for puree made from berry homogenate at production were multiplied by 1.09 [= 9.3/(8.6−0.07)].

Loss of total anthocyanins during processing, after adjustment for dilution, was 15% (**Table 2**). Substantially higher anthocyanin degradation, about 40%, was observed during processing of strawberry jam (*37*). There were minor changes in the levels of ellagic acid glycosides, *p*-coumaroyl glycosides, and flavonols after processing (**Table 3**). The influence of jam processing on flavonols in berries has also previously been reported to be small (*19*), although a decrease (15%) of flavonols in strawberries after cooking with sugar, has been reported (*18*). The concentrations of ellagic acid and (+)-catechin were 20% higher in the purees than in the berries. In raspberries, the content of free ellagic acid increased 2-fold with processing to jam (*16*). The increase of ellagic acid may be explained by release of ellagic acid after hydrolysis of the ellagitannins present. In the present experiment, the ellagitannins were reduced by 20% and total ellagic acid derivatives (ellagic acid + ellagic acid glycosides + ellagitannins) were reduced by 17% during processing to strawberry puree. A similar decrease in total ellagic acid (20%) was reported during strawberry jam processing (*17*).

Loss of total ascorbic acid during strawberry puree production was 18% (AA decreased 45%, whereas DHAA increased 2.4-fold) (**Table 4**). Previously, 12% loss of total ascorbic acid has been reported when strawberries were processed to puree (*35*), whereas 36% loss of AA was observed during processing to jam (*18*). Losses of TP (20%) and FRAP (24%) during strawberry puree production were as previously reported (*35*), and as in our study, TP and FRAP values were highly correlated. The loss in antioxidant activity determined as ORAC was lower (12%). In another study a minor decrease in TP (11%) and a 2-fold increase in antiradical activity (DPPH) was reported after jam processing (*19*).

Impact of Achene Level on Puree Composition at Production. Percentages of achenes in strawberry purees made from flesh, berry, and achene-enriched homogenate were 0, 1.2, and 2.9%, respectively (**Table 5**). Dry weights and pH in the purees at production were not influenced by achene level and were similar in all purees. Soluble solids decreased from 8.1 to 6.0% with increasing achene levels in the purees.

Achene level significantly affected concentrations of all anthocyanins, except cyanidin-3-glucoside (**Table 2**). In newly made puree of achene-enriched homogenate, anthocyanin levels were lower compared to purees made from flesh and berry homogenate. The anthocyanin composition (percent distribution) was quite similar regardless of achene level. In a previous study, different anthocyanin compositions were found in flesh and achenes, with substantially higher contents of cyanidin-3-glucoside in achenes than in flesh (*5*). In contrast to the present study, however, the achenes were milled prior to extraction, allowing components from the interior of the achenes to be released.

As expected from previous studies (*5*, *21*, *22*), more ellagic acid and its derivatives were present in purees with higher achene content (**Table 3**). Flavonol levels were also significantly higher in purees when more achenes were present. (+)-Catechin and proanthocyanidin concentrations were apparently not influenced by achene content, whereas the concentrations of *p*-coumaroyl glycosides were highest in purees made from flesh.

AA content in newly made puree from flesh was 3-fold higher than AA content in puree made from achene-enriched homogenate, whereas DHAA levels were not significantly influenced by achene level (**Table 4**). TP and FRAP increased significantly with increasing achene level, whereas there was a tendency ($p = 0.069$) toward higher ORAC values in purees with higher achene content. These results were as expected on the basis of previous findings for strawberries, showing high TP and antioxidant activities in achenes compared to flesh; that is, achenes contributed about 11 and 14% of TP and antioxidant activities, respectively, whereas the berries contained only about 1% achenes on a fresh fruit basis (*5*).

Impact of Time and Temperature during Storage on Constituents in Strawberry Purees. The statistical analysis revealed that the experimental factors achene level, storage time, and temperature significantly affected levels of most of the parameters investigated (**Tables 2−4**). There were also significant interactions between the experimental factors for most of the parameters investigated. Thus, the main effects should be interpreted together with the interaction effects.

There were significant interactions between achene level and storage time for most constituents in strawberries. The exceptions were cyanidin-3-glucoside, ellagic acid glycosides, (+)-catechin, flavonols, and ORAC, on which achene levels had no influence on changes during the storage period (time). There were also interactions between achene level and storage temperature for the majority of the parameters investigated. The exceptions were, again, cyanidin-3-glucoside, (+)-catechin, flavonols, and ORAC, in addition to pelargonidin-3-malonylglucoside, AA, DHAA, and FRAP. For the remaining parameters the impact of storage time and temperature was dependent on achene levels in the purees.

The findings of more extensive degradation of strawberry anthocyanins during storage at high temperature (**Table 2**) are in accordance with results reported previously (*20*, *37*). After 8 weeks at 6 °C, total anthocyanin content was between 73 and 84% of the content in the purees at production. After 16 weeks, the content had decreased to 56−64% of the initial values. After storage at room temperature for 8 and 16 weeks, total anthocyanin contents in the purees were 13−24 and 5−11% of the initial values, respectively. A higher retention of total anthocyanins was observed in strawberry jam stored at 20 °C for 9.5 weeks (33−47%), whereas retention (6−9%) similar to that in the purees in the present study was observed after about 17 weeks of storage (*37*). The decay of pelargonidin-3-glucoside, the main anthocyanin in strawberry purees (initially 78%), was similar to that of total anthocyanins. Pelargonidin-3-malonylglucoside was the least stable among the anthocyanins in the strawberry purees. Under storage at 22 °C, only 9−16 and 3−5% of the pelargonidin-3-malonylglucoside were left after 8 and 16 weeks, respectively. Significant demalonation has also previously been observed for malonyl esters of flavonoid

glycosides (*38*). It is worth noting that the amount of 5-carboxypyranopelargonidin-3-glucoside, present in minor amounts in strawberry puree at production (averaging 0.6% of total anthocyanins), increased in purees made from berry homogenate and achene-enriched fraction during storage and contributed 3−4 and 7−10% of total anthocyanins in strawberry purees stored at 22 °C in 8 and 16 weeks, respectively. The higher stability of the pyrano form may be explained by the restricted formation of the unstable, colorless equilibrium forms reported for ordinary anthocyanins (*31*). The degradation of anthocyanins was lower in purees with higher achene levels. When initially more anthocyanins were present in purees made from flesh, this relationship changed during storage. The increased stability observed when the purees contained more achenes could be caused by stabilization of compounds originating from the achenes, probably ellagic acid-containing compounds, or could be due to the lower content of ascorbic acid, known to accelerate anthocyanin decay (*39*, *40*).

The decline in ellagitannins during storage was dependent upon achene level; that is, a faster decline was observed when more achenes, and also more ellagitannins, were present (**Table 3**). The concentrations of ellagitannins in purees made from flesh did not change during storage. In purees made from berry homogenate and achene-enriched fraction, 80 and 72% of the initial ellagitannins remained in the purees after 16 weeks at 22 °C, respectively. After 16 weeks at 6 and 22 °C, 75−89 and 57−61% of the ellagic acid glycosides present in the purees at production remained, respectively. The concentration of ellagic acid in the purees increased steadily during storage and was nearly doubled after 16 weeks at 22 °C. Yet, the level of ellagic acid might be underestimated because of the low solubility of free ellagic acid. The increase in free ellagic acid may be explained by release of ellagic acid after hydrolysis of the ellagitannins. In raspberry jam, free ellagic acid was almost doubled after the first month of storage at 20 °C and then the concentration decreased during the next 3 months (*16*). In strawberry jam, total ellagic acid content did not change during storage for 9 months at −20 °C or at 5 °C (*17*). In the present study, the only significant decrease in content of combined ellagic acid-containing compounds during storage was observed in purees made from achene-enriched fraction stored for 16 weeks at 22 °C.

Except for the anthocyanins, (+)-catechin was the least stable among the phenolic compounds. After 16 weeks at 22 °C, 0.8 mg/100 g of fw, averaging 28% of the start levels, remained in the purees. The rapid degradation of (+)-catechin may be explained by enzymatic oxidation, as this ortho-diphenolic compound is shown to be a major substrate for oxidases (*14*, *41*). Even if heat treatment, as in the current experiment, normally will inactivate oxidative enzymes (*42*), a partially thermostable peroxidase, which has been detected in strawberries (*43*), may have contributed to the degradation. There was a slight decline in *p*-coumaroyl glycosides in stored purees made from flesh; that is, the concentration after 16 weeks of storage at 22 °C was 92% of the levels in the purees at production. *p*-Coumaroyl glycosides in purees containing more achenes were less stable, with 86 and 72% left in purees made from berry homogenate and achene-enriched fraction, respectively. Flavonol content in stored purees increased slightly, but not significantly, when stored at 6 °C. Also, Häkkinen et al. (*18*) reported a gradual increase in flavonol content in strawberry jam during storage. The increase can be due to decomposition of labile flavonol malonylglycosides present in strawberries (*7*), but not determined in the purees, followed by formation of the corresponding flavonol glycosides (*38*).

Table 6. TP, FRAP, and ORAC Values of Standards of Pelargonidin-3-glucoside, Cyanidin-3-glucoside, and Ascorbic Acids (AA and DHAA)

compound	TP[a] (mg of GAE/mg)	FRAP[b] (mmol of Fe/mg)	ORAC[c] (μmol of TE/mg)
pelargonidin-3-glucoside	0.46 (1.17)	0.007 (3.0)	12.05 (5.2)
cyanidin-3-glucoside	0.81 (2.13)	0.014 (6.3)	12.74 (5.7)
AA	0.63 (0.65)	0.011 (2.0)	0.57 (0.1)
DHAA	0.10 (0.10)	0.001 (0.2)	1.15 (0.2)

[a] Values in parentheses are given as mmol of GAE/mmol of compound. [b] Values in parentheses are given as mmol of Fe/mmol of compound. [c] Values in parentheses are given as μmol of TE/μmol of compound.

After 8 weeks storage at 6 °C, the AA concentrations were about 2 mg/100 g of fw (67−89% loss) in all purees, regardless of initial AA levels and achene contents in the purees (**Table 4**). In strawberry jam the decrease in AA was less extensive, that is, 27% after 3 months of storage at 5 °C (*18*). After storage at room temperature for 8 weeks, no detectable AA and only minor amounts of DHAA remained in the purees. TP and antioxidant activities in the purees were quite stable during storage at 6 °C. After 16 weeks, on average, 93, 88, and 91% of the TP, FRAP, and ORAC values, respectively, were retained in the purees. After storage at room temperature for 16 weeks, about 68% of the TP and FRAP values remained in the purees made from flesh, whereas for purees made from achene-enriched homogenate the corresponding number was about 82%. Development of ORAC values in purees during storage was not dependent upon achene level. TP and antioxidant activities in purees were better retained in the purees during storage than the anthocyanins and ascorbic acids. Similar results were obtained in a dessert made from berries, where AA and anthocyanins decreased rapidly during storage, whereas levels of other phenolic compounds and antioxidant activity were only slightly modified (*20*).

The minor changes of TP and FRAP in purees with more achenes may be explained by hydrolysis of conjugated compounds, as ellagitannins, to compounds with more free hydroxyl groups and thus higher antioxidant activities. Another explanation may be that purees made from flesh initially contained more ascorbic acids and anthocyanins than purees with higher achene content; thus, these compounds contributed considerably to TP and FRAP in newly made purees from flesh. Because the content of ascorbic acids and anthocyanins decreased rapidly, TP and FRAP values in purees with fewer achenes declined more rapidly than those in purees with more achenes, where the contribution to TP and FRAP values from other compounds was higher.

Contributions of Different Compounds to TP and Antioxidant Activities. To calculate the contributions from pelargonidin-3-glucoside, cyanidin-3-glucoside, AA, and DHAA to TP, FRAP, and ORAC values in strawberries and strawberry purees, TP and antioxidants activities of these compounds were determined (**Table 6**). The ORAC value of AA determined in the current assay was lower than expected. The anthocyanins, mainly pelargonidin derivatives, made an important contribution to TP (11%), FRAP (11%), and ORAC values (27%) in strawberries. Estimated contributions of total ascorbic acids to TP, FRAP, and ORAC values in fresh strawberries were 10, 12, and 1%, respectively. The calculated contributions of ascorbic acids to TP and antioxidant activities were not strictly correct, because the contents of AA and DHAA were determined

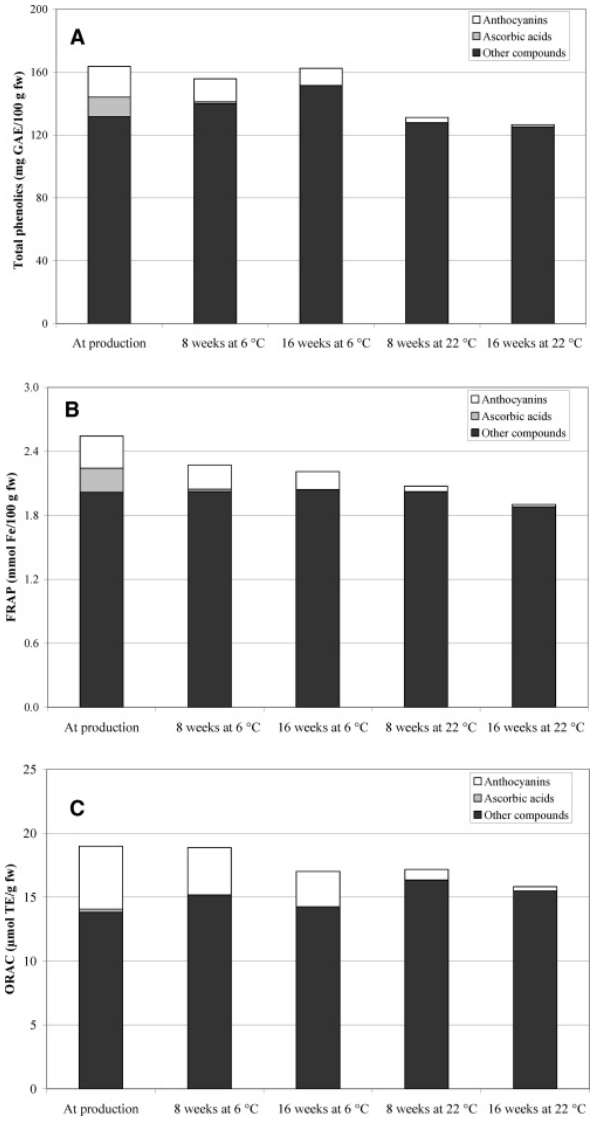

Figure 4. Changes in (**A**) total phenolics (TP), (**B**) FRAP, and (**C**) ORAC values in strawberry purees made from berry homogenate during storage. Contributions of anthocyanins and ascorbic acids to TP and antioxidant activities are shown.

in metaphosphoric acid extracts, whereas TP and antioxidant activities were determined in the acetone extracts. When antioxidant activity was determined as reducing properties with an electrochemical detector, a higher contribution from ascorbic acid to antioxidant activities in strawberries (23%) was found (*7*).

The percentage contribution of anthocyanins to TP and antioxidant activities in purees made from berry homogenate at production was very similar to that in strawberries, that is, TP (12%), FRAP (12%), and ORAC (26%) (**Figure 4**). The contributions to TP, FRAP, and ORAC values from total ascorbic acids were 8, 9, and 1%, respectively. The contributions from anthocyanins and ascorbic acids to TP and antioxidant activities in strawberry purees during storage are shown for purees made from berry homogenate (**Figure 4**). Similar development was observed in purees made from flesh and achene-enriched fraction (not shown). Because the concentrations of both ascorbic acids and anthocyanins in the purees decreased rapidly during storage, their contribution to the TP, FRAP, and ORAC values declined; for example, after 16 weeks of storage at 22 °C, only about 1% of the TP and antioxidant activities could be assigned to anthocyanins and none to ascorbic acids. The remaining compounds contributing to TP and antioxidant activities were probably phenolic compounds other than anthocyanins, with major contribution from the ellagitannins, shown to have high radical scavenging activity (*44*) and reported to be major contributors to antioxidant activities in fresh strawberries, pomegranate, and raspberries (*7*, *45*, *46*).

In conclusion, the study revealed that the presence of achenes in strawberry purees significantly contributed to increased levels and stability of many phenolic compounds. Furthermore, whereas processing and subsequent storage conditions caused decay in levels of anthocyanins and ascorbic acid, other phenolic compounds such as ellagic acid derivatives, as well as antioxidant activities, remained quite stable. The effects of including achenes and the stability of several compositional features may be interesting from a health perspective, as substantial proportions of strawberries are consumed as processed, stored products rather than fresh from the plant.

ABBREVIATIONS USED

AA, L-(+)-ascorbic acid; AAPH, 2,2′-azobis(2-amidinopropane)dihydrochloride; ANOVA, analysis of variance; DAD, diode array absorbance detector; DHAA, dehydro-L-(+)-ascorbic acid; ESI; electrospray ionization; FRAP, ferric reducing activity power; fw, fresh weight; GAE, gallic acid equivalents; HPLC, high-performance liquid chromatography; MS, mass spectrometer; ORAC, oxygen radical absorbance capacity; TE, Trolox equivalents; TCEP, tris[2-carboxyethyl]phosphine; Trolox, 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid; UV−vis, ultraviolet−visible light.

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