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Effect of Ascorbic Acid and Dehydration on Concentrations of Total Phenolics, Antioxidant Capacity, Anthocyanins, and Color in Fruits

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The purpose of this investigation was to report on the total phenolics, anthocyanins, and oxygen radical absorbance capacity (ORAC) of strawberry, peach, and apple, the influence of dehydration and ascorbic acid treatments on the levels of these compounds, and the effect of these treatments on fruit color. Results showed that fresh strawberry had the highest levels for total phenolics [5317.9 mg of chlorogenic acid equivalents (CAE)/kg], whereas lower levels were found in fresh apple and peach (3392.1 and 1973.1 mg of CAE/kg, respectively), and for anthocyanins (138.8 mg/kg), whereas lower levels were found in fresh apple and peaches (11.0 and 18.9 mg/kg, respectively; fresh strawberry had an ORAC value of 62.9 mM/kg Trolox equivalents. The fresh apple and peach were found to have ORAC values of 14.7 and 11.4 mM/kg of Trolox equivalents, respectively. The color values indicated that the addition of 0.1% ascorbic acid increased the lightness (L^*) and decreased the redness (a^*) and yellowness (b^*) color values of fresh strawberry, peach, and apple, sliced samples, and the puree made from them. Also, results showed that dehydration is a good method to keep the concentrations of total phenolics and anthocyanins and ORAC values at high levels.

KEYWORDS: Total phenolics; anthocyanins; antioxidant capacity

INTRODUCTION

Phenolic compounds are large, heterogeneous groups of secondary plant metabolites that are widespread in the plant kingdom (*1*). Phenolics have a wide variety of structures. Flavonoids, tannins, and phenolic acids are the main phenolic compounds. The structural basis for all flavonoids is the flavone nucleus (2-phenylbenzo-γ-pyrane). The flavonoid group can be divided into numerous categories based on hydroxylation of the flavonoid nucleus as well as the linked sugar (*2*). Tannins are the general name for phenolic substances capable of tanning leather or precipitating gelatin from solution. They can be divided into condensed proanthocyanidins, for which the fundamental structural unit is the phenolic flavan-3-ol (catechin) nucleus, galloyl and hexahydroxydiphenoyl esters and their derivatives, gallotannins, and ellagitannins (*3*). The main two groups of phenolic acids are hydroxybenzoic acids and hydroxycinnamic acids, both of which are derived from nonphenolic molecules benzoic and cinnamic acid, respectively (*4*). Due to toxicological concerns of synthetic antioxidants, there have been increasing interests in identifying phenolic compounds in plants to minimize or retard lipid oxidation in lipid-based food products (*5*). Most of these natural antioxidants come from fruits, vegetables, spices, grains, and herbs (*6*).

Anthocyanins, which act as pigments to give some fruits and vegetables their deep color, are a major component of the phenolic/flavonoid class (*7*). Recent research documents anthocyanins acting as antioxidants, providing many potential health benefits (*8*). Researchers are currently linking anthocyanin activity to improving vision, controlling diabetes, improving circulation, preventing cancer, and retarding the effects of aging, particularly loss of memory and motor skills (*9*, *10*). Oxygen radical absorbance capacity (ORAC) values are a measure of the antioxidant activity. Specifically, this assay measures the degree and length of time it takes to inhibit the action of an oxidizing agent. The ORAC was measured for red raspberries, black raspberries, and blackberries and found to be high (*11*).

Food dehydration is the process of removing water from food by circulating hot air through it by using different kinds of dehydration, which prohibits the activity of enzymes and growth of bacteria (*12*). The major problem associated with enzymes in fruits is the development of brown color and loss of vitamins. Using chemical compounds that interfere with deteriorative chemical reactions inactivates the enzymes in fruits. The most common control chemicals used are sodium bisulfite and

ascorbic acid (vitamin C). Ascorbic acid may be used in its pure form or in chemical mixtures (*13*).

It is hoped that information on the total phenolics, antioxidant activities, and anthocyanins and their treatments of several fruits can be used as criteria in a variety of food products. The objectives of this study were to evaluate strawberry, apple, and peach for total phenolics, antioxidant activities, and anthocyanins and to report on the effect of adding ascorbic acid on the total phenolics, anthocyanins, and antioxidant activities and to investigate the effect of dehydration on the concentrations of these compounds.

MATERIALS AND METHODS

Materials. Fruits (strawberry, apple, and peach) were purchased from a local supermarket. 6-Hydroxy-2, 5,7,8-tetramethyl-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI). 2,2-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA, Inc. (Richmond, VA). All other chemicals were of reagent grade and purchased from Fisher Scientific (Springfield, NJ).

Methods. *Phenolic Compounds Extraction.* About 5 ± 0.2 g of fruit samples was blended to attainable particle size using a blender (Osterizer Galaxie Dual Range 14, Oster Corp., Milwaukee, WI). Twenty-five milliliters of extraction solvent (400 mL of acetone/400 mL of methanol/200 mL of water/10 mL of acetic acid) was added exactly, and the container was stoppered tightly. The sample with the extraction solvent were incubated at 60 °C for 1 h and cooled to room temperature. The sample with extraction solvent was tissumized for 30 s and filtered through microcloth into a screw-cap test tube and capped immediately. The samples were kept frozen until the time of analysis.

Determination of Total Phenolics. Total phenolics were determined according to the Folin−Ciocalteu method (*14*). Filtrates from each extract (1 mL, three replicates) were introduced into screw-cap test tubes; 1.0 mL of Folin−Ciocalteu reagent and 1.0 mL of sodium carbonate (7.5%) were added. The tubes were vortexed and allowed to stand for 2 h. Absorbance at 726 nm was measured (Perkin-Elmer λ15 UV−vis spectrophotometer, Norwalk, CT). The total phenolic content was expressed as chlorogenic acid equivalents (CAE) in milligrams per kilogram of dry weight. A calibration curve was prepared in the same manner using CAE as standard

$$\text{total phenolics concentration (mg/kg)} = (A/b) \times [(\text{SW} + 25)/\text{SW}]$$

where A = absorbance at 726 nm, SW = sample weight (kg), and b = the slope of the standard curve of chlorogenic acid.

Determination of Total Anthocyanins. Total anthocyanin content was estimated using the pH differential assay of Giusti and Wrolstad (*15*). Absorbance was measured in a Hewlett-Packard 8452A photodiode array spectrophotometer (Palo Alto, CA) at 520 and 700 nm in buffers at pH 1.0 and 4.5, using absorbance (A) = $(A_{520} - A^{7}_{00})_{\text{pH1.0}} - (A_{520} - A_{700})_{\text{pH4.5}}$, with a molar extinction coefficient of cyanidin-3-glucoside (c3g) of 29 600. Results were expressed as milligrams per kilogram of dry weight (c3g equivalents).

Determination of Antioxidant Capacity. ORAC was measured using a modified version of the method of Cao and others (*16*) utilizing a Perkin-Elmer HT Soft 7000 Plus Bio Assay Reader (Norwalk, CT). The concentrations of reagents were identical to those of Cao and others (*16*) except for the working Trolox standard, which was diluted to 10 μM prior to use in the assay. For the assay, 20 μL of each sample diluted 100-fold with phosphate buffer was mixed with 160 μL of β-phycoerythrin (3.73 mg L^{-1}) in a clear 48-well Falcon microplate, and a baseline reading was obtained. As rapidly as possible, 20 μL of 320 mM AAPH was added to each well using a multichannel pipet. The plate was agitated for 20 s prior to reading and for 5 s before each reading at 2 min intervals for 70 min. Excitation and emission filter wavelengths were 535 and 560 nm, respectively. Data were expressed in millimolar Trolox equivalents (TE) per kilogram of dry weight.

Dehydration. Fresh fruits were cleaned and their cores taken out, and they were cut into slices using a knife. Fruit puree also was prepared using a blender. Each treatment was divided into two halves. Half was mixed with 0.1% ascorbic acid (for 2 min), and the other received no ascorbic acid. After that, samples were placed in the dehydrator (Harvest Saver, model R-4, manufactured by Commercial Dehydrated System, Inc.) at 40.4 °C for 24 h.

Color Measurement. The color of samples was measured by a Minolta colorimeter CR-300 (USA) and recorded as $L^*a^*b^*$ color system. The $L^*a^*b^*$ color system consists of a luminance or lightness component (L^*) and two chromatic components: the a^* component for green ($-a$) to red ($+a$) and the b^* component for blue ($-b$) to yellow ($+b$) colors. The colorimeter was calibrated using a standard white plate. Values of the white standard were $L = 97.10$, $a = +0.13$, $b = +1.88$. Color was measured at three positions.

Statistical Design and Analysis. All experiments were triplicated using completely randomized design. Analysis of variance was performed by the JMP statistical package (JMP Institute Inc., Cary, NC). Means were compared by least significant difference (LSD) at $P < 0.05$.

Table 1. Total Phenolics, Anthocyanin Concentrations, and ORAC in Fruits in the Presence and Absence of 0.1% Ascorbic Acid (AA)[a]

treatment	total phenolics (mg/kg)	anthocyanins (mg/kg)	ORAC (mM TE/kg)
fresh strawberry	5317.9 ± 242 d	138.8 ± 6.6 c	62.9 ± 2.9 e
fresh apple	3392.1 ± 109 e	11.0 ± 0.5 g	14.7 ± 0.8 f
fresh peach	1973.1 ± 78.9 f	18.9 ± 0.7 f	11.4 ± 0.4 f
strawberry puree	16689.5 ± 288 a	436.9 ± 14 b	195.6 ± 7.2 b
apple puree	8117.4 ± 131 bc	35.3 ± 0.8 e	84.5 ± 3.8 d
peach puree	7546.5 ± 279 c	37.8 ± 1.5 e	63.7 ± 1.5 e
dreid strawberry	16553.3 ± 394 a	533.9 ± 12 a	235.8 ± 9.1 a
dried apple	8574.5 ± 190 b	38.1 ± 1.7 e	97.1 ± 2.1 c
dried peach	7590.4 ± 200 c	50.9 ± 2.8 d	67.6 ± 3.1 e
fresh strawberry + AA	5677.1 ± 132 d	126.2 ± 3.8 c	64.4 ± 3.6 e
fresh apple + AA	3235.4 ± 147 e	9.2 ± 0.4 g	17.7 ± 0.8 f
fresh peach + AA	1624.4 ± 90.2 f	16.6 ± 0.7 f	12.4 ± 0.5 f
strawberry puree + AA	17073.6 ± 328 a	428.3 ± 10 b	185.0 ± 5.6 b
apple puree + AA	8462.6 ± 229 b	33.6 ± 1.2 e	78.6 ± 3.1 d
peach puree + AA	7589.4 ± 230 c	35.9 ± 1.9 e	68.7 ± 3.8 e
dried strawberry + AA	17148.7 ± 365 a	518.9 ± 24 a	248.6 ± 6.1 a
dried apple + AA	8779.9 ± 274 b	36.1 ± 2 e	101.8 ± 3.8 c
dried peach + AA	8412.9 ± 205 b	47.6 ± 1.8 d	71.7 ± 2.2 e

[a] Means ± standard deviation in the same column with the same letter are not significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

Total Phenolics. Table 1 shows the amounts of total phenolics, which was found to vary significantly in strawberry, peach, and apple, fresh, dried, and pureed, with and without addition of ascorbic acid and ranged from 17148.7 to 1624.4 mg of CAE/kg. This variation is expected in these fruits due to other constituents as well as the variation in the phenolic type among different fruits. Fresh strawberry was found to contain relatively higher levels of phenolics (5317.9 mg of CAE/kg), whereas lower levels were found in fresh apple and peach (3392.1 and 1973.1 mg of CAE/kg, respectively). The levels of phenolics in strawberry, peach, and apple (fresh, dried, and pureed) with the addition of ascorbic acid were very similar to those without ascorbic acid addition. Levels of total phenolics were higher in dried fruits followed by pureed and fresh products. Addition of ascorbic acid was found to have no effect on total phenolics concentrations. The differences in phenolics content in fruits could be attributed to the wide range of phenolic compounds species and their derivatives, which include simple phenolics and long-chain carboxylic acids (*8*).

Anthocyanins. The anthocyanins contents varied in strawberry, peach, and apple, fresh, dried, and pureed, with and without the addition of ascorbic acid and ranged from 533.9 to 9.2 mg of CAE/kg as shown in **Table 1**. This variation was

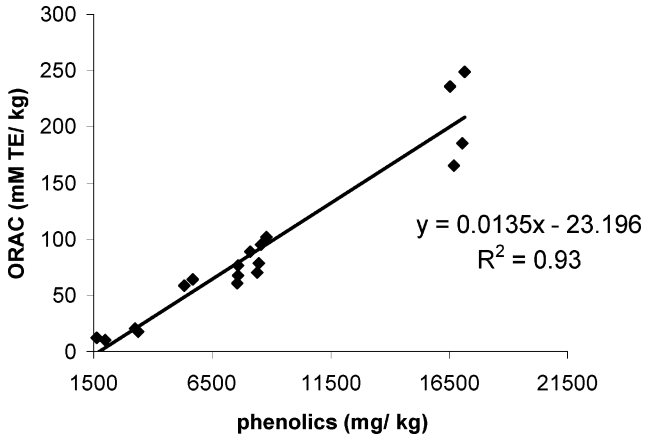

Figure 1. Relationship between ORAC and phenolics in fruits.

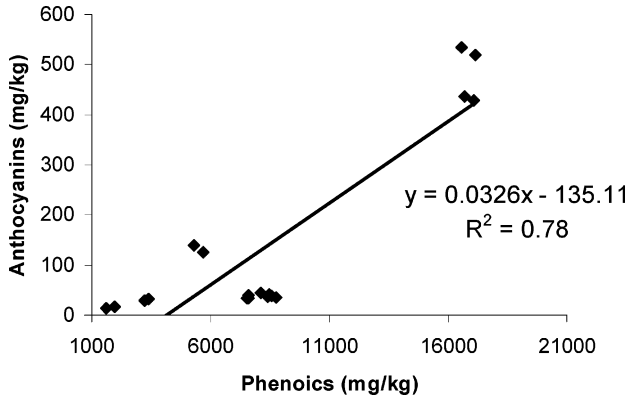

Figure 2. Relationship between anthocyanins and phenolics in fruits.

expected in the investigated fruits due to different concentrations of anthocyanins. Among plant extracts, fresh strawberry contained relatively higher levels of anthocyanins (138.8 mg/kg), whereas lower levels were found in fresh apple and peach (11.0 and 18.9 mg of CAE/kg, respectively). The concentrations of anthocyanins in strawberry, peach, and apple, fresh, dried, and pureed, with the addition of ascorbic acid were very similar to the samples without ascorbic acid addition. The total anthocyanins were higher in dried fruit followed by puree and fresh fruits. Addition of ascorbic acid was found to have no influence on anthocyanins concentrations.

Antioxidant Capacity (ORAC). Antioxidant activities of strawberry, peach, and apple, fresh, sliced, and pureed, with and without the addition of ascorbic acid as measured by ORAC ranged from 248.6 to 11.4 mM/kg of Trolox equiv as shown in **Table 1**. A higher level of antioxidant activity was observed in strawberry (62.9 mM/kg of Trolox equiv). The antioxidant activities of fresh apple and peach were 14.7 and 11.4 mM/kg of Trolox equiv, respectively. The results of ORAC are in agreement with the results reported by Wang and others (*17*). Shahidi and Marian (*8*) reported that differences in antioxidant activities of fruits could be due to their different structures from phenolic acids and flavonoid compounds as well as their derivatives. For instance, antioxidant activities of phenolic acids and their derivatives such as phenolic esters depend on the number of hydroxy groups in the molecules. Results showed that the concentrations of antioxidants in strawberry, peach, and apple, fresh, dried, and pureed, mixed with 0.1% ascorbic acid were very similar to those without addition of ascorbic acid. The total antioxidants were higher in dried followed by pureed and fresh fruits. Addition of 0.1% ascorbic acid was found to have no effect on levels of antioxidants.

As illustrated in **Figure 1**, a good linear relationship was found between ORAC and total phenolics contents ($r^2 = 0.93$), implying that the antioxidant activity of these fruits is largely due to the presence of phenolic compounds. The relationship between total phenolics and anthocyanins as shown in **Figure 2** was not as strong as with phenolics ($r^2 = 0.78$). This observation is in agreement with the findings reported previously by Prior and others (*18*).

The results indicated that dehydration was found to have a significant effect on the levels of these compounds (total phenolics, anthocyanins, and ORAC). Adding ascorbic acid (0.1%) increased the level of ORAC and total phenolics, whereas it decreased the amount of anthocyanins (but not significantly so). This may be attributed to ascorbic acid influence in degrading anthocyanins. The mechanism with the ascorbic acid may be a result from an intermediate peroxide produced by the degradation of the ascorbic acid itself. Also, a probable explanation might be due to the presence of trace amounts of iron and copper, which induce ascorbic acid to degrade anthocyanins. The results of the current investigation indicate that dehydration is considered to be a good method to maintain higher retention of these compounds.

Table 2. Color Values (*L**, *a**, and *b**) of Different Fruits in the Presence and Absence of 0.1% Ascorbic Acid (AA)[a]

treatment	*L**	*a**	*b**
fresh strawberry	46.51 k	3.57 g	9.41 j
fresh apple	50.22 j	0.07 j	16.87 h
fresh peach	64.51 e	–1.09 l	31.75 a
strawberry puree	40.26 m	16.68 a	7.60 k
apple puree	54.24 i	7.58 d	20.31 f
peach puree	58.81 g	8.77 c	28.19 bc
dried strawberry	69.48 d	6.33 e	19.05 g
dried apple	71.36 b	1.07 i	27.82 c
dried peach	50.45 l	6.02 e	19.20 g
fresh strawberry + AA	50.10 j	2.08 h	8.01 k
fresh apple + AA	53.31 i	–0.99 k	10.48 i
fresh peach + AA	70.15 c	–0.06 i	28.39 b
strawberry puree + AA	44.25 l	12.88 b	6.54 l
apple puree + AA	55.94 h	6.81 e	17.10 h
peach puree + AA	59.54 f	7.32 d	22.02 e
dried strawberry + AA	71.54 bc	5.38 f	17.56 h
dried apple + AA	74.25 a	–1.80 l	26.48 d
dried peach + AA	54.13 i	5.31 f	18.10 h

[a] Means in the same column with the same letter are not significantly different ($P < 0.05$).

Color Measurements. The lightness (*L**), redness (*a**), and yellowness (*b**) values of strawberry, peach, and apple, fresh, dried, and pureed, treated with and without ascorbic acid are shown in **Table 2**. Results showed that strawberry had the highest *a** values followed by peach and apple. These results are in agreement with the values presented in **Table 1**, which showed that strawberry had the highest anthocyanin values compared with apple and peach. Peach had the highest *L** and *b** values followed by apple and strawberry. The lightness, redness, and yellowness were significantly different. The explanation for this variation could be due to the nature of the pigments in these fruits. Addition of ascorbic acid was found to increase *L** values and to decrease *a** and *b** color values of strawberry, peach, and apple, fresh, dried, and pureed, and this could be attributed to enzyme inactivation due to moisture loss.

Conclusion. The purpose of this investigation was to investigate the effectiveness of dehydration and ascorbic acid addition on total phenolics, anthocyanins, and ORAC levels in different fruits. The results of this study showed that strawberry had the highest values of ORAC, anthocyanin, and total phenolics followed by apple and peach. The color measurements

indicated that addition of ascorbic acid increased L^* and decreased a^* and b^* color values of strawberry, peaches, and apple, fresh, dried, and pureed. The results supported the assumption that dehydration is a good method to maintain high retention of these compounds.

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