Phytochemical Composition and Antioxidant Stability of Fortified Yellow Passion Fruit (*Passiflora edulis*)

STEPHEN T. TALCOTT,* SUSAN S. PERCIVAL, JENNIFER PITTET-MOORE, AND CHARITY CELORIA

Department of Food Science and Human Nutrition, University of Florida, P.O. Box 110370, Gainesville, Florida 32611-0370

Yellow passion fruit juice (PFJ, *Passiflora edulis f. flavicarpa*) is an important component of many tropical fruit beverages, but limited data exist on its antioxidant chemical composition and stability during processing and storage. PFJ fortified with ascorbic acid (450 mg/L) and sucrose (10%) was compared to a nonfortified control, and each was evaluated with and without vacuum deaeration to remove dissolved oxygen. Following pasteurization, juices were stored for 28 days at 37 °C to accentuate physicochemical changes. Pasteurization (85 °C for 30 min) resulted in minor changes to physicochemical attributes, but appreciable changes occurred during storage that resulted in termination of the study after 28 days. Oxygen control strategies proved to be ineffective for quality retention and indicated oxygen-independent reactions affecting juice color, phytochemical content, and antioxidant activity. Ascorbic acid and sucrose fortification had an overall preservation effect on total carotenoids, the former resulting in hyperchromic shifts in absorbance, indicating their chemoprotection. Pasteurization resulted in a 25% loss in L-ascorbic acid, which was completely destroyed after 14 days of storage; losses coincided with increased juice browning and formation of 5-hydroxymethylfurfural. Numerous polyphenolics were present in PFJ, and 16 of them were tentatively characterized on the basis of spectral similarities to known standards. Individually, polyphenolics increased during pasteurization, only to decline during storage at elevated temperatures. Antioxidant activity was measured in PFJ and in two subfractions (hydrophilic and lipophilic) after processing and storage, but antioxidant values were nonadditive. A significant chemical interaction affecting antioxidant capacity was found for hydrophilic juice components, but none was observed in the presence of lipophilic phytochemicals. Physicochemical attributes and overall quality of PFJ were retained following pasteurization but were significantly impacted by degradative reactions during accelerated storage.

KEYWORDS: Passion fruit; antioxidant activity; polyphenolics; carotenoids; stability

INTRODUCTION

Passion fruit (*Passiflora edulis f. flavicarpa*) is a tropical fruit produced throughout the world with distinctive aromas and flavors making it a popular additive to many tropical fruit juice blends. Primarily two fruit types are commercially produced, yellow and purple, and both are commonly consumed throughout the world. Yellow passion fruits are thought to be hybrids of purple varieties (*1*) and are most commonly used for processed juices due to their more acidic taste and higher juice yield. Purple varieties are typically consumed fresh due to their sweeter taste. Yellow passion fruit juice (PFJ) is popular as an integral flavor component in tropical juice blends due to its unique flavor properties, but it is commonly consumed sweetened and diluted due to its acidic taste. Yielding ca. 30% juice by weight, the estimated fruit yield was 780 000 million tons

in 2001, with Brazil and Ecuador accounting for over 70% of the world market (*2*). Characterization of polyphenolics is limited for yellow passion fruit, but characterization of ascorbic acid (*3*, *4*) and carotenoids (*5*) has been reported. Other phytochemicals conclusively identified include aroma compounds such as volatile thiols, terpenes, fatty acid esters, alcohols, and various other aromatics (*6*−*8*).

The characteristic color of yellow passion fruit rind, flesh, and juice is due to provitamin A carotenes and xanthophylls which are typically sensitive to oxygen, heat, and light, and their stability may be influenced by thermal pasteurization, environmental conditions, and duration of storage. Thirteen carotenoids were identified in passion fruit, with ζ-carotene identified as the predominant compound (*5*); these compounds give visual appeal to juices and are important for their provitamin A and antioxidant activity. Carotenoids are widely regarded as effective quenchers of singlet oxygen, triplet oxygen, and peroxyl radicals (*9*−*11*); the polarity of carotenoids may

* To whom correspondence should be addressed. Tel.: (352) 392-1991. Fax: (352) 392-9467. E-mail: sttalcott@mail.ifas.ufl.edu.

influence antioxidant functioning since xanthophylls should function more efficiently against polar radicals (*12*). Additionally, exposure to light, heat, or oxygen may induce carotenoid isomerization, with cis-isomer configurations reported to have greater antioxidant capacity than their parent compounds (*13*–*16*). The affects of processing and storage on carotenoids retention have not been investigated in PFJ, and their retention is likely a critical factor affecting overall quality and potential health benefits.

The purpose of this investigation was to determine the thermal processing and storage stability of antioxidant compounds in yellow PFJ as influenced by fortification with ascorbic acid and sucrose. Monitoring major physicochemical changes associated with PFJ will identify factors influencing overall juice quality and establish a basis for potential health benefits of its consumption.

MATERIALS AND METHODS

Materials and Processing. Frozen, nonpasteurized single-strength Ecuadorian PFJ was obtained from ITI Tropicals, Inc. (Lawrenceville, NJ) and kept frozen (−20 °C) until needed. Juice was thawed at room temperature for ~24 h and kept cold (<5 °C) throughout preprocessing procedures. From a homogeneous pool of juice, three aliquots were fortified with 450 mg/L ascorbic acid from an aqueous stock solution or 10% granular sucrose and compared to an unfortified control. Each juice was then adjusted to a constant weight by adding deionized water (ca. 10%) to account for weight differences created by fortification. Juices were again subdivided, and half of the samples were immediately sealed into 50-mL screw-cap vials while the remaining juice was deaerated (635 mm Hg) for 10 min using a vacuum pump as described previously (*17*). Following deaeration, each juice was sparged with nitrogen through an air diffuser and filled into 50-mL plastic vials; the sample headspace was then flushed with nitrogen prior to capping. Juices (six treatments) were pasteurized in a water bath at 85 °C for 30 min, refrigerated for 24 h, and then placed into a 37 °C incubator for 28 days to accentuate physicochemical changes. Nonpasteurized juices containing 50 mg/L sodium azide (antimicrobial) were retained and kept refrigerated until analysis (~24 h). Juice treatments were evaluated after processing and after 14 and 28 days of storage for physicochemical attributes.

Physicochemical Analyses. A phytochemical isolate, referred to as a "ubiquitous" extract since it contained all soluble phytochemicals including carotenoids, polyphenolics, and ascorbic acid, was obtained by extracting PFJ (5 g/20 mL) with acetone–ethanol (1:1), shaded from direct light, and filtered through Whatman No. 4 filter paper. This extract was used for total carotenoid and antioxidant capacity determinations. Total carotenoids were determined directly from this isolate by recording absorbance values at 378, 401, 427, and 470 nm and quantifying using an extinction coefficient of 2500 (*18*).

Antioxidant activity was also determined on non-deaerated PFJ using the same acetone–ethanol (1:1) extract containing primarily carotenoids, polyphenolics, and ascorbic acid. These antioxidants were additionally partitioned into two distinct isolates from the ubiquitous extract on the basis of solubility (lipophilic and hydrophilic) by mixing in a known volume of petroleum ether and waiting for phase separation. An aliquot of the petroleum ether was evaporated under a gentle stream of air and then redissolved in acetone–ethanol, and all three isolates (lipophilic, hydrophilic, and ubiquitous) were evaluated for antioxidant capacity. Background interference was corrected by running a blank of acetone–ethanol to compensate for the slight radical inhibition by ethanol. Antioxidant capacity was determined using the oxygen radical absorbance capacity (ORAC) assay, described initially by Cao et al. (*19*, *20*) and later modified by Ou et al. (*21*), with fluorescein as the fluorescent probe. Peroxyl radicals were generated by 2,2′-azobis(2-amidinopropane) dihydrochloride, and fluorescence loss was monitored on a Molecular Devices fmax 95-well fluorescent microplate reader (485 nm excitation and 538 nm emission). Each fraction was diluted 50-fold in pH 7.2 phosphate buffer prior to pipetting into a 96-well microplate. A 4-fold dilution factor was used in the ORAC assay that

corresponded to an in-well standard concentration ranging from 6.25 to 50 μM Trolox.

Isolates for total soluble phenolics were obtained from methanolic extracts of PFJ (5 g/5 mL) and analyzed using the Folin–Ciocalteu assay as described previously (*22*), with data expressed as gallic acid equivalents (GAE). An additional isolate was obtained for HPLC analysis due to the presence of interfering compounds by extracting PFJ (5 g/15 mL) three times with ethyl acetate (EA). Solvent extracts were pooled, evaporated under reduced pressure, and redissolved in 50% methanol for separation by HPLC according to the HPLC conditions of Talcott et al. (*23*) using a Waters 2690 Alliance HPLC system with a Supelcosil LC-18 column (250 × 4.6 mm); detection was at 280 nm with a Waters 996 PDA detector.

Ascorbic acid was determined by homogenizing PFJ with 3% aqueous citric acid (2 g/5 mL), centrifuging, and filtering through Whatman No. 4 filter paper. An aliquot was diluted 3-fold with 3% citric acid and passed through an AccuBond ODS-C$_{18}$ cartridge (J&W Scientific, Folsom, CA), previously washed with methanol and 3% citric acid. The first 1 mL was discarded from the cartridge, and the remaining isolate was filtered and separated by HPLC according to Gokman et al. (*24*) using the same Supelcosil LC-18 column (detection limit ~5 mg/L). Standard recovery from PFJ was 98%.

Objective color (CIE-Lab) was measured on a 40-mL aliquot of PFJ after processing and storage. Tristimulus color measured lightness (*L**), hue angle (degrees), and chroma color intensity using a BYK-Gardner Colorgard colorimeter (Columbia, MD). Calibration with black and white tiles and verification of accuracy against standard tiles were performed before evaluations.

Investigations into chemical interactions affecting antioxidant capacity were assessed on the same three fractions obtained from acetone–ethanol as previously described, while three additional fractions were obtained from a subsequent extraction procedure by utilizing EA, which simultaneously extracted both lipophilic and EA-soluble compounds. Pooled EA extracts were filtered through Na$_2$SO$_4$ and evaporated under reduced pressure, leaving a dry residue. Lipophilic compounds (primarily carotenoids) were first removed from the residue with petroleum ether, evaporated in a separate flask, and redissolved in 100% methanol; remaining EA-soluble compounds in the residue were redissolved in 100% methanol. Juice components not soluble in EA (EA-insoluble) were blended with methanol to precipitate soluble pectin. The slurry was filtered and methanol removed under reduced pressure. This EA-insoluble fraction contained primarily water-soluble phytochemicals that were tested against lipophilic and EA-soluble fractions for potential chemical interactions affecting antioxidant capacity. All three fractions were adjusted to 50% methanol prior to antioxidant assessments and background subtracted using a blank of the same solvent.

Statistical Analysis. Data represent the means of triplicate analyses tested as a treatment by time factorial and blocked by the deaeration treatment. Three preprocessing treatments (ascorbic acid, sucrose, and control) and two deaeration treatments (with and without) were evaluated before and after thermal processing and following storage for 14 and 28 days at 37 °C. Linear regression, analysis of variance, and Pearson correlations were conducted using JMP software (*25*) and mean separation using the LSD test ($P < 0.05$).

RESULTS AND DISCUSSION

Yellow passion fruit juice (PFJ) was evaluated following fortification with sucrose and ascorbic acid and deaeration to remove dissolved oxygen for comparison to a nonfortified, non-deaerated control. The thermal pasteurization regime used in this study was relatively extreme compared to commercial processes and was hypothesized to result in the greatest change to physicochemical attributes, but overall only small changes in phytochemicals and objective color measurement were found. Significant changes in phytochemicals and juice color were subsequently observed during storage under accelerated conditions. Storage resulted in juice browning and development of sulfury, pungent aromas that resulted in discontinuation of the storage study after 28 days. No evidence of microbial spoilage
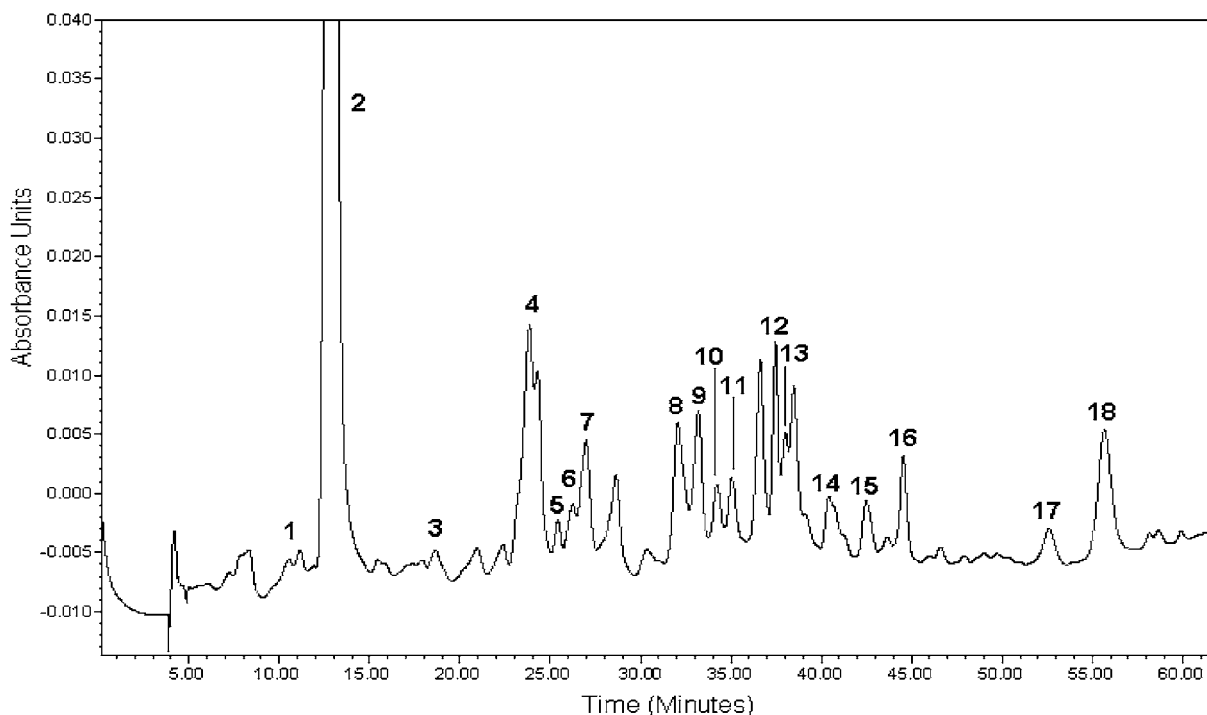
Antioxidants in Yellow Passion Fruit Juice

J. Agric. Food Chem., Vol. 51, No. 4, 2003   **937**

Figure 1. HPLC chromatograph of phenolic acids obtained from ethyl acetate extracts of passion fruit juice recorded at 280 nm (peak identification in **Table 1**).

was apparent since juice pH remained constant throughout the study (pH ~3.2). Although not formally evaluated through chemical or sensory evaluations, negative odors were hypothesized to result from heat-induced degradation of thiol-containing aroma volatiles.

Polyphenolics. Polyphenolic compounds are important constituents in many fruits and vegetables, and their quantification can give vital information relating to antioxidant functioning, food quality, and potential health benefits. Predominant polyphenolics were tentatively identified (**Figure 1**) and quantified (**Table 1**) in PFJ before and after thermal processing and after storage for 28 days at 37 °C. Deaeration treatments were found to be ineffective in retaining polyphenolics over time, and fortification had only a minor impact on polyphenolic retention, indicating a minor role of phenolic autoxidation. Juice extraction with EA was the only way to obtain quantifiable peaks by HPLC due to spectral interference and incomplete resolution from interfering compounds. Treatment of PFJ with tannase (EC 3.1.1.20) or β-glucosidase (EC 3.2.1.20) did not alter HPLC chromatographs, indicating that compounds hydrolyzed by these enzymes were not present (data not shown). Although over 40 compounds were detected in PFJ, only 16 polyphenolics, 5-hydroxymethylfurfural (HMF), and galacturonic acid were tentatively characterized and quantified on the basis of standard curves and spectral properties of closely related compounds. Concentrations of all compounds increased during pasteurization, and four compounds continued to increase during storage (compounds **1**, **10**, **12**, **18**), while the remaining compounds decreased as a result of thermal degradation or polymerization reactions. Formation of HMF during storage was especially dependent on fortification and was highest in ascorbic acid, followed by sucrose-fortified juices. HMF levels increased only slightly after pasteurization but increased appreciably during storage as a result of ascorbic acid and/or reducing sugar degradation or from Maillard browning reactions. Subsequent evaluation of PFJ polyphenolics extracted with EA and redissolved in water (pH 3.2) did not produce visible color when

Table 1. Tentative Identification and Quantification (μg/L) of Predominant Phenolic Acids Present in Non-deaerated Passion Fruit Juice before and after Processing and after Storage for 28 Days at 37 °C ($n = 2$)

peak no.	tentative identification	λ_{max} (nm)	nonpasteurized	after pasteurization	stored for 28 days
1	HMF	285.5	214 ± 17	572 ± 20	4972 ± 63
2	galacturonic acid	285.5	nq[a]	nq	nq
3	*p*-hydroxybenzoic acid	257.2	267 ± 41	334 ± 14	122 ± 25
4	syringic acid (deriv)[b]	276.1	3210 ± 120	4280 ± 450	1400 ± 300
5	syringic acid (deriv)	276.1	77 ± 23	116 ± 5	<10
6	caffeic acid	323.5	288 ± 54	318 ± 49	304 ± 15
7	*p*-coumaric acid (deriv)	309.3	519 ± 25	563 ± 27	610 ± 46
8	tryptophan (deriv)	279.6	2600 ± 175	3800 ± 150	208 ± 70
9	*p*-coumaric acid	309.3	623 ± 45	724 ± 59	477 ± 34
10	tryptophan (deriv)	279.6	579 ± 114	680 ± 20	1220 ± 111
11	tryptophan	279.6	733 ±132	960 ± 52	598 ± 35
12	flavonoid glycoside[c]	347.4	4640 ± 140	5090 ± 160	5490 ± 60
13	sinapic acid	323.5	626 ± 43	726 ± 48	321 ± 75
14	ferulic acid (deriv)	323.5	883 ± 30	1140 ± 97	882 ± 40
15	*o*-coumaric acid	271.3, 328.3	410 ± 90	518 ± 88	125 ± 17
16	ferulic acid	323.5	787 ± 77	890 ± 94	127 ± 21
17	syringic acid (deriv)	276.1	265 ± 38	310 ± 39	175 ± 20
18	syringic acid (deriv)	276.1	1020 ± 79	1210 ± 33	2170 ± 17
	total (peaks 3–18)		17 527	21 659	14 229

[a] nq, characterized but not quantified. [b] Derivatives (deriv) have spectral characteristics similar to those of the parent compound used for quantification. [c] Quantified as quercetin equivalents.

the samples were subjected to identical processing and storage conditions in vitro (data not shown); therefore, polyphenolics were not directly implicated as significant contributors to browning reactions in PFJ.

Quality Attributes. Physicochemical analyses were conducted to determine overall changes in antioxidant properties of PFJ during processing and storage, since no reports can be found in the literature. Discoloration due to formation of brown pigments, generally recognized as a quality defect in a variety of foods, was a major problem following storage of PFJ.

Table 2. Total Soluble Phenolics (Folin–Ciocalteu assay) and Lab Color Values of Passion Fruit Juice As Influenced by Ascorbic Acid (500 mg/L) and Sucrose (10% w/v) Fortification As Compared to a Nonfortified Control[a]

	lightness		hue angle (°)		chroma		total soluble phenolics (mg/L)	
	air	deaerated	air	deaerated	air	deaerated	air	deaerated
				Control				
unprocessed	53.4 a[b]	53.8 a	85.7 a	86.4 a	64.8 a	64.9 a	435 b	453 a
day 0	52.8 a	53.2 b	85.6 a	85.9 a	64.5 a	62.7 b	464 a	465 a
day 14	49.4 b	48.8 c*	83.4 b	83.4 b	54.1 b	55.4 c	426 b	426 a
day 28	47.2 c	47.4 d	82.2 c	83.1 b	51.3 c	52.2 d	422 b	422 a
				Ascorbic Acid				
unprocessed	53.6 a	53.7 a	84.6 a	85.9 a*[c]	65.8 a	66.2 a	619 b	723 a*
day 0	52.7 b	53.2 b	84.8 a	85.6 a	64.7 a	63.6 b	702 a	693 a
day 14	42.9 c	43.3 c	79.4 b	79.7 b	50.5 b	50.7 c	542 c	539 b
day 28	40.9 d	41.0 d	78.2 c	78.6 c	46.9 c	49.0 d	540 c	530 b
				Sucrose				
unprocessed	51.2 a	51.2 a	84.6 a	85.8 a	64.1 a	63.6 a	471 a	490 a
day 0	50.5 b	50.4 b	85.5 a	85.6 a	62.6 a	62.4 a	450 a	463 a
day 14	46.8 c	46.4 c	83.1 b	83.2 b	55.2 b	56.3 b	453 a	490 a*
day 28	45.1 d	44.6 d	82.4 c	81.7 c	51.6 c	52.0 c	459 a	457 a

[a] Passion fruit juice was analyzed prior to pasteurization (unprocessed), after pasteurization (day 0), and after 14 and 28 days of storage at 37 °C. [b] Similar letters in each column indicate that the overall effect due to storage within each treatment was not significantly different (LSD test, $P < 0.05$). [c] Values between columns marked with an asterisk indicate a significant difference due to deaeration for a given treatment and storage time.

Fortification with ascorbic acid or sucrose did not improve color values of PFJ during storage (**Table 2**), but rather ascorbic acid accentuated color degradation, as evidenced by HMF formation. Removal of dissolved oxygen (actual concentration not determined) had little effect on color retention and thus indicated a non-oxidative role for brown pigment formation. Pasteurization resulted in negligible changes to juice color, an important factor for overall quality since PFJ is widely incorporated into many processed tropical fruit blends. Lightness values were equivalent in control and sucrose-fortified juices (−6 units) but decreased 13 units for ascorbic acid fortified juices. Corresponding decreases in hue angle and chroma intensities were also observed, indicating that the characteristic bright yellow juice was increasingly masked with brown pigments during storage, appreciably affecting the perceived quality of the juice.

Total Soluble Phenolics and Ascorbic Acid. In contrast to color changes, total soluble phenolics (Folin−Ciocalteu assay) were found to be stable during processing and storage for control and sucrose-fortified juices, with no significant difference found between deaerated and non-deaerated juices (**Table 2**). Polyphenolic stability after processing and storage provides additional evidence that dissolved oxygen had a minor role in phytochemical stability, eliminating an autoxidative mechanism for destruction of reducing compounds. However, fortification with ascorbic acid increased total soluble phenolics by 13% immediately after processing, due to its reducing capacity, only to decrease by 23% after 14 and 28 days of storage. This decrease in total phenolics corresponded to increased juice browning ($r = 0.82$; lightness) and subsequent decreases in ascorbic acid. A 25% loss of ascorbic acid was observed during pasteurization and was absent after 14 days storage at 37 °C due to its instability at elevated temperatures. No naturally occurring L-ascorbic acid was present in the initial stock juice (<5 mg/L), possibly reflective of PFJ extraction or storage conditions prior to arrival for processing; however, dehydroascorbic acid was not determined. Natural ascorbic acid levels have been reported at 40−65 mg/100 g for fresh fruit (*3, 4*). The presence of low HMF concentrations following processing likely indicated that L-ascorbic acid was converted to dehydroascorbic acid during processing, eventually forming brown pigments and HMF in all treatments during storage.

Total Carotenoids. In addition to its unique sensory characteristics, yellow PFJ is characterized by its brilliant yellow color due to its diverse carotenoid content. Although major carotenoids have been identified in passion fruit (*5*), no studies have demonstrated their stability during processing or storage. Total carotenoids were quantified spectrophotometrically ($\epsilon = 2500$) at four wavelengths (378, 401, 427, and 470 nm), each corresponding to one or more predominant carotenoid (**Table 3**). ζ-Carotene, for example, reported to be the predominant carotenoid in PFJ, has absorbance maximums at 378, 400, and 425 nm (*26, 27*) and is likely responsible for predominant spectral bands in the ubiquitous extract. However, various carotenoids exhibit overlapping absorbance maximums at these wavelengths, so only general trends following processing and storage are discussed. Similar to soluble phenolics and color, PFJ carotenoids were not appreciably altered by thermal pasteurization, justifying its use in various juice blends. During storage, decreases were observed in both control and sucrose-fortified juices at all quantification wavelengths. The greatest losses occurred during the first 14 days of storage, and additional losses were not observed with continued storage. Carotenoid losses were independent of deaeration treatments and subjectively coincided with development of negative aromas in the juice that resulted in shelf life termination after 28 days of storage. Sucrose fortification had only a minor protective affect on carotenoids, which were 8−14% higher than in control juices after 14 days of storage. The presence of ascorbic acid fortification gave an impression of increased carotenoid concentrations during storage (+31−63%) due to the hyperchromic shift in absorbance at 378, 401, and 470 nm. Oxidative protection of carotenoids by ascorbic acid has been previously reported (*28−30*) and may be attributed to direct oxidative protection or isomerization reactions resulting in the spectral shifts. Since many tropical fruit juice blends are fortified with ascorbic acid, this protective effect on carotenoids is important for quality and nutritional retention during storage.

Antioxidant Activity. A novel fractionation technique was employed following extraction of a ubiquitous phytochemical isolate that aided in determining overall trends in antioxidant capacity during pasteurization and storage (**Figure 2**). When combined with petroleum ether and water naturally present in

Antioxidants in Yellow Passion Fruit Juice

J. Agric. Food Chem., Vol. 51, No. 4, 2003 **939**

Table 3. Total Carotenoids (mg/L; $\epsilon = 2500$) Present in Passion Fruit Juice Measured at Various Visible Wavelengths As Influenced by Ascorbic Acid (500 mg/L) and Sucrose (10% w/v) Fortification As Compared to a Nonfortified Control[a]

	carotenoid 378 nm		carotenoid 401 nm		carotenoid 427 nm		carotenoid 470 nm	
	air	deaerated	air	deaerated	air	deaerated	air	deaerated
				Control				
unprocessed	22.4 a[b]	24.0 a*[c]	26.2 a	28.9 a*	25.9 a	29.1 a*	9.25 a	10.5 a*
day 0	22.9 a	23.4 ab	27.2 a	28.3 ab	26.9 a	28.4 ab	9.41 a	10.0 ab
day 14	19.6 b	20.6 b	19.1 b	20.9 b	16.8 b	19.0 c	7.19 b	7.89 b
day 28	19.5 b	19.3 b	18.2 b	18.1 b	15.7 b	15.7 d	6.71 b	6.57 b
				Ascorbic Acid				
unprocessed	23.2 b	26.2 a*	27.7 ab	31.9 a*	27.6 a	31.8 a*	9.90 c	11.2 a*
day 0	23.8 b	26.3 a*	28.6 a	32.4 a*	28.5 a	32.7 a*	10.2 bc	11.6 a*
day 14	25.2 a	25.9 a	26.8 b	28.2 b	25.1 b	26.8 b	10.8 ab	11.4 a
day 28	25.8 a	25.1 a	27.5 ab	26.8 b	25.8 b	25.1 b	11.2 a	10.7 a
				Sucrose				
unprocessed	22.6 a	26.1 a*	28.0 a	32.1 a*	27.9 a	32.2 a*	10.1 a	11.7 a*
day 0	22.7 a	25.9 a*	27.5 a	32.2 a*	27.6 a	32.6 a*	9.67 a	11.8 a*
day 14	21.2 b	21.3 b	21.7 b	21.9 b	19.8 b	20.0 b	8.08 b	8.16 b
day 28	21.0 b	21.4 b	20.7 b	21.7 b	18.5 b	19.7 b	7.80 b	8.30 b

[a] Passion fruit juice was analyzed prior to pasteurization (unprocessed), after pasteurization (day 0), and after 14 and 28 days of storage at 37 °C. [b] Similar letters in each column indicate that the overall effect due to treatment and storage was not significantly different (LSD test, $P < 0.05$). [c] Values between columns marked with an asterisk indicate a significant difference due to deaeration for a given treatment and storage time.
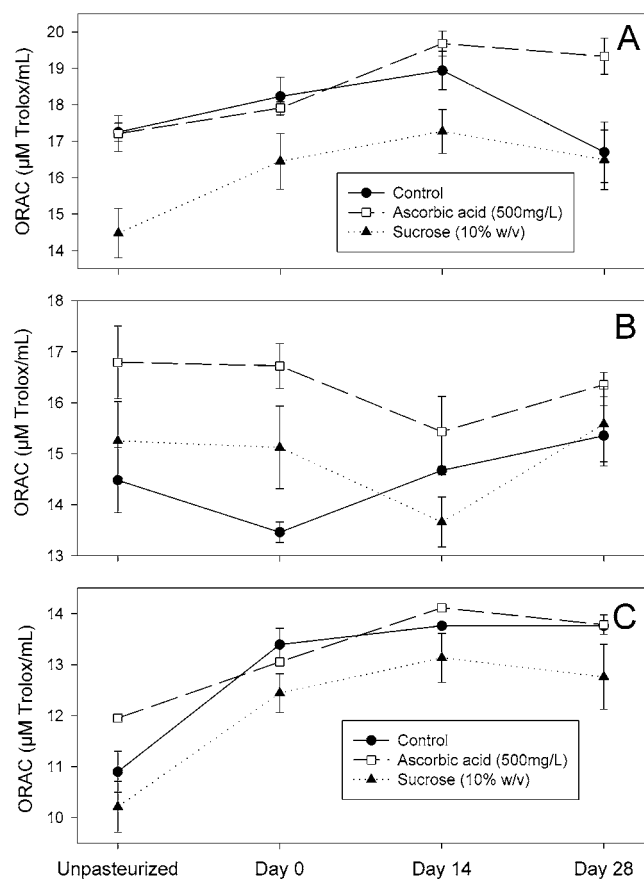

Figure 2. Antioxidant activity (μM Trolox equivalents/mL) of phytochemical fractions obtained from yellow passion fruit juice as affected by ascorbic acid (500 mg/L) and sucrose (10%) fortification. Measurements were taken before and after thermal pasteurization and after storage for 14 and 28 days at 37 °C ($n = 3$). (A) Ubiquitous isolate containing all extractable phytochemicals. (B) Hydrophilic fraction containing primarily polyphenolics and ascorbic acid. (C) Lipophilic fraction, obtained from petroleum ether, containing primarily carotenoids.

the juice, phytochemicals were readily partitioned into ether-soluble (lipophilic) or hydrophilic phases, and each was tested

for radical-scavenging properties. Thermally processed foods may have higher antioxidant capacity than fresh or unprocessed foods due to physicochemical changes that occur during heating, and resultant changes may occur before, during, or after processing, depending on phytochemical composition and storage conditions. Prior to fractionation, antioxidant capacity was assessed on the ubiquitous phytochemical isolate. The unpasteurized, nonfortified PFJ had an antioxidant capacity of 17.2 ± 0.25 μM Trolox equivalents per milliliter that subsequently increased for all juice treatments as a function of thermal processing. Control and ascorbic acid-fortified juices were not significantly different from each other until the end of the shelf life, but sucrose fortification unexpectedly lowered antioxidant capacity values by nearly 2 μM equivalents on average throughout processing and in the first 14 days of storage.

Hydrophilic fractions from unpasteurized, nonfortified PFJ had an initial antioxidant capacity of 14.48 ± 0.64, imparted primarily from polyphenolics and/or other water-soluble compounds. As expected, higher initial antioxidant capacity values were present in ascorbic acid-fortified juices as compared to control and sucrose fortification treatments. Although added ascorbic acid increased initial activity, a corresponding decrease in antioxidant capacity was not observed in response to ascorbic acid losses during storage. However, since nonfortified juices naturally increased in antioxidant capacity after pasteurization (14%), any losses in ascorbic acid affecting antioxidant capacity were negated during storage. Changes in antioxidant capacity observed after pasteurization and storage were relatively small for each juice treatment, indicating a high degree of oxidative stability for hydrophilic antioxidants in PFJ.

Lipophilic antioxidants in PFJ, consisting primarily of carotenoids, followed a trend in antioxidant capacity during processing and storage similar to that observed in the ubiquitous extract ($r = 0.76$). Nonpasteurized control juices had an initial antioxidant capacity of 10.90 ± 0.40. Pasteurization resulted in an antioxidant capacity increase of 9% for ascorbic acid-fortified and 22% for control and sucrose-fortified juices. Increases continued throughout storage, and antioxidant capacity was always higher than that in unpasteurized juices. Fortification with ascorbic acid prevented carotenoid degradation, likely due to the polarity of xanthophylls present, and was reflected by

positive associations between carotenoids and antioxidant capacity at 378 and 470 nm. However, the antioxidant capacity of control and sucrose-fortified juices was negatively correlated to carotenoids on average ($r = -0.62$), indicating that carotenoid losses did not appreciably affect antioxidant properties.

Fractionation of hydrophilic and lipophilic antioxidants from ubiquitous extracts of PFJ revealed that lipophilic compounds were primarily responsible for observed antioxidant increases during pasteurization. When averaged across all treatments and times, the ubiquitous extract had an antioxidant capacity of 17.5 μM Trolox equivalents per milliliter of juice, while the sum for hydrophilic and lipophilic fractions was considerably higher, at 28 μM. Within each fortification and storage time, the sum of fractions ranged from 47 to 76% higher than the value for the ubiquitous extract, potentially indicating interactions affecting radical-scavenging properties.

In Vitro Models. Chemical interactions affecting free radical-scavenging properties between carotenoids and polyphenolics have not been extensively reported in fruits and vegetables, except for in vitro studies between carotenes and tocopherols (*29, 31, 32*), yet both synergistic and antagonistic interactions impacting antioxidant capacity were reported between phytochemicals in various model systems (*33, 34*). Due to a nonadditive effect observed for antioxidants fractionated from PFJ, it was hypothesized that antagonistic or competitive interaction may exist. Two liquid–liquid extraction protocols were separately investigated for phytochemical interactions in unpasteurized PFJ.

The first interaction evaluated the ubiquitous acetone–ethanol extract partitioned as previously described (for processed and stored PFJ) into hydrophilic (ca. 435 mg/L GAE) and lipophilic (ca. 9 mg/L carotenoids at 470 nm) fractions. The second protocol isolated fractions following EA extraction to yield EA-soluble (ca. 50 mg/L GAE), lipophilic (ca. 9 mg/L carotenoids at 470 nm), and EA-insoluble (ca. 400 mg/L GAE) fractions. Isolates from these two protocols were evaluated alone and in combination for potential interactions affecting antioxidant capacity by varying amounts of each fraction while keeping ORAC assay conditions constant.

Acetone–Ethanol Fractionation. Hydrophilic, lipophilic, and ubiquitous extracts of PFJ solubilized from acetone and ethanol were evaluated for potential chemical interactions affecting antioxidant capacity. Lipophilic fractions combined with either hydrophilic or ubiquitous fractions did not result in an interaction since actual antioxidant capacity was within 5% of values predicted from linear regression analysis of each fraction tested individually (data not shown). However, combining hydrophilic and ubiquitous isolates resulted in antioxidant values that were 16–20% lower than values predicted by linear regression (**Figure 3**), indicating either a competitive or an antagonistic response between polar components present in each isolate. Identification of the specified compounds responsible for these responses has not been elucidated, but data suggest a concentration-independent interaction, since amounts of hydrophilic antioxidants were identical (ca. 435 mg/L GAE) at each interaction ratio evaluated.

EA Fractions. Antioxidant phytochemicals extracted with EA differed from those previously evaluated since polyphenolics were limited by their solubility, leaving behind an EA-insoluble fraction with appreciable radical-scavenging properties. HPLC analysis of the EA-insoluble fraction revealed up to eight compounds ranging from 2.5 to 12 mg/L GAE that are currently under elucidation. These compounds were likely responsible for chromatographic interference affecting polyphenolic separation by HPLC. All the compounds exhibited similar spectral proper-
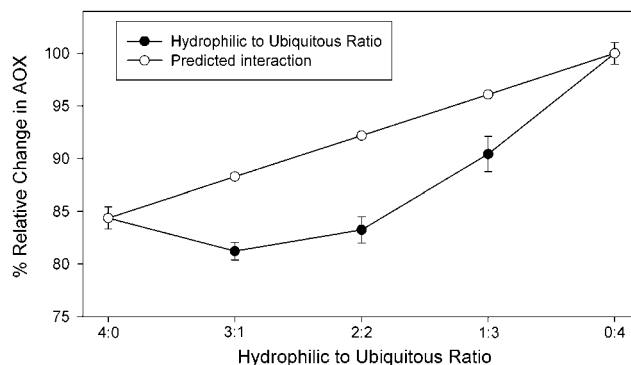

Figure 3. Significant interaction observed between a hydrophilic fraction and ubiquitous extract affecting relative antioxidant capacity (μM Trolox equivalents/mL) in nonpasteurized PFJ (see Materials and Methods). Total soluble phenolics in each combination was 435 mg/L GAE, yet decreases in antioxidant capacity were observed in relation to values that could be predicted from linear regression of each isolate tested individually.
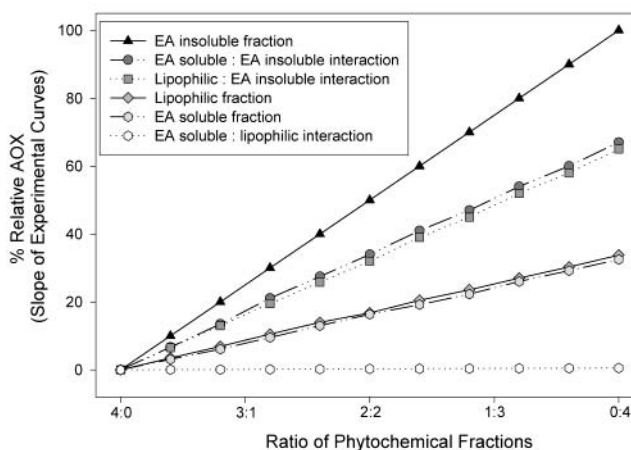

Figure 4. Nonsignificant interactions for antioxidant activity (μM Trolox equivalents/mL) between three phytochemical fractions obtained from ethyl acetate (EA) extracts of nonpasteurized yellow passion fruit juice (lipophilic, EA-soluble, and EA-insoluble; see Materials and Methods). Each interaction, plotted as slopes from linear regression, could be predicted on the basis of antioxidant activity of each fraction tested individually.

ties, with a characteristic absorption band for benzene at 220–250 nm and a single absorption band ranging from 276 to 300 nm. This second extraction protocol also created three fractions (lipophilic, EA-soluble, and EA-insoluble) that when tested individually exhibited a concentration-dependent response to antioxidant capacity. Total antioxidant capacity for these three fractions was 58% lower than values observed for ubiquitous acetone–ethanol extracts, indicating inherent differences in solubility based on solvent selection. Radical inhibition by EA-soluble and lipophilic fractions was nearly identical, accounting for 22 and 26% of total antioxidant capacity, respectively, with EA-insoluble compounds responsible for the remaining activity. Interactions were tested between fractions as previously described, but no discernible interactions were observed. Each combination of fractions resulted in an antioxidant value that could be predicted from linear regression of each fraction tested individually (**Figure 4**). Compounds that presented the competitive response in the previous fractions were likely combined in this second extraction protocol. The lack of interaction was indicative of the diversity of compounds present in PFJ that affect overall antioxidant properties.

Passion fruit contains a diversity of phytochemical compounds contributing to sensory, antioxidant, and quality characteristics

Antioxidants in Yellow Passion Fruit Juice

J. Agric. Food Chem., Vol. 51, No. 4, 2003 **941**

of processed juice. Ascorbic acid and sucrose fortification had an overall preservation effect on total carotenoids, while polyphenolics were found to be very stable during processing and storage. The time and temperature sensitivity of ascorbic acid, brown color formation, and the development of off-odors during storage are issues that affect product quality and should be considered as markers for quality retention. However, the inherent physicochemical stability of PFJ phytochemicals, along with potential health benefits of its consumption, are important factors leading to crop expansion and increased consumption as single-strength juice or as a key ingredient in tropical fruit beverages.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Morton, J. Passionfruit. In *Fruits of warm climates*; Dowling, C. F., Jr., Ed.; J.F. Morton: Miami, FL (distributed by Creative Resource Systems, Inc.: Winterville, NC), 1987; pp 320−328.

(2) Frei, B. *World Crop Reports: Passion Fruit*; Presented at the International Federation of Fruit Juice Processors, November 2001, Sydney, Australia.

(3) Vinci, G.; Botre, G.; Mele, G. Ascorbic acid in exotic fruits: a liquid chromatographic investigation. *Food Chem.* **1995**, *53*, 211−214.

(4) Suntornsuk, L.; Gritsanapun, W.; Nilkamhank, S.; Paochom, A. Quantitation of vitamin C content in herbal juice using direct titration. *J. Pharm. Biomed. Anal.* **2002**, *28*, 849−855.

(5) Mercadante, A. Z.; Britton, G.; Rodriguez-Amaya, D. B. Carotenoids from Yellow Passion Fruit (*Passiflora edulis*). *J. Agric. Food Chem.* **1998**, *46*, 4102−4106.

(6) Werkhoff, P.; Guntert, M.; Krammer, G.; Sommer, H.; Kaulen, J. Vacuum headspace method in aroma research: flavor chemistry of yellow passion fruits. *J. Agric. Food Chem.* **1998**, *46*, 1076−1093.

(7) Chassagne, D.; Boulanger, R.; Crouzet, J. Enzymatic hydrolysis of edible *Passiflora* fruit glycosides. *Food Chem.* **1999**, *66*, 281−288.

(8) Tominaga, T.; Dubourdieu, D. Identification of Cysteinylated Aroma Precursors of Certain Volatile Thiols in Passion Fruit Juice. *J. Agric. Food Chem.* **2000**, *48*, 2874−2876.

(9) Beutner, S.; Bloedorn, B.; Frixel, S.; Blanco, I. H.; Hoffmann, T.; Martin, H. D.; Mayer, B.; Noack, P.; Ruck, C.; Schmidt, M.; Schulke, I.; Sell, S.; Ernst, H.; Haremza, S.; Seybold, G.; Seis, H.; Stahl, W.; Walsh, R. Quantitative assessment of antioxidant properties of natural colorants and phytochemicals: carotenoids, flavonoids, phenols and indigoids. The role of *β*-carotene in antioxidant functions. *J. Sci. Food Agric.* **2001**, *81*, 559−568.

(10) Lowe G. M.; Young, A. J. Antioxidant and prooxidant properties of carotenoids. *Arch. Biochem. Biosyn.* **2001**, *385*, 20−27.

(11) Mascio, P. D.; Matos, H. R.; Medeiros, M. H. Protective effect of lycopene on lipid peroxidation and oxidative DNA damage in cell culture. *Arch. Biochem. Biosyn.* **2000**, *383*, 56−59.

(12) Miller, N. J.; Sampson, J.; Candeias, L. P.; Bramley, P. M.; Rice-Evans, C. A. Antioxidant activities of carotenes and xanthophylls. *FEBS Lett.* **1996**, *384*, 240−242.

(13) Nguyen, M. L.; Schwartz, S. J. Lycopene: Chemical and biological properties. *Food Technol.* **1999**, *53*, 38−45.

(14) Lavelli, V.; Peri, C.; Rizzolo, A. Antioxidant activity of tomato products as studied by model reactions using xanthine oxidase, myeloperoxidase, and copper induced lipid peroxidation. *J. Agric. Food Chem.* **2000**, *48*, 1442−1448.

(15) Takeoka, G. R.; Dao, L.; Flessa, S.; Gillespie, D. M.; Jewell, W. T.; Huebner, B.; Bertow, D.; Edeler, S. E. Processing effects on lycopene content and antioxidant activity of tomatoes. *J. Agric. Food Sci.* **2001**, *49*, 3713−3717.

(16) Bohm, V.; Puspitasari, P. L.; Feruzzi, M. G.; Schwartz, S. J. Trolox equivalent antioxidant capacity of different geometrical isomers of α-carotene, *β*-carotene, lycopene, and zeaxanthin. *J. Agric. Food Chem.* **2002**, *50,* 221−226.

(17) Talcott, S. T.; Howard, L. R. Phenolic autoxidation is responsible for color degradation in processed carrot puree. *J. Agric. Food Chem.* **1999**, *47*, 2109−2115.

(18) Gross, J. Carotenoids. In *Pigments in Vegetables: Chlorophylls and Carotenoids*; Van Nostrand Reinhold: New York, 1991; p 100.

(19) Cao, G.; Alessio, H. M.; Culter, R. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biol. Med.* **1993**, *14*, 303−311.

(20) Cao, G.; Verdon, C. P.; Wu, A. H. B.; Wang, H.; Prior, R. L. Automated assay of oxygen radical absorbance capacity with the COBAS FARA II. *Clin. Chem.* **1995**, *41*, 1738−1744.

(21) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* **2001**, *49*, 4619−4626.

(22) Swain, T.; Hillis, W. E. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *J. Sci. Food Agric.* **1959**, *10*, 63−68.

(23) Talcott, S. T.; Brenes, C. H.; Howard, L. R. Contribution of periderm material and blanching time to the quality of pasteurized peach puree. *J. Agric. Food Chem.* **2000**, *48*, 4590−4596.

(24) Gokman, V.; Kahranam, N.; Demir, N.; Acar, J. Enzymatically validated liquid chromatographic method for the determination of ascorbic and dehydroascorbic acids in fruit and vegetables. *J. Chromatogr. A* **2000**, *881*, 309−316.

(25) *JMP*; SAS Institute, Inc., SAS Campus Drive, Cary, NC, 1996.

(26) De Ritter, E.; Purcell, A. E. Carotenoid Analytical Methods. *Carotenoids as Colorants and Vitamin A Precursors: Technical and Nutritional Applications*; Academic Press Inc.: New York. 1981; pp 815−882.

(27) Britton, G. *Carotenoids, Volume 1B: Spectroscopy*; Birkhauser Verlag: Boston, 1995.

(28) Klaui, H.; Bauernfeind, J. C. Carotenoids as food color. In *Carotenoid as colorants and vitamin A precursors*; Bauernfeind, J. C., Ed.; Academic Press: New York, 1981.

(29) Bohm, F.; Edge, R.; Land, E. J.; McGarvey, D. J.; Truscott, T. G. Carotenoids enhance vitamin E antioxidant efficiency. *J. Am. Chem. Soc.* **1997**, *119*, 621−622.

(30) Biacs, P. A.; Daood, H. G. Lipoxygenase-catalysed degradation of carotenoids from tomato in the presence of antioxidant vitamins. *Biochem. Soc. Trans.* **2000**, *28*, 839−845.

(31) Palozza, P.; Krinsky, N. I. *β*-Carotene and a-tocopherol are synergistic antioxidants. *Arch. Biochem. Biophys.* **1992**, *297*, 184−187.

(32) Haila, K. M.; Lievonen, S. M.; Heinonen, M. I. Effects of lutein, lycopene, annatto, and *γ*-tocopherol on autoxidation of triglycerides. *J. Agric. Food Chem.* **1996**, *44*, 2096−2100.

(33) Howard, L. R.; Talcott, S. T.; Brenes, C. H.; Villalon, B. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* Species) as influenced by maturity. *J. Agric. Food Chem.* **2000**, *48*, 1713−1720.

(34) Saucier, C. T.; Waterhouse, A. L. Synergetic activity of catechin and other antioxidants. *J. Agric. Food Chem.* **1999**, *47*, 4491−4494.