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Pigments in Avocado Tissue and Oil

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Pigments are important contributors to the appearance and healthful properties of both avocado fruits and the oils extracted from these fruits. This study determined carotenoid and chlorophyll pigment concentrations in the skin and three sections of the flesh (outer dark green, middle pale green, and inner yellow flesh—nearest the seed) and anthocyanin concentrations in the skin of Hass avocado during ripening at 20 °C. Pigments were extracted from frozen tissue with acetone and measured using high-performance liquid chromatography. Pigments were also measured in the oil extracted from freeze-dried tissue sections by an accelerated solvent extraction system using hexane. Carotenoids and chlorophylls identified in the skin, flesh, and oil were lutein, α-carotene, β-carotene, neoxanthin, violaxanthin, zeaxanthin, antheraxanthin, chlorophylls a and b, and pheophytins a and b with the highest concentrations of all pigments in the skin. Chlorophyllides a and b were identified in the skin and flesh tissues only. As the fruit ripened and softened, the skin changed from green to purple/black, corresponding to changes in skin hue angle, and a concomitant increase in cyanidin 3-*O*-glucoside and the loss of chlorophyllide a. In flesh tissue, chroma and lightness values decreased with ripening, with no changes in hue angle. The levels of carotenoids and chlorophylls did not change significantly during ripening. As fruit ripened, the total chlorophyll level in the oil from the flesh sections remained constant but declined in the oil extracted from the skin.

KEYWORDS: Avocado oil; carotenoids; chlorophylls; anthocyanin; carotene; lutein; color; accelerated solvent extraction (ASE); (*Persea americana,* Mill.)

INTRODUCTION

Avocados are known to contain considerable quantities of plant pigments including chlorophylls, carotenoids, and anthocyanins (*1*−*4*). Among fruits, avocados are unusual in that they contain high amounts of oil and have been used for oil production (*5*). However, early commercial extraction of avocado oil was carried out using "harsh" systems such as high temperature and/or solvents and the resulting oil was then refined. These resulted in oils devoid of pigments and used primarily for cosmetics (*6*). Recently, New Zealand companies have commenced cold-pressed extraction of avocado oil for the high-value culinary market. In these oils, the concentration and nature of the pigments are important determinants of quality and marketability. The pigments in cold-pressed avocado oil may also have a range of health-related benefits. For example, lutein has been reported to be beneficial for the reduction of risk of age-related macular degeneration (*7*, *8*).

Lu et al. (*9*) found that ripe Hass avocado flesh contained the following pigments and concentrations: 2.93 μg g^{-1} lutein, 0.11 μg g^{-1} zeaxanthin, 0.25 μg g^{-1} β-cryptoxanthin and α-carotene, and 0.60 μg g^{-1} β-carotene. In Nabal avocados, lutein was the carotenoid present in the highest concentration, with total carotenoids ranging from 10 to 14 μg g^{-1} fresh weight (FW). In this cultivar, the skin contained 40 μg g^{-1} FW (*10*), while the lutein concentration in the flesh was about 50% of that in the skin. Cox et al. (*4*) characterized anthocyanin and chlorophyll pigments in the skin of Hass avocado during ripening. The concentration of a single anthocyanin (cyanidin 3-*O*-glucoside) was found to increase dramatically as the skin changed color ("purpling") and the fruit softened. They found that chlorophyll concentrations 1 day after harvest were a little higher (0.63 μg g^{-1}) than in ripe fruit (0.50−0.57 μg g^{-1}), while Woolf and Laing (*11*) observed relatively little difference, possibly because their first sample was taken 2−3 days after harvest. Cran and Possingham (*2*) examined the chlorophyll content in Fuerte and found that in "mature harvest-ripe" flesh,

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the chlorophyll contents of the different flesh sections were 316 (skin), 101 (dark green flesh), 51 (pale green), and 38 μg g^{-1} FW (yellow). However, other pigments, such as carotenoids, were not examined.

Cold-pressed avocado oil in New Zealand has been reported to have high concentrations of chlorophyll (40−60 μg g^{-1}) and carotenoids such as lutein (*12*−*14*). Lutein concentrations of 3.2 μg g^{-1} have been reported in cold-pressed oil extracted from the cultivar Hass, although concentrations can be less than 1 or more than 5 μg g^{-1} in other cultivars (*14*). In cold-pressed olive oil (a plant fruit oil extracted in a similar manner to avocado oil), the lutein concentration ranged from 0.2 to 3.9 μg g^{-1} and the β-carotene concentration ranged from 0.4 to 5.1 μg g^{-1} (*15*).

During the production of oil from avocado fruit, it is likely that not all pigments are extracted into the oil because of differences in the solubility of the pigments. For example, anthocyanins are water soluble whereas carotenoids and chlorophylls are lipid soluble. In addition, all oil soluble pigments in the tissues may not be completely extracted into the oil.

Thus, although there has been some study of pigments in Hass avocados, changes of all pigments during the ripening period and the amounts of pigments present in the oil have not been examined. From a commercial oil extraction perspective, these factors have implications in terms of the tissue types that are processed and their impact on oil quality. In this study, we have determined the pigment composition at different stages of ripening in Hass avocado skin and three sections of the flesh: dark green flesh (adjacent to the skin), pale green flesh, and yellow flesh (adjacent to the seed). In addition, we investigated the pigment composition of oil extracted from these tissue sections using hexane—a standard lipid extraction solvent.

MATERIALS AND METHODS

Experimental Overview. Hass avocados (*Persea americana* Mill.) were harvested and ripened at 20 °C, during which time the skin color and fruit firmness of intact fruits were assessed. During ripening, four types of tissue samples were collected as follows: skin, dark green flesh (adjacent to the skin), pale green flesh, and yellow flesh (adjacent to the seed). The colors of each of these tissue types were measured using a Minolta chromameter. Carotenoid (extracted by acetone) and chlorophyll (extracted by diethyl ether) concentrations were determined in the flesh and skin samples, and anthocyanins were measured in the skin only. Oil was extracted from each tissue type by hexane solvent extraction, and the pigment composition was then measured.

Reagents. The solvents used to extract pigments (diethyl ether, hexane, and acetone) were analytical grade, and HPLC (high-performance liquid chromatography) grade solvent was used for the HPLC mobile phases (methanol, acetonitrile, and ethyl acetate). Butylated hydroxyl toluene (BHT), $NaCO_3$, Na_2SO_4, and NaCl were analytical grade. Authentic standards of lutein, β-carotene, chlorophyll a, and chlorophyll b were purchased from Sigma (Sydney, Australia) and for anthocyanins, cyanidin 3-*O*-glucoside was purchased from Extrasynthese (Genay, France). Authenthic standards of antheraxanthin, violaxanthin, and zeaxanthin were a generous gift from Dr. P. Molnár, University of Pécs. Standards were prepared, and concentrations were determined using spectrophotometric measurements and published extinction coefficients (*19*).

Avocado Fruit. Hass avocados were harvested from a commercial orchard in Whangarei, New Zealand, in September (early summer—start of commercial harvest season) and transported to HortResearch, Mt. Albert Research Center, Auckland, for processing. At each sampling time, three replicates of 15 fruits were selected. Fruit firmness and skin color were measured on whole fruits before skin and flesh sampling. On arrival at HortResearch (2 days postharvest), fruits were randomized and graded for uniformity and quality, and the first sample was taken. Dry matter (DM; reflecting fruit maturity), initial skin color, and firmness measurements were made. The remaining avocados were treated with ethylene to accelerate ripening and reduce variability. The fruits were treated with ethylene (100 μL L^{-1} at 20 ± 1 °C) for 2 days. After ethylene treatment, the fruits were held at 20 ± 1 °C. Ethylene-treated fruits were sampled on days 3 (halfway through ethylene treatment), 4 (end of ethylene treatment), 6, 8, 9, and 13 after harvest.

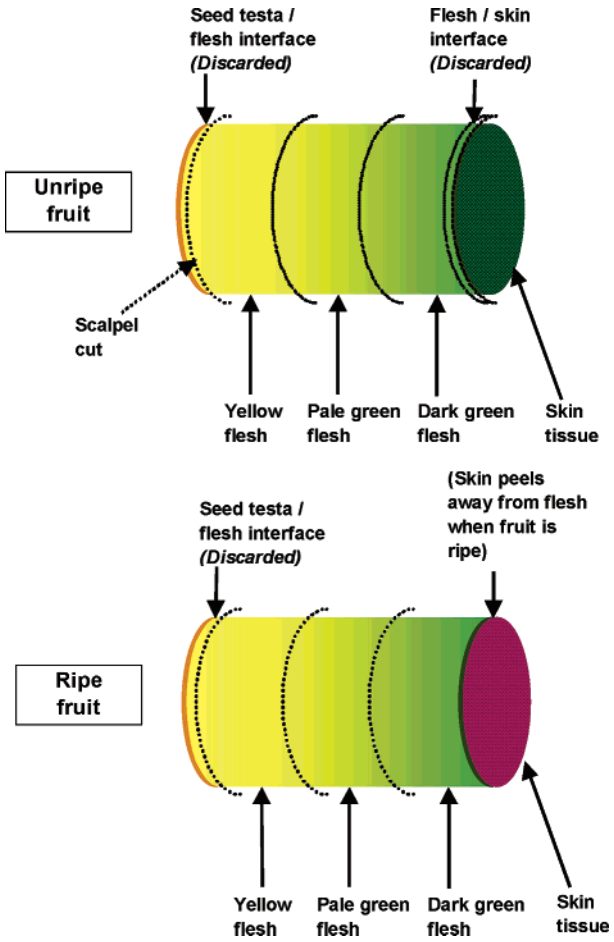

Figure 1. Diagram of tissue sampling system (7 mm diameter plug; area of 38.5 mm^2) and the tissue types sampled from unripe and ripe Hass avocados.

Skin and Flesh Sampling. The avocados were sampled by removing 6−8 plugs of skin (exocarp) and flesh (endocarp) with a 7 mm (i.d.) cork borer from the equatorial region. Each plug was divided into four sections: skin, dark green, pale green, and yellow flesh (**Figure 1**). The three flesh sections were of equal thickness (i.e., each of one-third of the flesh thickness). Sections from the 15 fruits of each of the three replicates were pooled for each flesh type, flash frozen with liquid nitrogen, and stored at −80 °C until analyzed.

DM Determination. DM (a standard measure of fruit maturity) was determined and found to be 35.2 ± 0.3% w/w, indicating a high level of fruit maturity (*16*).

Color Measurement. A Minolta Chromameter (CR300, Minolta, Osaka, light source D_{65}; white calibration plate) was used to measure lightness, chroma, and hue angles at three points around the equator of the whole fruit. For the skin and flesh sections, both sides of each section were measured with the chromameter immediately after sectioning, and the mean of the two values was determined.

Fruit Firmness Measurement. The firmness of whole fruit was measured using an Anderson digital Firmometer (Anderson Manufacturing, Tauranga, New Zealand) using a 200 g weight (*17*). The Firmometer reading (mm displacement) multiplied by 10 was the Firmometer value (FV). The FV was therefore a measure of fruit firmness, and as the fruit softened, the FV increased.

Oil Extraction. *Freeze Drying.* Frozen pooled sections of skin and flesh were freeze-dried (20 °C) and stored in oxygen-free N_2 (99.99%

purity) flushed laminated foil bags with an oxygen absorber sachet (Ageless, FX-20E, Mitsubishi Gas Chemical Co. Inc., Japan) at −25 °C until oil extraction. The sample was ground to a fine powder immediately before solvent extraction of the oil.

Accelerated Solvent Extraction (ASE). Solvent extraction of oil from freeze-dried tissue was carried out using the ASE (ASE 300 Dionex Corp., Sunnyvale, CA). A weighed ground sample of ≅20 g was placed in a 100 mL stainless steel closed cell fitted with a cellulose filter. Extractions were performed under minimal light (0.001 μmol s^{-1} m^{-2}) using 100% hexane (liquid chromatography LiChrosolv). Extraction conditions involved a 5 min sample heating time to 60 °C followed by 100 min of total extraction time at 10000 kPa. The run was split into five cycles of 20 min with a N_2 gas purge cycle of 90 s. Here and elsewhere, all N_2 used was oxygen-free (99.99% purity). The oil dissolved in the solvent was collected in dark glass bottles, which were N_2 flushed during and after extraction by the ASE 300.

Oil Drying/Storage. After oil extraction, hexane was removed over 2 h at 30 °C using a Rapid Vap unit (RapidVap N_2 Evaporation Systems, Labconco Corp., Kansas City, MO) under flowing N_2. The oil yield was expressed as % oil per dry weight of avocado tissue. The oil samples were poured into dark glass bottles, flushed with oxygen-free N_2, and stored at −80 °C until analysis.

Carotenoid and Chlorophyll Compositional Analysis. *Skin and Flesh.* Chlorophyll and carotenoid concentrations were determined in the skin and flesh sections. Because anthocyanins are only present in the skin (*4*) and as there were no obvious indications of red pigments in the flesh, only the skin was analyzed for anthocyanin concentrations. Extraction of carotenoids was carried out under yellow lighting to minimize degradation. Chlorophyll and carotenoids were extracted from avocado tissue using the methods described by Cano (*18*) with some modification. Five milliliters of 0.1% BHT in acetone and 100 mg of $NaCO_3$ were added to approximately 1 g of flesh or skin. The sample was macerated using an Ultra Turrax homogenizer at maximum speed for 1−2 min (Jancke and Kundel, Germany), and then, 500 mg of Na_2SO_4 was added. The test tube was flushed with N_2, sealed, and left overnight at 4 °C to ensure complete extraction. After it was rewarmed, 2 mL of diethyl ether and 8 mL of 10% NaCl were added to 2 mL of extract and mixed by shaking. The solution was centrifuged (Labofuge GL, Heraeus Christ, Germany) for 10 min at 2900*g*. The upper layer was collected into a separate test tube, and the extraction was repeated by adding a further 2 mL of diethyl ether to the remaining aqueous layer and recentrifugation. The combined diethyl ether extract was evaporated to dryness with N_2 at 30 °C. The concentrate was redissolved in 0.8 mL of 0.1% BHT/acetone, mixed with a vortex, and centrifuged for 20 min at 2900*g*. The supernatant was analyzed by HPLC.

Oil from Flesh Sections. Solid-phase extraction (SPE) was used to isolate carotenoids and chlorophylls from oil followed by HPLC to quantify the pigment concentrations. Avocado oil (approximately 0.8 g) was diluted with 4 mL of hexane containing 1% BHT and applied to a SPE cartridge (C18, 500 mg, p/n 305250, Alltech, Derrfiled, IL) that had previously been conditioned with 8 mL of acetone followed with 8 mL of hexane. The SPE cartridge was then washed with 4 mL of hexane, and then, the lutein and chlorophyll were eluted with 4 mL of acetone. The total volume of the extract was adjusted to 5 mL with acetone containing 0.8% BHT. During this procedure, lipids, including α- and β-carotene, were eluted with the hexane wash; therefore, α- and β-carotene in the oil was not measured by this method.

HPLC Procedure for Carotenoids and Chlorophylls. A Waters HPLC (model 2690, Milford, MA) equipped with PDA (model 996) and fluorescence (model 474) detectors was used. The separation column was a Waters Spherisorb 5 μm ODS2 4.0 mm × 250 mm. A ternary gradient system of A (20% 0.5M ammonium acetate, 80% methanol), B (10% H_2O, 90% acetonitrile), and C (ethyl acetate) as described by Wright et al. (*20*) was used. The gradient program started with 100% A. After 4 min, the mobile phase was 100% B, which then changed to 20% B and 80% C at 18 min and maintained until 22 min. The mobile phase then returned to the starting composition by changing 100% B by 25 min and then 100% A at 29 min. The total sample run time was 35 min. All solvent gradients were linear. The flow rate was 1 mL min^{-1}. Carotenoids were measured by extracting chromatograms at 455 nm and were quantified as lutein equivalents. Carotenoids were identified by comparison with authenthic standards; neoxanthin was tentatively identified from retention times and spectra (*19*). Chlorophylls were measured by fluorescence (excitation, 440 nm; emission, 460 nm) and quantified as chlorophyll b equivalents. Chlorophyll a and b peaks were identified by comparison of retention times with authentic standards. Pheophytins a and b and chlorophyllides a and b were identified at their expected retention times and by the similarity of UV/vis spectra to chlorophylls a and b, respectively (*19*).

Anthocyanin Compositional Analysis of Skin Tissue. Eight milliliter of ethanol/H_2O/acetic acid (80/20/1) was added to about 1 g of skin and macerated with an Ultra Turrax homogenizer. The test tube was capped and left at 4 °C for 48 h to extract. Following centrifugation (2900*g*), a portion of the supernatant was transferred to a 2 mL HPLC vial and the anthocyanins content was measured by HPLC.

HPLC Procedure for Anthocyanins. The Waters HPLC, as described before for carotenoid and chlorophyll analysis, and a Merck LiChrospher 100, RP18e 5 μm, 4.0 mm × 250 mm column, were used for anthocyanin separation. A binary gradient system consisting of A (1.5% phosphoric acid) and B (acetic acid/acetonitrile/phosphoric acid/H_2O (20:24:1.5:54.5) was used. The initial mobile phase composition was 80% A and 20% B. The composition changed with a linear gradient to 30% A and 70% B at 25 min and 10% A and 90% B at 30 min. The 10% A and 90% B composition was held for 5 min until 35 min and then returned to the initial mobile phase composition by 40 min. The total sample run time was 45 min. The flow rate was 1.0 mL min^{-1}. Anthocyanins were quantified as cyanidin 3-*O*-glucoside equivalents from chromatograms extracted at 530 nm.

Statistical Analysis. Separate multiple regression models were fitted to the chlorophyll and hue angle data sets to determine the linear effects of tissue type and days after harvest, since these attributes did not show evidence of an apparent trend over time. The chlorophyll data were transformed using the logarithmic transformation before model fitting to ensure that model assumptions were met. To account for variability over time in the composition of the other fruit attributes, separate exponential curves were fitted to the daily readings. Analysis of certain parameters of these curves allowed comparison of the tissue sections (*21*). The parameter estimates were analyzed using analysis of variance. The analyses were performed using GenStat (Release 8.1) [(PC/Windows XP) Copyright 2003, Lawes Agricultural Trust (Rothamsted Experimental Station, United Kingdom)]. Mean separation tests were carried out using Fisher's Protected LSD at the 5% level of significance. Model adequacy checks were performed by examining various plots (histograms, normal probability plots, and scatter plots) of the residuals.

RESULTS

Ripening of Hass. *Firmness.* The Firmometer value of the avocado measured from day 2 to day 13 increased from 9 to ≅100 as the fruit ripened (data not shown). A Firmometer value of 60 indicates "firm ripe", 80 "soft ripe", and 100 "over ripe", which were reached on approximately days 8, 9.5, and 13, respectively. Thus, fruit were ripened well past a fully eating ripe stage of firmness and the full range of fruit firmness that might exist in a commercial system was examined.

Skin and Flesh Color. The skin of the intact fruit decreased in lightness (from ≅40 to 27) and chroma (from ≅15 to 3) over the ripening period, but hue angle increased (from ≅125 to 250°) (**Figure 2a−c**). The color of the skin sections showed similar trends with decreased chroma (from ≅24 to 12) and increased hue angle (from ≅100 to 220°), although lightness showed first an increase and then a decrease. The difference between the skin color of the whole fruit and the skin sections was because the skin section values are an average of measures made on both inner and outer surfaces (as was carried out for sections of the flesh).

The color of the three flesh sections showed significant ($P < 0.001$) changes during the ripening period (**Figure 2a−c**). In all flesh tissue types, chroma decreased from ≅43 to 35. Similarly, lightness decreased in a linear manner from ≅74 to

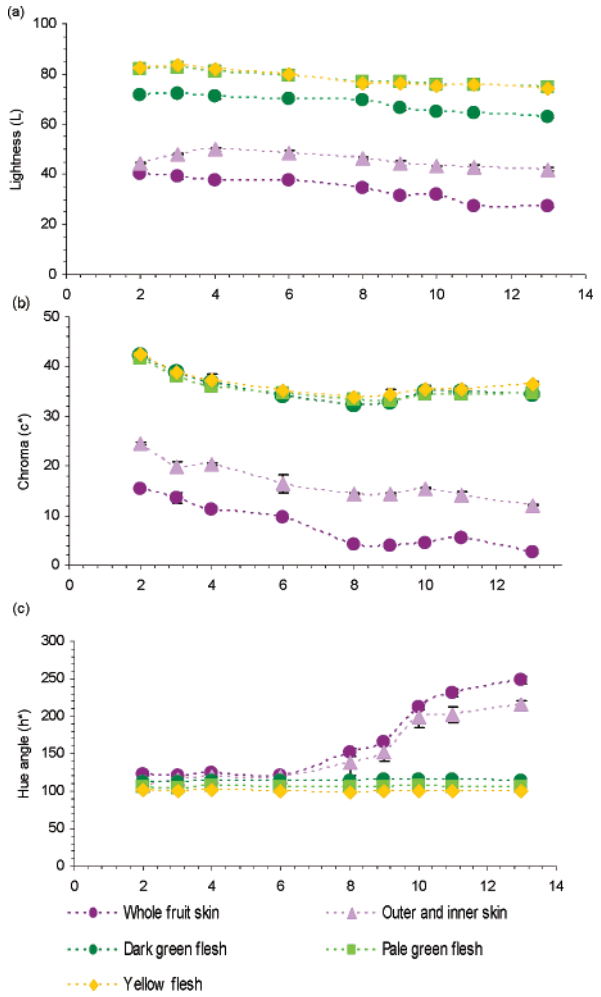

Figure 2. Color changes during ripening of Hass avocado whole fruit (skin) and four tissue sections: skin, dark green flesh, pale green flesh, and yellow flesh. (**a**) Lightness, (**b**) chroma, and (**c**) hue angle. Data are presented as means ± SEM ($n = 45$).

64 for dark green flesh, ≅83 to 75 for pale green flesh, and 83 to 75 for yellow flesh. At day 2, hue angles for each of the tissue types were significantly different (≅113, 105, and 101° for dark green, pale green, and yellow flesh tissue, respectively) and showed only slight decreases over time.

Pigments Extracted from Skin and Flesh Sections. *Carotenoids.* The concentrations of total carotenoids were different between the tissue types ($P < 0.001$) with the concentrations in the skin greater than concentrations in the flesh tissues (**Figure 3a−d**). Total carotenoid concentrations in the skin and flesh tissues declined in an approximately exponential manner as the fruit ripened ($P < 0.001$). Lutein was the most abundant of carotenoids found in Hass avocado skin, with an initial concentration of 20.5 μg g^{-1} declining to 8.0 μg g^{-1} at day 13 (**Figure 3a**). Other carotenoids in skin tissue at 2 days after harvest were, in decreasing order of abundance, β-carotene (≅half that of lutein, at day 2), neoxanthin, violaxanthin, α-carotene (≅5 μg g^{-1}), and antheraxanthin and zeaxanthin (≅1 μg g^{-1}). All carotenoids decreased with ripening.

In the three flesh sections, carotenoid concentrations were significantly lower, approximately 10% of those found in the skin (**Figure 3b−d**). Lutein was the most abundant carotenoid in the dark green, pale green, and yellow flesh sections throughout the ripening period, except at day 2 and 3 when there were higher concentrations of neoxanthin (which later declined to <0.4 μg g^{-1} with ripening). Lutein concentrations

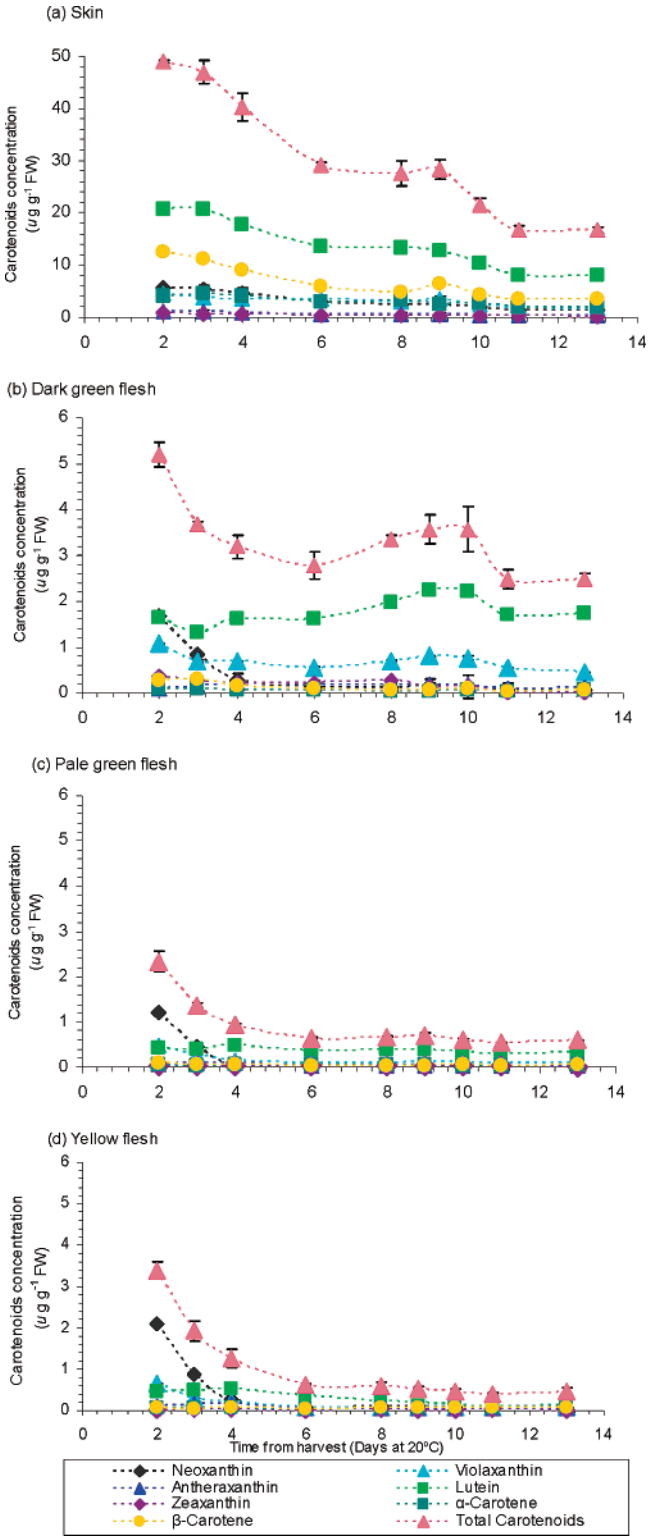

Figure 3. Carotenoid pigment concentrations in Hass avocado (**a**) skin, (**b**) dark green flesh, (**c**) pale green flesh, and (**d**) yellow flesh during ripening. Data are presented as means ± SEM ($n = 3$).

in the flesh tissues did not vary significantly over the ripening period with a mean concentration in the dark green, pale green, and yellow flesh of 1.8, 0.4, and 0.3 μg g^{-1}, respectively. β-Carotene, α-carotene, violaxanthin, antheraxanthin, and zeaxanthin remained relatively constant at very low concentrations (<0.1 μg g^{-1}).

Chlorophyll. Total chlorophyll concentrations were higher in skin tissue than in flesh tissue ($P < 0.001$; **Figure 4a−d**). The

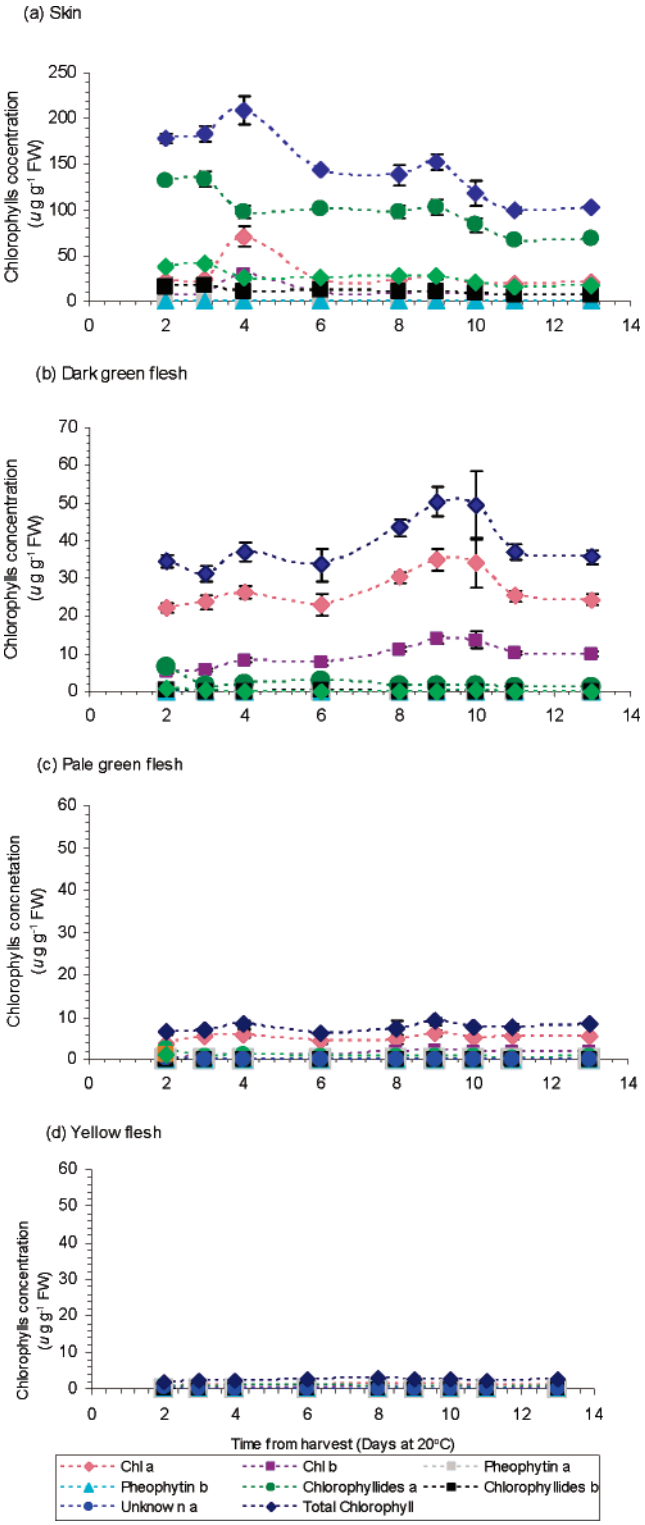

Figure 4. Chlorophyll pigment concentrations in Hass avocado (**a**) skin, (**b**) dark green flesh, (**c**) pale green flesh, and (**d**) yellow flesh during ripening. Data are presented as means ± SEM ($n = 3$).

chlorophyll content was highest in dark green flesh, lower in pale green, and lowest in yellow flesh.

The concentration of chlorophyll a was higher than that of chlorophyll b in the skin and flesh sections (**Figure 4a−d**). Chlorophyll a and b concentrations did not change in skin or flesh during ripening. Pale green and yellow flesh contained less chlorophylls a and b than the dark green flesh and skin.

Several metabolites of chlorophyll were also detected and quantified as chlorophyll b equivalents. Chlorophyllide a

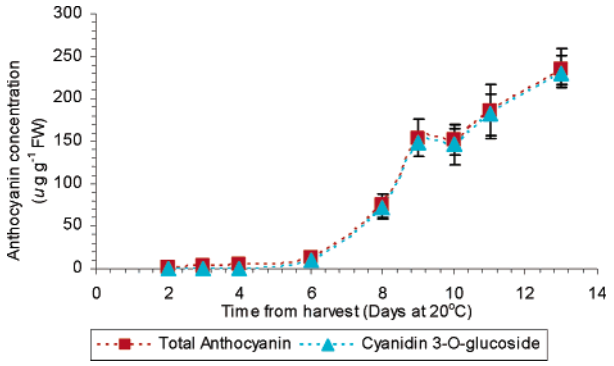

Figure 5. Total anthocyanin and cyanidin 3-*O*-glucoside concentrations in Hass avocado skin tissue. Data are presented as means ± SEM ($n = 3$).

(removal of the phytol group) was the highest in skin tissue 2 days after harvest (131.2 μg g^{-1}) and declined with ripening in all tissue types (except in the yellow flesh where it remained constantly low) (**Figure 4a−d**). Chlorophyllide a in all flesh sections was about 1−5% of that found in the skin. Chlorophyllide b concentrations were significantly lower (maximum 16 μg g^{-1}) than chlorophyllide a and declined to virtually zero during ripening, as did chlorophyllide a over time. Low concentrations (0.10 μg g^{-1}) of pheophytins a and b were detected in skin tissue but not in any flesh tissues (data not shown).

Anthocyanin. In skin tissue, anthocyanin concentrations increased continuously during ripening of Hass avocado after day 4 (**Figure 5**). Cyanidin 3-*O*-glucoside accounted for almost the entire observed anthocyanin concentration. For example, after 13 days, the cyanidin 3-*O*-glucoside concentration was 230 μg g^{-1} while the total anthocyanin concentration was 235 μg g^{-1}.

Pigments in Oil Extracted from Tissue Sections. *Carotenoids in Oil.* The highest concentrations of carotenoids were found in oil extracted from the skin, at approximately 10 times the concentrations of carotenoids in oil extracted from the flesh tissues (**Figure 6**). The total carotenoid concentration in oils extracted from skin and flesh tissues declined as the fruit ripened ($P < 0.001$). In oil from the skin, lutein was the carotenoid present at the greatest concentration of 151.0 μg g^{-1} for day 2 samples, declining to 79.7 μg g^{-1} by day 13 (**Figure 6a**). In day 2, oil samples from the skin, the other carotenoids, in decreasing order of concentration were antheraxanthin (37.3 μg g^{-1}), neoxanthin (13.9 μg g^{-1}), and violaxanthin (4.6 μg g^{-1}). Zeaxanthin was not detectable in oils extracted from either skin or flesh samples.

Over the ripening period, the lutein concentration in the oil from the dark and pale green sections did not vary significantly from day 2 to day 13 (average concentrations of 6.8 and 1.7 μg g^{-1}, respectively). In yellow flesh, lutein extracted into the oil decreased from ≅3 to <1 μg g^{-1}. Neoxanthin was present in relatively high concentrations in oil from all flesh sections at 6−8 μg g^{-1} at day 2 but declined to zero after 6 days. The concentrations of violaxanthin and antheraxanthin were lower at day 2 with ≅3−5 μg g^{-1}, in oil from dark green flesh, and 1−3 μg g^{-1} in oil from pale green and yellow flesh. Concentrations of both pigments declined rapidly to zero at day 6 (**Figure 6b−d**).

Chlorophylls in Oil. As found for fresh tissues, chlorophyll and chlorophyll-related compound concentrations were greatest in oil from the skin and decreased moving from skin into the interior flesh tissues (**Figure 7a−d**). In oil extracted from skin,

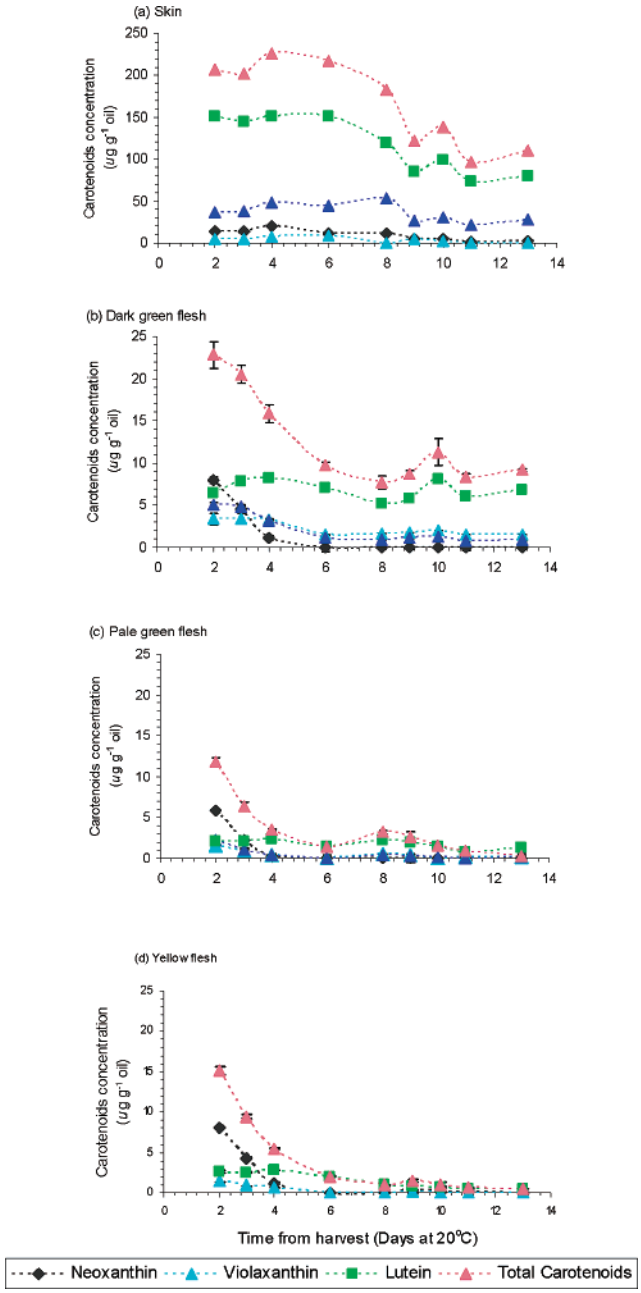

Figure 6. Carotenoid concentration in oil extracted from Hass avocado (**a**) skin, (**b**) dark green flesh, (**c**) pale green flesh, and (**d**) yellow flesh during ripening. Data are presented as means ± SEM ($n = 3$).

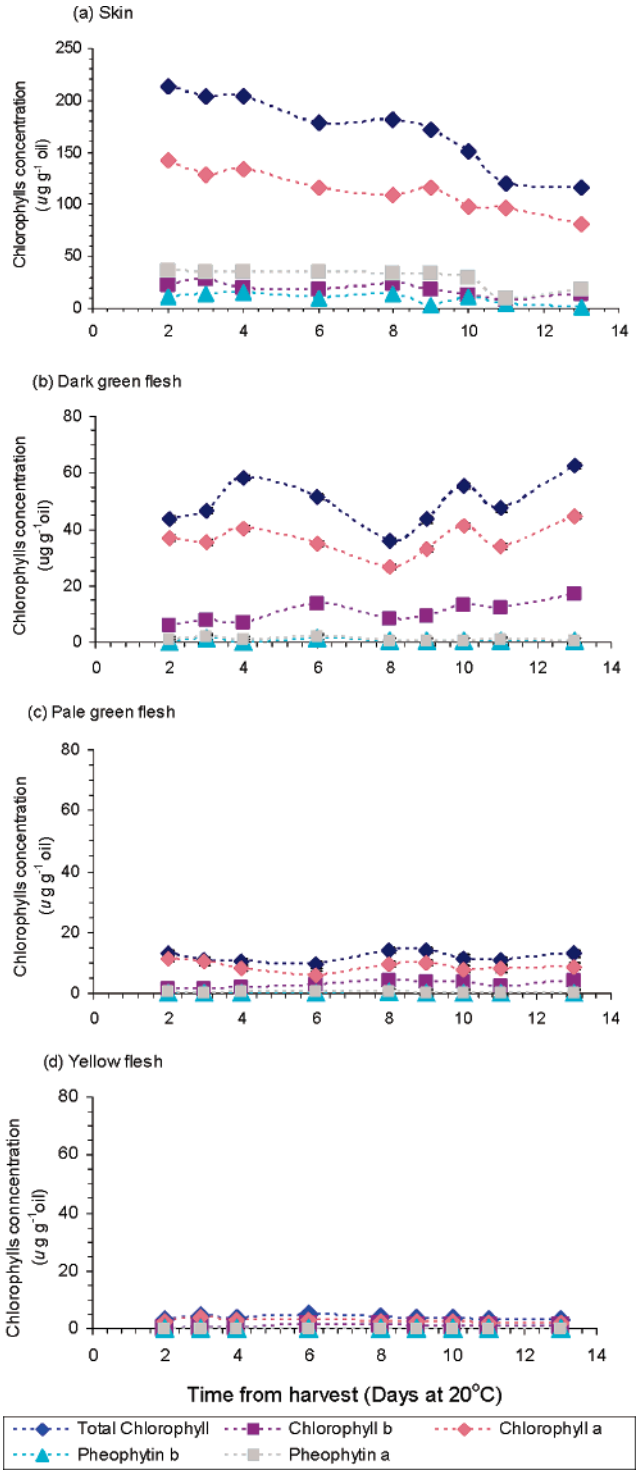

Figure 7. Chlorophyll concentration in oil extracted from Hass avocado (**a**) skin, (**b**) dark green flesh, (**c**) pale green flesh, and (**d**) yellow flesh during ripening. Data are presented as means ± SEM ($n = 3$).

the total concentration of chlorophylls decreased from 213.8 to 115.6 μg g^{-1} over the ripening period (**Figure 7a**). Chlorophyll a, the main chlorophyll, followed a similar trend to that of total chlorophyll, decreasing from 143.0 to 81.1 μg g^{-1} by day 13. At day two, the concentrations of pheophytin a, chlorophyll b, and pheophytin b were 37.0, 22.7, and 11.1 μg g^{-1}, respectively, and all pigments declined with ripening. No chlorophyllides were detected in oils extracted from either skin or flesh tissue.

The total chlorophyll content in oils extracted from flesh tissues did not change significantly during ripening (**Figure 7b**−**d**). The average total chlorophyll in oil from the dark green, pale green, and yellow flesh was 43.8, 13.1, and 3.0 μg g^{-1}, respectively. The chlorophyll a content averaged 37.0, 11.4, and 2.7 μg g^{-1} in the oil extracted from the dark green flesh, pale green, and yellow flesh, respectively, and did not change over ripening. The chlorophyll b concentration increased slightly in the oil from the dark green flesh over the ripening period, from 5.7 to 16.7 μg g^{-1}. The concentrations of chlorophyll b (<5 μg g^{-1}) and pheophytins a and b (<1 μg g^{-1}) in oils from the pale green and yellow flesh were low during the entire ripening period (**Figure 7c**,**d**).

Proportion of Tissue Pigments Extracted into Oil. Using the DM and oil content (determined from ASE extraction) of each tissue section, the proportion of pigments extracted into the oil was calculated (**Table 1**). Carotenoids were essentially completely extracted from flesh tissues (85−100%) but only partly extracted (28%) from the skin. In contrast, on average, 40−

Table 1. Calculated Values for the Proportion (%) of Pigments from Skin and Flesh Tissues Extracted into Oil by ASE Extraction

	% extraction of pigment from skin or flesh tissue[a]	
tissue section	total carotenoids (not including α- and β-carotenes)	total chlorophyll (not including chlorophyllides)
skin	28 ± 2	29 ± 8
dark green flesh	100 ± 9	40 ± 3
pale green flesh	98 ± 15	57 ± 4
yellow flesh	85 ± 9	65 ± 5

[a] Mean values of extractions from days 2 to 13, ± standard error for $n = 9$.

65% of the chlorophylls were extracted from avocado flesh tissues into the oil, and this reduces to only 29% from the skin.

DISCUSSION

This study shows that the concentrations of pigments in Hass avocado vary significantly between tissue types (skin and flesh), tissue position (inner to outer flesh), and during ripening. The pigment concentrations present in oils produced from these tissues reflect the tissue pigment composition for pigments that are oil soluble (water soluble pigments are not extracted from avocado tissue into the oil).

Fruit Tissue Color and Pigment Concentrations. Overall, these results confirm the visual changes seen in ripening Hass avocados, with skin color changing from a bright vivid green color to dark green and then purple/black. It was observed that higher hue angle values for tissue (more green color) were correlated with higher concentrations of chlorophylls and carotenoids. This agrees with observations in other avocado cultivars such as Fuerte (*2*), where chlorophyll concentrations declined from outer to inner flesh tissues. Similarly, the changes in flesh color with ripening are likely to be correlated with reduced pigment concentrations, particularly for chlorophyll in skin tissue. The skin tissue contained significantly greater concentrations of both chlorophylls and carotenoids than the less-green flesh tissue. This is consistent with observations in Nabal avocados where total carotenoid and lutein concentrations in the flesh are ≅half those of the skin tissue (*10*). In Hass, we observed lutein concentrations of 2.2−2.5 μg g^{-1} FW, similar to those reported by Lu et al. (*9*) (2.93 μg g^{-1} FW).

Pigment Concentration Changes during Ripening. This study was the first to examine pigment concentrations over the entire ripening period for Hass avocado. In flesh tissue, chlorophyll did not decrease significantly, while in the skin, total chlorophylls declined with ripening, although more quickly than observed by Cox et al. (*4*). Total carotenoids also showed a consistent decrease in the skin (where the majority of the carotenoids are located), and lutein, an important pigment from a health perspective (*8*), declined by ≅50% in the skin over the ripening time. Similarly, in the flesh, total carotenoids declined by over 50%, although the concentrations of lutein were much lower than in the skin (≅10% of that in the green flesh tissue).

The relative concentrations of chlorophylls a and b showed little change during ripening in any of the tissues, similar to our previous observations (*4*). The high concentrations of total chlorophylls in the skin were a result of high concentrations of chlorophyllide a and to a lesser extent chlorophyllide b. Chlorophyllides are water soluble pigments and are intermediates in chlorophylls a and b biosynthesis (*22*) and chlorophyll degradation (*22*, *23*). The high concentrations of chlorophyllide a found in avocado skin may be a result of chlorophyll synthesis or degradation in the skin.

Chlorophyllides are formed by removal of the phytol moiety from chlorophyll by the enzyme chlorophyllase and are not likely to have arisen during sample preparation since the samples were flash frozen in liquid N_2, stored at −80 °C, and extracted at chilled temperatures in the absence of light. Importantly, all samples were treated similarly and only skin samples from the earlier stages of ripening contained chlorophyllides, even though all skin samples contained similar concentrations of chlorophylls. Chlorophyll can also be degraded to pheophytins a and b during ripening and can be produced under conditions of mild acidity and heat, such as those used for extraction (*23*). Pheophytins were not detected in avocado skin suggesting that the dominant breakdown pathway of chlorophyll in avocado skin is the action of chlorophyllase on chlorophyll to generate chlorophyllides.

The most substantial change during ripening in pigment concentration was in the skin where anthocyanins increased. This was almost solely because of the dramatic increase in cyanidin 3-*O*-glucoside 4−6 days after harvest and was highly correlated with skin color change (hue angle) and fruit firmness. This is consistent with results previously observed with late season Hass (*4*), except that changes in pigment concentrations, skin color, and fruit ripening occurred ≅2 days earlier in this study. These differences are consistent with the lower maturity (harvested earlier in the season) of the avocados in the current study.

Proportion of Pigments in Tissue and Oil. The percentage of pigment extracted into the oil varied by pigment type and tissue type. Chlorophyllides a and b were not detected in oil as they are only water soluble, even though they are the major chlorophylls in skin during the early stage of ripening. The same is true for the water soluble anthocyanins. Total chlorophyllides present in the skin, dark, pale, and yellow flesh sections up to 9 days storage was on average 70, 8, 16, and 38% of the total chlorophylls present, respectively. Hence, the 40−65% from flesh and 29% from skin of all chlorophylls extracted would likely account for the majority of the oil soluble chlorophyll pigments in each tissue, including chlorophyll degradation products not identified. The method used to determine the carotenoid content of the oil did not detect α- and β-carotene. These may have been extracted into the oil and not accounted for, hence the slightly lower recovery of carotenoids. The concentrations of α- and β-carotene in the oils would be less than that of lutein, the most abundant carotenoid. Note that the values presented in **Table 1** were corrected for the nondetection of α- and β-carotene. α- and β-Carotene made up on average 31, 5, 12, and 23% of the total carotenoids in the skin, dark green, pale green, and yellow flesh tissue sections, respectively.

Pigment Concentration in Extracted Oil. The types and concentrations of pigments in avocado oil are important indicators of product quality (appearance) and health profile (*9*). Because the extraction of carotenoids from the flesh tissues into the oil was nearly 100% (**Table 1**), the carotenoid composition of oil was primarily determined by the composition in the avocado tissue used. Lutein, which was present in the greatest concentrations in avocado flesh, was therefore also present in the greatest concentrations in oil. Oils extracted from unripe flesh tissues contained greater concentrations of carotenoids than oils from ripe flesh tissues. In contrast, the chlorophyll composition varied substantially between the skin or flesh tissues and the oil, due to the hydrophilic nature of chlorophyllides, which are not extracted into the oil. Thus, the concentrations of total chlorophyll extracted into the oil were not as easily predicted

from the concentrations found in the skin and flesh tissues. Results presented here are for oils extracted using standard solvent extraction methods, which produce oils that will not be identical to those extracted by commercial cold pressed systems. We are undertaking research to compare pigment concentrations in solvent-extracted and cold-pressed extracted oils.

Practical Implications. This research suggests that, if a goal for avocado oil production is to maximize the pigment concentrations in the oil, then maximizing the proportion of green flesh tissue, and particularly the skin tissue, may be required. Our future work will examine the effects of the amount of skin used during cold-pressed extraction on oil pigmentation, oil quality, and its shelf life. The declining concentrations of lutein and other carotenoids with fruit ripeness mean that extracting oil from fruit at a later stage of ripeness will result in lower concentrations of pigments in oil.

Avocado fruit and avocado oil may confer considerable health benefits by decreasing the risk of coronary heart disease, cataracts, diabetes, chemoprevention, prostate cancer, and age-related macular disease (*9*, *13*, *24*−*27*). Some of these health effects may be due to the antioxidant pigments present in avocado. It is important therefore to maximize the effectiveness of these compounds that are present in Hass avocado fruit and derived products such as oil. It has been suggested that oil may enhance pigment bioabsorption in humans (*9*, *26*) and provide a method for increasing the health benefit of lipid soluble pigments. Consuming avocado oil may be one way of gaining "concentrated" amounts of avocado "health-promoting" pigments (e.g., lutein), since the oil extracted from avocado contains many of the pigments found in the flesh and skin of avocados.

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