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ORIGINAL PAPER

Influence of Proportion of Skin Present During Malaxing on Pigment Composition of Cold Pressed Avocado Oil

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Abstract The effect of the proportion of 'Hass' avocado skin tissue present during aqueous cold pressed avocado oil extraction on the pigment concentration and oil quality was determined. Increasing amounts of skin (i.e., from 0 to 100% of available skin) were included with the flesh before grinding and malaxing in a laboratory-scale cold pressed extraction process. The recovered oils were analyzed for oil quality and color. The pigment concentrations in the oil were determined by high performance liquid chromatography (HPLC). With increasing amounts of skin addition, there was an increase in the green color, as indicated by hue angle, of the avocado oils. There were also increased concentrations of carotenoids and chlorophylls in the oil as the proportion of skin during malaxing was increased. The lutein concentration in the oils increased from 1.13 to 3.21 μg g^{-1} as the amount of skin added to the malaxer increased from 0 to 100% skin, and the total chlorophyll concentration in the same oils increased from 7.3 to 27.5 μg g^{-1}. The green color of cold pressed avocado oil and the pigment concentrations can be increased by adding more skin to the malaxer during oil extraction.

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Introduction

Cold pressed avocado oil, with a distinctive green color, is a relatively new oil in the commercial culinary oil field [1, 2]. Cold pressed avocado oil is defined as oil extracted using mechanical or physical means at temperatures below 50 °C [2] and is extracted using methods similar to that used for extra virgin olive oil [3]. Avocado flesh is initially ground to a pulp, followed by malaxing with or without water at <50 °C. This temperature is considerably lower than has been used in alternative extraction methods for other avocado oils [2]. After malaxing, the solid and liquid phases are separated, and then oil recovered from the liquid phase (oil and water) by centrifugation [2, 4]. Before the commercial development of this aqueous cold pressed process, avocado oils were mainly extracted using organic solvents and/or heat along with refining, bleaching and deodorizing steps, resulting in a light colored oil with good stability, but with little taste and reduced beneficial health components [2].

Cold pressed avocado oil is beginning to be appreciated as a unique oil not only for culinary use, but also for its potential health benefits [1, 2], many of which relate to minor components present in the oil. For example, avocados have been reported to have one of the highest concentrations of the carotenoid lutein compared with other fruit [5], and the lutein, along with other carotenoid and chlorophyll pigments, is extracted into the oil [6]. Lutein has been reported to be beneficial for reducing age-related macular degeneration (AMD) [7, 8]. Pigments found in avocado flesh and skin include lutein, α-carotene, β-carotene, antheraxanthin,

neoxanthin, violaxanthin, zeaxanthin, chlorophyll *a* and *b,* pheophytin *a* and *b* and chlorophyllide *a* and *b* [5, 6, 9–11]. The anthocyanin, cyanidin 3-*O*-glucoside, has also been found in the skin [6, 10]. The majority of the carotenoid and chlorophyll pigments were found in avocado oils extracted from these tissues, with the exception of cyanidin 3-*O*-glucoside and chlorophyllides *a* and *b,* which are water soluble [6]. A key finding from Ashton et al. [6] was that the concentrations of many of the pigments were significantly higher (5 to 10-fold) in the skin than the flesh tissues, although the proportions of pigments extracted into the oil from the skin were less than those extracted from the flesh.

The rich green color and concentrations of many plant pigments in avocado oil are important from a range of perspectives including marketing, oil quality and stability, and in terms of healthfulness of the oil [2, 11]. As noted by Ashton et al. [6], the color of cold pressed oil can be manipulated by the proportion of avocado tissues that are processed. The proportion of skin tissue is the key factor that can be varied, which may affect the pigment composition of the oil. Extraction of pigments during avocado oil extraction has not been investigated previously, but has been investigated during the extraction of olive oil. Gallardo-Guerrero et al. [12] and Criado et al. [13] both reported that during olive oil extraction, chlorophyll and carotenoid pigments are degraded and lost. Criado et al. [13] also commented that if during olive oil extraction the tissue was not crushed sufficiently, then full release of pigments did not occur.

The objective of this study was to determine the effect of the proportion of skin tissue present during aqueous extraction procedures under laboratory-scale conditions on pigment concentrations and quality of avocado oil.

Materials and Methods

Avocado Fruit

Avocados (*Persea americana* Mill 'Hass') were harvested late in the New Zealand (NZ) season (January), from an orchard in Whangarei, NZ and delivered to the Mt Albert Research Centre, Auckland. One day after harvest, the fruit were randomized into trays and ethylene ripened (100 μL L^{-1} ethylene, CO_2 <0.5%) over 2 days at 20 °C. After removal from the ethylene chamber, the fruit were held for 3 days at 20 ± 2 °C to achieve the desired firmness, and then held overnight at 5.5 ± 0.5 °C until extraction of the oil. Firmness expressed as a Firmometer value (Fv) (mm × 10) was measured using an Anderson Firmometer, which measures displacement [14], and thus the units increase with softening. Extraction was carried out over 3 days, replicates were randomized over the 3 days, and average firmness values ranged between 71 and 80 Fv, which equates to a "fully eating ripe" stage [14].

Oil Extraction

Avocado oil was extracted from the ripened fruit over three successive days using a laboratory-based cold pressed extraction procedure based on procedures recommended by Woolf et al. [2]. For each trial, ten fruit were cut into quarters and the skin and flesh separated by hand peeling. The skins were cut into small pieces (≅2.5 cm^2) immediately before grinding and the flesh and skin were ground in a bench-top hammer-mill with a 4-mm screen. Distilled water was then added to the ground flesh and skin in a ratio of 3:10 water:avocado flesh and skin. The mixture of pulp was then malaxed in stainless steel jacketed vessels (malaxers) with overhead mixers designed to scrape the inside surface of the vessel. The mixture in the malaxer was mixed at 90 rpm for 60 min. The malaxers were maintained at 45–48 °C. After malaxing, water was added in a 1:1 ratio to the mixture of pulp. The mixture was then centrifuged, at 40 °C, for 20 min at 8,600 rpm (12,500*g*) (Sorval Dupont centrifuge, USA). The oil layer was re-centrifuged at 20 °C for 10 min at 5,000 rpm (2,720*g*) (Model MR 1822, Jouan, France). The oil was recovered and placed into dark brown glass bottles, flushed with oxygen-free nitrogen and stored at −80 °C until analysis. Oil yields obtained during the laboratory extraction procedure described were not determined.

Percentage of Skin Addition

Ripe 'Hass' avocado is reported to be made up of 68% flesh, 18% seed and 14% skin (by fresh weight) [4], with the proportion of skin to flesh remaining relatively constant across a wide range of fruit sizes. For the extraction trials, based on the above percentage of skin to whole fruit, the amount of skin added back to the flesh during malaxing was calculated based on the total mass of skin available for each batch of fruit. The percentages of the total available skin added back during malaxing in the laboratory extractions were 0 (no skin), 5, 10, 20, 40, 70 and 100% (all skin added to the malaxing process; by fresh weight).

Analysis of Oils

All reagents used were analytical grade. The free fatty acid % (FFA) value was expressed as w/w % (as oleic acid). The FFA was determined using the method described by the American Oil Chemists Society's Official Methods, Ca 5a-40 [15]. The peroxide value (PV) was determined using the AOCS Official Method Cd 8-53 [15], except dichloromethane was used as a solvent instead of chloroform.

Two grams of avocado oil was dissolved in 10 mL acetic acid/dichloromethane (3:2) solvent. The assay was then carried out as per the standard procedure. Both FFA and PV were analyzed in each sample in triplicate.

Color Measurement of Oil

The Minolta CR300 colorimeter (Minolta Japan) calibrated using a standard white plate, was used to measure the color of the avocado oil. Oil samples were placed into plastic cuvettes (10 mm path length) and placed into a blackened and dark sample holder for color measurements with the colorimeter. Samples were measured in triplicate.

Pigments by High Performance Liquid Chromatography

Carotenoid and chlorophyll pigments were quantified in the oil by high performance liquid chromatography (HPLC) using the method reported in Ashton et al. [6]. Before injection into the HPLC, the carotenoids and chlorophylls were isolated from the oil using solid-phase extraction (SPE). The oil was dissolved in hexane before it was passed through the SPE (C18) cartridges and eluted with acetone. The HPLC column used was a Waters Spherisorb 5 μm ODS2 (4 × 250 mm) with a ternary gradient system of A (20% 0.5 M ammonium acetate, 80% methanol), B (10% H_2O, 90% acetonitrile), and C (ethyl acetate) as described by Wright et al. [16]. Carotenoids were detected using UV/vis absorbance 455 nm and quantified as lutein equivalents. Chlorophylls were detected using fluorescence (excitation 440 nm, emission 460 nm) and quantified as chlorophyll *b* equivalents. All pigment components were near baseline resolved and the retention times for the HPLC peaks were as follows: neoxanthin 10.5 min; antheraxanthin 12.4 min; lutein 13.6 min; chlorophyll *b* 14.2 min; chlorophyll *a* 15.1 min; pheophytin *b* 16.7 min; and pheophytin *a* 17.4 min.

Statistical Analysis

Results were analyzed by ANOVA using SPSS 18.0 (PASW Statistics, USA) using one way analysis to determine any significant differences ($P < 0.05$), followed by Tukey's and Games-Howell tests to identify individual significantly different means.

Results and Discussion

Quality of Extracted Oil

The FFA% for avocado oil extracted in the laboratory for 0–100% skin additions ranged from 0.18 to 0.23% w/w (as oleic acid), with an overall mean value of 0.21 ± 0.01% w/w (as oleic acid) ($n = 21$) (Fig. 1). Oils extracted with 40 and 70% skin were found to have significantly less FFA% than found for all other % skin additions ($P < 0.05$). The peroxide values (PVs) for oils extracted for 0–100% skin addition were also within a narrow range of 1.82–1.99 meq/kg oil, with an overall mean value of 1.86 ± 0.03 meq/kg oil ($n = 21$), and there were no significant differences between the PVs of all the % skin additions ($P < 0.05$).

The color of the oil was determined by measuring lightness, chroma and hue angle and the chromaticity values of a^* and b^* were also measured. The color of the oil with increasing amounts of skin changed significantly ($P < 0.05$) from 0 to 100% skin addition for all chromaticity and color space values (Fig. 2). For 0–100% skin additions, the a^* value decreased from −0.12 to −5.47 (red to green) and the b^* values decreased from 9.31 to −1.87 (yellow to blue). The lightness (L) decreased from 99 to 89, chroma (c) decreased from 15 to 12 and the hue angle (h°) increased from 171 to 222 for 0–100% skin additions ($P < 0.05$) (Fig. 2). Overall, the changes in the chromaticities a^*, b^* and hue angle indicated that the color of the oil became a darker green with increasing amounts of skin addition. With lightness values close to 100, this indicated that the oils were clear and light against the black background of the measurements.

Pigments in Extracted Oils

Typical HPLC chromatograms for carotenoids and chlorophylls identified in the extracted oils are shown in Fig. 3. As the percentage of skin addition was increased from 0 to 100% during the extraction process, the lutein concentration

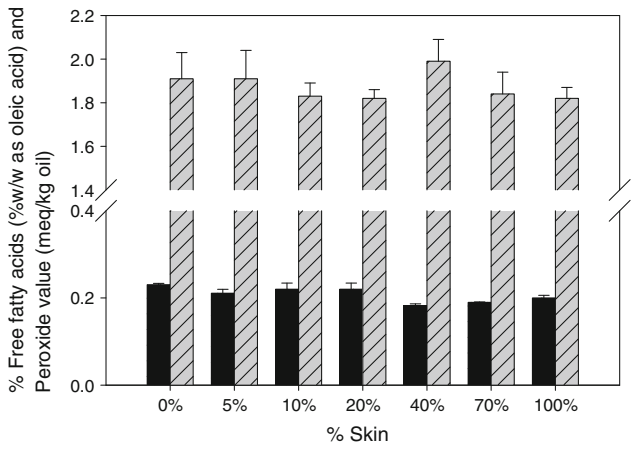

Fig. 1 Percentages of free fatty acids (*solid black*) and peroxide values (*grey stripes*) for oils extracted with different percentages of skin. Data are presented as mean ± SEM ($n = 3$)

in the oil more than doubled, increasing from 1.13 to 3.21 μg g^{-1} (Fig. 4). The concentration of lutein at 100% skin addition was significantly higher than for all other amounts of skin addition ($P < 0.05$). This would be expected, since the concentration of lutein in avocados is highest in the skin [6]. At 10–70% skin addition, the concentrations of lutein in the oils were not significantly different from one another but significantly more than at 0 and 5% but less than at 100% skin addition ($P < 0.05$). The concentrations of antheraxanthin and neoxanthin extracted into the oils did not change significantly ($P > 0.05$) with different amounts of skin addition. Antheraxanthin was on average 0.2 μg g^{-1} in all the oils extracted; this was only 10% of the concentration of lutein in the oil, and neoxanthin was present in the oils at an average concentration of 0.090 μg g^{-1}.

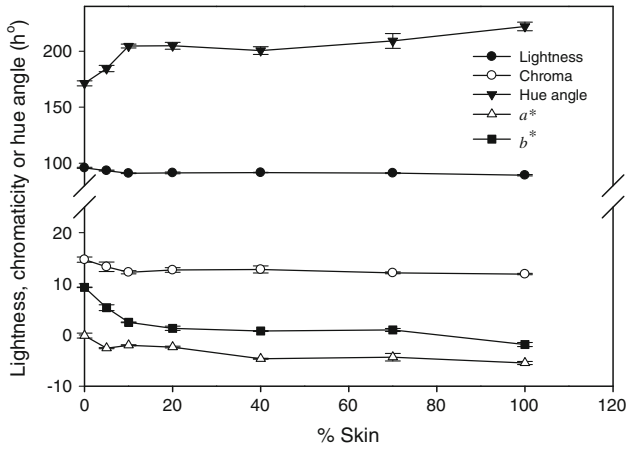

Fig. 2 Influence of the percentage of skin addition during extraction on the color (lightness, hue angle, chroma, a^* and b^*) of avocado oil. Data are presented as mean ± SEM ($n = 3$)

The total chlorophyll in the extracted oils increased as the percentage of skin addition increased (Fig. 5). The total chlorophyll concentration with 100% skin addition was 3.7 times greater than at zero skin addition, which was significantly higher than amounts for all other skin addition amounts ($P < 0.05$) (Fig. 5). In these oils, the chlorophyll *a* concentration tripled from 2.5 μg g^{-1} at zero skin addition to 7.5 μg g^{-1} at 100% skin addition. Chlorophyll

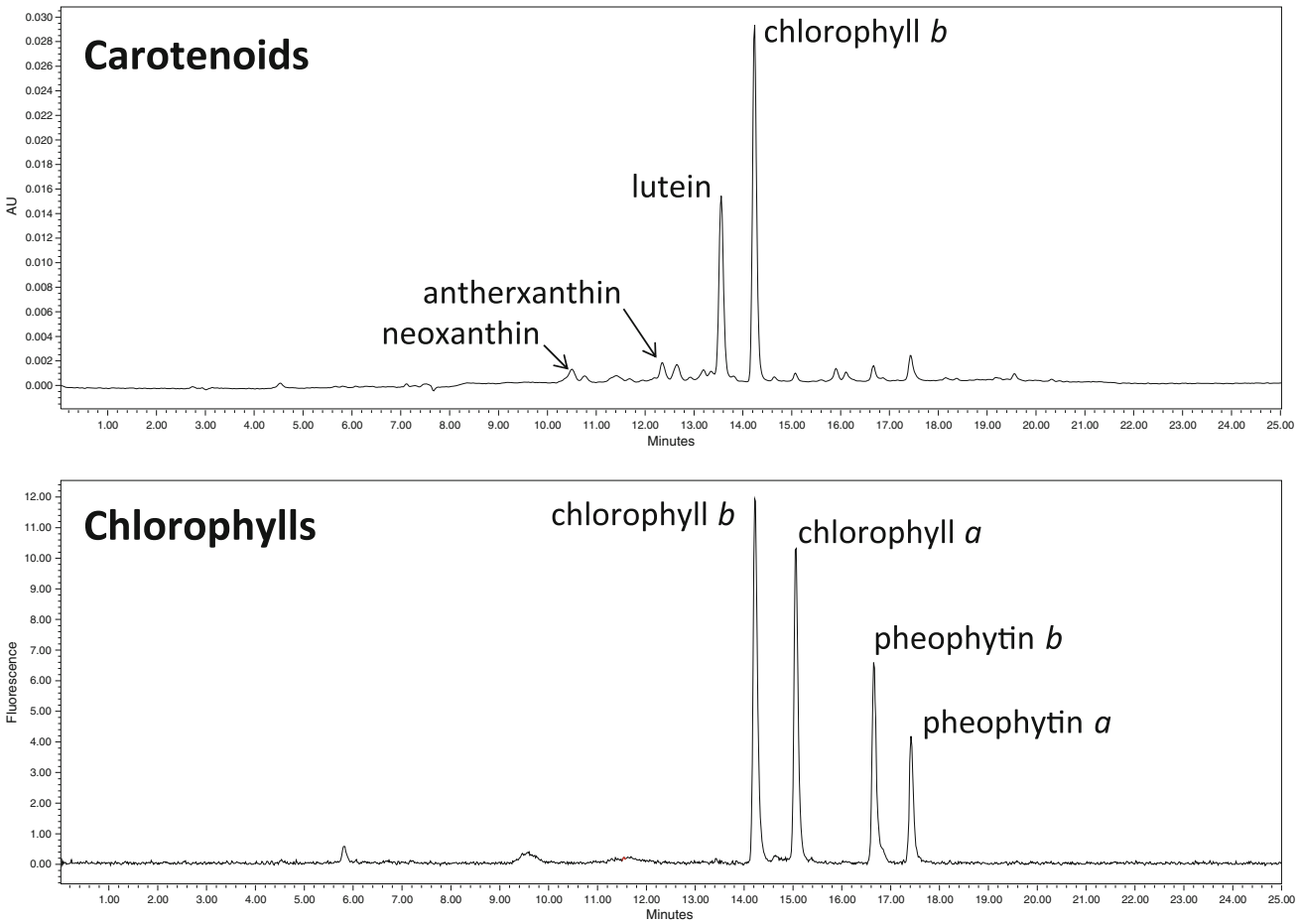

Fig. 3 Representative chromatograms of an avocado oil sample extracted with 100% skin. Carotenoids were detected at 455 nm, and chlorophylls using fluorescence (ex 440 nm, em 460 nm)

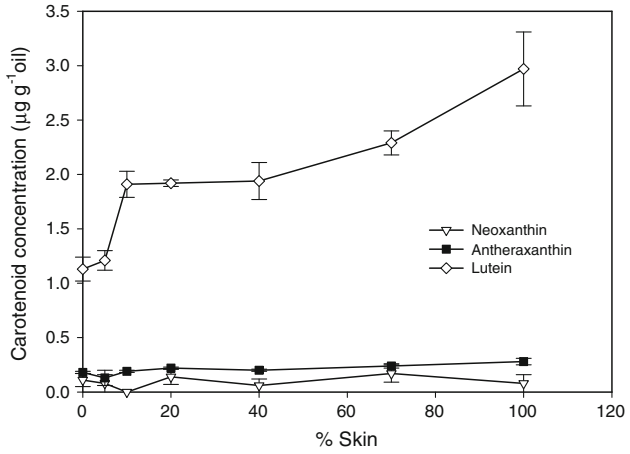

Fig. 4 Influence of the percentage of skin addition during extraction on the concentrations of carotenoid pigments in avocado oil, as determined by HPLC. Data are presented as mean ± SEM ($n = 3$)

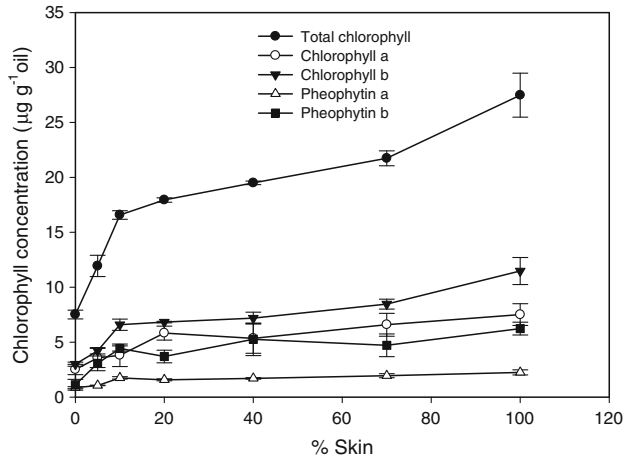

Fig. 5 Influence of the percentage of skin addition during extraction on the concentrations of individual and total chlorophyll pigments in avocado oil, as determined by HPLC. Data are presented as mean ± SEM ($n = 3$)

b increased in concentration from 3.0 μg g^{-1} for zero skin addition to 11.5 μg g^{-1} with 100% skin addition. The concentration of pheophytin *a* increased from 0.9 μg g^{-1} at zero skin addition to 2.3 μg g^{-1} at 100% skin addition. Likewise, the concentration of pheophytin *b* was 1.1 and 6.2 μg g^{-1} at 0 and 100% skin addition, respectively. All chlorophyll compounds were found to be significantly higher in oils with 100% skin addition than with no skin addition ($P < 0.05$).

Impact of Skin Added During Malaxing on Pigment Concentration in the Oil

The results show that higher proportions of skin present during malaxing led to more lutein extracted into the oil (Fig. 4). All carotenoids are found in unripe 'Hass' avocados, but as they ripen, the carotenoid concentrations decrease [6]. The carotenoids were found to have a decreasing order of abundance in skin and flesh: lutein, *β*-carotene, neoxanthin, violaxanthin, α-carotene, antheraxanthin, zeaxanthin. When fruit are ripened to a Firmometer value of 60 Fv, virtually all carotenoids other than lutein decrease to near-zero. The fruit used in this study had firmness values ranging from 71 to 80 Fv; therefore, many of the minor carotenoids were unlikely to be present in the skin and flesh and would therefore be unlikely to be extracted into the oils. Ashton et al. [6] reported that the avocado skin and dark green flesh (the thin layer immediately below the skin) contained the highest concentrations of chlorophyll pigments. When the skin is removed from ripe avocados, a portion of the dark green flesh adheres to the skin, which can lead to a potential loss of chlorophyll pigments. The more skin added during malaxing, the more chlorophyll extracted into the oil from the skin and green flesh layer, resulting in darker green oil, with a higher hue angle (Fig. 2).

Gallardo-Guerrero et al. [12] reported a loss of carotenoids and chlorophylls during extraction of oil from olive fruit. They found that as fruit ripened, the carotenoid concentration declined, but with this increase in ripeness, the carotenoids were released more readily into the oil, and a higher proportion of carotenoids were extracted into the oil instead of remaining in the pomace. Gallardo-Guerrero et al. [12] postulated that the chlorophyll fractions were either destroyed or remained in the pomace; not being released from the intracellular structures, they were said to be occluded in the pomace. They also found chlorophyll *b* was destroyed more than chlorophyll *a*. Results from this study showed the concentration of chlorophyll *b* present in the oils was greater than the concentration of chlorophyll *a* (Fig. 5); with increasing amounts of skin added to the malaxer, more chlorophyll *a* and *b* were extracted into the oil. As the fruit were 'fully ripe', pigments were released from the mesocarp cells, and the increase in pigment concentration was due to the increase in the amount of skin added to the malaxers.

The concentrations of pheophytin *a* and *b* were consistently 30 and 50%, respectively, of the chlorophyll *a* and *b* concentrations for all amounts of skin added during malaxing. This indicated there were similar degrees of chlorophyll degradation during grinding and malaxing. The ratio of chlorophyll to carotenoid pigments in the oils was similar for all skin addition percentages except for the extraction with zero skin. For laboratory extractions at 5–100% skin addition, the average chlorophyll to carotenoid ratio was 8.3 ± 0.2. For zero skin laboratory extraction, the ratio was 5.3. This indicates that the extractions of chlorophylls and carotenoids are highly

correlated and that presence of skin during oil extraction contributes to a higher proportion of chlorophyll pigments in the oil.

Impact of Skin Added During Malaxing on Extraction Economics

As mentioned previously, flesh makes up ≅68% of the total fresh weight of a ripe 'Hass' avocado and the skin only contributes 14% [4]. The flesh of a ripe avocado contains ≅30% oil while the skin only contains ≅5% oil by fresh weight (Woolf, Wong, Requejo-Jackman, unpublished data). Thus, with 100% skin addition to the malaxer (for a constant malaxer fill by weight), the oil yield per malaxer could drop by ≅14% compared with no skin addition. It is not known how easily the oil is extracted from the skin in a cold-pressed system. Hence, addition of skin during malaxing, although producing oil with higher concentrations of pigments, could lead to losses in yield per malaxer (but not on a per weight of fruit processed basis). Conversely, the addition of skin tissue may improve oil extraction due to increased abrasion effects which could help with cell wall disruption and oil release.

Conclusions

Increasing the proportion of skin included during the extraction of avocado oil results in oil with more green color, and higher concentrations of chlorophylls and lutein. The initial chemical quality (FFA%, PV) of the oil after extraction was not found to be affected by the proportion of skin included during extraction. Thus, oil color and pigment concentrations can be increased by adding more skin during malaxing. In future published work, the influence of skin addition on the stability and sensory nature of the oils during their shelf life will be presented.

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References

1. Eyres L, Sherpa NL, Hendriks G (2001) Avocado oil: a new edible oil from Australasia. J Lipid Technol 13:84–88
2. Woolf A, Wong M, Eyres L, McGhie T, Lund C, Olsson S, Wang Y, Bulley C, Wang M, Friel E, Requejo-Jackman C (2009) Avocado Oil. In: Kamel-Eldin A, Moreau R (eds) Gourmet and health-promoting specialty oils. AOCS Press, Urbana, pp 73–126
3. Kiritsakis AP (1998) Olive oil: from the tree to the table. Olive oil: from the tree to the table, 2nd edn. Food & Nutrition Press, Trumbull
4. Wong M, Ashton OBO, Requejo-Jackman C, McGhie TK, White A, Eyres L, Sherpa N, Woolf AB (2008) Avocado oil—the color of quality. In: Culver C, Wrolstad RE (eds) Color quality of fresh and processed foods. American Chemical Society, Washington, pp 328–349
5. Heinonen MI, Ollilainen V, Linkola EK, Varo PT, Koivistoinen PE (1989) Carotenoids in Finnish foods—vegetables, fruits, and berries. J Agric Food Chem 37:655–659
6. Ashton OBO, Wong M, McGhie TK, Vather R, Wang Y, Requejo-Jackman C, Ramankutty P, Woolf AB (2006) Pigments in avocado tissue and oil. J Agric Food Chem 54:10151–10158
7. Koh HH, Murray IJ, Nolan D, Carden D, Feather J, Beatty S (2004) Plasma and macular response to lutein supplement in subjects with and without age-related maculopathy: a pilot study. Exp Eye Res 79:21–27
8. Richer S, Stiles W, Statkute L, Pulido J, Frankowski J, Rudy D, Pei K, Tsipursky M, Nyland J (2004) Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (lutein antioxidant supplementation trial). Optometry 75:216–229
9. Gross J, Gabai M, Lifshitz A (1972) The carotenoids of the avocado pear—*Persea americana*, Nabal variety. J Food Sci 37:589–591
10. Cox KA, McGhie TK, White A, Woolf AB (2004) Skin colour and pigment changes during ripening of 'Hass' avocado fruit. Postharvest Biol Technol 31:287–294
11. Lu QY, Arteaga JR, Zhang QF, Huerta S, Go VLW, Heber D (2005) Inhibition of prostate cancer cell growth by an avocado extract: role of lipid-soluble bioactive substances. J Nutr Biochem 16:23–30
12. Gallardo-Guerrero L, Roca M, Minguez-Mosquera I (2002) Distribution of chlorophylls and carotenoids in ripening olives and between oil and alperujo when processed using a two-phase extraction system. J Am Oil Chem Soc 79:105–109
13. Criado MN, Motilva MJ, Goni M, Romero MP (2007) Comparative study of the effect of the maturation process of the olive fruit on the chlorophyll and carotenoid fractions of drupes and virgin oils from arbequina and farga cultivars. Food Chem 100:748–755
14. White A, Woolf AB, Hofman PJ, Arpaia ML (2005) The international avocado quality manual. ISBN: 0-478-06837-9
15. AOCS (1998) Official methods and recommended practices of the American Oil Chemists' Society. American Oil Chemists' Society, Champaign
16. Wright SW, Jeffrey SW, Mantoura RFC, Llewellyn CA, Bjornland T, Repeta D, Welschmeyer N (1991) Improved HPLC method for the analysis of chlorophylls and carotenoids from marine plankton. Mar Ecol Prog Ser 77:183–196