Oleosome Oil Storage in the Mesocarp of Two Avocado Varieties

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Abstract: Studies on avocado oil have focused on the most common commercial cultivars, Hass, Fuerte, and Bacon, rather than the less common varieties, *P. americana* var. drymifolia and *P. americana* var. americana, even though the drymifolia variety has a higher oil content and the americana variety is the most common avocado grown in the tropics. The most abundant storage structures for plant oils are the oleosomes, and the aim of this study was to determine the oleosome size, oil yield, and fatty acid composition of the americana and drymifolia varieties, using the Hass cultivar as a reference. Differences were found between the three avocado types for 1) oil yield, with drymifolia having higher and americana lower oil content (*p* < 0.05%), 2) oleosome size, with Hass having a larger (41.53 µm) and americana a smaller (11.96 µm) size, and 3) fatty acid composition, with the americana and drymifolia varieties showing less monounsaturated fatty acids (oleic) and more polyunsaturated fatty acids (linoleic) and saturated fatty acids (palmitic); while Hass had a high level (60%) of monounsaturated fatty acids. Small but significant differences were also found between oleosome and mesocarp oils isolated from the drymifolia and Hass types.

Key words: avocado, fatty acids, oleosome, GC/MS

1 INTRODUCTION

The main feature that distinguishes the avocado fruit (*Persea americana* Mill.) is the high content of oil in the mesocarp, which gives the fruit its creamy texture and contributes to its flavor. The oil is a source of saturated (palmitic and stearic acid), monounsaturated (oleic and palmitoleic acid), and polyunsaturated (linoleic and linolenic acid) fatty acids that impart health benefits through anti-cholesterolomic, antioxidant, and anti-inflammatory activities. Studies on avocado fruit oil have mostly focused on the commercial cultivars, such as Hass, Bacon, and Fuerte, whereas few studies have been carried out on the fruits of native Mexican (*P. americana* var. drymifolia) and American (*P. americana* var. americana) varieties, despite their excellent medicinal and nutritional properties.

The avocado tree belongs to the family Lauraceae, a primitive group of Angiosperms that was domesticated during the pre-Hispanic period in Mesoamerica as three botanical varieties (*P. americana* var. americana, *P. americana* var. guatemalensis, and *P. americana* var. drymifolia). The americana variety comes from Central America, where it is known as ‘palta’ or ‘pagua,’ and produces a large fruit that can reach 25 cm in length and weigh over 300 g. The fruit has a low oil content, ranging from 2.5% to a maximum of 8%, and the fruit pericarp is green in color, changing to brownish when ripe. The guatemalensis variety is native to the region between Chiapas (southern México) and Guatemala and produces a small fruit with a thick and corky pericarp. The oil content of the guatemalensis mesocarp ranges from 10 to 13%. The drymifolia variety, also known as the native Mexican avocado, comes from the central and eastern regions of México. Unlike the other two varieties, which are adapted to tropical and subtropical climates, it tolerates a cold temperate climate and higher altitudes, and is resistant to some diseases. The drymifolia fruits have an anise scent with a thin, edible, pericarp that is colored green to dark purple (almost black) when ripened and the fruits possess the highest oil content, reaching 15 to 20% and above.

In plants, oils are stored intracellularly as lipid droplets,
called oleosomes, which are structures similar to micelles. The oleosomes are composed of a triacylglycerol matrix surrounded by a phospholipid monolayer. Unlike micelles, the oleosomes have a surrounding protein coat (consisting primarily of oleosins). The coat proteins prevent the coalescence of oils and maintain the strong relationship between the oil and the oleosome surface. Alterations in the oleosin structure modifies the oleosome and can negatively affect the germination of some seeds\(^{12 - 14}\).

The oleosomes in avocado mesocarp have not been associated with germination, but oleosomes have been detected in the mesocarp of oil palm, olive, and avocado fruits. The oleosomes in fruit mesocarp are larger in size than seed oleosomes; for example, in olive fruits, the oleosomes range in size from 0.5 to 2 \(\mu\)m in the seeds and from 10 to 20 \(\mu\)m in the mesocarp. Similarly, in palm oil fruits, the oleosomes range in size from 4 to 26 \(\mu\)m in the seeds and from 4 to 32 \(\mu\)m in the mesocarp. Ho et al.\(^{18}\) related the oil content with the size and number of oleosomes in the oil palm fruit mesocarp, reporting that a higher oil content is associated with a higher number of smaller oleosomes\(^{15 - 18}\).

Although the avocado fruit of the Mexican drymifolia variety has a high oil content, no characterization studies have been conducted on its oil storage cellular structures, on the oil composition, or on the possible differences between the previously reported cultivars and the botanical varieties. In this work, we compared the location, profile, and fatty acid composition of the oil from the mesocarp and isolated oleosomes of \(P.\) americana var. drymifolia and \(P.\) americana cv. Hass to the oil extracted from \(P.\) americana var. americana.

## 2 MATERIALS AND METHODS

### 2.1 Plant materials

All avocado fruits were collected at the ripening stage. The fruits of \(P.\) americana var. drymifolia were collected in Petembo, Michoacán México (Latitude 19.1167, Longitude 101.493611); the fruits of \(P.\) americana var. americana were purchased at a local market in Mérida Yucatán México, and the fruits of \(P.\) americana cv. Hass were acquired at a local market in Morelia, Michoacán, México. The fruits were washed, peeled, and destoned to separate the pulp (mesocarp) from the seeds and peel (pericarp).

Some mesocarp slices from each avocado variety were used for histological analysis, while others were ground to obtain oleosomes or used for oil extraction.

### 2.2 Histologic analysis

The tissues were embedded in TissueTeck\textsuperscript{TM} and then frozen at \(-23^\circ\)C for component solidification in cryostat (Hyrax c25 Zeiss\textsuperscript{TM}) prior to slicing into 70 \(\mu\)m thick sections. The slices were fixed for 15 min in 4\% formaldehyde/15\% glycerol, thoroughly rinsed with distilled water, stained for 5 min with Sudan III, rinsed with 70\% ethanol, stained for 5 min with toluidine blue, and rinsed with distilled water. The slides were observed by light microscopy at 40\(\times\) magnification. The images were analyzed with ImageJ (https://imagej.nih.gov/ij/) software to obtain areas, perimeters, and Feret’s diameters for the red-stained oleosomes.

### 2.3 Oleosome isolation and oil extraction

Oleosomes were obtained by the Tzen method\textsuperscript{19} consisting of flotation on sucrose buffers followed by one wash with detergent (Tween 20\textsuperscript{TM}) and one wash with ionic solution (2M NaCl). The oleosome oils in 300 \(\mu\)l of oleosome suspension were recovered using 3 mL of chloroform/methanol 2:1 (C/M). The mesocarp oils were recovered from 500 mg of mesocarp finely ground in liquid nitrogen and rinsed three times with 3 mL of C/M per rinse. The solvent was evaporated in a nitrogen atmosphere and the oil was desiccated for 48 h at 37\(^\circ\)C. Triplicate oil extractions were conducted and the yield was calculated for each sample. Hass avocado oil was used as a reference.

### 2.4 Reversed phase thin layer chromatography

Reversed-phase thin-layer chromatography (RP-TLC) was carried out on RP-C18 thin-layer plates (Merck 60-RP-18) as the stationary phase and dichloromethane: acetic acid: acetone (20:40:50) as the mobile phase, according to a method obtained from the European pharmacopoeia\textsuperscript{20}. About 5 mg of oil was loaded per lane and the plate was developed once. The plates were treated with phosphomolybdic acid and heated for 3 min at 95\(^\circ\)C to reveal the triacylglycerol (TAG) patterns, which were compared with those obtained from commercial olive and avocado oils used as references.

### 2.5 GC/MS

The fatty acid composition was determined for the oil of each avocado variety by an acid derivatization method in 2 mL reaction vials. The reaction mixture, consisting of 100 \(\mu\)l of sample oil and 1 \(\mu\)l of 1M HCl in methanol, was heated at 80\(^\circ\)C for 1 h. The methyl esters were recovered with hexane and the solvent was evaporated with nitrogen gas. The methyl esters were resuspended in 200 \(\mu\)l of methanol, and 1 \(\mu\)l was injected into a GC/EIMS Agilent series 5975C instrument (splitless and 1 \(\mu\)l/min of helium as the carrier) equipped with an Agilent 122-0162U DB-1ms Ultra Inert column (60 m \(\times\) 250 \(\mu\)m \(\times\) 0.25 \(\mu\)m). The oven was held at 150\(^\circ\)C for 3 min and then heated to 300\(^\circ\)C at a rate of 4\(^\circ\)/min and held for 20 min at the final temp. The separated methyl esters were detected with a mass detector working in the electronic impact (EI) mode, and data were collected in the scan mode from 50–550 \(m/\)z.
2.6 Fatty acid identification and quantification

Chromatograms were analyzed with AMDIS software and the NIST database (http://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:amdis and https://www.nist.gov/srd/nist-standard-reference-database-1a-v17) for identification of signal peaks. The fatty acid peaks were identified by their retention times and mass spectra, and the percentage of each fatty acid was estimated using the area under the curve.

2.7 Statistics

All data were analyzed with R software and the percentage data were transformed using the formula:

\[ \text{adj\%} = \left( \frac{180}{\pi} \right) (\sin^{-1} (\sqrt{\%})) \]

where:
- \text{adj\%} = adjusted percent
- \% = original percent expressed as centesimal

to normalize the distribution of data and to allow variance analysis (ANOVA) and calculation of the mean differences (Tukey test).

3 RESULTS AND DISCUSSION

3.1 Oleosome staining

Sudan III staining allowed in situ observation of the oleosomes (Fig. 1). Toluidine blue gave non-specific purple-blue staining of proteins and a slight staining of the cell wall. Sudan III stains neutral lipids, such as TAGs, an orange-red color, so the oleosomes appeared as spheroids with an orange to reddish-brown color. The oleosome mean size differed between the three avocado varieties (Table 1): they were small (11.96 ± 1.44 μm) and dispersed in P. americana var. americana (Fig. 1 panel A), larger (41.53 ± 6.83 μm) and without form in P. americana cv. Hass (Fig. 1 panel C), and clustered in P. americana var. drymifolia (Fig. 1 panel B). The cluster size and shape were very similar to a single oleosome from the Hass cultivar; however, each oleosome that formed the drymifolia cluster had a smaller size (21.52 ± 8.49 μm) and a spheroidal shape; this difference was evident because the toluidine blue stained the protein coat that separated the oleosomes, so the oleosomes appeared as individual structures with dark purple borders. The protein in the Hass cultivar appeared as violaceous-blue stained patches on the oleosome surface, but no oleosome clusters were spotted; the size difference was evident despite the high variability. Hass avocado mesocarp had larger oleosomes (p < 0.05).

As reported by Platt (11) and Huang (16), avocado mesocarp cells possess oleosomes and the Sudan III staining reveals the presence of TAGs inside them, while toluidine blue stains the oleosome protein coat. This differential staining allowed the observation of the oleosome clusters in drym-

Fig. 1  Stained micrographs of different avocado varieties. A) American (americana variety), B) Mexican (drymifolia variety), C) Hass. Oleosomes are orange-red spheroids, the violet-blue stain indicates proteins on the oleosomes, and the lines indicate cell walls. D) Comparison of oil yields (% adj) and the Feret’s diameter of the oleosomes (μm); amer = Persea americana var. americana, drym = P. americana var. drymifolia, Hass = P. americana cv. Hass.
Oleosome sizes of avocado fruit mesocarp.

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</table>

Data obtained with ImageJ software.

The drymifolia variety had smaller oleosomes than the Hass cultivar, but a higher oil content. This result suggests that the mesocarp oil content does not directly relate to oleosome size or that the content varies across a range of sizes.

3.2 Oleosome isolation and mesocarp oil content

Oleosomes were only recovered from the fruits of *P. americana* var. drymifolia and cv. Hass. In *P. americana* var. americana, the oleosome phases were too thin and impossible to recover. The americana variety had the lowest oil content, at 2.56%, in agreement with the value of 2.5-8% previously reported by Whiteley. This low oil content could explain the difficulty of oleosome isolation from this fruit variety. The drymifolia fruit mesocarp had the highest oil content, at 13.27% (*p < 0.05*), while Hass mesocarp had a content of 9.57%. These values are lower than those previously reported because the present values were expressed on a fresh matter basis, while other reports refer to dry matter. Since the dry matter is about 30% (meaning a loss of 70% as water), the relevant dry weight percentages would be 8.53% for americana, 44.23% for drymifolia, and 31.9% for Hass.

Figure 1 panel D shows the correlation between mesocarp oil content and oleosome size. The analysis of variance (*p < 0.05*) confirmed that the americana variety has the lowest oil content and the smallest oleosome sizes, while the highest oil content was found for the drymifolia variety.

3.3 Reversed phase thin layer chromatography of oleosome triacylglycerides

Triacylglycerides are the main storage components in avocado oleosomes. Figure 2 shows the RP-TLC results for the TAG profiles for the different oleosome oils. The profiles for solvent-extracted oils show high RF bands (lanes 1-3). The profile banding for the americana variety has a low intensity (lane 3), whereas the commercial avocado and olive oil samples have a greater number of bands (lanes 4, 5). All the profiles are similar to the olive oil bands previously reported, because they all show low RF bands. The RP-TLC data indicate that the TAG profile is specific for each avocado variety and for specific extraction methods.

The method used here was obtained from the European pharmacopeia, which uses this method to compare samples against oil patterns to detect oil mixtures and adulterations. The fatty acids associated with triglycerides occur in several combinations, so RP-TLC is used for quality control, as it separates different TAG species depending on their physical and chemical properties. Phosphomolybdic
Acid treatment then oxidizes the unsaturated fatty acids to develop a banding pattern that is characteristic for the TAG composition of the oil. However, this pattern can be altered by the extraction method or by mixing or adulterating the oils.

This is the first report of avocado oil banding profiles for commercial avocado oil (extracted by extrusion from Hass fruits) and for fruits of the Hass, drymifolia, and americana varieties (extracted with a chloroform/methanol solvent mixture).

3.4 Avocado mesocarp fatty acid profiles

Although acid derivatization is a widely reported reaction for determining fatty acid profiles, most papers published on avocado oil have used alkaline methods. For validation purposes, we compared the fatty acid profile data previously reported for the Hass cultivar obtained using the alkaline method with the fatty acid profile obtained by the acid method. As shown in Fig. 3, in all cases, no significant differences (p > 0.05) were found, which validated the use of the acid method.

Figure 4 shows the fatty acid composition of the mesocarp tissues of the drymifolia and americana varieties and the Hass cultivar. A clear and inversely proportional difference is observed between oleic (ole)-palmitic (pal) and linoleic (lin)-palmitoleic (pol) fatty acids.

The Hass cultivar has a higher oleic content and a lower content of palmitic and stearic (ste), whereas the drymifolia and americana varieties have similar oleic and palmitic acid contents (respectively, 42% and 38% for drymifolia and 41.7% and 39.1% for americana). The americana variety has the highest linoleic and stearic content, whereas drymifolia has the highest palmitoleic acid content. Differences are evident between oils from the mesocarp and oils from the oleosomes. The palmitic and linoleic acid levels are higher and the palmitoleic acid levels

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**Fig. 2** Reversed-phase thin layer chromatography showing the triacylglyceride (TAG) profiles of 1) Hass avocados (*Persea americana* (X Mill.) cv. Hass), 2) Mexican avocados (drymifolia variety), 3) American avocados (americana variety), 4) commercial olive oil, and 5) commercial avocado oil. TAGs were detected with phosphomolybdic acid.

**Fig. 3** Fatty acid composition of oil from Hass avocado (*Persea americana* (X Mill.) cv. Hass), as reported by Villa 2011, Meyer 2008, Kilaru 2015, & Takenaga 2008 and measured with an acid method. ole = oleic, pal = palmitic, lin = linoleic, lnl = linolenic, and ste = stearic acids.
are lower in Hass oleosome oil than in Hass mesocarp oil. Conversely, the palmitoleic acid levels are higher and the linoleic and stearic acids are lower in drymifolia oleosome oil than in drymifolia mesocarp oil.

Avocado oil, as with most vegetable oils, is rich in palmitic and oleic acids. As previously reported, Hass avocados have far more oleic (>50%) than palmitic acid (<30%) and these levels are close to those reported for olive oil that has high oleic acid concentrations (~70%) and low palmitic and linolenic acid concentrations (<16%) [27]. This finding can explain the common bands observed for Hass oil and olive oil on the RP-TLC plates. The drymifolia and americana varieties have far more palmitic acid (accounting for as much as 39% of the fatty acids) and far less oleic acid, at just 42%.

Figure 5 shows the saturation profile for the analyzed oils. The saturated fatty acids (SFAs), palmitic and stearic acid, accounted for about 40% of the fatty acids in the mesocarp oil extract of the drymifolia and americana varieties, and had the highest levels (p<0.05). Conversely, the commercial avocado oil had lower levels, at 7.6%. The monounsaturated fatty acids (MUFA's), palmitoleic and oleic acid, had the highest values in the mesocarp extract of the Hass cultivar and the commercial avocado oil, accounting for about 80% of the total fatty acids, whereas the americana and drymifolia mesocarp extracts contained only about 45% of these MUFA's. The polyunsaturated fatty acids (PUFA's), linoleic and arachidonic acid, were highest in the mesocarp of the americana and drymifolia varieties (accounting for about 13%) and lowest in the Hass cultivar (at 3.6%). The Hass cultivar and the drymifolia variety also displayed few differences between the oleosome and mesocarp extracts in terms of their unsaturated fatty acid profiles.

The findings reported here offer new insights into the composition of avocado oils and indicate useful commercial traits for the different avocado types, as the saturation profiles of the americana and drymifolia avocados demonstrate a lower percentage of monounsaturated fatty acids (MUFA's) and a higher percentage of polyunsaturated fatty acids (PUFA's). Recent work on saturation profiles and health effects now suggests that replacing dietary SFAs with PUFAs can have a modest effect on coronary heart disease risk (although the effect of replacing SFAs with MUFA's is less certain) [28]. The saturation level of membrane fatty acids is also related to cold acclimation, and this can explain the differences between mesocarp and oleosome oils, as the temperate-climate drymifolia has more PUFAs in the mesocarp oil than in the oleosome oil while Hass variety has more MUFA's in the mesocarp oil than in the oleosome oil (Table 2).

The composition of Hass pulp and isolated idioblasts (oil cells) has recently been reported [29] and the fatty acid composition was similar for both mesocarp and idioblast oils. This previous work focused on the acetogenins of avocado but investigated the fatty acid composition as well. The results clearly showed that for cv. Hass the oleosome fatty acids were enriched in palmitic acid, while the idioblasts contained the same fatty acids as the mesocarp. Other studies of idioblasts and their chemical composition have indicated that these oil cells contain a wide range of chemical compounds, including alkaloids, sesquiterpenes, and...
hydroperoxides\textsuperscript{[21]}, indicating that fatty acids are not the most abundant components in these structures.

4 CONCLUSION
Avocado mesocarp oil is stored in oleosomes and a higher oil content (13\% fresh weight) is observed in \textit{P. americana} var. drymifolia, which has a large oleosome size of 21.52 μm. The RP-TLC band profiles of avocado oils show three common bands that are conserved in all avocado types and in olive oil, but the rest of the bands are specific for each avocado variety and depend on the extraction method. The fatty acid oil composition of the drymifolia and americana avocado varieties is lower in oleic and higher in palmitic when compared to the oil from the Hass cultivar. This higher palmitic and linoleic content can be a useful trait that can extend the commercial use of avocado oils.

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References

\begin{table}[h]
\centering
\caption{Avocado mesocarp and oleosome oil yield and composition.}
\begin{tabular}{lcccccc}
\hline
\textbf{Tissue} & \textbf{Yield} & \textbf{palmitoleic} & \textbf{palmitic} & \textbf{linoleic} & \textbf{oleic} & \textbf{stearic} \\
\hline
\textit{Persea americana} & & & & & & \\
var. drymifolia Mesocarp & 13.05\textsuperscript{a} & 12.49\textsuperscript{b} & 38.73\textsuperscript{c} & 18.74\textsuperscript{c} & 40.73\textsuperscript{d} & 3.90\textsuperscript{e} \\
var. drymifolia Oleosome & 13.49\textsuperscript{b} & 16.30\textsuperscript{a} & 37.42\textsuperscript{c} & 16.36\textsuperscript{c} & 43.29\textsuperscript{d} & 2.49\textsuperscript{e} \\
var. americana Mesocarp & 2.56\textsuperscript{a} & 6.52\textsuperscript{a} & 39.15\textsuperscript{c} & 21.40\textsuperscript{a} & 41.72\textsuperscript{d} & 5.94\textsuperscript{e} \\
cv. Hass Mesocarp & 9.82\textsuperscript{a} & 11.80\textsuperscript{a} & 24.28\textsuperscript{a} & 10.59\textsuperscript{e} & 60.16\textsuperscript{a} & 1.94\textsuperscript{e} \\
cv. Hass Oleosome & 9.32\textsuperscript{a} & 4.53\textsuperscript{a} & 30.92\textsuperscript{a} & 15.83\textsuperscript{a} & 53.77\textsuperscript{a} & 1.65\textsuperscript{e} \\
Avocado & NA & NA & 13.90 & 13.22 & 64.87 & 7.67 \\
\hline
\end{tabular}
\footnotesize{\textsuperscript{a,b,c,d,} groups with significant differences; \(p = 0.05\)}
\end{table}


