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First report on quality and purity evaluations of avocado oil sold in the US

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ABSTRACT

The demand for avocado oil has increased significantly as consumers resonate with its potential health benefits, however, due to the lack of enforceable standards, consumers are unprotected from fraud (i.e., economic motivated adulteration). This study analyzed avocado oils currently on the market in the US to evaluate their quality (e.g., free fatty acidity, peroxide value, UV absorbances, vitamin E) and purity (e.g., fatty acids, sterols, triacylglycerols). Our results showed that the majority of commercial samples were oxidized before reaching the expiration date listed on the bottle. In addition, adulteration with soybean oil at levels near 100% was confirmed in two "extra virgin" and one "refined" sample. These findings demonstrate there is an urgent need to develop standards for avocado oil not only to ensure the consumers receive high quality and authentic products but to establish a level playing field to support the continuing growth of global avocado oil industry.

1. Introduction

The world's production of avocados increased one million tonnes from 2014 to 2017 and is projected to continue rising with Mexico accounting for one third of the world's production (Altendorf, 2019). Consumer demand for the fruit is largely due to the health benefits associated with avocados, which have high amounts of monounsaturated fatty acids and antioxidants (Fernandes, Gómez-Coca, Pérez-Camino, Moreda, & Barrera-Arellano, 2018; Wang et al., 2019; Wong, Requejo-Jackman, & Woolf, 2010). The rising popularity of avocados has also led to the rise in avocado products, namely avocado oil.

Competition in the market place for avocado oil continues with one major boundary, there are currently no standards to determine if an avocado oil is of the quality advertised and authentic. Oils that are of poor quality or blended with cheaper edible oil can be traded and sold at lower prices than high quality or authentic products leaving bulk buyers, food service professionals and consumers unprotected. With no standards available, there is no way to ensure avocado oil is safe. Standards developed for edible oils commonly fall into two categories, quality and purity. Quality can be controlled by the fruit used to make the oil, extraction process, storage; it's mostly related to level of hydrolysis of the fruit and oxidation of the oil (Woolf et al., 2009). An oil is considered pure or authentic if there are no other additives or oils present other than what is listed on the label.

So far, much of literature has focused on improving extraction methods for avocado oil (Corzzini, Barros, Grimaldi, & Cabral, 2017; Dos Santos, Alicieo, Pereira, Ramis-Ramos, & Mendonça, 2014; Krumreich, Borges, Mendonça, Jansen-Alves, & Zambiazi, 2018; Ortiz Moreno, Dorantes, Galíndez, & Guzmán, 2003; Ramírez-Anaya, Manzano-Hernández, Tapia-Campos, Alarcón-Domínguez, & Castañeda-Saucedo, 2018; Tan & Ghazali, 2019; Werman & Neeman, 1987). There have also been multiple studies chemically characterizing avocado oil based on cultivar (Fernandes et al., 2018; Manaf, Rahardjo, Yusof, Desa, & Nusantoro, 2018; Yanty, Marikkar, & Long, 2011) and region (Donetti & Terry, 2014; Tan, Tan, & Tan, 2017). However, there is a need to understand the range in quality and purity of the avocado oils currently on the market and how chemical composition of these oils compare to avocado oils characterized in literature. A few studies have done this on a small scale (Fernandes et al., 2018; Flores, Perez-Camino, & Troca, 2014; Werman & Neeman, 1987), however, to our knowledge no study has comprehensively evaluated the quality and purity of avocado oils available in the US, which is one of the largest consuming countries in the world (Altendorf, 2019).

Here we present an analysis of the quality and purity of avocado oils available in the US market with the goal of starting a database to support standards development for this industry. Twenty-two samples were collected from six grocery stores (14 samples) and two online sources (eight samples), efforts were made to cover all the major brands and types of oil (extra virgin/unrefined and refined). Oil quality was determined using free fatty acidity (FFA), peroxide value (PV), and specific extinction in ultraviolet (UV) absorbances in addition to

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Table 1
Sample information for the oils used in this study.

Sample Code	Purchasing Method	Expiration Date (month-year)	Product Origin	Cost/fl oz ($)	Packaging Type
EV1	Online	Oct-21	California	2.23	Dark glass
EV2	In store	Jun-21	California	1.29	Dark glass
EV3	In store	Feb-21	Mexico	0.65	Dark glass
EV4	In store	Sep-20	California	1.53	Dark glass
EV5	Online	Jul-21	California	1.57	Dark glass
EV6	Online	NA	Brazil	0.49	Clear plastic
EV7	Online	Jun-21	California	2.35	Dark glass
R1	Online	Jun-21	Spain or Mexico	0.44	Dark plastic
R2	In store	Aug-20	Mexico	0.74	Dark glass
R3	In store	Nov-20	Mexico	0.43	Dark glass
R4	Online	Dec-20	Mexico	0.35	Clear plastic
R5	In store	May-20	Mexico	0.25	Dark plastic
R6	In store	Jul-20	Mexico	0.77	Dark glass
R7	Online	Dec-19	Mexico	0.80	Dark glass
R8	In store	Apr-21	Mexico	1.44	Clear glass
R9	In store	Apr-21	Mexico, USA, or Spain	0.29	Clear plastic
U1	In store	NA	Mexico	0.29	Dark plastic
U2	In store	Apr-21	Mexico, USA, or Spain	0.66	Tin bottle
U3	In store	Mar-21	Mexico, USA, or Spain	0.71	Tin bottle
U4	In store	May-21	Mexico	0.47	Dark glass
U5	In store	Jun-21	Mexico	0.79	Dark glass
U6	Online	Feb-21	Mexico	0.34	Clear plastic

chlorophyll and tocopherol content. The authenticity of the oils was assessed using the fatty acids, sterols, and triacylglycerols (TAG) profiles. This study aimed to better understand the quality and purity of avocado oils available in the US and to demonstrate that there is an urgent need for standards in this industry.

2. Materials and methods

2.1. Avocado oil samples

A total of 22 avocado samples consisting of both extra virgin and refined oils were collected from six grocery stores (14 samples) and two online sources (eight samples). Each oil sample was wrapped in aluminum foil and stored in the dark at 20 °C. Samples were purged with nitrogen after each opening. Table 1 contains information such as purchasing method, expiration date, product origin, cost and packaging type for each oil. Samples were separated into three groups according to their label. Extra virgin oil was coded as "EV" in front of the sample number, refined avocado oil as "R", and unspecified oils "U". The unspecified oils were samples that either did not specify the type of avocado oil or, samples that had unclear and ambiguous labels on the bottle.

2.2. Quality parameters

FFA, PV, UV specific extinction at 232 nm, 270 nm, and ΔK were determined using AOCS methods Ca 5a-40 (09), Cd 8b-90 (09), and Ch 5–91 (09) (American Oil Chemist's Society, 1998), respectively.

2.3. Minor components

Chlorophylls were determined according to AOCS method Cc 13d-55 (09) (American Oil Chemist's Society, 1998). Tocopherols were determined according to Gimeno, Castellote, Lamuela-Raventós, de la Torre, and López-Sabater (2000) with some modifications. Oil (40 μL) and hexane (160 μL) were briefly vortexed. The internal standard, α-tocopheryl acetate (purity 98%, Fisher Scientific Company LLC, USA) in ethanol at a concentration of 300 μg/mL, was then added in addition to 600 μL of methanol. The sample was vortexed for 1 min and centrifuged (5000 rpm, 5 min, Beckman GS-15R). Samples were stored at −20 °C for 2 h to allow oil to separate from the organic phase. The organic extract was filtered (0.45 μm, nylon). Analysis was performed on an Agilent 1290 Infinity II LC system with a diode-array detector using an Agilent ZORBAX Eclipse Plus C18 column (3.5 μm, 3 × 100 mm). The mobile phase was methanol:water (96:4), isocratic. A 20 μL injection volume and flow rate of 1.0 mL min^{-1} were used giving a total run time was 12 min. DAD signal was recorded at 292 nm. All solvents used above were HPLC grade, from Fisher Scientific LLC, USA. Standards α-tocopherol (>96%), and α-tocopheryl acetate (98%) were purchased from Fisher Scientific LLC, USA. Analytical grade standards δ-tocopherol and γ-tocopherol were purchased from MilliporeSigma, USA.

2.4. Purity parameters

The IOC official method for the determination of the fatty acid methyl esters by gas chromatography (COI/T.20/Doc. No 33/Rev.1, 2017) was used for fatty acid profile analysis (International Olive Council, 2017). The GC-FID analysis was conducted on an Agilent 7890A GC (Agilent Technologies, USA). A 20 m × 180 μm × 0.20 μm DB-23 capillary column (Agilent Technologies, USA) was used to achieve the separation of individual fatty acids. The injection volume was 1.0 μL and helium, ultra-high purity, Airgas, USA was used as a carrier gas at a flow rate of 1 mL min^{-1}. The injector temperature was held at 250 °C at a split ratio of 50. The GC oven program was initially held at 80 °C for 0.5 min; then ramped at 65 °C min^{-1} to 175 °C, followed by a ramp of at 10 °C min^{-1} to 185 °C, which was held for 0.5 min. The last ramp was at 7 °C min^{-1} to 230 °C and held for 5 min, giving a total run time of 14.89 min. The FID temperature was 260 °C. The detector gas consisted of hydrogen, ultra-high purity, Praxair, USA (flow rate: 40 mL min^{-1}), air, specialty grade zero air, Praxair, USA, (flow rate: 400 mL min^{-1}), and helium, ultra-high purity, Airgas, USA make up gas (flow rate: 25 mL min^{-1}). Peak identification was performed using a FAME C8–C22, certified reference material, TraceCERT, MilliporeSigma, USA.

The IOC official method for the determination of the composition and content of sterols (COI/T.20/Doc. No 30/Rev.1, 2013) was used with modifications (International Olive Council, 2013). The unsaponifiable fraction was prepared by drying 0.5 mL of internal standard 0.2% α-cholestanol, analytical grade standard, MilliporeSigma, USA, ethyl acetate solution under nitrogen before adding 50 mL of 2 mol L^{-1} ethanolic potassium hydroxide, >85%, Fisher Scientific LLC, USA, to 5 g of the avocado oil sample. The mixture was heated to gentle boiling and kept under reflux for 20 min. The organic/aqueous mixture was extracted three times, 200 mL ethyl ether in total, washed with DI

water, dried with anhydrous sodium sulfate, >99%, Fisher Scientific LLC, USA, evaporated to dryness, and further dried in an oven. The sterols were separated from the other unsaponifiable fractions on a silica gel $60F_{254}$-coated aluminum-backed thin-layer chromatography (TLC) sheet (MilliporeSigma, USA) with hexane/ethyl ether (60:40, v/v). The sterols band was made visible by spraying the plate with 0.2% 2, 7-dichlorofluorescein, ~90% (TLC), MilliporeSigma, USA, ethanolic solution and was then dissolved in 10 mL hot ethyl acetate and 30 mL ethyl ether and evaporated to dryness. All solvents used above were of HPLC grade from Fisher Scientific LLC, USA. Finally, 300 μL of the silylation reagent (pyridine, >99%, Fisher Scientific LLC, USA/hexamethyl disilazane, >99%, MilliporeSigma, USA/trimethylchlorosilane, >99%, MilliporeSigma, USA, 9:3:1, v/v/v) was added to prepare the trimethylsilyl ethers for GC injection. The GC-FID analysis was conducted on an Agilent 7890A GC (Agilent Technologies, USA). A 30 m × 0.25 mm × 0.25 μm DB-5 capillary column (Agilent Technologies, USA) was used with an injection volume of 1.0 μL and helium, ultra-high purity, Airgas, USA, as the carrier gas at a flow rate of 1.2 mL min^{-1}. The injector temperature was held at 280 °C at a split ratio of 25. The GC oven program was held isothermally at 150 °C for 8 min; then ramped at 20 °C min^{-1} to 290 °C and held for 20 min to obtain a total run time of 37.33 min. The FID temperature was 300 °C. The detector gas consisted of hydrogen, ultra-high purity, Praxair, USA (flow rate: 30 mL min^{-1}), air, specialty grade zero air, Praxair, USA (flow rate: 400 mL min^{-1}), and helium, ultra-high purity, Airgas, USA, make up gas (flow rate: 25 mL min^{-1}). Peak identification was carried out with standards campesterol (65%), stigmasterol (95%), β-sitosterol (95%), each from MilliporeSigma, USA and by comparing the generated chromatograms against the sample chromatograms provided in the IOC official method and their relative retention times while the quantification was performed using the peak area and concentration of the internal standard.

TAGs were separated and analyzed using the method described in Green et al. (2020). In brief, each oil was diluted to a final concentrate of 1% with chloroform and then analyzed with the Vanquish™ Flex UHPLC-CAD system (Thermo Fisher Scientific, Waltham, MA, USA). Analytes were separated on a Thermo Scientific™ Accucore™ C18 column (100 mm × 2.1 mm; 2.6 μm). The injection volume was 1 μL and the flow rate was 0.5 mL min^{-1}. Mobile phase A was acetonitrile and mobile phase B was isopropanol using the solvent gradient conditions: start, 10% B; 2 min, 10% B; 25 min, 40% B; 30 min, 60% B; 35 min, 90% B; 40 min, 50% B and 45 min 10% B. All solvents were HPLC grade from Fisher Scientific LLC, USA.

2.5. Statistical analysis

Statistical analysis was accomplished using Originlab Corporation software version "OriginPro 2016 Sr2." This program was used to run PCA on all samples analyzed with the UHPLC-CAD. Principal component scores were computed by Originlab.

3. Results and discussion

3.1. Quality parameters

Free fatty acids in the oil are caused by lipolysis where the fatty acids are separated from the TAG and are commonly used as a measurement for oil quality (CODEX, 2017; Woolf et al., 2009). The free fatty acid content of the oils is summarized in Fig. 1a. Overall, samples labeled as "extra virgin" had higher free fatty acidity than "refined" which is expected as the refining processes remove free fatty acids. The unspecified avocado oils had similar values to the refined, aside from U2 and U3, which had an FFA of 0.59% and 0.97%, respectively. Woolf et al. (2009) proposed the refined avocado oil should have values that are less than 0.1% FFA while Werman and Neman et al. (1987) saw about 0.55% FFA for refined oils (Werman & Neeman, 1987; Woolf et al., 2009). The refined oils in this study were all at or under 0.1%. Samples labeled as "extra virgin" had an FFA range of 0.03–2.69%, with an overall average of 1.31%. Commercial samples labeled as "virgin" analyzed in Flores et al. (2014) had FFA values ranging from 0.45 to 0.56%, while avocado oils made in-house in literature range from 0.12 to 2.84% (Bora, Narain, Rocha, & Queiroz Paulo, 2001; Krumreich et al., 2018; Manaf et al., 2018; Ortiz Moreno et al., 2003). The high values seen in this study could indicate use of poor-quality fruit and/or poor handling during processing, particularly for EV1, EV4 and EV5, which had values near 2.5%. Unhealthy fruits that are damaged, bruised, overripe, insect infested; prolonged time between harvest and processing; overheating during processing are all factors that can contribute to a rise in FFA (Woolf et al., 2009).

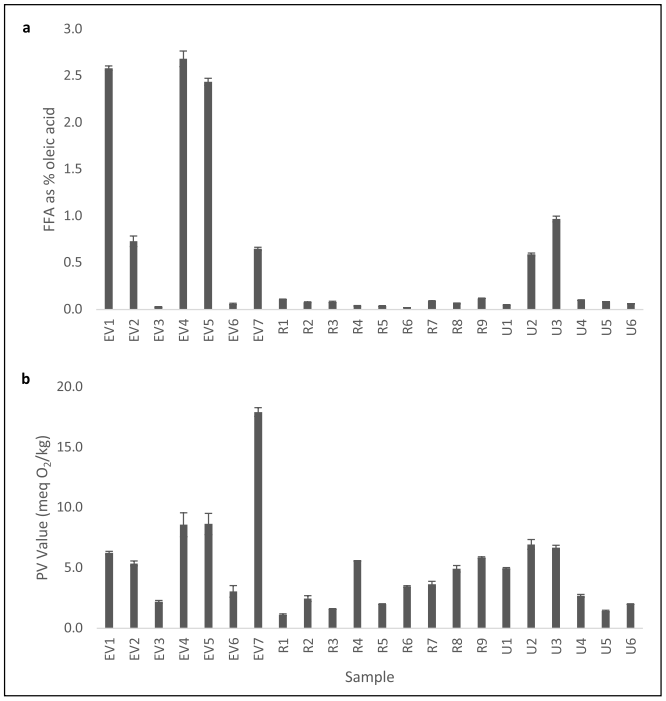

Fig. 1. (a) Free fatty acid content reported as % oleic fatty acid. (b) Peroxide value expressed in meq O2/kg. Each bar is an average of triplicate measurements and error bars are calculated using the standard error of the mean (SEM). EV stands for extra virgin, R for refined, and U for unspecified avocado oil.

Peroxides are the primary oxidation products formed when an oil is exposed to oxygen and produce undesirable flavors and odors. The peroxide value results are shown in Fig. 1b. Although trends within the three sample groups are less obvious than with the FFA results, overall, the refined oils had the lowest PV values averaging at 3.42 meq O_2/kg. The unspecified samples had a slightly higher average (4.13 meq O_2/kg); extra virgin samples were the highest at 7.4 meq O_2/kg. As with FFA, the refining process removes peroxides, therefore, lower values are expected for refined oils than those labeled extra virgin. However, many of the refined oils in this study still have notably high PV values. Woolf et al. (2009) proposed 0.5 meq O_2/kg to be the upper limit for PV in refined avocado oils and standards proposed by Mexico for CODEX cap the acceptable PV at 2 meq O_2/kg. All of the oils except for R1, R3, and R5 were above these limits (CODEX, 2017; Woolf et al., 2009).

Table 1 contains sample information including purchasing method (in store or online), expiration date, product origin, cost and packaging type for each oil. Interestingly, the three refined oils with the highest PV values (R4, R8, and R9) were stored in clear instead of tinted packaging, which is not protective against photooxidation. Another factor that can contribute high PV values is storage time. The closer an oil is to the best by date on the bottle, the more likely it has had a long storage time. In this study, however, no correlation was found between

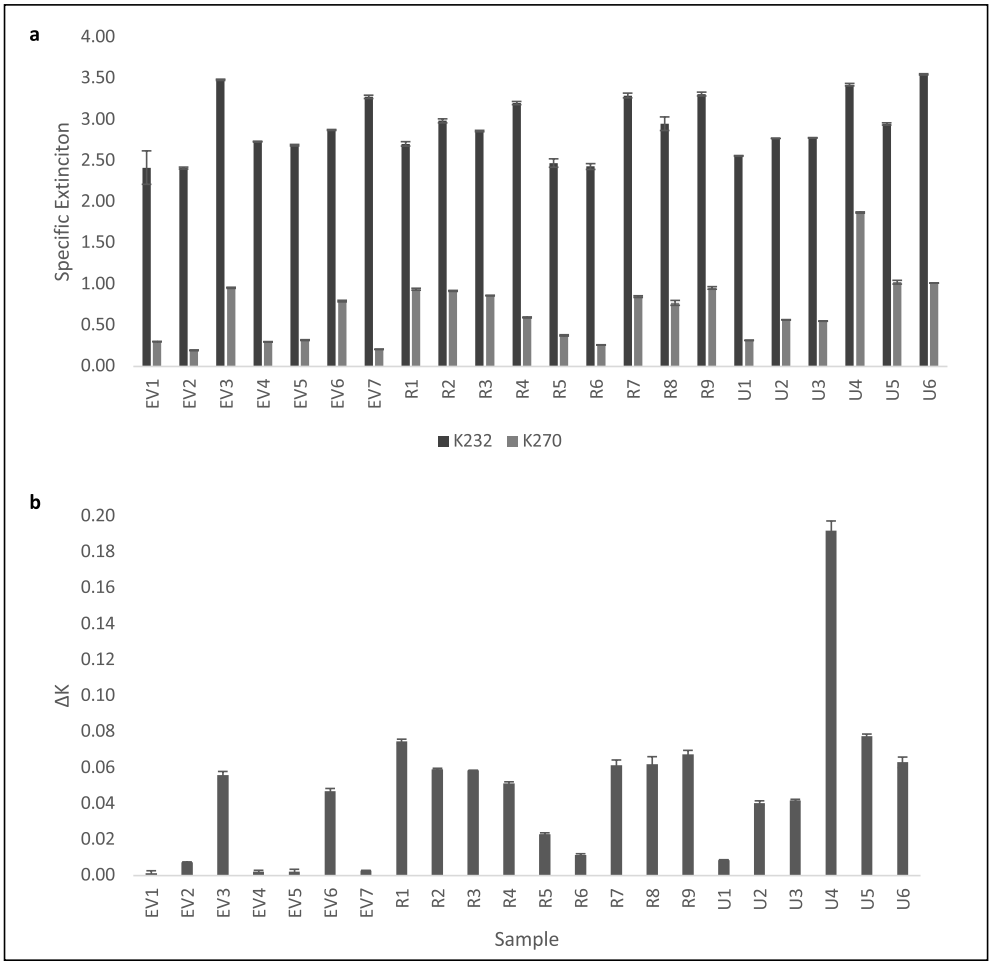

Fig. 2. (a) Values for the primary oxidation products (K232) and secondary oxidation products (K270) in each oil. (b) Values for ΔK. Bars are an average of triplicate measurements and error bars show SEM. EV stands for extra virgin, R for refined, and U for unspecified avocado oil.

the expiration date on the bottle and the PV values and all the samples were tested before reaching the expiration date. Literature values range from 1.4 to 12.74 meq O_2/kg for lab-made avocado oil samples (Bora et al., 2001; Elez-Martinez et al., 2005; Jorge, Polachini, Dias, Jorge, & Telis-Romero, 2015; Krumreich et al., 2018; Manaf et al., 2018; Ortiz Moreno et al., 2003). A study looking at two commercial virgin avocado samples in Chile, storage time unknown, saw higher PV values of 8 meq O_2/kg and 12.95 meq O_2/kg (Flores et al., 2014). All of the samples tested in this study were in those ranges, aside from EV7, at 17.9 meq O_2/kg. Coincidentally, EV7 was the most expensive sample ($2.35/fl oz) out of the 22 samples purchased for this study.

K_{232} is another measure of the primary oxidation products present in an oil while K_{270} measures secondary oxidation products. Fig. 2a shows the K_{232} values range from a low of 1.4 for sample R6 and EV1 to a high of 3.5 for sample U6. These ranges are comparable to values observed in the limited studies that have measured the specific extinction in UV in avocado oils. Ramírez-Anaya et al. (2018) saw K_{232} values of 1.8–2.8 for centrifuge extracted oil at different malaxation temperatures (Ramírez-Anaya et al., 2018). Another study looking at commercial oils in Chile saw K_{232} values in the range of 3.16–4.19 (Flores et al., 2014). It is likely the increase of primary oxidation products seen in commercial samples from both this study and Flores et al. (2014) compared to the values seen in Ramirez-Anya et al. (2018) are because long storage time results in an increase of autoxidation.

Refined oils have a higher K_{270} because refining processes create conjugated trienes, which absorb at about 270 nm. Storage time can also increase K_{270} in avocado oils; Elez-Martinez, Soliva-Fortuny, Gorinstein, & Martin-Belloso (2005) demonstrated that a fresh sample had a value of 0.4, which increased to 1.6 after 24 weeks (Elez-Martinez et al., 2005). In this study, the K_{270} was higher for many of the refined (average 0.725) and the unspecified oils (average 0.865) compared to the 0.459 average of the extra virgin samples. No correlation was seen between the expiration dates and K_{270} values. There was one unspecified oil, U4, with a particularly high K_{270} value of 1.84, which could indicate poor quality or harsh refining processes. When looking at the extra virgin samples EV3 and EV6 had higher K_{270} than the rest of the samples in this group. However, a K_{270} range of approximately 0.1–0.8 was seen in fresh in-house made oils under varying malaxation conditions (Ramírez-Anaya et al., 2018). This range was also seen in a study that analyzed commercial oils labeled as virgin (best-by date unknown), and is similar to the extra virgin oils in this study (0.16–0.77) (Flores et al., 2014).

The ΔK value can help distinguish virgin or extra virgin oil from one that is refined. The difference between a poor-quality virgin or extra virgin oil and one adulterated with refined oil can often be seen using ΔK (Vossen, 2007). Fig. 2b summarizes the ΔK values for the oils in this study. To the best of our knowledge, this is the first report of ΔK values for avocado oil and we are therefore unable to compare values in this study with other literature. In the standards for olive oil from the International Olive Council, extra virgin olive oil must have a ΔK below 0.01 (Vossen, 2007). As anticipated, all of the refined oils are either at or above this limit as are all of the unspecified avocado oils with U1 having the lowest value of 0.01. U4 has gone under significant refining, with a value of nearly 0.2. For the extra virgin samples EV1, EV2, EV4,

EV5, and EV7 are all under the extra virgin olive oil limit of 0.01. These are also the same samples that had a low K_{270}. This indicates it is likely that these oils are not adulterated with refined oils; however, some are of poor quality as they had high FFA and PV values. Interestingly, EV3 and EV6 which had low FFA and PV values and seemed to be the highest quality of the extra virgin samples had higher K_{270} and notably high ΔK values of 0.056 and 0.047, respectively compared to the other extra virgin samples. This indicates that it is possible that these two samples are refined or are blended with refined oils; the ΔK values for these two samples are still within the standard for refined olive oils, which must be below 0.16 (CODEX, 2017). In addition, the prices of these two samples were significantly lower than other extra virgin samples and more comparable with the refined oils.

3.2. Minor components

Chlorophyll pigments are what give extra virgin avocado oil its classic green color. In addition to the extra virgin labeled samples, three unspecified oils (U2, U3, and U6) were also tested as they appeared light green in color unlike the other refined oils and unspecified oils, which were light, pale yellow. The chlorophyll content ranged from 6.62 mg/kg to 98.8 mg/kg as shown in Fig. 3. EV1, EV2, EV4, EV5, and EV7 contained ~95 mg/kg chlorophyll; these oils were noticeably dark green in appearance. The chlorophyll content seen in literature ranges greatly from 1.0 mg/kg to 69.8 mg/kg (Ashton et al., 2006; Jorge et al., 2015; Krumreich et al., 2018; Werman & Neeman, 1987; Wong et al., 2011). The inclusion of skin during processing could be responsible for the high values seen in this study. However, the values seen in Wong et al. (2011) are lower than those seen in this study and in Ashton et al., 2006, which saw a chlorophyll content of to 214 mg/kg from the skin (Ashton et al., 2006; Wong et al., 2011). These variations are not unusual as the cultivar and ripeness of the fruit, extraction method, storage can all greatly impact the amount of chlorophyll in the oils. It's important to note that EV3 and EV6, which had the lowest chlorophyll content, were also the same oils that had low FFA and PV but high ΔK and K_{270}. This also supports the hypothesis that these oils are either refined or blended with oils that are refined.

There are eight compounds that make up vitamin E content, four tocopherols (α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol) and four tocotrienols. In this study, the four tocopherol compounds were quantified with beta and gamma values summed together (Table 2). Woolf et al. (2009) proposed that the tocopherol content in extra virgin avocado oil should be between 70 and 190 mg/kg (Woolf et al., 2009). Refined oils were not included in this range, as tocopherols are largely removed in the refining process. For all but three samples (EV3, EV6 and U6) in this study, alpha tocopherol was the highest concentration, followed by gamma, then delta which is consistent with literature (Fernandes et al., 2018; Madawalaa, Kochharb, &

Table 2
Individual and total tocopherol content, expressed in mg/kg, for each avocado oil.

	α-Tocopherol	γ + β-Tocopherol	δ-Tocopherol	Total tocopherols
EV1	155.2 ± 11.8^{def}	ND	ND	155.2^{efghi}
EV2	116.0 ± 4^{fgh}	ND	ND	116.0^{ghi}
EV3	87.3 ± 3.2^{hi}	412.5 ± 55.4^{b}	145.6 ± 5.7^{c}	645.4^{b}
EV4	120.7 ± 4^{fgh}	ND	ND	120.7^{ghi}
EV5	143.3 ± 1.5^{efg}	ND	ND	143.3^{fghi}
EV6	95.9 ± 0.5^{ghi}	581.3 ± 67.1^{a}	229 ± 9.7^{a}	906.2^{a}
EV7	140.9 ± 11.9^{efg}	ND	ND	140.9^{fghi}
R1	396.7 ± 8.7^{a}	108.8 ± 4.7^{cd}	ND	505.5^{c}
R2	178.2 ± 2.9^{cde}	ND	ND	178.2^{efgh}
R3	194.2 ± 7.6 ^{cd}	102.6 ± 21.3^{cd}	ND	296.8^{de}
R4	34.0 ± 1.9^{j}	ND	ND	34.0^{i}
R5	116.9 ± 2.6^{fgh}	ND	ND	116.9^{ghi}
R6	194.7 ± 13.1^{cd}	ND	ND	194.7^{efg}
R7	209.3 ± 12.2^{c}	ND	ND	209.3^{efg}
R8	276.8 ± 15.3^{b}	ND	ND	276.8^{ef}
R9	49.9 ± 6.7^{ij}	ND	ND	49.9^{hi}
U1	156.8 ± 6.2^{def}	ND	ND	156.8^{efghi}
U2	52.2 ± 0.2^{ij}	42.4 ± 1.4^{cd}	ND	94.6^{ghi}
U3	60.1 ± 9.3^{ij}	41.5 ± 5.4^{cd}	ND	101.6^{ghi}
U4	317.6 ± 20.9^{b}	106.8 ± 5.3^{cd}	ND	424.4^{cd}
U5	388.0 ± 12.7^{a}	129 ± 5.2^{c}	ND	517.0^{c}
U6	91.1 ± 0.2^{hi}	440 ± 48.1^{b}	161.8 ± 6.6^{b}	692.9^{b}

ND = not detected. Data shown as the average of triplicate measurements plus minus standard error of the mean (mean ± SEM, n = 3). Different letters (a, b, c, etc.) in each column indicate significant differences calculated using Tukey test.

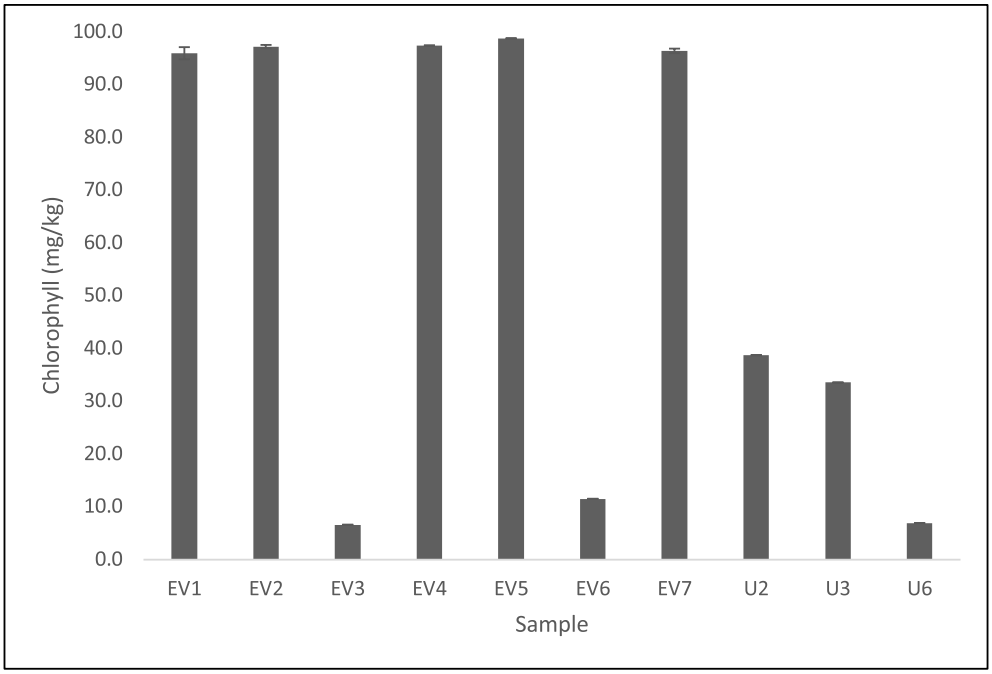

Fig. 3. Total chlorophyll content determined by AOCS official method Cc 13d-55. Measurements are done in triplicate with error bars indicating SEM. EV stands for extra virgin, R for refined, and U for unspecified avocado oil.

Duttaa, 2012; Manaf et al., 2018). However, the varietal can significantly impact the tocopherol content, for the Bacon avocado variety gamma tocopherol is higher than alpha (Fernandes et al., 2018). The lowest total tocopherol contents in this study were seen in R4 (34.0 mg/kg) and R9 (49.9 mg/kg). This study shows multiple samples (EV3, EV6, R1, U4, U5, U6) had total tocopherol contents over 400 mg/kg, which is interesting as the highest documented total tocopherol content in literature, to our knowledge, is 282 mg/kg (Corzzini et al., 2017). In particular, there are three samples with a notably high total tocopherol content, EV3, EV6 and U6 at 645.4 mg/kg, 906.2 mg/kg, and 692.9 mg/kg, respectively. These samples had significantly higher levels of gamma and delta tocopherols compared to the other samples in this study and to values seen in literature for avocado oils. A study that reported on the tocopherol content in fruits and vegetables (Chun, Lee, Ye, Exler, & Eitenmiller, 2006), showed soybean oil has similar tocopherol levels and distributions to those seen in EV3, EV6 and U6, therefore, it is possible these samples contain soybean or had soybean tocopherols added after processing for preservation.

3.3. Purity parameters

Fatty acid profile is commonly used as a part of purity parameters to determine if an oil is adulterated. Table 3 shows the fatty acid profiles of all the samples which are consistent with literature with the exception of EV3, EV6 and U6. These three oils had a linolenic acid (C18:3) values of 8.2–9.8%, while one of the highest values seen in literature was 3.19% in Hass variety (Tan et al., 2017). These oils also had a linoleic acid (C18:2) content of ~55%, substantially higher than seen in the other avocado oils in this study and from literature values, which were approximately 20% (Manaf et al., 2018; Tan et al., 2017). These oils also had high stearic acid (C18:0); low oleic (C18:1) and palmitic (C16:0) acids and their values for the fatty acid profile fit in the parameters for soybean oils from the CODEX standards for named vegetable oils (CODEX, 2017). The other oils in this study all had values comparable to literature with the exception of stearic acid (C18:0), which is higher in R1, R2, R3, R7, R8, U1, U4, and U5 than has been seen previously in literature (Berasategi, Barriuso, Ansorena, & Astiasarán, 2012; Bora et al., 2001; Fernandes et al., 2018; Forero-Doria, García, Vergara, & Guzman, 2017; Noorzyanna, Marikkar, Mustafa, & Mat Sahri, 2017; Ortiz Moreno et al., 2003; Woolf et al., 2009). Samples R1, U4 and U5 also had lower palmitoleic acid (C16:1) compared to what has been reported in literature (Berasategi et al., 2012; Bora et al., 2001; Fernandes et al., 2018; Forero-Doria et al., 2017; Ortiz Moreno et al., 2003; Ozdemir & Topuz, 2004; Tan et al., 2017). These deviations seen in the fatty acid profile could be a result of economic adulteration, however, due to lack of standards, one cannot easily make such claims. To support the establishment of standards, we need to build a database that includes natural variances such as climate, varietal, and growing region can impact the fatty acid profile of avocado oil.

The sterols profile is another purity parameter often used in conjunction with the fatty acid profile. Table 4 shows the sterols in all the samples. Samples EV3, EV6, and U6 had lower value of β-sitosterol of ~55% and higher values of campesterol and stigmasterol of ~20% and ~15%, respectively, which matched the sterols profile of soybean oil according to the CODEX standards. All other oils had values comparable to what has typically been seen in literature (Fernandes et al., 2018; Jorge et al., 2015; Madawalaa et al., 2012) with the exception of R1, U4 and U5. These oils are characterized by slightly higher amounts of campesterol, stigmasterol, Δ-7 stigmaseterol and Δ-7 avensterol and lower β-sitosterol. However, it has been shown avocado oil can have a β-sitosterol content as low as 73.9 mg/kg (Berasategi et al., 2012) and changes in extraction conditions can increase campesterol to values comparable to those seen in R1, U4, and U5 (Dos Santos et al., 2014). Like with the fatty acid profile results, a standard that accommodates natural variables such as cultivar, fruit maturity, irrigation and extraction methods and discriminates pure avocado oil from adulterated one is needed in order to use sterols as a purity indicator for samples like R1, U4, and U5.

TAG profiles were determined for each oil and plotted using PCA as in Green et al. (2020) (Green et al., 2020). Fig. 4 shows samples EV3, EV6, and U6 are located around the soybean oil cluster indicating they are likely 100% soybean oil and corroborating the fatty acid and sterols profiles. All other avocado samples are in a separate group, close to the olive oils. This is expected as avocado, like olive oil, is high in TAGs containing oleic fatty acid and low in linoleic and linolenic. However, there are three samples R1, U4, and U5 are slightly removed from the other avocado oils in the cluster. These samples also have multiple values for their fatty acids and sterols profiles that are outside the range of 2xSD from pure samples in this study. This could be due to natural variance of the avocado fruits, processing conditions, or economic adulteration with high oleic sunflower or safflower oils. Preliminary analysis using the CODEX standards for vegetable oils suggested that

Table 3
Fatty acid profile expressed as percent of total fatty acids for each avocado oil.

	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C24:0
EV1	ND	16.5 ± 0.1	6.9 ± 0	0.5 ± 0	55.6 ± 0.1	19.2 ± 0.1	1.2 ± 0	ND	0.1 ± 0.1	ND	ND
EV2	0.1 ± 0	15.6 ± 0	6.5 ± 0	0.5 ± 0	61.0 ± 0	15.2 ± 0	1.0 ± 0	ND	0.2 ± 0	ND	ND
EV3	0.1 ± 0	10.9 ± 0	0.1 ± 0	4.0 ± 0	21.4 ± 0.1	54.4 ± 0.1	8.2 ± 0	0.3 ± 0	0.2 ± 0	0.3 ± 0	0.1 ± 0
EV4	0.1 ± 0	15.5 ± 0	6.4 ± 0	0.5 ± 0	59.3 ± 0.1	17.0 ± 0.1	1.1 ± 0	ND	0.2 ± 0	ND	ND
EV5	0.1 ± 0	15.6 ± 0	6.4 ± 0	0.5 ± 0	58.6 ± 0	17.5 ± 0	1.1 ± 0	ND	0.2 ± 0	ND	ND
EV6	0.1 ± 0	10.4 ± 0	0.1 ± 0	3.8 ± 0	19.7 ± 0.5	55.4 ± 0.4	9.8 ± 0	0.4 ± 0	0.2 ± 0	0.3 ± 0	0.1 ± 0
EV7	ND	16.0 ± 0	6.6 ± 0	0.5 ± 0	62.4 ± 0	13.4 ± 0	0.9 ± 0	ND	0.2 ± 0	ND	ND
R1	ND	10.0 ± 0	1.7 ± 0	2.3 ± 0	69.1 ± 0	15.2 ± 0	0.5 ± 0	0.3 ± 0	0.3 ± 0	0.4 ± 0	0.2 ± 0
R2	ND	14.7 ± 0	5.8 ± 0	1.4 ± 0	64.4 ± 0.1	12.2 ± 0	0.7 ± 0	0.2 ± 0	0.3 ± 0	0.2 ± 0	0.1 ± 0
R3	ND	13.2 ± 0	4.2 ± 0	1.4 ± 0	63.8 ± 0.1	16.0 ± 0.1	0.7 ± 0	0.2 ± 0	0.3 ± 0	0.2 ± 0	0.1 ± 0
R4	ND	15.8 ± 0	6.8 ± 0	0.5 ± 0	63.8 ± 0	12.0 ± 0	0.8 ± 0	ND	0.2 ± 0	ND	ND
R5	ND	15.0 ± 0	6.5 ± 0	0.8 ± 0	63.6 ± 0	12.8 ± 0	0.8 ± 0	0.1 ± 0	0.2 ± 0	0.1 ± 0	ND
R6	ND	17.8 ± 0	8.6 ± 0	0.6 ± 0	61.0 ± 0.1	10.9 ± 0	0.8 ± 0	0.1 ± 0	0.2 ± 0	ND	ND
R7	ND	14.4 ± 0	5.2 ± 0	1.4 ± 0	64.8 ± 0	13.0 ± 0	0.7 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.1 ± 0
R8	ND	13.4 ± 0	5.1 ± 0	1.6 ± 0	67.5 ± 0	10.9 ± 0	0.6 ± 0	0.2 ± 0	0.2 ± 0	0.3 ± 0	0.1 ± 0
R9	ND	14.1 ± 0	5.2 ± 0	1.0 ± 0	63.2 ± 0	15.0 ± 0	0.8 ± 0	0.2 ± 0	0.2 ± 0	0.1 ± 0	0.1 ± 0
U1	ND	16.5 ± 0	7.4 ± 0	1.3 ± 0	63.9 ± 0	9.8 ± 0	0.7 ± 0	0.2 ± 0	0.2 ± 0	ND	ND
U2	ND	16.4 ± 0	7.2 ± 0	0.6 ± 0	60.0 ± 0	14.7 ± 0	0.9 ± 0	ND	0.2 ± 0	ND	ND
U3	ND	16.5 ± 0	7.4 ± 0	0.6 ± 0	60.4 ± 0	13.9 ± 0	0.8 ± 0	0.1 ± 0	0.2 ± 0	ND	ND
U4	ND	10.4 ± 0	2.0 ± 0	2.1 ± 0	66.5 ± 0	17.4 ± 0	0.5 ± 0	0.4 ± 0	0.3 ± 0	0.2 ± 0	0.1 ± 0
U5	0.1 ± 0	11.2 ± 0	0.6 ± 0	2.8 ± 0	68.3 ± 0	15.4 ± 0	0.5 ± 0	0.4 ± 0	0.3 ± 0	0.4 ± 0	0.2 ± 0
U6	0.1 ± 0	10.9 ± 0	0.1 ± 0	4.0 ± 0	21.0 ± 0	54.7 ± 0	8.2 ± 0	0.3 ± 0	0.2 ± 0	0.3 ± 0	0.1 ± 0

ND = not detected. Data shown as (mean ± SEM, n = 2).

Table 4
Sterols profile for each avocado oil expressed as percent total sterols. Total sterols in mg/kg.

	Brassicasterol	Campesterol	Stigmasterol	Δ7-campesterol	Clerosterol (II)	β-sitosterol (III)	Δ5- Avenasterol	Δ7- Stigmasterol	Δ7-Avenasterol	Total Sterols
EV1	0.4 ± 0.4	5.5 ± 0	0.8 ± 0.2	ND	1.9 ± 0.1	85.6 ± 0.5	5.7 ± 0.3	ND	ND	5955 ± 110
EV2	ND	5.4 ± 0.3	ND	ND	1.9 ± 0.1	86.8 ± 0.7	5.8 ± 0.3	ND	ND	4670 ± 200
EV3	ND	20.3 ± 0.1	15.8 ± 0.1	ND	ND	56.3 ± 0.1	2.7 ± 0	2.8 ± 0.2	2.1 ± 0.3	2601 ± 75
EV4	ND	5.6 ± 0.1	0.6 ± 0	ND	1.8 ± 0	86 ± 0.3	6.0 ± 0.3	ND	ND	5649 ± 200
EV5	ND	5.8 ± 0	0.6 ± 0	ND	1.9 ± 0	85.4 ± 0.3	6.3 ± 0.3	ND	ND	5245 ± 140
EV6	ND	23.3 ± 0.1	15 ± 0.2	ND	ND	55.2 ± 0.1	3.8 ± 0.2	1.5 ± 0.1	1.3 ± 0.1	3306 ± 0
EV7	ND	6.3 ± 0	ND	ND	1.9 ± 0	86.3 ± 0.1	5.6 ± 0.1	ND	ND	4263 ± 31
R1	ND	8.6 ± 0.2	4.6 ± 0.1	ND	0.9 ± 0	75.6 ± 0.2	4.5 ± 0.2	4.3 ± 0.1	1.4 ± 0.2	2906 ± 10
R2	ND	5.7 ± 0	1.4 ± 0	ND	1.2 ± 0	85.7 ± 0.1	4.6 ± 0.1	1.5 ± 0.1	ND	3356 ± 48
R3	ND	7.6 ± 0.3	2.2 ± 0.2	ND	1.3 ± 0.1	81.4 ± 1.9	5.2 ± 0	2.2 ± 2.2	ND	3362 ± 56
R4	ND	4.9 ± 0	0.4 ± 0	ND	1.4 ± 0	87.1 ± 0	5.6 ± 0.1	ND	ND	3850 ± 3.0
R5	ND	5.6 ± 0	0.9 ± 0	ND	1.3 ± 0	86.0 ± 0	5.2 ± 0	0.5 ± 0	ND	3926 ± 14
R6	ND	6.3 ± 0	0.6 ± 0	ND	1.5 ± 0	86.5 ± 0.1	5.1 ± 0.1	ND	ND	3553 ± 25
R7	ND	5.8 ± 0	1.3 ± 0	ND	1.2 ± 0	87 ± 0.1	4.8 ± 0.1	ND	ND	3344 ± 74
R8	ND	6.1 ± 0.1	2.5 ± 0	ND	1.3 ± 0.1	81.1 ± 0.3	4.6 ± 0.2	3.4 ± 0	1.2 ± 0.1	3168 ± 170
R9	ND	9.1 ± 0	2.1 ± 0	ND	1.4 ± 0	81.4 ± 0.1	5.9 ± 0.1	ND	ND	4125 ± 73
U1	0.4 ± 0.4	6.0 ± 0	0.6 ± 0.2	ND	1.2 ± 0	88.4 ± 0.4	3.5 ± 0.2	ND	ND	2859 ± 70
U2	ND	7.7 ± 0.6	1.1 ± 0	ND	1.6 ± 0	83.5 ± 1.1	6.0 ± 0.5	ND	ND	4066 ± 250
U3	ND	6.8 ± 0.2	1.1 ± 0	ND	1.6 ± 0	84.9 ± 0	5.6 ± 0.2	ND	ND	4340 ± 69
U4	ND	10.1 ± 0.1	3.8 ± 0.1	0.7 ± 0	0.9 ± 0.1	74.7 ± 0	4.6 ± 0.2	4.0 ± 0.2	1.2 ± 0.1	3341 ± 95
U5	ND	9.2 ± 0.1	4.8 ± 0.1	ND	ND	77 ± 0.2	3.6 ± 0.1	4.2 ± 0.2	1.2 ± 0.1	3465 ± 66
U6	ND	20.6 ± 0.2	16.2 ± 0.4	ND	ND	56 ± 0.4	2.5 ± 0.3	2.8 ± 0.3	1.8 ± 0.1	2678 ± 130

ND = not detected. Data shown as (mean ± SEM, n = 2).

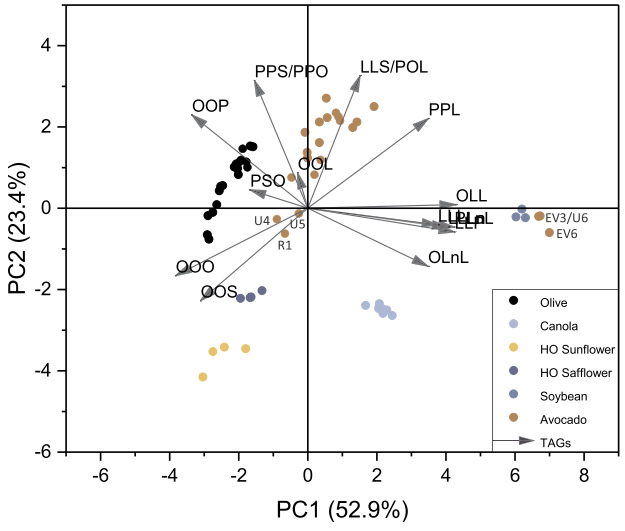

Fig. 4. TAG profiles plotted using PCA. The six avocado oils that differed from other samples are labeled according to their sample codes. All other avocado oils from this study are labeled as avocado, shown in dark orange. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

50:50 adulteration of avocado oil: high oleic sunflower could yield similar profiles as samples R1, U4, and U5.

4. Conclusions

This study demonstrates, for the first time, there are problems in both quality and purity in the store-bought extra virgin and refined avocado oil. The majority of the samples were of low quality with five of the seven oils labeled as "extra virgin" having high FFA values and six of the nine "refined" oils had high PV. FFA, PV, and specific extinction in UV data demonstrated that these oils have undergone lipolysis and oxidation, respectively. This likely resulted from improper or prolonged storage, using damaged or rotten fruits, or extreme and harsh processing conditions. Extra virgin oils often are more expensive and distinguished from lower grades such as virgin or crude oils using the above quality parameters.

Adulteration with soybean oil was found in two samples labeled as "extra virgin" avocado oil (EV3 and EV6) and one labeled as "pure" avocado oil (U6). Tocopherol, fatty acid, sterols, and TAGs data show this adulteration is occurring at or near 100% for all three samples. This not only is a potential health hazard for consumers but creates unfair competition in the market. EV3 and EV6 cost $0.65/fl oz and $0.49/fl oz, compared to the other extra virgin oils, which averaged at $1.73/fl oz. Authentic extra virgin avocado oils are clearly being outcompeted by this economically motivated adulteration. In the case of samples EV3, EV6, and U6 the adulteration was confirmed in addition to the adulteration percent and adulterant oil. However, the need for standards is also demonstrated by the samples R1, U4, and U5. The variance seen in their fatty acid, sterols, TAGs, and tocopherols profiles could be due to natural variance of the avocado fruits, processing conditions, or unnaturally, economic adulteration with high oleic sunflower or safflower oils. In order to establish fair standards, it is also imperative to know how these parameters change with varietal, harvest time, and processing conditions to determine the appropriate ranges for avocado oil, ensuring authentic products are not flagged incorrectly. This study gives a timely overview of the quality and authenticity of the avocado oils available on the US market and a call to action for the standards establishment.

Author contributions

S.W. and H.G. prepared the study. H.G. performed the experiments and both contributed to the writing.

CRediT authorship contribution statement

Hilary S. Green: Investigation, Data curation, Writing - original draft, Writing - review & editing. **Selina C. Wang:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing - review & editing.

Declaration of competing interest

The authors have no competing interests to declare.

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