A Simple and Sensitive Method for Detecting Avocado Seed Oil in Various Avocado Oils

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ABSTRACT: The presence of avocado seed oil in various avocado oils was detected by thin-layer chromatography (TLC). An unsaponifiable compound, called Component-C, was identified in the avocado seed oil. This compound is unique to avocado seed and has a blue color when developed on the TLC plate. At least 9.2 μg of avocado seed unsaponifiables were needed at a single TLC spot to detect the unique blue color definitely. Admixtures of as little as 0.16% avocado seed oil in various avocado oils can be detected by the proposed procedure.
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KEY WORDS: Avocado seed oil, thin-layer chromatography, unsaponifiables.

Avocado *(Persea americana)* is a fruit of unusually high oil content and is relatively rich in chlorophyll, the latter lending the oil an attractive emerald-green color (1). The oil is unsaturated, and the predominant fatty acid is oleic (2). Avocado oil is produced by either organic-solvent extraction or centrifugal separation (3). Mature, hard or soft, either pitted or intact fruits are used in both methods. The crude unrefined oil is used mainly in the cosmetics industry, while the refined, clear oil is generally preferred as a comestible. Previous studies, carried out in our laboratory, have shown that the presence of the lipidic fraction of the avocado seed in edible avocado oils causes growth inhibition and certain abnormalities in lipid metabolism in growing rats (4,5). Because oil from the seed may be present in some commercial avocado oils, it is in the public interest to consume oils that lack the lipidic fraction of the avocado seed. We demonstrate here a simple procedure for the detection of avocado seed oil (ASO) by identifying a unique component in its unsaponifiable fraction.

MATERIALS AND METHODS

Commercial refined avocado oil, derived from pitted fruits (Hass variety) and produced by centrifugal separation (P-RAO-C), was obtained from Avochem (Santa Paula, CA). Unrefined avocado oil, derived from either intact (I-URAO-C) or pitted (P-URAO-C) fruits (Fuerte variety) and produced by centrifugal force separation, was obtained from E. Shmueli Industries Ltd. (Ashdod, Israel). Unrefined avocado oil, derived from intact fruit (Fuerte variety) and produced by hexane extraction (I-URAO-H), was obtained from Miluot (Haifa, Israel). Fresh avocado extracts were prepared from fruit (Fuerte variety) peels, flesh, and seeds. The fruit parts and avocado leaves were freeze-dried, triturated, and extracted with petroleum ether (60–80°C) in a Soxhlet apparatus. Moisture and lipids content in the flesh and seeds were measured gravimetrically. Fatty acid profiles were analyzed by gas–liquid chromatography as previously described (3). Unsaponifiables (US) of avocado oils and extracts were prepared according to Method 6b-53 of the American Oil Chemists' Society (AOCS) (6) and measured gravimetrically. ASO admixtures were prepared by mixing various amounts of ASO with commercial avocado oils prepared from pitted fruits. Avocado oils, extracts, and admixtures were subjected to cold ethanol precipitation. Five volumes of ethanol were added, and the mixture was mixed thoroughly on a magnetic stirrer for 30 min, then placed at 4°C for 12 h, following which the precipitate was discarded and the ethanol was evaporated. Avocado oils, extracts, US, and admixtures were separated by thin-layer chromatography (TLC). Samples, 10 mg each, in 40 μL chloroform were applied as a single spot on a TLC plate and eluted with equal amounts of petroleum ether (60–80°C) and ethyl ether. Standards of β-sitosterol, α-tocopherol, squalene, β-carotene, and β-amyrin were used to characterize the US components. Components were identified by spraying the plates with a 50% (w/w) aqueous sulfuric acid solution, and color was developed at 115°C for 10 min. The content of the unique Component-C was determined gravimetrically as previously described (7).

RESULTS

The avocado flesh and seed make up 80.5 and 19.2% (w/w) of the intact fruit, respectively. Based on wet weight, the avocado flesh contains 15.10% oil, whereas the seed contains only 1.05%. Thus, the calculated concentration of seed oil in the whole avocado oil is 1.63%. Oleic acid is the predominant fatty acid in commercial avocado oils, ranging from 59 to 70%. The fatty acid profile of the ASO differs significantly from that of commercial oils. ASO contained only 30% oleic acid but substantial amounts of short-chain (<16:0), palmit-

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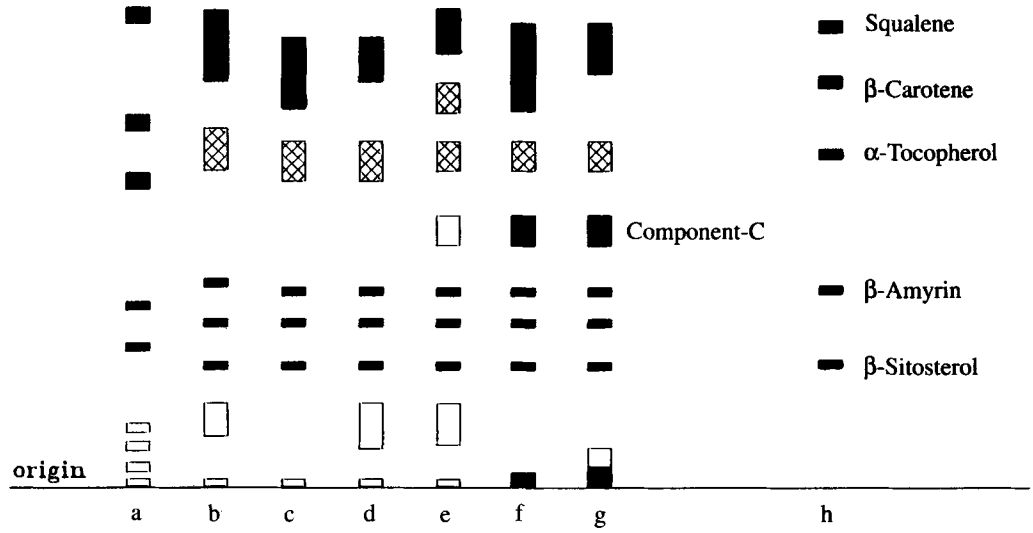

FIG. 1. Thin-layer chromatography of unsaponifiable matter from various avocado oils. Developing solvent: petroleum ether (60–80°C)/ethyl ether (1:1). Spraying reagent: 50% (w/w) H_2SO_4. P-RAO-C (Avochem, Santa Paula, CA), pitted refined avocado oil, centrifugal separation; P-URAO-C (E. Shmueli Industries Ltd., Ashdod, Israel), pitted unrefined avocado oil, centrifugal separation; I-URAO-C (E. Shmueli Industries Ltd.), intact unrefined avocado oil, centrifugal separation; I-URAO-H (Milnot, Haifa, Israel), intact unrefined avocado oil, hexane extraction: a, leaves; b, peels; c, P-RAO-C; d, P-URAO-C; e, I-URAO-C; f, I-URAO-H; g, seed; h, standards.

oleic (16:1), and linolenic (18:3) fatty acids compared with the tested oils. I-URAO-H contained only traces of the short-chain fatty acids, and they were not detected in I-URAO-C, or in oils derived from pitted fruits.

TLC separation of US revealed several fractions (Fig. 1). Part of these fractions were identified as hydrocarbons represented by squalene and β-carotene, tocopherols by α-tocopherol, triterpenes by β-amyrin, and sterols by β-sitosterol. Additionally, ASO, its US, and those obtained from oils of intact fruits (I-URAO-C and I-URAO-H) contained another fraction, designated as Component-C (7), which was located between the tocopherol(s) and the triterpene(s), and had a blue color. This Component-C, however, was not detected in the US of P-RAO-C and P-URAO-C or in the flesh, peels, or leaves. To detect the unique blue color of Component-C, at least 9.2 μg of the seed US is needed at a single TLC spot. US content in P-RAO-C, P-URAO-C, and I-URAO-C was 1.35, 1.41, and 1.57%, respectively. In contrast, I-URAO-H contained 2.06%, whereas US content in ASO reached 57.44%. Component-C content in the seed US was 6.27%, 2.84% in I-URAO-H, and only 1.24% in I-URAO-C. Table 1 summarizes the minimal concentrations of added ASO in P-RAO-C and P-URAO-C that are necessary for the detection of Component-C as a unique marker of the seed lipidic fraction. Without any treatment, Component-C was detected only after adding ASO at a final concentration of 8.2% in P-RAO-C, and 16.3% in P-URAO-C. Ethanol precipitation enabled the detection of ASO at levels of 0.82 and 1.64% in P-RAO-C and P-URAO-C, respectively. However, saponification allowed the detection of ASO in both oils, even at concentrations as low as 0.16%. In untreated oils, likely to contain the lipid fraction of the seed (I-URAO-H and I-URAO-C), to some extent, Component-C was not revealed by TLC. On the other hand, ethanol precipitation did allow the detection of Component-C, even though only in I-URAO-H, whereas after saponification the presence of the seed was verified in both oils.

TABLE 1
Minimal Concentrations (%) of ASO Added to Refined or Unrefined Avocado Oils for the Visual Detection of Component-C by TLC Before and After Ethanol Precipitation or Saponification[a]

	With ASO		Without ASO	
Treatment	P-RAO-C	P-URAO-C	I-URAO-H	I-URAO-C
Untreated	8.20	16.30	ND	ND
Ethanol precipitation	0.82	1.64	D	ND
Saponification	0.16	0.16	D	D

[a]D, detected; ND, not detected; ASO, avocado seed oil; P-RAO-C, pitted refined avocado oil, centrifugal separation; P-URAO-C, pitted unrefined avocado oil, centrifugal separation; I-URAO-C, intact unrefined avocado oil, centrifugal separation; I-URAO-H, intact unrefined avocado oil, hexane extraction; TLC, thin-layer chromatography.

DISCUSSION

There are several analytical methods whereby oils can be distinguished and characterized. Common parameters for this purpose are refractive index, specific weight, iodine number, saponification number, and US content. According to these parameters, different oils may reveal similar values. Oils also can be characterized by their fatty acid profile, but despite the relative sensitivity of this method, some oils (e.g., avocado

and olive) still demonstrate similar fatty acid profiles (8). The fatty acid profile of ASO differs significantly from that of the oil extracted from the flesh. The unique discriminator is the presence of short-chain fatty acids in ASO. Presence of the seed during hexane extraction (I-URAO-H) or centrifugal separation (I-URAO-C) yielded oils with significantly different fatty acid profiles. However, using the fatty acid profile for the detection of ASO is not recommended because it fails to reveal the presence of seed-derived short-chain fatty acids in the intact oil (I-URAO-C). The detection of short-chain fatty acids only in I-URAO-H is attributable to the more efficient oil extraction capacity of hexane. In contrast, reliance on the US composition to detect the presence of ASO proffers more promise. Some investigators noticed that US furnish a "fingerprint" of the oil, useful for the detection of foreign admixtures and any adulteration (8). Indeed, the present study demonstrated that, apart from the main US components, there is an additional component, Component-C (Fig. 1), only in I-URAO-H and I-URAO-C that contain ASO, to a certain extent. To verify that Component-C originates from the ASO, the US from avocado peel, leaves, and flesh were also separated by TLC, and indeed, Component-C was found only in the US of the seed. Its quantity comprised 3.6% of ASO, thus suggesting its possible use as an "indicator" of ASO. The ability to improve the detection of ASO impurities was tested on untreated avocado oils, and also after ethanol precipitation and saponification. ASO was added at several concentrations to refined and unrefined avocado oils (P-RAO-C and P-URAO-C, respectively). Early TLC analysis of these two untreated oils, as well as of their ethanol precipitates or US, yielded no blue spots. Hence, we reasoned that any appearance of a blue color at the relevant relative fraction migration of Component-C should indicate the presence of added ASO. The blue color of Component-C appeared only when the ASO reached levels that were five and ten times greater than the natural calculated content of ASO in the refined and unrefined avocado oils, respectively. That such high levels of ASO are necessary to detect the blue spot of Component-C is probably due to the masking effect of various lipidic fractions that have a higher relative migration distance than those of Component-C. The blue color is better detected in refined than unrefined avocado oils because, during refining, some of the masking fractions are probably removed (9). Removal of waxes by ethanol precipitation increased the ability tenfold to detect added ASO in both refined and unrefined oils (Table 1). Further enhancement was observed by saponification. We showed that the lowest concentration of ASO that could be detected was 0.16%, which is one-tenth the natural calculated content of ASO in avocado oil. However, saponification equalizes the detection ability in refined and unrefined oils. The present study demonstrated the efficacy of this method in detecting the presence of ASO in two commercial avocado oils (Table 1). Both were prepared from intact fruits, by hexane extraction (I-URAO-H) or by centrifugation (I-URAO-C). Thus, both oils are expected to contain, to some extent, the seed lipidic fraction. Yet, I-URAO-C is expected to contain less ASO because of the low extraction efficiency (70–75%) of the centrifugal procedure compared with that of hexane (85–90%) (3). TLC separation of these untreated oils was not successful in detecting the blue spot. This result was expected because untreated crude oils have to contain at least ten times the natural concentration of ASO for the characteristic blue spot to be detected. Ethanol precipitation enabled the detection of ASO only in the hexane-extracted oil. However, use of the US of these oils revealed the existence of ASO also in the centrifugally treated oil.

In conclusion, avocado oils are used in both the cosmetic and the food industries. Because ASO exerts some toxic effects, its presence in edible avocado oils is not recommended. We have described herein a simple, low-cost procedure suitable for the detection of ASO. Our procedure identifies, through TLC separation, a unique unsaponifiable component of ASO, namely Component-C, which makes it possible to detect relatively low levels of the seed oil.

REFERENCES

1. Swisher, H.E., Avocado Oil, from Food Use to Skin Care, *J. Am. Oil Chem. Soc. 65*:1704–1706 (1988).
2. Rainey, C., M. Affleck, K. Bretschger, and P.B. Alfin-Slater, The California Avocado. A New Look, *Nutr. Today 29*:23–27 (1994).
3. Werman, M.J., and I. Neeman, Avocado Oil, Production and Chemical Characteristics, *J. Am. Oil Chem. Soc. 64*:229–232 (1987).
4. Werman, M.J., S. Mokady, I. Neeman, and L. Auslander, The Effect of Avocado Oils on Some Liver Characteristics in Growing Rats, *Food Chem. Toxicol. 27*:279–282 (1989).
5. Werman, M.J., I. Neeman, and S. Mokady, Avocado Oils and Hepatic Lipid Metabolism in Growing Rats, *Ibid. 29*:93–99 (1991).
6. *Official and Tentative Methods of the American Oil Chemists' Society*, 3rd edn., American Oil Chemists' Society, Champaign, 1974, Method Ca 6b-53.
7. Werman, M.J., S. Mokady, and I. Neeman, Partial Isolation and Characterization of a New Natural Inhibitor of Lysyl Oxidase from Avocado Seed Oil, *J. Agric. Food Chem. 38*:2164–2168 (1990).
8. Gutfinger, T., and A. Letan, Studies of Unsaponifiables in Several Vegetable Oils, *Lipids 9*:658–663 (1974).
9. Bailey, A.E., *Industrial Oil and Fat Products*, Interscience Publishers Inc., New York, 1951, pp. 611–672.

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