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The Sulphuric Acid Oil Digestion Method for Avocados

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There is a need for a reliable rapid method for oil determination A in avocado fruits. Lesley and Christie (4) have published on the refractometer method as related to this subject, but workers at the Florida Station (6) have found this method subject to errors ranging from 2 per cent to 40 per cent when applied to Florida grown avocados. The work here reported was therefore begun in 1935-36, and the results have been checked for two additional seasons, 1936-37 and 1937-38. The method is based on the principle of sulphuric acid digestion on which Babcock (2) based his method for the determination of butter fat in cream. Other workers have based methods of fat determination on this same principle (3, 5, 7). The range of the experiment includes Florida grown fruits of varieties of the three races of avocados, as well as three kinds of hybrids, Mexican race: Northrup, San Sebastian, Capec, Mexican seedling; Guatemalan race: Taylor; West Indian race: Trapp; and the hybrids : Winslowson (West Indian x Guatemalan, Gottfried (West Indian x Mexican), Lula (Mexican x Guatemalan). Both immature and mature fruits were included in each of the three seasons.

The procedure has been standardized and the error of the amount determined is well below *2½* per cent, if the precautions regarding the minimum amount of oil recovered under these conditions are observed. If an 18-gram, 6-inch size Babcock bottle is used, the size of the sample must be regulated so as to secure at least .40 gram of oil in order to reduce the error of the amount determined to 1 per cent. If the amount determined is .25 gram, the error is around 2 per cent.

The standard procedure was adopted after considering the factors, concentration of acid, the ratio of acid to amount of tissue, temperature of water bath, time of digestion, centrifuge speed, and time of centrifuging. More than 381 determinations were made during the 3 years, and each was checked against the standard ether extraction method. In order to compare results obtained by this method with those of the ether extraction method on the basis of oil quality, the refractive index, the iodine number, and Kreis' test for rancidity were determined for oil obtained by both methods from green and mature fruits of varieties of the three races and three types of hybrids. In every case Kreis' test for rancidity was negative for oils extracted by both methods. There was also close agreement in the case of the iodine number and the refractive index. Since the complete results will appear in the Journal of Agricultural Research, only the following brief summary of the procedure is presented here:

Apparatus:—Food chopper with peanut butter attachment; centrifuge ; water bath which can be regulated at 55 degrees C; Babcock milk test bottles 8 per cent, 18-gram, 6-inch size.

Reagents:—Sulphuric acid C.P., sp. gr. approximately 1.84, diluted to the strength of 1.5 parts sulphuric acid to 1 part of water (1.5: 1); sea sand, C.P.

Procedure:—Use a sufficient number of fruits so as to obtain a representative sample. Extract seeds by cutting fruit in half lengthwise, peel halves and pass avocado tissue through a food chopper with peanut butter attachment. Mix the ground tissue thoroughly and keep it in a closed container. Complete weighing operations as quickly as possible to avoid loss of moisture.

Weigh out *5*- to 6-gram samples of the avocado tissue into tared 50 ml beakers. Transfer the sample into a porcelain mortar with the aid of a stirring rod. Add 5 to 6 ml 1.5-1 H_2SO_4 first into the beaker to collect remaining portions of the tissue and then pour into the mortar. Add 1 gram of sea sand (C.P.) and grind to a paste. Add 15 to 16 ml more of the sulphuric acid (1.5 to 1) and mix thoroughly. Transfer the resultant mixture into an 8 per cent, 18-gram, 6-inch Babcock milk test bottle. Use a funnel with a long slender neck for this purpose. Wash the mortar and pestle with three successive portions of 6 to 7 ml of acid, transferring each portion to the bottle. The Babcock bottle holds approximately 48 ml up to the neck. Therefore, the total amount of acid added would be between 38 and 43 ml, which would bring the level of the mixture to within a short distance of the bottom of the neck of the bottle. Place these bottles in a constant temperature water bath for a period of 30 minutes at 55 degrees C. The acid digests all of the avocado tissue, leaving the avocado oil.

Separate this oil from the mixture by whirling the bottles in a centrifuge equipped with Babcock-test attachment and geared to a speed of 1500 revolutions per minute, for 5 minutes. Remove the bottles, fill to the bottom of the neck with the dilute acid, whirl again for 3 minutes. Then remove the bottles, fill to the first graduation of the neck of the bottle with the dilute acid, and whirl a final time for 1 minute. Transfer the bottles back to the constant temperature water bath for a period of 10 minutes, after which the amount of oil can be read in the neck of the bottle. If the limits of the oil cannot be distinguished easily, which happens sometimes with oil from very immature fruits, this difficulty may be overcome by the addition of a few drops of the dilute acid at 55 degrees C down the side of the neck of the bottle to separate the oil from the digested portion.

Calibration of Babcock Bottles:—Since the Babcock bottles are calibrated to read in per cent butter fat, it is necessary to determine a factor for use in calculating the amount of avocado oil. The following procedure has been found convenient: Fill a Babcock bottle with sulphuric acid (1.5: 1) so that the acid meniscus is just below the first graduation at 55 degrees C. Add avocado oil little by little until the oil approaches the first graduation. Place in a water bath at 55 degrees C for 10 minutes, transfer to a centrifuge and whirl for 3 minutes at 1500 rpm, and return to the water bath. After a period of 10 minutes read the upper meniscus of the oil. Cool the bottle to room temperature by placing the bottle under running tap water and dry the outside thoroughly with a clean dry towel. Place on analytical balance and weigh accurately to the fourth place. By means of a medicine dropper add the oil, calibration factor of which is desired, so that it occupies

from 2.5 to 5 spaces at 55 degrees C. Re-weigh and the difference in weight will l)e equal to the amount of oil introduced into the bottle. Place in a water bath at 55 degrees C for 10 minutes, transfer to the centrifuge for 1 minute at 1500 rpm and again place in the water bath. After an interval of 10 minutes read the upper meniscus. This reading minus the previous meniscus reading will be equal to the spaces occupied by the weighed amount of oil introduced into the Babcock bottle. From these data calculate the weight of oil contained in one Babcock space and use this as the calibration factor.

Dry Weight Determinations:—The amount of oil in avocados is usually expressed on a fresh weight basis for time-of-harvesting studies. If the determinations are to be expressed on a dry weight basis, 5 to 6-gram samples (in duplicate) are spread in glass weighing bottles and dried to constant weight at 62.5 degrees *C* in a vacuum oven. Twelve hours' drying is required to bring to constant weight under these conditions.

Sulphuric Acid Treatment of Ether Extracted Oils:—After the experiment was begun it was found that in the case of quite immature avocado flesh the readings were considerably higher for the ether extraction as compared with the sulphuric acid digestion method. It is known that ether extracts other substances than pure oil from plant tissues, and apparently in the case of immature avocado fruits this fraction is relatively large. It was found that when such oils were retreated with 1.5 to 1 sulphuric acid the readings were comparable to those secured by the sulphuric acid digestion method. These fat-like substances extracted with avocado oil are apparently destroyed by H_2SO_4. The following procedure was used: Samples between .75 and 1 gram of the ether-extracted oil are weighed into a Babcock bottle, and 38 to 40 cc of sulphuric acid (1.5:1) added. Shake well, transfer to a water bath at 55 degrees C for 30 minutes. Centrifuge for 5 minutes, add sulphuric acid up to the bottom of the neck, re-centrifuge for 3 minutes, add sulphuric acid up to the first graduation, and centrifuge again for 1 minute. Transfer to a water bath at 55 degrees C for 10 minutes and read the limits of the oil. Compare the results with those secured by the sulphuric acid extraction method.

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